EV685 - VIRUCIDAL ACTIVITY OF CHEMICAL BIOCIDES AGAINST MIMIVIRUS, A PUTATIVE PNEUMONIA AGENT


Universidade Federal de Minas Gerais, UFMG, Av. Antonio Carlos 6627, Pampulha Belo Horizonte/ MG CEP 31270-901 Cx Postal 486 E-mail: rafaelkcampos@gmail.com

Acanthamoeba polyphaga mimivirus (APMV), the largest known virus, has been studied as a putative pneumonia agent, especially in hospital environments. Despite the repercussions of the discovery of APMV, there has been no study related to the control of APMV and the susceptibility of this virus to disinfectants. This work investigated the virucidal activity against mimivirus of chemical biocides commonly used in clinical practice for the disinfection of hospital equipment and rooms. APMV was dried on sterilized steel coupons, exposed to different concentrations of alcohols (ethanol, 1-propanol and 2-propanol) and commercial disinfectants (active chlorine, glutaraldehyde and benzalkonium chloride) and titrated in amoebas using 22 the TCID50 value. The stability of APMV on an inanimate surface was also tested in the presence and absence of organic matter for 30 days. APMV showed a high level of resistance to chemical biocides, especially alcohols. Only active chlorine and glutaraldehyde were able to decrease the APMV titers to undetectable levels. Dried APMV showed long-lasting stability on an inanimate surface (30 days), even in the absence of organic matter. The data presented herein may help health and laboratory workers plan the best strategy to control this putative pneumonia agent from surfaces and devices.

EV707 - CHARACTERIZATION OF THE FIRST BRAZILIAN VIROPHAGE ISOLATED FROM RIO NEGRO WATER SAMPLES, AMAZON

Boratto, P.V.M., Campos, R.K., Ferreira, P.C.P., Kroon, E.G., Abrahão, J.S.

Laboratório de Virus- Depto. de Microbiologia - ICB- UFMG, UFMG, Av. Antônio Carlos, 6627 - Pampulha - C.P. 486 - CEP 31270-901 - BH,MG E-mail: pvboratto@gmail.com

Virophages are agents that use replication machinery of other virus to produce its own progeny, reducing the infectivity of these “hosts”. They are usually associated with infections caused by giant viruses (130-750nm). The first described virophage, the Sputnik virus, was discovered in 2008, in a water-cooling tower located in Paris, in association with a virus of the Mimiviridae family. Further studies described the characterization of other phycodnaviruses virophages in antarctic lakes. The purpose of this study was to prospect virophages in Rio Negro, AM, Brazil. Water samples were collected and enriched in water-rice medium and, subsequently, submitted to successive filtrations. Molecular and biological assays were performed with suspect samples, including real-time PCR, transmission electron microscopy, infectivity reduction tests and one-step-growth-curve assays using mimivirus and Acanthamoeba castellanii cell culture. The PCR results indicated the amplification of a gene fragment that encode for the proteins...
of the virophage capsid. Icosahedral structures (40nm) were observed by the electron, associated with mimivirus factories. Infectivity reduction tests and one-step-growth-curve assays showed a decrease of 99.9% of mimivirus cytopathic effects and replication, which is consistent with virophages biology. Our data indicate the isolation of the first Brazilian virophage, the fifth in the world. Although the Amazonian biome has one of the richest biodiversity in the planet, the diversity of viruses is poorly studied, as such as their ecological relationships. Based on our data and others studies, we can speculate that the virophages could participate on the biological control of giant viruses in the Amazonian biome.

**EV721 - SURVEILLANCE OF ENTEROVIRUS, POLIOVIRUS AND HUMAN ADENOVIRUS IN SAMPLES FROM SPRING WATER SOURCES IN FLORIANÓPOLIS/SC**

Nascimento, M.A., Fongaro, G., Barardi, C.R.M.

Universidade Federal de Santa Catarina, UFSC, Campus Trindade, MIP, UFSC CEP: 88040-900 E-mail: mari_aln@hotmail.com

The microbiological quality of environmental waters is still evaluated by means of fecal indicator bacteria. Human adenoviruses (HAdV) are viral agents of multiple pathogenesis and they have been found in surveys of polluted waters. Poliovirus (PV) belongs to enteroviruses group and must be monitored on environment, once the wild virus has not been eradicated and individuals immunized with attenuated oral vaccine shed vaccine-derived poliovirus in their feces, which can suffer genetic drifts and regain neurovirulence. The present study aimed to evaluate the incidence of HAdV, enteroviruses and PV in spring water sources from six sites of Florianópolis/SC, with a total of 60 samples analyzed. Four sites were located in different points of Lagoa do Peri, which is one of the main drinking water supplier in Florianópolis; one site was a surface water source used for drinking without treatment by a small school and the last one a surface water source treated by a community association before consumption. The viruses were detected by RT-PCR (enteroviruses) and qPCR (HAdV). Positive samples of enteroviruses by RT-PCR were tested for viral infectivity and distinction of enteroviruses from polioviruses by cell culture using passages on RD and L20b cells respectively. Positive HAdV samples by qPCR were submitted to DNase treatment, to check viral integrity. The results showed that HAdV was present in 95% of the analyzed samples with an average of 2.93x10^7 gc/L with 74% of integrity by DNase and enteroviruses was present in 40% of the samples. All of the enteroviruses showed to be infectious and non-polio, since they showed cytopathic effects (ECP) on RD cells and not on L20b cells. The high incidence of HAdV and infectious enteroviruses indicates contamination of these water sources with human effluents, and portrays the lack of health care, justifying the urgent need to add viral parameters in the water quality surveillance. Financial support: CNPq Universal 470808/2009

