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Microcosm environment models for studying the stability of adenovirus and murine norovirus in water and sediment

M.E.I. Elmahdy[a,c], M.E. Magri[a,b], L.A. Garcia[a], G. Fongaro[a], C.R.M. Barardi[a,*]

[a] Laboratório de Virologia Aplicada, Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, Brazil

[b] Departamento de Engenharia Sanitária e Ambiental, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, Brazil

[c] Environmental Virology Lab., Department of Water Pollution Research, National Research Centre (NRC), 33 El-Buhouth st., P.O. BOX 12622, Dokki, Giza, Egypt

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ABSTRACT

Microcosms are useful tools for understanding the survival and fate of enteric viruses in aquatic environments. This study set out to determine the stability of infectious enteric viruses in an aquatic environment using a laboratory scale microcosm. Sediment and overlaying water were collected from a lagoon and inoculated with known concentrations of recombinant adenovirus (AdV-GFP) and murine norovirus (MNV-1). Infectious particles of these viruses were measured using fluorescence microscopy (AdV-GFP) or the plaque assay method (MNV-1), over 85 days in two different conditions: under natural sunlight and in fully darkened environments. The time required to reach one log reduction in viral titres (T_{90}) of viable viruses in a natural condition microcosm for AdV-GFP and MNV-1 was shorter than in a dark condition microcosm. There was also a negative correlation between the temperature and infectivity of these viruses in both water and sediment samples. Considering that microcosms aim to mimic natural environment conditions and that AdV-GFP and MNV-1 are excellent surrogates for measuring the infectivity of the respective viruses strains, the results presented here have the potential to be applied in future health hazard studies and also would be useful for future climate scenarios.

1. Introduction

Enteric viruses are an important group of pathogenic agents, which are responsible for a large number of human diseases. Some of these viruses are nowadays considered to be waterborne pathogens, causing diseases such as hepatitis, gastroenteritis, meningitis, respiratory diseases, conjunctivitis or asymptomatic infections (Wyn-Jones and Sellwood 2001), and are considered a major threat to public health. The majority of these pathogens are transmitted via the faecal-oral route.

These viruses can occur naturally in aquatic environments or can be transported via sewage discharge or rainfall. They cannot replicate in aquatic environments (Wyn-Jones and Sellwood 2001) but can remain stable for long periods, and can be detected year-round especially when they are adsorbed into organic materials (Prevosta et al., 2015),. These pathogens are present in sediment in large concentrations, as well as in water columns, and can remain infectious for prolonged periods (Bosch et al. 2008; La Rosa et al., 2012; Elmahdy et al., 2016).

The viability of enteric viruses and their persistence in different aquatic environments (i.e., waste, groundwater, surface or even treated water) is a very important issue to be considered, especially with regards to public health. Supplying adequate and safe drinking water to the population is essential for ensuring human health. Disinfection and treatment steps used in drinking water treatment plants (DWTP) are not efficient to remove all viruses due to technical failure or high viral concentration (Waldman et al., 2017). Several factors are responsible for controlling the stability of these viruses in aquatic environments such as salinity, temperature, UV exposure, predators, and association with organic materials (Fong et al. 2005, Bosch et al. 2006, Suttle, 2007, Lugoli et al. 2009). These factors can inactivate viruses by different routes, causing damage to viral genomes or causing denaturation or damage in capsid proteins.

Many studies have reported that viral pathogens can increase their stability at lower temperatures, maintaining their infectivity (Rzeżutka and Cook, 2004; Bosch et al., 2006). It is therefore important to gather more information on the stability of enteric viruses, either in sediment or in water columns, in natural aquatic environments. Analysis of aquatic ecosystem in nature is usually difficult due to many obstacles. Another way to avoid this problem by using laboratory microcosms which can be used as tools to study the stability of waterborne pathogen and their behaviour including viruses by mimicking natural environments under controlled conditions, providing valuable information and helping for enhancing predictive models that can facilitate

* Corresponding author.
E-mail address: celia.barardi@ufsc.br (C.R.M. Barardi).

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understanding of the situation and interactions that happen on a real scale.

Considering the significant importance of evaluating viral stability in water and sediments, the aim of this study was to determine the stability of human adenovirus (AdV) and murine norovirus (MNV-1) in laboratory microcosms as representatives of very prevalent viruses in aquatic environments, representing DNA and RNA viral genomes respectively.

Human adenoviruses are pathogens that can be transmitted via fecal-oral and respiratory routes. They are DNA viruses, which have greater resistance to environmental stressors, such as UV radiation, temperature, chlorine concentration and pH variation, including sewage treatment procedures (PINA et al., 1998; CARTER, 2005; Lechevallier and Au, 2004; Fong and Lipp, 2005). These viruses replicate in the gastrointestinal tract and can be excreted in high concentrations (10^8–10^{11} particles/g of faeces). Presence of HAdV in the aquatic environment is well documented in the literature (BOSCH et al., 2008; OGORZALY et al., 2015; RAMES et al., 2016; STAGGEMEIER et al., 2017). Also some of these viruses (AdV40 and AdV 41) are responsible for many sporadic cases and outbreaks of childhood diarrhea all over the world (OKA et al., 2015; OUÉDRAOGO et al., 2016).

Recombinant human adenovirus serotype 5 (AdV-GFP), has some modification in its genome region and can be potentially used as a bioindicator for stability studies. This strain is characterized by the E1 gene, replaced by the gene coding for green fluorescent protein (GFP). HEK 293 A (human embryonic kidney cell line) is genetically transformed to express the viral protein E1, therefore this cell line is susceptible and permissive to AdV-GFP. Therefore, when AdV-GFP replicates, GFP is transcribed along with the viral genome, emitting a green fluorescence that can easily be detected by fluorescence microscope (DAN et al., 2010; Weaver and Kadan, 2000). By using this model, there is no need for further immunological methods, making this model faster, inexpensive, and very specific (Li et al., 2010). However, in literature, there are few reports related to the use of AdV-GFP to study the stability and infectivity of human adenovirus in aquatic environments (GARCIA et al., 2015;Nascimento et al., 2015).

