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HOUSEHOLD-BASED BIODIGESTERS PROMOTE THE REDUCTION OF ENTERIC VIRUSES AND BACTERIAS IN VULNERABLE AND POVERTY RURAL AREA

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Resumo

The present study evaluated the river water quality improvement by implementation of household-based biodigesters in vulnerability and poverty rural area, in Minas Gerais State-Brazil. Biodigesters were installed for domestic wastewater treatment. Wastewater was collected before and after treatment and the physicochemical parameters and pathogens removal (human adenovirus (HAdV), hepatitis A (HAV) virus, *Salmonella* sp. and *Escherichia coli*) were evaluated; Additionally, river water was sampled before and after the household-based biodigesters implementation, to verify the contamination reduction and the positive impact of domestic wastewater treatment on waterborne pathogen reduction, considering HAdV, HAV, *Salmonella* sp. and *E. coli* quantification. The applicability in real-scale of decentralized treatment systems using household-based biodigesters promoted reduction of 90, 99, 99.99 and 99.999% from HAV, *Salmonella* sp., *E. coli* and HAdV from domestic wastewater, respectively; The river water quality improvement before the wastewater treatment application was highlight in the present study, considering that the reduction of waterborne pathogens in this water in 90, 99.99 and 99.999% of *E. coli*, HAV and HAdV, respectively (*Salmonella* sp. was not detected in river water). In general, this is an important study for encouraging the decentralized sanitation in vulnerable and poverty area, as well in rural sites, considering the positive impact of this implementation on public health.

Palavras-chaves: Public health, Waste Treatment, Circular Economy, One Health

MICROBIOLOGICAL EVALUATION OF MAMPITUBA RIVER - TORRES / RS

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Resumo

Mampituba River is in the northeast of Rio Grande do Sul, and its waters are used for rice irrigation, fishing, tourism and recreation. The assessment of bathing is performed through Resolution Nº 274/2000 of the National Council of the Environment (CONAMA), and the presence of bacteria such as total coliforms (TC), *Escherichia coli* (FC) and *Enterococcus* spp. (ENT).Other microorganisms are also present in water, such as enteric viruses. *Human Mastadenovirus* (HAdV) belongs to *Adenoviridae* family, with double stranded DNA and absence envelope. Objective of this study was to evaluate the presence of TC, FC, ENT and HAdV-C. Eight points (P) were delimited, P1 is closer from river mouth e P7 from river source and 500ml of water were collected in December 2018, January and February 2019. For bacterial analysis of TC, FC and ENT,100mL aliquots were tested by Colilert® and Enterolert® (IDDEX) kits. The presence of HAdV was evaluated appling the ultracentrifugation method, with the extraction of genetic material by the commercial kit BioPur® followed by Real-Time Polymerase Chain Reaction (qPCR) targeting the hexon protein of the capsid. All groups of bacteria were positive at all points and collections, while the presence of HAdV-C showed variation. TC established all points in all collection as unfit for recreation, with quantification >2000/100ml. In December, FC above acceptable values at one point (P1) and ENT at two (P1, P5). Furthermore, in 3 points ENT (P5, P6, P7) was higher than FC, and HAdV was only negative at 1 point (P7). In January, 5 points (P3, P4, P5, P6, P7) ENT was highest than FC. FC above acceptable values at 1 point (P1) and ENT at 3 (P1, P4, P5).HAdV was positive in 3 points (P1, P2, P7).In February, ENT established all points as unfit for recreation, in addition to FC above acceptable values at 2 points (P1, P2). In 4 points (P4, P5, P6, P7) ENT was greater than FC, and HAdV was positive at 4 points (P1, P2, P3, P4).At some points it was possible to observe the relationship between the high prevalence of bacteria and virus positivity, as well as the increase of all parameters in the month of February.From the analysis of the samples with different indicators it was possible to observe the low microbiological quality of the water highlighting the presence of viral indicator and the relation with levels of bacteria, being important to emphasize that ENT was more efficient compared to the FC. Financial Support: CNPq, Feevale University.

Palavras-chaves: Bacteria, HAdV, Surface water, qPCR

MICROBIOLOGICAL EVALUATION OF WATER AND SAND OF NORTH RIO GRANDE DO SUL BEACHES

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Resumo

North coast of Rio Grande do Sul has economy associated with tourist activity. The assessment of water bathing had done by Resolution of the National Environment Council (CONAMA) Nº 274/2000, which uses markers such as total coliforms (TC), *Escherichia coli* (FC) and *Enterococcus* spp. (ENT) to define fecal contamination. Enteric viruses are present in waters and sand too. The *Human Mastadenovirus* (HAdV), is a non-enveloped virus and double-stranded DNA. The aim of this study is to evaluate the presence of TC, FC and ENT, as well as HAdV-C in water and sand samples from four beaches: Torres, Capão da Canoa, Imbé and Tramandaí. The collection had performed in December 2018, January and February 2019, where 500ml of water and 50g of wet and dry sand were collected. Bacteria were evaluated using 100 mL aliquots, tested by the Colilert® and Enterolert® kits. Concentration of water samples was by the ultracentrifugation method, and the sand was eluted with MEM pH 11,5, followed by extraction of the genetic material by the BioPur® kit and Real-Time Polymerase Chain Reaction (qPCR). In water samples, TC showed values above the limits in 4 samples, with quantification >2000/100ml, while FC and ENT were within the limits. HAdV was positive in 75% (3/4) samples in December, with loads from $3.17x10^4$ to $4.15x10^4$ genomic copies/5µL (gc/5µL), 50% (2/4) in January ranging from $3.41x10^4$ and $1.50x10^5$ gc/5µL, 25% (1/4) in February with a load of $5.78x10^4$ gc/5µL. In wet soil, TC, FC and ENT were within the reference values, and in December ENT was higher than the FC value in Capão da Canoa. HAdV was positive in 100% (4/4) samples in December, with loads from $2.93x10^4$ to $1.16x10^5$ gc/5µL, and positive in 25% (1/4) in February, with a load of $6.69x10^4$ gc/5µL. In dry soil, TC above in two samples, FC and ENT within the values, but with ENT above FC at one point in January. HAdV was positive in 100% (4/4) samples in two months, ranging from $3.68x10^4$ to $8.28x10^4$ gc/5µL in December, $4.36x10^4$ to $6.93x10^4$ gc/5µL in February. In January it was positive in 50% (2/4), with loads of $5.07x10^4$ and $3.65x10^4$ gc/5µL. With bacteriological values above the references it is possible to classify water as unfit for bathing and ENT, because it is more resistant to seawater, can be a more efficient marker when compared to FC. Beyond that, it is also possible to assess that in December there were the largest detections of microorganisms. Financial Support: CNPq, Feevale University.