**EV770 - SPATIAL AND TEMPORAL ANALYSES OF BACTERIOPHAGES IN TWO TROPICAL MARINE ENVIRONMENTS OF RIO DE JANEIRO STATE,**
Indubitably, Earth’s ocean represents the world’s largest biosphere of marine phages. However, in a spite of the abundance little is yet known about this distribution and diversity in tropical aquatic ecosystems. Here, we evaluated for the first time marine phages and their relationship with host and environments variables in two coastal regions of Rio de Janeiro, Brazil. In this research was analyzed the presence of phages in surface seawater samples (27) collected from the eutrophic Guanabara Bay (GB) and the upwelling region of Arraial do Cabo (AC), during winter, spring and summer of 2011. A detailed study of physical-chemical and nutrients parameters were done (T=18.7-28.1°C, S=30-35, pH=7.86-8.73, Conductivity=47.1-52.9 ms, DO=5.36-12.5 mgL-1, Chl-a =2.32-22.58μg.L-1, NH4+=1.69-28.98μM.L-1, NO2-=0.14-4.15 μM.L-1, NO3-=0.12-8.15μM.L-1 and PO43-=0.06-3.29 μM.L-1). A strong positive correlation (PC) were observed for bacteria (BAC) and Chl-a (r= 0.84) in AC stations, showing the dominance of primary productivity in this region. Otherwise, in GB stations were verified a weak PC for BAC and Chl-a (r=0.36) and a high PC for BAC and P043 (r=0.70) indicating a possible “Bottom-Up” control. It was observed in summer samples the highest bacterial abundances for AC (13.4 ±0.7 x107cells ml-1) and for GB (11.8 ±0.5x107cells ml-1). These data were strongly correlated with bacterial biomass production, as measured by 3H-thymidine incorporation for AC (18.7±0.2x10-5gCl-1h-1) and GB (23.4±0.4x10-5gCl-1h-1). The 20L of seawater samples were filtered based on virus concentration through the method of adsorption and elution in polarized membranes allowing molecular analyses. In this study, PCR primers CPS1/CPS2 were successful in yielding PCR products of approximately 165 bp from virus communities concentrates from both sites studied. Besides, marine phages were also examined by TEM and until now we observed only Podoviridae family. This is the first report of marine phage in seawater samples collected from coastal regions of Rio de Janeiro and a better characterization of tropical phages diversity is still needed.
Several enteric viruses are present in aquatic environments due to contamination by sewage effluents, even in the absence of fecal coliforms, which are the microbial indicators of water quality assessment. These viruses are frequently associated to waterborne viral gastroenteritis, including group A rotaviruses (GARV). Infection by GARV is a significant public health problem, especially in developing countries. The present study aimed to investigate the presence of GARV in surface waters of the Bacia Hidrografa do Corrego de Sao Pedro (BHCSP), in the city of Juiz de Fora, Minas Gerais state, correlating with microbiological and physico-chemical parameters for water quality. From July 2011 to May 2012, 2L of surface water were collected at 8 sites along the basin, in six campaigns, totaling 48 samples. Putative present viral particles were concentrated by adsorption-elution in negatively charged membrane, followed by centrifugation. The viral RNA, extracted by the silica method, was submitted to RT-PCR for detection of the virus genome. Fecal coliforms were quantified and physico-chemical parameters (conductivity, chlorine, pH, salinity, temperature and turbidity) were determined in each site in all campaigns. The presence of genetic material of GARV was detected in 25.0% (12/48) of the studied samples. Bacteriological analyses showed that 53.7% (27/48) of analyzed water samples exceeded the values established by the CONAMA N357/05 for class 1 and 2 waters. GARV were detected in 19.0% (4/21) of the water samples considered into the values acceptable of the microbiological quality. Statistical analyzes only showed significant correlation between GARV detection and turbidity (p=0.000). The data of this study confirm that the lack of coliforms does not necessarily exclude other pathogens, such GARV and point to the need the establishment of viral parameters to assess water quality. "Financial support": CNPq, CAPES, FAPEMIG, EMBRAPA and Propesq-UFJF.
constitute an ecosystem with high biodiversity and productivity, and can even be compared to rainforests. In recent decades, coral reefs have suffered an unprecedented decline due to human intervention compromising the reef systems health. It is therefore necessary to establish the mechanisms responsible for the loss of their quality of life systems. The main objective of this study was to observe micro-organisms presence in coral Siderastrea stellata with the scope of understanding the microbial loop functioning and assess marine viruses participation in coral diseases. The samples were taken in a site within the waters of a Brazilian state park coastal seas conservation unit. The participation of marine viruses as regulators of bacterial metabolism and marine life diversity is the basis of our research on interactions between diseases of coral reefs and microbial loop. The evaluation of the presence of virus in coral was performed through epifluorescence microscopy and polymerase chain reaction (PCR) techniques with promising results for G20 primer that is a conserved fragment of the virus capsid, incorporation of H³-thymidine, virus quantification and physical-chemical analyses of water column. Results showed an amplification of DNA from marine virus demonstrating association of virus to coral. Likewise there was incorporation of H³-thymidine supporting the results obtained in quantification and PCR. There was a satisfactory result of bacterial culture which was assessed by epifluorescence microscopy that allowed to calculate bacterial total biovolume. Analyses of transmission electron microscopy (MET) was performed and genome analyses will be realized by sequencing in near future.

**EV940 - USE OF FLOW CYTOMETRY AND PLAQUE ASSAY TO DETECT INFECTIOUS HUMAN ADENOVIRUSES IN WATER MATRICES**

Moresco, V., Damazo, N.A., Barardi, C.R.M.