Norovirus is very contagious and is composed of a single-stranded RNA positive sense genome. It can be transmitted by direct contact with infected people and contaminated water or food. Norovirus infection can lead to serious gastroenteritis, especially in young children and elderly people. The most common norovirus strain (genogroup II genotype 4) has been the dominant cause of outbreaks worldwide, is transmitted by the fecal-oral route and by aerosol (SUFFREDINI et al., 2012; MESQUITA et al., 2011; TERIO et al., 2010; Gentry et al., 2009). Human norovirus is difficult to grow in cell cultures; however, murine norovirus (MNV-1) was the only norovirus that showed *in vitro* replication capacity in cell culture (WOBUS et al., 2006). MNV-1 has been widely used as a surrogate for human norovirus. It belongs to the same family and has considerable similarity in structure, transmission route and disease characteristics in mice (CANNON et al., 2006; OKA et al., 2015).

2. Material and methods

2.1. Collection of samples and microcosm design

Fresh surface water and sediment samples were collected at the centre of Peri Lagoon, Florianopolis, Santa Catarina State, Brazil (27°4′48.3″S / 48°31′16.7″W). Microcosms were established in triplicates by using glass containers not sealed but just covered with gauze to allow the gases exchanges (NADER-Brazil, 50 mL) filled with a 5 cm sediment layer (10 g fresh wet sediment) and an overlaying water column of 5 cm (10 mL fresh surface water). The microcosms were stored in two conditions: i) Full dark environment (24h dark); ii) Natural environment (+/−12 h of natural sunlight/+/−12h of dark moon light). The experiment lasted 85 days. The evaporation was compensated by the addition of the same type water (surface water collected from Peri Lagoon) either in natural or full dark environment to avoid dryness as a key inactivation factor for microbes.

Infectivity studies were undertaken with AdV-GFP, and MNV-1 and the tests were revealed using fluorescence microscopy and plaque assay, respectively, as stated below.

2.2. Physical-chemical analysis

Water temperature (WT), conductivity (Cond), pH, and dissolved oxygen (DO) were measured *in situ* with specific probes (WTW-Multi350i) for all samples after collection, as outlined in the standard methods for the examination of water and wastewater - American Public Health Association (APHA, 1998). Also the average of UV intensity was calculated according to data base of Metrologic Base of Institute of Planning and Economics of Agronomy in Santa Catarina State, Florianópolis (EPAGRI, 2016).

Water samples were taken to the laboratory to quantify Ortho-Phosphate (PO_4), Total Phosphate (TP), Nitrite (NO_2.N), nitrate (NO_3.N), ammonium nitrogen (NH_4.N), and total Nitrogen (TN), which were measured in filtered water samples using a Millipore AP40–47 mm glass fiber (GOLTERMAN et al., 1978; KOROLEFF, 1976). The sediment samples were collected with an Ekman-Birge grab (15 × 15 cm; 0.0225 m2) and the different components of the sediment were determined by sieving and organic matter content by loss on ignition according to Lemes-Silva et al. (2016).

2.3. Cell lines and preparation of viral stock of AdV and MNV-1 for seeding experiment in water and sediment samples

The cell lines HEK 293 A (human embryonic kidney) and RAW 264-7 (*Mus musculus*, mouse macrophages), permissive to AdV-GFP and MNV-1, respectively, were used for the experiments. Both were cultivated in sterile cell culture flasks (75cm^2), with Dulbecco's Minimum Essential Medium Eagle`s salts (DMEM) (Sigma) supplemented with 10% fetal bovine serum (FBS) and 1% HEPES, until confluence was reached (24 h, 37 °C, and 5% CO_2). The growth media were removed and 1.0 mL of each viral suspension was inoculated separately into each cell culture flask. After 1 h for viral adsorption at 37 °C, 20 mL of the cell maintenance media were added consisting of the same growth medium, but with 2% FBS. Cell cultures were always monitored and compared with an uninfected control cell to check the appearance of cytopathic effect (CPE). In the case of AdV-GFP, the cells were monitored every day, and observed using an inverted fluorescence microscope, which allowed the cells to be visualized with green fluorescence (GFP) due to the transcription and expression of GFP protein, which reveals viral replication. In the case of MNV-1, the cells were observed under inverted microscopes until 100% cytopathic effect (CPE) was achieved, characterized by rounded cells and even detachment from the surface, destroying the cell monolayer.

The cell culture flasks containing infected cells with AdV-GFP or MNV-1 were frozen at −80 °C and thawed at 25 °C three times for cell lysis. Then, the virus suspension was transferred, separately, to a tube and centrifuged at 3500 × *g* for 4 min at 4 °C to remove the cell debris and separate the supernatant. The supernatants were used to infect other flasks with the appropriated cells in order to expand the amount of virus stocks to be used for further experiments.

2.4. Artificial inoculation and sampling of microcosms

Stock viral suspensions were prepared as previously described, either for AdV-GFP (2×10^7 FFU/mL) or MNV-1 (5×10^6 PFU/mL). One mL from each viral stock was inoculated into each microcosm jointly and mixed for 5 min for homogenization followed by 1 h of proper settling. A total of 54 microcosms were prepared at T_{zero} to be evaluated in triplicates (destructive samples) for each collection time. Half of the