Palavras-chaves: Beach, HAdV, Sand, Water, qPCR

A NEW DSRNA MYCOVIRUS INFECTING THE PHYTOPATHOGENIC FUNGI MYCOSPHAERELLA FRAGARIAE

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Resumo

Mycoviruses are widely distributed in the major taxonomic groups of filamentous fungi including phytopathogens and can be associated with serious disorders in the natural physiology of the host such as debilitation, hypovirulence, toxin production and morphological changes. In this work we identify a new mycovirus infecting *Mycosphaerella fragariae*, etiological agent of Common Leaf Spot Disease in strawberry. *M. fragariae* was isolated and grown in PDA, and total nucleic acid were extracted from mycelia. After treatment with DNase I and S1 nuclease, five dsRNA elements were detected, which indicated viral infection. Viral particles were purified, and extraction of dsRNA from the purified viral particles showed the same pattern of dsRNA found in fungi mycelia. The dsRNAs were sequenced, the reads were trimmed and quality filtered and the virus genome was assembled using de novo assembly. The contigs obtained ranging between 600 and 2000 nucleotides were selected and submitted to BLAST analysis. The genomic dsRNA1 has 1,829 nucleotides and codes a putative RNA-dependent RNA Polymerase, dsRNA2 has 1,588 nucleotides and codes a putative Coat Protein (CP). Blast analysis showed that RdRp sequence shares 82.81% with amino acid sequence of *Ustilaginoidea virens partitivirus* and CP sequence shares 71.36% with amino acid sequence of *Discula Destructiva virus 1*, both species of *Partitiviridae* family. The dsRNAs 3, 4 and 5 (1073, 937 and 620nt in size) was identified as RdRp and CP defective sequences. According with International Committee on Taxonomy of Viruses (ICTV), the taxonomic criteria for the demarcation of a new species in the genus *Gamma- partitivirus* is identity ≤80% for the aa sequence of CP and ≤90% for the aa sequence of RdRp. Our results suggest that a mycovirus isolated from *M. fragariae* belongs to *Partitiviridae,* being a new specie in the *Gammapartitivirus* genus.

Financial Support: CNPQ, CAPES.

Palavras-chaves: Characterization, Fungi, Mycosphaerella, Mycovirus, Partitiviridae

WIDESPREAD DISTRIBUTION OF PROPHAGES SIGNALING THE POTENTIAL FOR ADAPTABILITY AND PATHOGENICITY EVOLUTION OF RALSTONIA SOLANACEARUM COMPLEX GENOMES

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Resumo

Prophages can have a positive or negative effect on the host cell, affecting its lifestyle, genomic diversity and bacterial fitness. However, many basic aspects of how these organisms affect the host cell remain poorly understood. *Ralstonia solanacearum* is a gram-negative plant pathogenic bacterium, encompassing a great diversity of ecotypes regarded as a species complex (*R. solanacearum complex* - RSC). *Ralstonia* genomes have a mosaic structure containing numerous elements, signaling to the potential for its evolution through horizontal gene transfer. In this context, we have made a screening in 120 RSC complete genomes from the NCBI database in order to identify prophage sequences integrated into RSC genomes. In total, 374 prophage-like elements were found in both the chromosome and megaplasmid. These elements encode several genes, including some related to host fitness, virulence factors, antibiotic resistance and niche adaptation that might contribute for RSC adaptability. Putative complete prophages belonging to the families *Inoviridae, Myoviridae* and *Siphoviridae*, were found, the most abundant being the members of *Inoviridae* family. Similar prophage-like elements are widespread into the complex at different species and/or geographic origin, suggesting that RSC phages are ancestrally acquired. Also, an analysis of CRISPR-Cas spacer sequences demonstrated the presence of viral sequences that indicate successive infection events during the bacteria evolution. Among the complete prophages, we found 14 novel putative viruses integrated into RSC genomes. These genomes have hallmark proteins from bacteriophages, and might be active. Altogether, our results provide insights about the diversity of prophages in RSC genomes and suggest that these elements may deeply affect the shape of the genome evolution among the strains impacting the virulence and host-range adaptation. Financial support: CAPES, CNPq, FAPEMIG.

Palavras-chaves: Prophages, Ralstonia, Genome evolution

EXPLORING THE VIRAL DIVERSITY OF SINGLE-STRANDED (SS) DNA VIRUSES IN DAIRY CATTLE RUMEN

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Resumo

Ruminant animals stand out for their ability to efficiently convert food into milk, meat and derivatives. This efficient use of ingested energy is directly linked to the symbiotic relationship of the microorganisms present in the rumen of these animals. Microorganisms are essential because they can produce enzymes to degrade the food ingested by the animal. It is well known that rumen is not only important for digestion, but it also plays a central role in ruminant growth, high production performance and health in general. Studying and promoting strategies that optimize the functioning of the rumen has been the objective of several studies. There are several studies about rumen microbiome. Recently, interest in rumen virome has been increasing due to the important ecological role of viruses in different environments such as soil and seawater Studies using the metagenomic approach associated with the advent of high throughput sequencing r have been disseminated as a method to investigate the virome associated with various animal species. This approach has proven to be efficient in identifying new as well as previously described viruses from various animals. Here, we used a metagenomic approach associated with an enrichment for circular viral DNA using rolling circle amplification (RCA) to recover ssDNA genomes from ruminal fluid collected from 2 dairy cattles. Analysis of BLASTn and BLASTx revealed genomes of Anelloviridae, *Circoviridae*, *Geminiviridae*, *Inoviridae*, *Microviridae, Parvoviridae* and *Smacoviridae, Geminiviridae*, *Inoviridae*, *Microviridae*, *Parvoviridae* and *Pleolipoviridae*. Partial sequencing of clones showed sequences of capsid and replication initiator proteins M*icroviridae* family. Finally, we recovered three complete virus genomes from the M*icroviridae* family using back to back primers. ssDNA viruses are common and ubiquitous in nature and studies are needed to evaluate the impact and the relationship of these viruses with their hosts.

Financial support: CNPq, CAPES, FAPEMIG

Palavras-chaves: CATTLE RUMEN, METAGENOMIC, MICROVIRIDAE, ssDNA GENOMES, VIRAL DIVERSITY

THE INVOLVEMENT OF VIRAL SRNAS IN THE CONVERSION OF THE PHYTOPATHOGENIC RALSTONIA PSEUDOSOLANACEARUM INTO A COMMENSAL BACTERIUM BY AN INOVIRUS

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Resumo

In a previous work we isolated and charaterized an inovirus Ralstonia solanacearum inovirus brasil 1 (RSIBR1), which is capable to infect *Ralstonia* spp., and modulates bacteria pathogenesis. We hypothesized that this strong phenotypic alteration in virus infected bacteria should be modulated by a global pathogenicity regulator coded or induced by virus replication. Several studies have pointed out the role of sRNAs in regulating pathogenicity mechanisms in bacteria. By computational analysis (RNA space) we were able to predict six putative sRNAs, coded by RSIBR1 genome, and sRNAs structures were predicted using mfold. The possible targets of putative sRNAs coded by RS1BR were predicted using IntaRNA and TargetRNA2 and we identified 144 and 145 targets in *R. pseudosolanacearum* chromosome and megaplasmid, respectively. We observed that targets are involved or related with protein folding, phage protein, signal peptide protein, DNA-binding protein, secretion, transmembrane transporter protein, transcription regulator protein and several hypothetical proteins, suggesting that sRNAs can be used by RS1BR1 to convert the plant pathogenic *R. pseudosolanacearum* into a commensal bacterium. The analysis of expression of sRNAs will be performed by Northern blot which, along with a transcriptome analyses of infected bacteria, will help us to better understand the phenotypic alteration induced by RS1BR1 in *Rasltonia* spp.