Universidade Federal de Santa Catarina, UFSC, Laboratório de Virologia Aplicada, MIP, CCB, Florianópolis, SC E-mail: vanessamoresco@hotmail.com

Human adenoviruses (HAdV) are one of the most prevalent and persistent enteric viruses in the aquatic environment and are responsible for several waterborne outbreaks. The current methods used to detect HAdV in water samples are usually based on molecular techniques, although these methods do not predict viral infectivity. An alternative to evaluate the presence of infectious viruses is to infect permissive cells in vitro. In order to determine if different water matrices can interfere on in vitro HAdV replication, the aim of this study was to evaluate the detection limit of HAdV2 in two water matrices: fresh water and treated drinking water using flow cytometry (FACS) and plaque assay (PFU). For both assays, A549 cells were infected with known amounts of HAdV2, using serial tenfold dilutions either in PBS or in the water matrices. FACS and PFU were performed as described by Barardi et al., 1998 and Cromeans et al., 2008 respectively, with some modifications. The results obtained in the FACS assay showed no significant differences between the HAdV2 diluted in PBS or in the water matrices. FACS and PFU were performed as described by Barardi et al., 1998 and Cromeans et al., 2008 respectively, with some modifications. The results obtained in the FACS assay showed no significant differences between the HAdV2 diluted in PBS or in the water matrices, with a percentage of fluorescent cells ranging from 32 to 1.48% according to virus
dilution, corresponding respectively to 1.30 E+06 and 1.13 E+05 infected cells/mL. PFU evaluation showed a similar detection limit of HAdV2 diluted in PBS and in treated drinking water, with viral titres of 1.4 E+03 and 1.7 E+03 PFU/mL respectively. On the other hand, the detection of HAdV2 in fresh water decreased 2 logs (9.0 E+01). This lower value is probably due to water components which can inhibit cell infection. The FACS method provided faster (72h) and more sensitive analysis when compared to plaque assay (7 days), allowing the immunodetection in the early stages of virus replication, while PFU requires cell to cell spread. These two methods will be further applied to evaluate the presence and infectivity of HAdV2 in water matrices. Financial support: CNPq Universal 471755/2011-7; CAPES

EV962 - DETECTION AND QUANTIFICATION OF HEPATITE A VIRUS (HAV) AT THE MAIN SOURCE OF WATER SUPPLYING IN BELEM, BRAZIL AND ITS RELATION WITH THE CONCENTRATION OF COLIFORMS


1. Instituto Evandro Chagas, IEC, Laboratório de Microbiologia Ambiental-Rodovia BR 316 SN, Ananindeua-Pa

2. Instituto Oswaldo Cruz-Fiocruz, IOC/FIOCRUZ, Av. Brasil, 4365 Manguinhos-Rio de Janeiro-RJ E-mail: dd_amorim@yahoo.com

The survey for waterborne pathogens is currently an important mission of the health surveillance teams, especially with regard to water intended for human consumption. Brazil lacks data concerning the occurrence of enteric viruses in public water supplies, especially about HAV which is highly endemic in the North of the country. For this study, monthly samples were collected in three points of the Utinga water reserve. The chromogenic substrate method was used for colimetric assays. Viral particles were concentrated and eluted in filtering membranes, RNA was extracted and reverse transcribed. Viral cDNA was detected by nested PCR and quantified by real-time PCR. In the collection point located at the Bolonha Lake, colimetric assays revealed values that varied from 1.97x102 (MPN/100 mL) to 7.7x103 (MPN/100 mL). HAV was detected in 4 of 12 samples by Nested-PCR, but only two of these were positively quantified by real-time PCR (average of 521,702.95 copies/µL). At the point located at the Água Preta channel, thermotolerant coliforms varied from 2.01x102 (MPN/100 mL) to 2.91x103 (MPN/100 mL). HAV was found in 6 of 12 samples, of which only one was successfully quantified by real-time PCR, yielding an average of 312,983.39 copies/µL. No samples from the point located at ETA Bolonha were positive for coliforms, nevertheless, HAV was detected in 2 samples through nested-PCR, yielding copy numbers of 285,278.89 and 44,256.35, respectively. Nested PCR proved to be more sensible to detect viral RNA. High concentrations of thermotolerant coliforms in the surface waters of the Utinga supply highlighted the need to protect this important water source from local environmental impacts,
such as the release of sewage in nature. The quality of water from the Bolonha output is seriously compromised in virological terms, highlighting the absence of an obligatory relation between the presence of coliforms and viruses in water.

**EV975 - IS IT NECESSARY TO CONCENTRATE VIRAL PARTICLES FROM SOIL MATRICES FOR MOLECULAR DETECTION OF ENTERIC VIRUSES?**


Universidade Feevale, Feevale, ERS-239, 2755, Novo Hamburgo, RS, CEP 93352-000 E-mail: rstaggemeier@gmail.com

The protocols available for recovery and detection of viral particles from soil and sediment samples are highly variable regarding in sensitivity, costs and complexity. The most of virus concentration methods are based on acid precipitation, organic flocculation, and polyethylene glycol precipitation (PEG-6000). The development of methodologies that allow for proper detection of viral pathogens in the environment is of great importance in studies of environmental virology. The present study developed a methodology for virus concentration in sediments able to extract the virus from particle material. This study compared the standard PEG concentration method with no concentration. The PEG method was used as often reported on literature and for the non-concentration technique 1 g of the solid (sediment) was added to 1 ml of Eagle's minimum essential medium (E-MEM, Nutricell; pH7,4). The solution was homogenized by vortexing for 1 minute and then centrifuged at 14,000 rpm for 10 minutes. The supernatant was used for viral DNA / RNA extraction. The identification of viral genomes present in the samples was performed by conventional PCR for adenovirus (AdV), rotavirus (RV), enteroviruses (EV). We analyzed 20 sediment samples from farms in both methodologies. Through the technique of PEG, only 3 samples were positive for AdV. Using the non-concentration technique 7 samples were positive for AdV and 6 for RV. In both techniques there was no identification of positive samples for EV. The protocol with no previous concentration showed greater sensitivity, effectiveness and shorter execution time. Furthermore, it requires low cost of reagents and equipment commonly found in laboratories, making it an attractive alternative than virus concentration soil and sediment samples. Financial support: CNPq, CAPES, FAPERGS, Feevale.