Financial support: FAPEMIG, CNPq, CAPES, Suzano

Palavras-chaves: Inovirus, Ralstonia pseudosolanacearum , sRNAs

RELATIONSHIP BETWEEN ZIKA VIRUS INFECTION AND PLUVIOMETRIC PRECIPITATION IN MATO GROSSO, 2016.

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Resumo

Zika virus (ZIKV) is an arbovirus, transmitted by Aedes mosquitoes and its incidence may be related to factors resulting from human activity in the environment, such as deforestation, increasing urbanization, low socioeconomic levels, and also related to natural factors, such as rapid climate change. Taking into account the high incidence of the disease in the state of Mato Grosso, we sought to analyze if the cases of ZIKV infection are correlated with rainfall. This is a descriptive, ecological study. Reported case data comes from the Reporting Disease Information System (SINAN) for 2016, which was made available by the State Health Department of Mato Grosso (SES-MT), for population-based data were used 2016 estimates provided by the Brazilian Institute of Geography and Statistics (IBGE) and rainfall data were collected from the Tropical Rainfall Measuring Mission (TRMM) database, thanks to the 3B42 sensor, with average weekly values per millimeter (mm). For the correlations, we used the data of number of reported cases and the average precipitation by Epidemiological Weeks (SE). It was also used the concept of time lag, with a lag period of 0 to 6 weeks, taking into account the larva development time until the adult mosquito life time. Spearman's correlation coefficient was used and a significance level of 5% and $p <= 0.05$ was adopted. It was found that the highest proportion of cases was concentrated between the 1st to 13th SE (91%), which includes summer in the state, with high temperatures and greater precipitation volume. It was observed that in only two periods analyzed the correlations were significant, being the 06 weeks of accumulated rainfall that preceded the cases of SE 1 ($r = -0.18$) and the 3 weeks of accumulated precipitation that preceded the SE 4 cases ($r = 0.03$). Although the largest proportion of cases occurred during the rainy season in the state, even with the use of different lag times, the correlations between the variables were weak. Thus, these results suggest that rainfall in the state of Mato Grosso is not a determinant of ZIKV proliferation, but allows the ideal conditions for mosquito proliferation through containers that can serve as breeding grounds.

Financial Support - Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq)

Palavras-chaves: Climate Processes, Determining Factors, Epidemiology , Health Surveillance, Zika Virus Infection

INFECTION RISK ASSESSMENT OF HUMAN MASTADENOVIRUS SPECIES C AND F IN CONTAMINATED WATERS FROM SOUTHERN BRAZIL

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Resumo

In the region of Vale dos Sinos, four streams of great importance are located for the communities that developed around them, which are Pampa stream (A1), Estância-Portão stream (A2), Luis Rau stream (A3) and Schmidt stream (A4). The lack of basic sanitation, industrial and domestic sewage disposal and the irregular urban occupation, make them important objects of study for this region. The absence of resolution to these important environmental and infrastructure issues results in the spread of microorganisms. The purpose of the study was to determine the presence of *Human mastadenovirus* (HAdV) genomes of species C and F, and estimate the risk of infection from exposure to these viruses. HAdV-C causes respiratory tract diseases, while HAdV-F causes enteric diseases. The collections were performed bimonthly in the springs (S), at intermediate points (I) and mouths (M), between 2017 and 2018. A total of 96 samples were concentrated by ultracentrifugation. Viral DNA was extracted with the commercial Biopur® kit and quantified by real-time polymerase chain reaction (qPCR) using oligonucleotides flanking the hexon capsid protein region. Also, qPCR was using to calculate quantitative microbial risk assessment (QMRA) that was used to estimate risk of infection when the population are exposure to this contaminated environment. All samples were negative for HAdV-C in their S points, but viral loads of HAdV-F were detected in 72% of the samples, with A1 presenting the highest concentration ($1.59x10^8$ gc/L). In the I points, 75% of the samples had the HAdV-C genome, and A4 had the highest viral concentration ($1.99x10^8$ gc/L). Still, on the I point, 75% was positive for HAdV-F, and the highest viral load was found in A3 ($1.08x10^8$ gc/L). In the M points, 50% of the samples of the stream showed positive results for HAdV-C, A1 presented $3.58x10^8$ gc/L and 78% of the samples were positive for HAdV-F, with A3 containing 1.95 $x10^8$ gc/L. The HAdV-C contaminated samples present the average of $6.33x10^{-1}$ for daily infection risk and in 32% of total samples was estimated the daily infection risk at $9.99x10^{-1}$. HAdV-F present the average of $7.14x10^{-1}$ for daily infection risk and 14% samples were $9.99x10^{-1}$ for daily risk. The results of qPCR, corroborating the high risk obtained by QMRA, suggest that the waters of the streams are becoming mere open sewers that endanger daily the lives of the surrounding residents.

Developers: FEEVALE, CNPq, FAPERGS E FINEP.

Palavras-chaves: Infection risk, HAdV-C , HAdV-F, QMRA, Streams

MICROBIOLOGICAL EVALUATION IN THE COMPOSTING PROCESS FROM A DRY TOILET UNIT

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Resumo

In order to increase access to basic sanitation and reduce the environmental impact, the sustainable and low cost technology of dry toilet has been adopted in developing countries. It is an alternative that uses the composting process to treat waste produced before disposal. Thus, it is necessary to verify the efficiency of the process to avoid contamination by pathogenic microorganisms that may be present in this organic matter. The samples of this work were collected in April 2019 in the dry toilet installed at the Arca Verde Institute in São Francisco de Paula - RS, covering all phases of the composting process (7 points). The presence, quantification and infectivity of the enteric viruses represented by the *Human mastadenovirus* C and F serotypes (HAdV-C and F) were evaluated. Also bacteria of the fecal coliforms group (CF), represented by *Escherichia coli* (E. coli), total coliforms (CT) and *Enterococci* (ENT). For viral analysis, the extraction of viral DNA by Biopur® kit and the amplification of genomic copies by real time polymerase chain reaction (qPCR) were performed. Colilert® and Enterolert® kits were applied for bacteriological analysis, following manufacture instructions. The samples showed a 17.98% reduction in the CT parameter of the third phase of the process and ENT in the second phase (0.7%). *E. coli* (13.30%) was reduced in the third phase and was absence in the final compost (fertilizer). HAdV - C genomes were detected throughout the process, ranging from $2.16x10^3$ to $2.16x10^4$ genomic copies (gc) / 5uL, while HAdV - F was present in lower concentrations (average of $4.8x10^2$ gc/ 5uL). After performing the integrated cell culture qPCR assay (ICC - qPCR), samples showed absence of infectivity for HAdV - C and F. It is important to highlight that this is a preliminary study and the evaluated system needs to be better monitored through larger sampling and collection at different periods, in order to verify the safety in the use of the dry toilet and subsequent disposal of the fertilizer.
Financial Support: CAPES. FAPERGS. FEEVALE.