**EV977 - VIRAL DETECTION IN WATER AND SEDIMENTS SAMPLES FROM RURAL AREAS OF ROLANTE AND RIOZINHO, RS, BRAZIL**


1. Universidade Feevale, Feevale, ERS-239, 2755, Novo Hamburgo, RS, CEP 93352-000

2. Empresa de Assistência
The Rio dos Sinos watershed is located in the northeastern of Rio Grande Sul. The municipalities of Rolante and Riozinho are part of the watershed and located right above the Guarani Aquifer. Enteric viruses in the soil have the ability to migrate through it by the successive adsorption-desorption phenomena, thus, providing risk of contamination of groundwater by the ease of penetration of viral particles in the soil. Among these viruses are the adenovirus (AdV), enterovirus (EV) and the rotavirus genogroup A (GARV), which causes diseases in humans and animals. This study aimed to evaluate the presence of AdV, EV and GARV in water and sediment samples from rural properties of Riozinho and Rolante, Rio Grande do Sul, Brazil. Twenty sediment (100 g) samples and 55 water (500 mL) samples from springs, artesian wells, dams and streams were collected and submitted to extraction of DNA/RNA, followed by, when necessary, the synthesis of cDNA by reverse transcription. The viral detection for EV and GARV was performed by polymerase chain reaction (PCR), and detection for AdV by Real Time PCR (qPCR). Of the 55 water samples, 87,3% (48/55) were positive for AdV, 25,5% (14/55) for GARV and 1,8% (1/55) for EV. From sediment samples, 80% (16/20) were positive for AdV and 30% (6/20) for GARV. The results suggest intense contamination of groundwater, surface water and soil in the region, furthermore, the presence of these pathogens in soil may contribute to an increased risk of contamination of groundwater. Financial support: CNPq, CAPES, FAPERGS, Feevale.

EV984 - DETERMINATION OF THE RECOVERY EFFICIENCY OF HUMAN AND MURINE NOROVIRUS ON BERRIES

Melgaco, F.G., Victoria, M., Corrêa, A.A., Miagostovich, M.P.

1. Oswaldo Cruz Institute-Oswaldo Cruz Foundation, IOC-Fiocruz, Av. Brazil, 4365, Manguinhos, Rio de Janeiro, Brazil
2. Universidad de la República Regional Norte, unorte, Universidad de la República Regional Norte, Salto, Uruguay
3. Laboratory of Comparative and Environmental Virology, LVCA, Av. Brazil, 4365, Manguinhos, Rio de Janeiro, Brazil E-mail: fabianagilmelgaco@gmail.com

Foodborne illness is an important problem of global public health, leading to a large number of hospitalizations mainly due to acute gastroenteritis (AG). Norovirus (NoV) is the most important agent of outbreaks of AG and their identification in foods is difficult due to the complexity of the food matrix, the presence of inhibitors, low levels of viral contamination and genetic diversity of these viruses. There is not currently a single viral detection method validated internationally suitable in foodstuffs. In order to determine the efficiency of a viral concentration method for NoV by organic flocculation from samples of red fruits (strawberries), two different variables were evaluated and compared: different elution buffer (PBS 1X, pH 7.0 and Glycine 0.05M/Tris-HCl 0.1M, pH 9.5); a Becker and filter bag as containers to mix samples. Strawberries from local commercial sources were divided in 25 g samples.
Experiments on artificial contamination with NoV GII.4 and murine norovirus 1 (MNV-1) were performed by using flocculation with 1% skimmed milk. The viral RNA was extracted from 140 µL of the concentrated sample with the QIAamp viral RNA mini kit® (Qiagen). For complementary DNA synthesis, SuperScript® III Reverse Transcriptase and random primers were used; the viral detection was performed by real time RT-PCR. The best recovery for NoV GII.4 was achieved by combining elution with Tris-glycine in filter bags (3.6 to 43.3%). The recovery of MNV-1 eluted with PBS buffer in Becker presented the best recovery (14.7 to 50.5%), probably due to the presence of pigments and pH value, as well as inhibitors of enzymatic reactions. The establishment of methods for viral concentration from food matrices will assist epidemiologic investigations of outbreaks associated with NoV, previously hampered by the lack of appropriate methodologies, assisting in mapping the routes of transmission and demonstrating the importance of these viruses in the occurrence of foodborne outbreaks. Financial support: POM LVCA, CNPq, APQ1/FAPERJ.