Palavras-chaves: Basic sanitation, Enterococci, Escherichia coli, HAdV, Qpcr

HUMAN MASTADENOVIRUS IN ENVIRONMENTAL WATER SAMPLES DETECTED BY REAL TIME PCR AND IMMUNOCHROMATOGRAPHIC

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Resumo

Human enteric viruses are largely excreted in feces and current wastewater treatment methods are sometimes not efficient to eliminate viruses. *Human mastadenovirus* (HAdV) are the important causes of acute respiratory tract infections, conjunctivitis, hemorrhagic cystitis and gastroenteritis. The present study aimed to evaluate two commercially available immunochromatographic (IC) kits to identify the presence of HAdV in environmental samples in comparison with real time polymerase chain reaction (qPCR). Environmental water samples were collected at two points in Novo Hamburgo city (sewage and drinking water), before and after treatment every 15 days during 2 months, totaling 16 samples. The samples were concentrated by ultracentrifugation and tested for HAdV with two different IC kits: Bionexia (Biomerix) and Coris (Serion). After concentration, viral DNA was extraction using the Biopur® kit and the amplification of genomic copies was performed by qPCR targeting the hexon capsid protein of HAdV serotype C. However, when qPCR was performed the HAdV-C genome was present in 100% (4/4) of raw sewage samples, 75% (3/4) in treated sewage samples, 50% (2/4) in raw water samples and 75% (3/4) in treated water samples. The viral loads in positive samples ranged from $7.47x10^4$ to $1.75x10^6$ genomic copies/5uL, achieving the highest concentration in non-treated sewage samples. From these preliminary study is possible to stressed that the IC kits were unable to detect the presence of viral antigens in the concentration range presented in the analyzed samples. Moreover demonstrated the need for further research in the development of IC kits, enabling its future use as low cost methodology and simple handling possible viral detection method in environmental samples. Finnancial Support: University FEEVALE, FAPERGS/MS/CNPq/SESRS, CAPES.

Palavras-chaves: HAdV, Lateral flow, qPCR, Sewage

WATER AND SEWAGE COLIFAGES AS MICROBIOLOGICAL INDICATORS OF FECAL CONTAMINATION

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Resumo

Introduction: Waterborne diseases are an important public health problem, and it is essential to prove and maintain the water's quality available for human consumption. Such verification is usually done by coliforms, preferably *Escherichia coli*. However, it has been suggested theexpansion of indicators used due to the presence of different viruses in the water, including bacteriophages. The quantification of coliphages, viruses that infect *E. coli*, as a parameter of microbiological water quality has already been recognized by the American Public Health Association (APHA), and the presence of such viruses is related to faecal contamination of water resources. **Objective**: With that in mind, the objective of this study was to research coliphages in raw and treated water and sewage samples as indicators of fecal contamination. **Methodology**: To this end, two water samples (one raw and one treated) from one treatment plant (ETA) and two sewage samples (raw and treated) from one treatment plant (ETE) were collected on the same day in sterileflaks andcarried under refrigeration. Phage quantification was performed on 1 out of a total of 6 collections that will be performed at ETA and ETE in the municipality of Novo Hamburgo during 2019. The coliphages were screened by agar overlay lysis plate assay using *E. coli* ATCC 13706 as a host. For the tests, 1 mL aliquots of raw water and treated sewage samples were used, while for the raw sewage only 100 µL was used. Besides, the treated water sample was enriched in order to amplify the presence of phages. Three independent experiments were performed, in duplicate, for each water / sewage sample. **Partial Results**: The average coliphage found, expressed in Plaque Forming Units per mL (PFU / mL), was: i) raw sewage = 688; ii) sewage after treatment = 5; iii) raw water = 7; iv) post treatment water = 3. **Conclusion**: The results showed that sewage treatment was very effective in reducing viable phages as it decreased over 99% of the present coliphages. On the other hand, in raw water (pretreatment) a small amount of phage was detected, but surprisingly viable coliphages were found in the treated water sample, and after treatment no coliform bacteria (coliphage hosts) were detected. The presence of coliphages in water considered fit for human consumption corroborates the importance of research related to alternative water quality indicators aimed at maintaining public health. **Financial support**: Feevale University

Palavras-chaves: Bacteriophages, Potable water, Water quality

MOLECULAR DETECTION OF OROPOUCHE VIRUS IN MOSQUITOES FROM MATO GROSSO, BRAZIL, 2017

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Resumo

Arboviruses represent a public health problem in Mato Grosso every year. We conducted a survey for arboviruses in adult female mosquitoes 19.110 collected in four municipalities of Mato Grosso, Midwestern Brazil, between 2017 and 2018, after Zika and Chikungunya virus introduction and spread in the State. Female pools (n=261) were processed to RNA extraction followed by RT-PCR targeting alphavirus, flavivirus and orthobunyavirus species. Our partial results permitted the identification of three pools of *Culex quinquefasciatus* collected in Caceres, Sinop and Cuiabá positive for Oropouche virus (OROV) in March, June and July, 2017, respectively. Maximum likelihood estimation (MLE) was of 3.25 (IC 4.61-8). Phylogenetic analysis indicated 100% similarity with samples from a study previously carried out in the state, belonging to subgenotype Ia, clustering with other isolates obtained from humans and animals in the Brazilian Amazon, and 99.4% identity with the subgenotype Ic also from northern Brazilian samples. The results corroborate previous findings showing the circulation of this arbovirus in the state.

Financial Support: Mato Grosso State Research Support Foundation

Palavras-chaves: Arbovirus, Oropouche, Mosquitoes

RISK OF ZIKA VIRUS INFECTION IN MATO GROSSO MUNICIPALITIES, BRAZIL, 2016.