EV1057 - ROTAVIRUS DETECTION IN DIFFERENT AQUATIC ECOSYSTEMS FROM THE METROPOLITAN AREA OF BELEM, PARA, BRAZIL


2. Instituto Evandro Chagas, IEC,

Introduction: The concentration of bacteria of the coliform group often determines the microbiological quality of water for consumption; however, minimum standards of bacteria do not indicate the absence of virus. The enteric viruses are highly stable in the environment while maintaining their infectivity even after exposure to treatment processes. These viruses excel at etiology of waterborne diseases such as acute gastroenteritis, especially among children under five years of age being the rotavirus (RV) considered the major cause of these diseases. Considering that the metropolitan region of Belém, Pará State, has a great influence of rivers, lakes and streams, this emphasizes the importance of researching RV in water samples from different ecosystems. Methods: Water samples were monthly collected from six points of the metropolitan region of Belém (Port of Açai, Port of Ver-o-Peso, Tucunduba River, Black Water Lake Canal, Bolonha Lake and UNA sewage), from November 2008 to October 2010 and 24 samples were collected from each point. The search of RV was performed in duplicate by polymerase chain reaction in real time using the region of the NSP3 gene as target. Results: Of the 144 water samples analyzed 51.4% (n=74) were positive for RV with no difference between the frequencies of two years. The major and minor events were observed in October (n=12) and January (n=2), respectively. The place with the higher number of positive samples was the UNA sewage with 75% (n = 18), followed by Tucunduba River with 54.16% (n=13) in addition to the Port of Ver-o-Peso.
and Bolonha Lake with 50% (n = 12), each. Conclusion: The high presence of RV in important aquatic ecosystems for the metropolitan region of Belém alert to the need of monitoring viral contamination of these sites in order to improve water quality and consequently the health of the population. Financial support: PIBIC-UFPA/FAPESPA/CNPq, PIBIC-IEC/FAPESPA/CNPq, Fundação de Amparo a Pesquisa do Estado do Pará - (FAPESPA).

**EV1163 - KINETICS OF VIRAL VIABILITY DECAY IN OYSTERS CRASSOSTREA GIGAS SUBJECTED TO DEPURATION UNDER UV TREATMENT**

Souza, D.S.M., Fongaro, G., Pilotto, M.R., Moresco, V., Delfino, N., Barardi, C.R.M.

Universidade Federal de Santa Catarina, Laboratório de Virologia Aplicada, MIP, Florianópolis, SC - E-mail: dorissms@hotmail.com

Mollusks depuration using ultraviolet (UV) disinfection can be employed for their decontamination before consumption. In the present study, oysters remained 14 days in four different places in the Florianópolis Bay, SC, in order to be naturally contaminated by a variety of microorganisms and particles. The strategy for oysters allocation was: two sites approved by Brazilian regulations for oyster’s cultivation (1 and 2) and two sites highly impacted by pollution (3 and 4). The aim of this study was to evaluate the interference of a diversity of contamination on the viral depuration of these oysters using UV as disinfection method (43.85mJ/cm²). Plaque forming unit (PFU) assay was the method of choice for viral infectivity evaluation. As positive controls, some animals were artificially contaminated with known amounts of Human Adenovirus 2 (HAdV2) and Murine Norovirus 1 (MNV1) after 14 days in each selected environment. All the oysters were distributed in depuration tanks and analyzed after 96 and 168h of depuration. Three oyster/time were collected, the tissue extracts were produced and inoculated, in non-cytotoxic dilutions, either in A549 or in RAW cell monolayers for HAdV2 or MNV1 quantification respectively. After 96h in all sites, HAdV did not show significant reduction; after 168h, reduction was of 1log (90%) on sites 1, 2 and 3 and 2 logs (99%) on site 4. MNV reduction was of 3 logs (99.9%) after 96h on sites 1 and 3 and less than 1 log on site 4. After 168h, MNV1 was not detected in all the samples. The results showing a higher disinfection of HAdV in oysters from site 4 could be false negatives since the great amount of contaminants on the oyster’s meat can inhibit cell infection. These previous results allowed us to conclude that HAdV was more resistant than MNV to disinfection by UV on depuration tanks. Further studies are necessary to support these conclusions. Financial support: MAPA/CNPq/2008-2; Universal 2009 MCT/CNPq; MCT/CNPq CT-Agronegócio/MPA nº 25/2010 - Formação de Recursos Humanos em Pesca e Aquicultura

**EV1168 - PRESENCE OF ADENOVIRUS (ADV) IN SAMPLES OF HUMAN FECES FROM PATIENTS IN THE REGION OF THE SINOS RIVER WATERSHED, SOUTHERN BRAZIL**

Enteric viruses are distributed worldwide and are responsible for different diseases that affect humans, such as gastroenteritis, conjunctivitis, pneumonia and hepatitis infections. Outbreaks of viral gastroenteritis have been reported, having been the adenovirus (AdV) found in high concentrations in feces of infected individuals. The main route of transmission of these pathogens is the fecal-oral route, however, an alternative route of transmission from environmental samples, including, drinking water, has been increasingly considered a risk to public health. The man is usually affected by virus diseases of human origin, however, may be an intermediate host of viral agents of animal origin as Canine AdV (CAV-1 and -2), Bovine AdV (BAV) and Avian AdV (EDS-76). The objective of this study was to identify viral particles AdV and their species, in samples of human feces. A total of 150 feces samples were collected during the winter 2011 and summer of 2012, derived from patients aged mixed in Esteio municipality. From the extraction of viral DNA, viral detection was performed by Polymerase Chain Reaction in Real Time (qPCR) for AdV. The results showed that 36% (55/150) of samples were positive for EDS, 46% (70/150) for CAV, 14% (22/150) for BAV, 6% (9/150) for HAdV and 26% (39/150) for AdV species is unknown. The maximum amounts of DNA copies / ml for the EDS were 9.4 x 10^7 x 6.7 for 108 CAV, to BAV 7.6 x 10^7 to 5.33 x 10^7 HAdV and to AdV undetermined species , 42 x 10^7. In addition, it was possible to identify co-viral infection in 45% (68/150) samples, and 21% (32/150) had no positivity for any AdV. In addition, the infection rate was higher in the summer (72%), compared to the winter (57%). The results suggest a great spread of adenovirus not restricted to host specificity, but related to the means by which is shed through the environment. Those infections may have occurred, either by consumption of contaminated water or contact with other species carrying this agent. Financial Support: FAPERGS, CAPES, CNPq.
are host specific in most cases and highly resistant to conventional treatments for drinking water. Adenoviruses (AdV) are frequently found in environmental samples, and their presence in water may indicate contamination from human or different animal sources since they are species-specific. The objective of this study was to detect these viral agents and their species in samples of treated water for public supply. A total of 60 samples were collected monthly from 10 Water Treatment Plants (WTP), located along the Rio dos Sinos watershed, from March to December 2011. The viral genomes were detected using the qPCR technique (Polymerase Chain Reaction in real time) and species differentiation was made using high resolution melting after the amplification steps (qPCR-HRM). The primers used were originally designed and are capable of binding to conserved regions from different species of AdVs (human, canine, bovine, swine, and avian). A total of 23.3% of the samples (14/60) were positive for AdV avian, 8.3% (5/60) for AdV canine and 11.6% (7/60) for AdV species is unknown. There was co-contamination in 26.6% of the samples (16/60) and 30% of samples (18/60) were negative for any AdV. The results confirm the spread of adenovirus and its species in treated water at the watershed level. In this sense, it may be of fundamental importance to invest in new technologies for water and sewage treatment in order to reduce the incidence of these agents and its impacts on water quality. Financial Support: FAPERGS, CAPES, CNPq.

**EV1218 - VIRAL CONTAMINATION OF SURFACES AND OBJECTS IN A HOSPITAL FROM RIO GRANDE DO SUL**

dos Santos da Silva, V.S., Henrique de Mello, M., Staggemeier, R., Fabres, R., Soliman, M., Bianchi, E., Rodrigues, M.T., Spilki, F.R.

Universidade Feevale, FEEVALE, RS-239, 2755 - CEP93352-000 - Novo Hamburgo, RS E-mail: joseanesilva@feevale.br

Viruses can invade the human body through the conjunctiva, genital tracts, intestinal or respiratory tracts. Enteric viruses have been increasingly recognized as causes of nosocomial infections (NI), however specific studies on viral pathogens on hospital surfaces rarely occur. This study aimed to detect by molecular methods Human adenovirus (HAdV) and Rotavirus A (RV-A) on hospital surfaces and to establish a preliminary estimate of the rate of occurrence of these viruses on surfaces in hospitals. 32 samples were collected at hospital clinics, emergency room and surgical ward. Each point was defined based on the possibility of contact of the hands of health workers (such as light switches, table for medication preparation, bench tops, computer mouse, sinks for hands washing, handle, anesthesia cart and support desks in surgical room). The samples were subjected to molecular biology methods, having gone through the steps of extracting nucleic acid, cDNA, PCR / qPCR and electrophoresis. From the 32 samples analyzed, 20/32 (62.5%) were positive for HAdV by qPCR method. None of the samples were positive for the presence of RV-A. It was concluded that HAdV can be considered a biological marker of contamination of the hospital environment, and highlights the importance of hand washing and the
use of aseptic techniques and standard operating procedure as a way to prevent infection of a susceptible host by this virus.

**EV1313 - STABILITY OF HUMAN ENTERIC VIRUSES IN SEAWATER SAMPLES FROM MOLLUSKS DEPURATION TANKS COUPLED WITH ULTRA-VIOLET IRRADIATION**


Universidade Federal de Santa Catarina, UFSC, 88040-970 Florianópolis, Santa Catarina, Brasil
E-mail: lucasabu@gmail.com

Viruses have been associated with episodes of illnesses related to the consumption of contaminated shellfish, and this risk may be reduced by mollusk depuration. In this work, the stability of human adenovirus, murine norovirus and hepatitis A virus in natural seawater in a closed system depuration tank with and without ultra-violet treatment was investigated. Three hundred liters of seawater was artificially seeded with these viruses and disinfected using a 36 W lamp. Samples of 1 L of seawater were collected at 24, 48, 72, 96 and 120 h, and viral particles were concentrated by the skimmed milk flocculation method. The viral decay was evaluated by quantification of the genome copy number by quantitative real time PCR and quantitation of infectious viral particles (except for hepatitis A virus) in three independents assays. Based on the molecular detection results, there was a 5log10 and 3log10 reduction in viral load for human adenovirus and hepatitis A virus after 120 h of ultra violet treatment, respectively. For murine norovirus, a 4.5log10 reduction was observed at 72 h. Cell culture results, similar to those of the molecular detection assays, showed that murine norovirus was not detected after 72 h of treatment, while human adenovirus remained infectious for up to 72 h. Assays of viral stability without ultra violet irradiation demonstrated a progressive 1.5 to 2.5 log10 reduction in the number of viral particles after 120 h of seawater recirculation for the three viruses tested. In conclusion, the ultra violet treatment effectively reduced the number of viral particles in seawater, and the natural decrease in the concentration of each virus could be due to seawater composition, viral aggregation and the existence of environmental factors such as ionic strength and compounds naturally found in seawater.


**EV1349 - LONGITUDINAL SURVEY OF HUMAN ADENOVIRUS ON TREATED AND UNTREATED WATER FROM RIO DOS SINOS, RIO GRANDE DO SUL, BRAZIL**


UNIVERSIDADE FEEVALE, FEEVALE, ERS-239, 2755 , Novo Hamburgo, RS , CEP 93352-000 E-mail: manu@feevale.br

Enteric viruses transmitted by fecal-oral route, such as adenovirus (ADV) are associated with various pathologies such as acute gastroenteritis, especially...
in children under four years old. ADV viruses belong to the family Adenoviridae, possessing a double-stranded DNA genome and are non-enveloped viruses. The present study investigated the occurrence of human ADV samples of water from the Rio dos Sinos watershed, the major source of public water supply for a population of approximately 1.5 million people. From July to December 2011, samples were collected in 500 mL of water treatment plants along seven points of the Rio dos Sinos river and affluents. A total of 78 samples were analyzed, being 39 samples of untreated water and 39 samples of treated water. The samples were concentrated to isolate the viral genome with a membrane filtration system with negatively charged, followed by extraction and amplification of DNA by qPCR (polymerase chain reaction in real time). The viral genome was detected in 76.9% (30/39) of treated water samples and 74.3% (29/39) samples of untreated water. In general, the number of copies were elevated in both types of samples for all points analyzed, which the highest values reaching 7.02 x 10^9 copies of DNA per ml of treated water and 3.77 x 10^10 DNA copies per mL for untreated water. The results show that the presence of these viral agents may overpass the water treatment systems. Financial Support: CAPES, CNPQ, FAPERGS, FEEVALE.