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Resumo

Since 2014, Zika Virus (ZIKV) infection has been spreading rapidly in Brazil. The state that reported the most cases in 2016 was Mato Grosso, with an incidence of 671 cases per 100,000 inhabitants. Thus, a study was conducted to identify priority areas for ZIKV infection control actions. This is an ecological study, with data from reports of cases of ZIKV infection by Epidemiological Weeks, which took place in 2016, in the municipalities of the state of Mato Grosso. Case notification data were extracted from the Reporting Disease Information System (SINAN) and population data were based on 2016 estimates provided by the Brazilian Institute of Geography and Statistics (IBGE). Relative risk (RR) was calculated as the ratio between the number of observed cases and the expected number of cases. To perform the RR calculation, the spatial scanning technique was used with the SatScan software. The periods of the year and the municipalities of the state where the risk for developing the disease was highest were identified. It was found that the highest proportion of cases was concentrated between the 1st to 13th epidemiological weeks (91%), with a decrease in notifications in the following weeks. In the last week of the year (epidemiological week 52) there was an 89% increase in cases compared to the number of cases reported in the previous week. During the scan, 3 clusters were identified, 2 high risk (RR = 11.66 and 6.99) and 1 low risk (R = 0.03). The high-risk primary cluster (RR = 11.66) was located in the North and Northeast mesoregions of the state of Mato Grosso. The municipalities with the highest RR in the northern mesoregion were Lucas do Rio Verde (RR = 12.22), Nova Mutum (RR = 18.10) and Brasnorte (RR = 8.10). The municipalities with the largest RR of the Northeast mesoregion were Água Boa (RR = 18.16), Nova Mutum (RR = 18.10) and Lucas do Rio Verde (RR = 12.22). In conclusion, the highest rates of the disease were recorded in the period that includes the summer in the state, marked by higher volumes of rainfall, higher relative humidity and high temperatures. The priority areas for health surveillance, which had higher RR, are bordering municipalities, located in the North and Northeast mesoregions of Mato Grosso. In order to control the disease, it is essential that intersectoral actions that transcend the exclusive chemical control actions of the vector are agreed upon.

Financial Support:Conselho Nacional de Desenvolvimento Científico e Tecnológico

Palavras-chaves: Zika Virus, Risk, Mato Grosso, Relative risk, Brazil

CONTAMINATION OF THE SOIL FROM A PUBLIC PARK BY HUMAN AND CANINE MASTADENOVIRUSES

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Resumo

Public squares and parks are places often used by adults and children for leisure and recreation, usually accompanied by their dogs. Circulation of these animals in these environments may cause soil contamination by pathogens eliminated through feces, as is the case of enteric viruses of the families *Adenoviridae* and *Parvoviridae*. The goal of this work was to evaluate the presence of *Carnivore protoparvovirus 1* (CPV-1) and different species of *Mastadenovirus* in the soil of a public park with animal recreation area and the sand of the children's playground during a period of 6 months. Soil and sand were sampling bi-monthly. Samples were eluted in Eagle's minimum essential médium. DNA extraction were made with ReliaPrep™ Blood gDNA Miniprep System (Promega®), samples were then analyzed through polymerase chain reaction and later submitted to sequencing by Sanger method and to phylogenetic tree construction. During first three samplings all samples were negative, however after works that were carried out in streets near the park and after some rainy days, next samplings presented 30% (64/216) of positivity for *Human mastadenovirus C* (HAdV-C), 1,4% (3/216) for HAdV-E and still 0,4% (1/216) for *Canine mastadenovirs A* (CAdV-A), no sample was positive for CPV-1. Contamination with viruses of human origin in the park soil may be caused due to exposition of the water pipes in the works that were done in the streets near the park, since that no viral inativation treatment is done on the water. Rain may have been responsible for spreading these contaminants to the park soil and to the playground sand. Thereby, once again it is noticeable how anthropic actions may be interfering with the environment even indirectly.

Palavras-chaves: anthropic actions, children playground, microbial contamination

COMPARISON OF IMMUNOMAGNETIC SEPARATION AND ULTRACENTRIFUGATION AS CONCENTRATION METHODS FOR HUMAN MASTADENOVIRUS IN WATER SAMPLES

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Resumo

Even though viruses are discarded in large quantities in water resources by domestic sewage releases, they may be present in very low amounts in water samples, making direct analysis and detection a challenge, thus it is often needed concentration of large sample volumes. Therefore, rapid and reliable methods are needed to detect a small number of viral particles, especially infectious, in environmental samples. Immunomagnetic separation (IMS) is a method that concentrates viral particles through the use of an antibody-antigen complex. Paramagnetic particles are coated with a specific antibody for the target pathogen. The pathogen binds to the specific antibody and the antigen-antibody complex can easily be concentrated in a small volume by applying an external magnetic field. The goals of this study were to standardize IMS combined with real-time polymerase chain reaction (qPCR) (IMS-qPCR) to detection of *Human mastadenovirus* (HAdV) in water samples and to compare difference in the viral concentration methods between ultracentrifugation and IMS. Fifteen sites with different kinds of superficial water were sampled in a city of the South of Brazil. The samples were concentrated by ultracentrifugation and IMS. In the IMS step, monoclonal and polyclonal antibodies against HAdV were used to coat the paramagnetic beads. For viral detection, qPCR assays were performed using a specific primer (VTB1) to detected HAdV species F (HAdV-F). No samples that were concentrated by ultracentrifugation showed contamination by HAdV-F. However, in the water samples concentrated by IMS were detected 33% (5/15) of positive samples for each antibody, being only one site positive for both antibodies, totalizing nine different sites with HAdV-F contamination. The rate of detection varied from 1.39E+05 to 3.03E+06 genomic copies/L. Until now, IMS showed to be a concentration step to viral particles more effective than ultracentrifugation. IMS is considered a versatile assay with very high specificity, so special attention needs to be given for this method. The use of IMS-qPCR demonstrated to improve the assessment of HAdV in water resources.

Financial Support: CAPES, CNPq, DCIT – Ministério da Saúde – Brasil, Programa Institucional de Bolsas de Doutorado Sanduíche no Exterior (PDSE) / CAPES (processo nº: 88881.187818/2018-01).

Palavras-chaves: Immunomagnetic Separation, Human mastadenovirus , Antibodies

PROSPECTION OF NEW ENTEROBACTER AEROGENES BACTERIOPHAGES FOR BACTERIA CONTROL PURPOSE

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Resumo

Enterobacter aerogenes is a gram-negative bacteria with clinical significance as an opportunistic pathogen, responsible for causing hospital-acquired infections in intensive care patients. This species can be found on the urinary, blood, gastrointestinal and respiratory human tract, been especially dangerous for patients on mechanical ventilation. Over the last three decades, this pathogen has been recognized as a multidrug resistant bacteria, increasing the challenges on its elimination and on the infections treatment. Although bacteriophages have been discovered more than one century ago, their use is still very low. Phages can be a good alternative to treat multidrug-resistant pathogens, as Enterobacter aerogenes, due the capacity of lyse bacteria, its specific host range, rapid self-proliferation and low intrinsic toxicity. In addition, the advantages of this viruses include the possibility of eliminating the pathogen from the environment, since this enterobacteria has a fecal-oral route and can be spread through the wastewater for example. In the present work the main aim was to isolate Enterobacter aerogenes phages from human wastewater for application in environmental bacteria control. The bacteriophage isolation process was not described due to intellectual protection product and process. After 12h at 37ºC the plates were analyzed seeking for lysis plate. Two different profile of phages capable of causing lysis in Enterobacter aerogenes were found, with two different lysis plate patterns. The first of them caused a significant number of small plates (diameter < 1 mm) while the second one formed a few plates with bigger size (diameter > 8 mm). The first phage presented lysis plates similar to those formed by E. aerogenes phages previously described on the putative Siphoviridae family, however other phage infecting the same bacteria was classified on the putative Myoviridae family. This phages have icosahedral capsids, long tails and double-stranded DNA, they are also non-enveloped and hence very stable on the environment. Both phages found presented great power of lysing the bacterial host and favorable features for environmental application, indicating potential for biocontrol of Enterobacter aerogenes.

Financial support: CNPq, Embrapa and CAPES.

Palavras-chaves: Enterobacteria control, lysis plate, multidrug-resistant pathogens

VIRUSES AND BACTERIA ADSORPTION FROM SWINE WASTEWATER USING Moringa oleifera SEED SHELL

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Resumo

Moringa oleifera is a plant originally from Asia, but it is found in many countries, including Brazil (common name - "Acácia branca"). It is called "miraculous tree" as a result of the various medicinal properties (in seed, flowers, leaves and roots) that it has, which made its use spread in several countries. Besides its medicinal use, its crushed seed has active coagulating agents with the ability to reduce bacterial contamination and water turbidity. The ***M. oleifera*** seed is composed of a globular and a three winged part, however only its globular part is used in water treatment and the winged parts are discarded. The aim of this study was to evaluate the use of these whole seeds (globular and three winged parts) in reducing the turbidity and pathogens contamination in swine wastewater. Before the tests started, the swine wastewater samples were screening for some bacteria and viruses. HAdV-2 was inoculated into each sample and functioned as a positive control for the viral recovery Samples were added with different concentrations of ***M. oleifera*** whole seed (10mg/L 100mg/L and 1000mg/L) and analyzed until 8 h. Every 2 h, 2 ml of each sample were collected and pathogens [***Escherichia coli***, DNA viruses (PAdV and PCV2) and RNA viruses (HEV and RVA) viruses] were quantified in swine wastewater. After 8 h, the bacteria and viruses adsorbed to the seeds were eluted in PBS and quantified by bacterial culture and RT-qPCR, respectively. The results showed that the adsorption pattern among the pathogens studied were different. The higher quantity of ***E. coli*** in the three treatments was measured after 8 h in swine wastewater and it was inversely proportional to the seed quantity added in the samples (1.25g>2.5g>5.0g). ***E. coli*** was detected in the seeds shells after 8 h of adsorption and showed the same proportion observed in wastewater samples (1.25g>2.5g>5.0g). HAdV-2 decayed over time, but did not bind to seeds, in all treatments applied. Increase detection of PAdV and PCV2 were observed after 2 h compared to time-zero of incubation (before adding the seeds), point to an inhibition effect reduction in the samples. The adsorption process (viral and bacterial) from animal effluent are still pioneered studies using ***M. oleifera*** seed shells. These results will support future biomembrane studies for animal effluents hygienization, aiming environmental health by decentralized sanitation. Financial support: Projeto Universal - CNPq n. 420398/2016-3; CAPES.

Palavras-chaves: Virus inativation, environmental health, hygienization, bacteria, biomembrane

BACTERIOPHAGES ISOLATION AGAINST KLEBSIELLA PNEUMONIAE AND PROTEUS MIRABILIS FOR ENVIRONMENTAL APPLICATION

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Resumo

The indiscriminate use of antibiotics in poultry pigs and poultry chickens had lead to an increase of multidrug-resistant bacteria (MDR) through the years. Bacteriophages are specific viruses to infect bacteria and can be used as an alternative to biocontrol bacteria cells and destabilize biofilm. Phages can be found and isolated from wastewater and other sources of the environment. In this context, the main objective of this study was to isolate bacteriophages which can infect *Klebsiella pneumoniae* and *Proteus mirabilis* from samples of the environment and use that as an alternative against biofilm control. Samples of the environment include wastewater, the effluent of wetland, poultry pig and poultry chicken. The bacteriophage isolation process was not described due to intellectual protection product and process. After 12h of incubation at 37ºC in order to optimize replication of bacteria cells and bacteriophages, the plaque lysis plaque were measured, indicating activity of bacteriophages in samples with the capability of lyse the hosts. The plates ranged from 3 to 9 mm in diameter approximately. Phages of *Proteus m*. and *Klebsiella p*. usually belongs to order *Caudovirales*, can belong to family *Podoviridae* and *Myoviridae*, respectively. The phages isolated proved themselves to be biocontrol options to colonies of *Klebsiella pneumoniae* and *Proteus mirabilis*, being necessary to conduct experiments to evaluate the activity against biofilm in controlled and real samples. It's still unknown if one or more of the isolated viruses can infect multiple bacteria such as both *Proteus m*. and *Klebsiella p*., future experiments may be created to evaluate such aspects to find a phage which can degrade multiple biofilmes and therefore be used in phages cocktails. Financial support: Projeto Universal - CNPq n. 420398/2016-3; CAPES.

Palavras-chaves: Bacteriophage, Environmental technology, bacteria control

BRAZILIAN CATTLE BACTERIOPHAGES ISOLATION

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Resumo

According to the Secretary of Health Surveillance annual data, in 2017, Escherichia coli and Salmonella spp. were the manly etiological agents causing human outbreaks of food poisoning in Brazil. Antibiotic therapy has been used in livestock, to prevent the spread of pathogenic bacteria among the shoot and zoonotic transmission. It has shown to be a useful and efficient method, however, the number of bacterial resistance is increasing. In addition, medicating all the animals (sick and health) is costly and can produce environmental contamination. In this scenario, new methods of decontamination were necessary. One efficient method is the bacteriophage cocktail discovery. Just a small fraction of phage, about 0.0002%, are already known in the biosphere, this shows the importance of studies like this. The aim of this study was to search for new phages that have lytic activity and with a wide host range for different strains of enterobacteria in cattle (as swine and poultry residues). The bacteriophage isolation process was not described due to intellectual protection product and process. Various plates units were isolated on Salmonella enteritidis, Aeromonas allosaccharophila, and Shigella sonnei. Two possible families of viruses (Siphoviridae and Myoviridae) that infect enteric bacteria were isolated in this study. Myoviridae consists in viruses with a contractile tail long (113×16 nm) and relatively thick (80–455×16–20 nm), compared with other tailed virus and bacteriophages, the myoviruses often have larger heads, in an icosahedral capsid, with 152 capsomers, and higher particle weights, the genome is DNA usually double-stranded, genome size range from ~24 kb to ~316 kb. Siphoviridae are bacteriophage with a non-contractile tail (150×8 nm), the head is icosahedra, about 60 nm in diameter, and consist of 72 capsomers, genome (DNA) size range from ~16.5 to ~80 kb. Next-generation sequence pipeline will proceed to determine phage taxonomy. This phage cocktail can be used in bacterial control in an environmental sample in One Health approach (human, animal, and environmental health). Financial support: Projeto Universal CNPq n.420398/2016-3, CAPES.