Y.B.

1. SAVIR, Instituto Evandro Chagas, IEC, Br 316, Km 7, Leibilândia, Ananindeua, PA SAMAM, Instituto Evandro Chagas, IEC, Br 316, Km 7, Leibilândia, Ananindeua, PA

2. Universidade Federal do Pará, NMT, UFPA, Generalissimo Deodoro, Umarizal, Belém, PA E-mail: dielleteixeira@gmail.com

Norovirus (NoVs) and Human Astrovirus (HAstV) are associated with gastroenteritis outbreaks worldwide and their presence in aquatic environment, principally in source waters, represent a great risk to human health. The continuous monitoring of these waters is extremely important. This study aimed the monitoring of a water supply system composed for two lakes (Bolonha e Água Preta) and a water treatment plant (WTP), which are located in the Utinga Environmental Park, Belém, PA, responsible for water supply of the Metropolitan Region of this city. Sample collection occurred monthly, from November 2010 to October 2011, in each lake and in the output WTP. The viruses were concentrated from 2L of each water samples by adsorption-elution, followed of RNA extraction by silica method. NoVs detection was carried out using semi nested RT-PCR and real time PCR. The semi nested was performed using in the first step the primers JV13I/JV12Y, and in the second the pairs JV13I/GI and JV12Y/NoroII-R specific for GI and GII, respectively. Specific primers and probes, targeting the ORF1-2 junction region were used to detect NoV GI and GII in the real time PCR. For HAstV detection, the cDNA obtained after reverse transcription was subjected
to conventional PCR using the primers 269/270. A total of 36 samples were analyzed for NoVs and HAstVs. In semi nested, NoVs was present in 25% (9/36), all belonging to GI, except two samples in which the co-circulation of both genogroups was found. Only one sample was classified as NoV GII by real time, which was also detected by semi nested. The nine NoVs positive samples were obtained from lakes Bolonha (n=4) and Água Preta (n=5). HAstV were not found in any sample. Our results showed the circulation of NoVs in the two main lakes located in Belém, indicating the existence of a fecal contamination source in these lakes. However, all samples from WTP’s output were negative demonstrating that the treatment applied has been effective. Financial support: Fundação de Amparo à Pesquisa do Estado do Pará (FAPESP), IEC/SVS/MS

EV1359 - ENVIRONMENTAL SURVEILLANCE OF ENTEROVIRUSES FROM SEWAGE WATER IN RIO DE JANEIRO: A PRELIMINARY ANALYSIS

Pereira, J.S.O., Costa, E.V., Silva, L.R., Silva, E.M., Da Silva, E.E.

Fundação Oswaldo Cruz/Laboratório de Enterovírus, Fiocruz, Av. Brasil, nº 4365, Pav. Hélio e Peggy Pereira, sala B211, Manguinhos - RJ. E-mail: jpereira@ioc.fiocruz.br

Human enteroviruses are primarily transmitted via the fecal-oral route, by direct contact with virus shed from gastrointestinal tract of infected individuals. Detection of these viruses in river waters and sewage can be an alternative method to monitor circulating viruses in humans and the environment. This study was carried out to evaluate the presence of enteroviruses in a sewage treatment plant (ETE Alegria/CEDAE) located in the city of Rio de Janeiro, Brazil. From December 2011 to June 2012, 14 samples were collected weekly by Grab Sample method and tested using three concentration methods: Adsorption by Chloride of Sodium, Two-phase Separation and Silica Carrier. Following virus isolation using RD and L20B cells, isolates were identified and typed by RT-PCR. Enteroviruses were isolated from 14 (100%) of specimens. A total of 7 out of 14 isolates were positive for polioviruses (Sabin 2 = 6 isolates, Sabin 3 = 1 isolate) while the others were non-polio enteroviruses. Up to now, three out of 7 non-polio enterovirus isolates were analyzed in more detail by sequencing a fragment of the gene coding for the viral protein, VP1. The following enteroviruses were identified: 1 Echovirus 6, and 2 Echovirus 7. Environmental surveillance has been used successfully in monitoring enteric virus circulation and is of crucial importance at the final stages of the WHO global polio eradication initiative. These results show a continuous presence of Sabin-related poliovirus and non-polio enteroviruses in the analyzed area. Financial support: CNPq, CGLAB/MS, Fiocruz.