Palavras-chaves: BACTERIOPHAGES , ISOLATION, CATTLE , ENTEROBACTERIA, MULTIDRUG-RESISTANT PATHOGENS

VIRUCIDAL POTENCIAL OF MICROALGAE EXTRACTS CULTIVATED IN SWINE MANURE

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Resumo

Use of microalgae for swine effluents treatment and for energy purposes has been widely reported in the context of nutritional recycling and biomass valuation, having enormous potential for environmental sanitation purposes and for obtaining biocidal products. The present study aimed to evaluate the virucidal action of microalgae extracts cultivated in swine manure against the herpetic model (Herpes Simplex Virus type 1, KOS strain). For this, microalgae *Chlorella* sp. was obtained from a field-scale lagoon used to remove nutrients from swine wastewater digestate originated from an anaerobic biodigester (Brazilian Agricultural Research Corporation, EMBRAPA, Concórdia, SC, Brazil). After 11 days following inoculation, the growth medium containing the microalgae biomass was harvested via centrifugation. The harvested biomass was immediately frozen (-40 °C) and lyophilized under vacuum. It was performed a liquid-liquid fractionation of the lyophilized biomass using solvents of increasing polarity, such as *n*-hexane, dichloromethane and methanol. Mixtures of the *n*-hexane, dichloromethane and methanol extracts, in different concentrations, and $4x10^4$ PFU of HSV-1(KOS strain) in serum-free MEM, were co-incubated for 15 min at 37°C, prior to the dilution of those mixtures to non-inhibitory concentrations of them (1:100). The residual infectivity was determined by viral plaque number reduction assay. Dichloromethane and methanol extracts decreased 100% of HSV-1 infectious capacity at the lowest tested concentration (3.125 µg/mL) when compared to the untreated control. This assay was conducted in order to determine if the microalgae extracts were capable of inactivating the virus in the absence of cells, thereby measuring the virucidal potential of these samples on viral particles and their consequent decreasing in HSV-1 infectious capacity.

Financial support: CNPq

Palavras-chaves: Biocidal products, Effluents treatment, HSV, Microalgae

DOE OPTIMIZATION PROCESS OF A T4-LIKE BACTERIOPHAGE USING THE ROTATIONAL CENTRAL COMPOSITE DESIGN (RCCD) METHODOLOGY TO DETERMINE OPTIMAL CARBON SOURCES AND CULTIVE CONDITIONS

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Resumo

Phage therapy is the application of virus that kill bacteria, also called phages, to control the bacterial growth. The use of phages to combat bacterial diseases has been used since the description of these viruses, about 1917. However, with the discovery of antibiotics and the relatively inconsistence in the treatment of patients with phage cocktails (probably due to the poor understanding of phage biology that the researchers had in that time), the phage therapy was forgotten. Recently, due to the increase of bacterial strains resistant to the action of antibiotics, phage therapy has been reclaiming its space in the treatment of diseases and environmental problems caused by bacteria. There is still the possibility of using phage enzymes such as depolymerases and lysines, to disrupting bacterial biofilm. When cells are in a biofilm array, they become much more resistant to the action of biocides, and cause major damage in the industrial and hospital area. Despite the full potential of bacteriophages as bacterial growth suppressing agents, little is discussed about the optimization of viral particles for the use of these organisms on a large scale. The aim of this work was to evaluate the progeny of a T4-like phage, the vB_EcoM_UFV09, in seven culture media (LB rich medium and M9 minimal medium with the carbon sources: acetate, lactic acid, pyruvate, glycerol, succinate and glucose) together with the influence of temperature, incubation time, agitation and Multiplicity of infection (MOI) from the Rotational Central Compound Design (DCCR) methodology. The results indicated that the culture parameters influenced the viral production differently in each sources. Furthermore, some of them were insensitive to the environmental stimuli. We believe that this happens due to the overloaded physiological state of the bacterial cell growing in a medium with carbon sources that are more difficult to obtain energy. The determination of the best growth condition for the optimization of vB_EcoM_UFV09 production took into account the economic viability of the carbon source, the stability of the source in the culture medium and the impact of the cultivation parameters on viral progeny production. The practical evaluation of the ideal conditions predicted by the DCCR demonstrated that the model was efficient in determining the optimal cultivation conditions.

Financial support: PETROBRAS, CAPES, CNPq, FAPEMIG

Palavras-chaves: Bacteriophage, Central Composite Design (CCD), T4-like phage

EXPANDING THE REPERTOIRE OF AMOEBA GIANT VIRUSES: ISOLATION AND CHARACTERIZATION OF ORPHEOVIRUS BRASILIENSIS IN VERMOAMEBA VERMIFORMIS

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Resumo

Since the discovery of the first giant virus in 2003, prospecting studies have promoted the isolation of these viruses from a wide range of environments, such as soil and water samples from rivers, lakes, oceans and sewers. Characterization studies of these isolates sparked several debates and revealed features never before described, expanding the boundaries of the then virosphere. We attempted to isolate new amoeba giant viruses from water samples collected from lagoons in Diamantina city, MG, Brazil. A total of 30 samples were inoculated in *V. vermiformis* monolayers. A total of 19 samples induced cytophathic effect in the cells. Molecular assays revealed the isolation of Kaumoebavirus (in 18 samples) and Orpheovirus (in one sample). We went further in the characterization of the second Orpheovirus isolated in the world, which we named Orpheovirus brasiliensis. The viral particle and cycle were analyzed by transmission (TEM) and scanning (SEM) electron microcopy and by immunofluorescence (IF) assays. We sequenced and analyzed some gene markers to phylogenetically characterize our isolate. SEM images revealed oval shape particles, ranging around 1172nm, presenting a unilateral depression in the particle longitudinal portion. TEM images revealed that Orpheovirus brasiliensis particles present an ostiole in one of the apices, and viral capsid is composed by at least 2 outer layers covering an inner membrane. Asynchronous viral infection induces the formation of a large (about 5,22µm in length) electron-lucent viral factory in the cytoplasm, frequently aside of cell nucleus and surrounded by mitochondria. Synchronous infection was performed to study viral cycle by IF. The viral entry occurs in less than 1 hour, and more than one particle can be incorporated by amoeba. Particle's genome uncoating was observed close to amoeba nucleus and newly-formed virus begin to be observed 8h PI followed by a massive viral release observed from 12h to 24h PI. Particles seem to be released from cells both by exocytosis and lysis. Furthermore, the analysis of DNA Pol δ reveals 12 polymorphisms in comparison to Orpheovirus IHUMI-LCC2, and phylogenetic analyses show that both Orpheovirus cluster with viruses belonging to the putative "Pithoviridae" family. Taken together, our results represent a step-forward in the understanding of the diversity and the biology of Orpheoviruses.

Financial Support: CNPq, CAPES, FAPEMIG, Ministério da Saúde, Pró-Reitoria de Pesquisa da UFMG

Palavras-chaves: Orpheovirus, Vermamoeba vermiformis, giant viruses, NCLDV

INFLUENCE OF FAECAL CONTAMINATION FROM THE CAMBORIÚ RIVER ON THE MICROBIOLOGICAL QUALITY OF WATER IN A BIVALVE SHELLFISH PRODUCTION AREA

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Resumo

The state of Santa Catarina is a major shellfish producer in Brazil, responsible for 95% of the national production of bivalves such as *Crassostrea gigas* oysters and *Perna perna* mussels. A significant part of the shellfish production areas (SPAs) in this state is located close to rivers draining urban areas lacking sewage collection and treatment systems (SCTS). This is a concern since bivalves are filter feeders and may accumulate human pathogens in their tissues, becoming vectors of diseases. One of these SPAs is located ~1.5 km far from the Camboriu River (CR) mouth. The river catchment covers two municipalities with very different profiles: Camboriú, upstream, has agriculture and industry as main activities, an estimated human population of 80,830 and no coverage with STCS; and Balneário Camboriú (BC), downstream, has tourism as its main activity, hosting every year around 3 million tourists during the summer. BC has an estimated baseline population of 138,730, and high STCS coverage, 94%. The aim of this study was to evaluate the prevalence of faecal indicator bacteria and enteric viruses along the CR and analyze the impact of the river discharge on the microbiological quality of water in the adjacent SPA. The levels of Thermotolerant coliforms (TC), Human Adenovirus (HAdV), Hepatitis A virus (HAV), Norovirus (NoV) genogroups I and II were monitored fortnightly during one year at 5 sites: 4 points along the CR and 1 at the SPA. TC were quantified by culture and the enteric viruses by qPCR. A gradient in the TC levels was observed, with the highest results obtained in the most upstream point (geometric mean-GM= $3.9*10^4$ MPN.100 mL^{-1}) and decreasing levels were detected in the points located downstream. The TC levels in the SPA were the lowest (GM= 4.5 and 90^{th} percentile = 29 MPN.100 mL^{-1}), within the legal limits for shellfish direct consumption without post-harvesting treatment (Approved area under US criteria). NoV and HAV were not detected, while HAdV was detected exclusively in points within the CR. The genome of this virus was detected in one to three samples among the 24 samples collected per site. The study confirms the faecal contamination of the CR and suggests that the pollution arising from this river does not affect significantly the microbiological water quality in the SPA located in the coast of BC. Modelling studies are needed to further understand the pollution dynamics in this area.

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Palavras-chaves: Adenovirus, Bacteria, Hepatitis A virus, Oyster, Norovirus

EVALUATION OF LYTIC PHAGE POTENTIAL IN DECREASE BIOFILM FORMATION OF ENTEROBACTERIA

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Resumo

Phage therapy is the application of virus that kill bacteria – known as bacteriophages - to control bacterial growth. The use of phages to combat bacterial diseases such as dysenteries, typhoid and cholera has been used since the description of such viruses in the late 1910's. However, with the discovery of antibiotics and the relative inconsistence/ineffectiveness of treatments of patients with phage cocktails - probably due to the poor understanding of phage biology by that time -, the phage therapy was left aside. Recently, due to the increase of bacterial strains resistance to the action of antibiotics, the need for alternative methodologies to control the growth of pathogenic bacteria 'gained force', making phage therapy return to be a good alternative for this purpose. While application of bacteriophages for treatment in humans is still being tested, their use in veterinary diseases such as mastitis and environmental problems associated with biofilm formation has been recurrent and with excellent results. The aim of this study was to isolate specific bacteriophages against *Escherichia coli, Serratia marscencens, Citrobacter freundii* and *Shigella flexneri,* and to evaluate their influence on the growth and biofilm formation of these bacteria. The phages were isolated from samples of sewages from the municipality of Viçosa-MG. We found two specific phages for *E. coli*, one for *S. marscencens*, one for *C. freundii*, and one for *S. flexneri*. The effectiveness of phages in inhibiting bacterial growth was assessed from the 96-well plate growth curve incubated at 37 ° C for 24 hours. Phages were added to the culture medium (approximately $5x10^5$ PFU / mL final concentration) containing previously activated hosts and at initial OD of 0.1. Biofilm quantification was performed after the incubation period by Violet Crystal colorimetric method and the absorbance was measured at 590 nm. The results indicate that the phages were able to decrease bacterial growth by approximately 60% and inhibited, on average, 80% of the biofilm formed by the hosts during the evaluated period. Studies of morphological characterization, viral infection kinetics and genome of these phages are being developed, so that they can be effectively used as phage therapy agents in the future.

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Palavras-chaves: Bacteriophages, Biofilm, Enterobacteria

DETECTION OF HUMAN BOCAVIRUS RECOMBINANT STRAINS IN SEWAGE FROM URUGUAYAN CITIES

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Resumo

Group A Rotaviruses (RVA) are the main cause of acute gastroenteritis (AG) in children under 5 years of age worldwide. This study aims to detect, quantify and assess the microbial risk of RVA in the watersheds of the Santa Lucia and Uruguay rivers in Uruguay. Monthly sampling (June 2015 to May 2016) were carried out for one year in six points in the watershed of the Santa Lucía River and four in the Uruguay River, totalizing 120 samples. Viral concentration was performed with the adsorption-elution method and detection and quantification of RVA was carried out by quantitative PCR (qPCR). RVA was detected in 41% (49/120) of the analyzed samples for both watersheds, being 42% (20/48) detected in the Uruguay River and 40% (29/72) in the Santa Lucía River. The virus was present in all the analyzed points in both watersheds and in the coldest months of the year in the Uruguay River, however, no clear pattern of seasonality was observed in the Santa Lucía River. The mean concentration for RVA was $1,3x10^5$ genomic copies/L (g.c./L). The microbiological risk assessment shows that Santa Lucía watershed presented the highest risk of infection (6.41E-01) and illness (3.20E-01) estimated for the point downstream of Florida city in July 2015 meanwhile for Uruguay River, the highest probabilities of infection (6.82E-01) and illness (3.41E-01) were estimated for the point of drinking water intake in Salto city in August 2015. These results suggest that the RVA contamination of these important rivers negatively impact on their microbiological quality since they are used for recreation and drinking water intake, demonstrating that the disposal of waste from cities located in their riverside confers a constant threat of infection for the general population, especially for children.

Área de virología: Ambiental

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EDETECTION, QUANTIFICATION AND MICROBIAL RISK ASSESSMENT OF GROUP A ROTAVIRUS IN RIVERS FROM URUGUAY

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Resumo

Human Bocaviruses (HBoV) are mainly associated with respiratory and gastroenteric infections. These viruses belong to the family *Parvoviridae*, genus *Bocaparvovirus* and are classified in four subtypes (HBoV1-4). Recombination and point mutation have been described as basis of parvovirus evolution. In this study, three viral sequences obtained from HBoV positive sewage samples collected in two Uruguayan cities were characterized by different methods as recombinant strains. The recombination event was localized in the 5´ extreme of VP1 gene and the parental strains were identified as members of HBoV3 and HBoV4 subtypes. Uruguayan HBoV recombinant strains have a high percentage of similarity among them, suggesting a successful dispersion throughout the country. As far as we known, this study represents the first detection of HBoV recombinant strains in the Americas.

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