EV1385 - REMOVAL OF ADENOVIRUSES FROM ACTIVATED SLUDGE USING ORGANIC AND INORGANIC COMPOUNDS


Universidade Fevale, Fevale, ERS-
Environmental Virology: EV

239, 2755 | Novo Hamburgo, RS | CEP 93352-000 E-mail: rafafabres@hotmail.com

The effluent treatment systems are composed by the integration of methods of treatment. The system is usually divided into preliminary treatment, primary, secondary and possibly tertiary. The tertiary treatment is not always used, can be accomplished by physical and chemical processes such as coagulation/flocculation/decantation and is particularly good alternative for the removal of heavy loads coliforms. In this study we compared the effects of removal of enteric viruses, including nine coagulants, at 100 ppm and 1000 ppm each, aluminum sulfate with and without iron, ferric chloride, polyaluminum chloride (inorganic) commonly used coagulants in water treatment plants (WTP), and commercial formulations of tannin,WW ACQUAPOL®, ACQUAPOL® C1 18, ACQUAPOL® oF 18, ACQUAPOL® T832, ACQUAPOL® 893/11 (organic compounds). Enteric viruses have the characteristic of being resistant to the methods currently used in Brazil for treating sewage, being of importance use for monitoring water quality along with the detection of total and fecal coliforms. In the present study used the adenovirus (ADV) gastroenteritis-causing and disease in humans. Samples were collected from 50 L of sewage soon after the preliminary treatment and 50L of treated sewage in activated sludge (secondary treatment). Samples were collected in duplicate, one in March in the year 2012 and another collection in May of that year, the sewage treatment plant in Canoas, RS. Coagulation tests were performed using a jar-test system. Then, its RNA were extracted from which was subjected to polymerase chain reaction in real time (qPCR), using primers to conserved regions of potential alignment in the genome of the viral species, corresponding to the hexon protein gene of AdV, HAdVCf (VTB2Fw; 5’-GAGACGTACTTCAGCCTGAAT--3’) and HAdVcr- (VTB2Rev; 5’-GATGAACGCAGCGTCAA-3’). Of 54 samples analyzed, the viral copy number diminished in 41 (76% of total samples). The better results were observed for the inorganic coagulant, ferric chloride, which can remove all of the enteric viruses. In the case of organic coagulant was the most effective ACQUAPOL® WW. Support: CNPq, Fapergs, Seta S/A, Universidade Feevale, Çapes

EV1476 - EFFICACY OF AUTOMATED COMPOSTING FOR REMOVAL OF ADENOVIRUSES FROM SWINE MANURE


1. Universidade Feevale, FEEVALE, Rodovia RS 239, no. 2755, Vila Nova, CEP: 93352-000 - Novo Hamburgo, RS, Brazil

2. Universidade Federal de Santa Maria, UFSM, Av. Roraima nº 1000 - Cidade Universitária, CEP: 97105-900, Santa Maria - RS E-mail: mayra_soliman@hotmail.com

The swine husbandry is a potentially polluting activity to the environment due to pollutants that may be contained in their effluents. Aiming to reduce the environmental impact, there are several...
manure treatment systems, including automated composting. However, little is known about the efficiency of this method for the removal of microorganisms. Among the possible microorganisms present in manure are the adenoviruses (AdV), members of the Adenoviridae family, consisting of double-stranded DNA genome, are often found. The purpose of this work was to evaluate the efficiency of automated composting system in the elimination of AdV from swine manure. The adenoviral species present on swine manure were characterized through the amplification by real-time PCR followed by a differential step of high resolution melting. (CanineAdV, AvianAdV, BovineAdV, HumanAdV, and of course Porcine AdV were detected and quantified before and after composting. An automatic composting unit was developed the manure applied was from the swine termination units and wood shavings as substrate. The frequency of application of the waste in the windrow and its revolving occurred each five days. Twelve samples were collected from liquid waste before its addition to the substrate and six samples the compost (waste and wood shavings) for viral analysis. The samples were diluted with Minimum Essential Medium (MEM), to be held the extraction of viral DNA and the polymerase chain reaction in Real Time (qPCR). The reaction utilized primers Mastadeno AdV (F1-5'GCAGTGGTCGTACATGCACAT-3', 5'-TCGGTGGTGACGTCGTGG R1-3') which allowed the detection of different species AdV. These primers were designed from the sequences of full target viral genes, with the aid of the Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). From the twelve samples analyzed before composting, all were contaminated by BAV, whereas the compost resulted negative for the different species of AdV. These results indicate the efficiency of the process. Further experiments will be conducted to assess the efficacy of treatment allowing the use of slurry for agronomic and avoiding contamination of water sources.

EV1486 - DETECTION OF BK AND JC POLYOMAVIRUSES BY NESTED-PCR FORM THE ENVIRONMENTAL ISOLATES OF ACANTHAMOEBA SP

Comerlato, J., Arantes, T., Kulman, M., Caumo, K., Campos, F., Roehe, P.M., Franco, A.C.A.

Universidade Federal do Rio Grande do Sul, UFRGS, Sarmento Leite, 500/208, Centro Porto Alegre, RS E-mail: jucomerlato@gmail.com

The human polyomaviruses BK and JC are double-stranded, naked DNA viruses. They are transmitted mainly through urine-oral route and up to 80% of the human population excretes one or both viruses in the urine. Immunossupression induces virus replication and nephropathy, one of the main causes of graft rejection in renal transplant patients. Acanthamoeba sp are free-living and ubiquitous amoebae found in air, soil and aquatic environments. They are resistant to disinfection procedures and responsible for the transmission of different pathogens to humans (e.g. Legionella sp and fungi). This study aims to evaluate the presence of BKV and JCV DNA in isolates of Acanthamoeba sp obtained from environmental samples. Fifty samples of Acanthamoeba sp, isolated from swimming pools and dust of hospital
were obtained and morphologically characterized. The isolates were submitted to DNA extraction and subsequently detection of BKV and JCV DNA by nested-PCR. The first PCR targeted a DNA fragment shared by both viruses. The JCV PCR was performed with the following primer pairs: JLP16 (PF 5’-TAAAGCCTCCCCCAACAGAAA-3’ and JLP15 (PR 5’-ACAGTGGCCAGAATTCACCTACC-3’), expected to give rise to a JCV product that is 215 bp long. To detect BKV, the primer pair BK6 (PF 5’- CCAAGGCGAGCTCCCCAAAAAG-3’) and BK4 (PR 5’-AGTAGATTCCACAGGTAGTCCTC-3’) was employed to give rise to a 296 bp long amplicon. Of the 50 isolates analyzed, 28% (15/50) amplified a fragment compatible with BKV and 24% (12/50) with JCV DNA. The presence of BKV and JCV in Acanthamoeba sp may indicate the ability of this microorganism to promote the spread of viruses already widely resistant in the environment as human polyomaviruses. Finep, Capes, CNPq