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Behaviour and recovery of human adenovirus from tropical sediment under simulated conditions

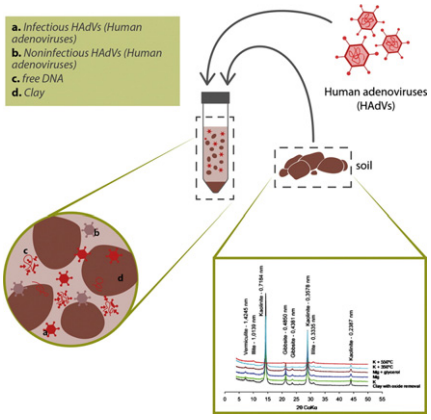
Hugo Delleon Silva [a,b], Marco Aurélio Pessoa-de-Souza [c], Gislaine Fongaro [d], Carlos E. Anunciação [e], Elisângela de P. Silveira-Lacerda [f], Célia Regina Monte Barardi [d], Marco Tulio Antonio Garcia-Zapata [a,*]

[a] Núcleo de Pesquisas em Agente Emergentes e Re-emegentes, Instituto de Patologia e Saúde Pública, Universidade Federal de Goiás, Brazil
[b] Instituto Brasil de Ciência e Tecnologia, Anápolis, Brazil
[c] Departamento de Zootecnia, Pontifícia Universidade Católica de Goiás, Campus II, Goiânia, Brazil
[d] Laboratório de Virologia Aplicada, Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, Brazil
[e] Laboratório de Diagnóstico Genético e Molecular, Departamento de Bioquímica e Biologia Molecular, Instituto de Ciências Biológicas II, Universidade Federal de Goiás, Brazil
[f] Laboratório de Genética Molecular e Citogenética, Departamento de Biologia Geral, Instituto de Ciências Biológicas I, Universidade Federal de Goiás, Brazil

HIGHLIGHTS

- Tropical solids decreased genome copy numbers and viral infectivity.
- Organic matter did not influence genome copy numbers but decreased viral infectivity of HAdV-5.
- Acidic pH hinders viral inactivation.

GRAPHICAL ABSTRACT



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ABSTRACT

This study assessed the contributions of pH and organic matter (OM) on the recovery of infectious human adenovirus 5 (HAdV-5) and genome copies (GCs) in waters that were artificially contaminated with tropical soil. The use of a mathematical equation was proposed based on the flocculation index of clay to assess the recovery of total GCs in these controlled assays. The results suggest that solids in the water reduced the viral genome copy loads per millilitre ($GC \cdot mL^{-1}$) and viral infectivity. OM did not influence the $GC \cdot mL^{-1}$ recovery rate ($p > 0.05$) but led to a 99% ($2\ \log_{10}$) reduction in plaque-forming unit counts per millilitre (PFU/mL), which indicates that infectivity and gene integrity were non-related parameters. Our findings also suggest that acidic pH levels hinder viral inactivation and that clay is the main factor responsible for the interactions of HAdV-5 with soil. These findings may be useful for future eco-epidemiological investigations and studies of viral inactivation or even as parameters for future research into water quality analysis and water treatment.

* Corresponding author at: Núcleo de Pesquisas em Agente Emergentes e Re-Emegentes, Instituto de Patologia e Saúde Pública, Universidade Federal de Goiás, 74643-970 Goiânia, Goiás State, Brazil.
E-mail address: nupereme@gmail.com (M.T.A. Garcia-Zapata).

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1. Introduction

Human adenoviruses (HAdVs) belong to the family *Adenoviridae*, genus *Mastadenovirus*, which contains 57 different serotypes distributed across seven species (A–G) (ICTV, 2013). They are icosahedral, nonenveloped viruses containing a double-stranded linear DNA genome (Enriquez, 2002).

These viruses are of substantial public health importance (Silva et al., 2010), as they are excreted in faeces, urine, and respiratory droplets (Metcalf et al., 1995; Jiang et al., 2001), and can cause a series of disease states in infected individuals by the respiratory and faecal–oral routes. Examples include upper respiratory tract infections (pharyngitis and tonsillitis), lower respiratory tract infections (bronchiolitis and pneumonia), conjunctivitis, cystitis, and gastroenteritis (Mena and Gerba, 2008).

HAdVs are stable in the environment and resistant to water treatment methods (Thompson et al., 2003), particularly to UV irradiation (Liden et al., 2007). Furthermore, they are ubiquitous in the environment year-round (Katayama et al., 2008).

These pathogens are prevalent in both treated and untreated water (Jiang et al., 2001; Silva et al., 2010; Fongaro et al., 2013) and are often detected in higher concentrations than other enteric viruses (Wong et al., 2010). Thus, HAdVs are indicated for use as viral biomarkers of environmental water and drinking water quality (Wyn-Jones et al., 2011; Silva et al., 2011).

Detection of HAdVs in water destined for human consumption can be accomplished either by molecular techniques alone (Silva et al., 2011) or in combination of these techniques with cell cultures (Wyn-Jones et al., 2011; Garcia et al., 2012) that allows access infectivity (ability of the virus to replicate in permissive cells) (Herzog et al., 2008). Despite the great sensitivity of PCR, the main limitation is the lack of the correlation between the detected viral genome and viral infectivity, which limits conclusions about the significance for public health (Hamza et al., 2011).

Despite advancements in the detection of HAdVs in different water sources, information is lacking on the relationship of these viruses with the solids, sediments, or suspended solids in the waters in which they are present.

Viral adsorption to solids is known to be an extremely complex process (Schijven and Hassanizadeh, 2000), and variations can be observed even among different serotypes of the same virus (Singh et al., 1986). Studies that report viral adsorption behaviour to solids usually employ phages as models (Schijven and Hassanizadeh, 2000). These studies are valid, but only as predictive models, and cannot infer the true permanence of infectious viruses, such as HAdVs. This is due to variations in the isoelectric point (pI), virion size, hydrophobicity, and capsid proteins (Schijven and Hassanizadeh, 2000), among other factors that have not yet been discovered.

HAdVs are more prevalent than norovirus, enterovirus, hepatitis A virus, and human poliovirus in biosolids (Wong et al., 2010). Fong et al. (2010) found that 100% of sewage and effluent samples were infectious with HAdV. These findings suggest that matrix solids may play a crucial role in the gene integrity and infectivity of HAdVs.

The interactions of HAdVs in soil solutions were recently analysed in two studies. In the first, Wong et al. (2012) investigated the influence of different concentrations of inorganic ions on the aggregation and deposition behaviours of HAdVs in sandy soil. In the second, Wong et al. (2013) evaluated the role of soil organic carbon (SOC) and solution-phase dissolved organic carbon (DOC) on sorption capacity and reversibility of organic carbon on adsorption of HAdVs. These two studies assessed adsorption behaviour using real-time quantitative PCR (qPCR) but did not evaluate the influence of solids and total organic matter on HAdV infectivity.

qPCR is a highly sensitive technique, capable of detecting a small number of microorganisms (Botes et al., 2013), but when used alone, it is unable to distinguish between infectious and non-infectious viral particles. Furthermore, viral interactions with solid particles may lead to viral inactivation by loss of capsid integrity, with the consequent release of genetic material (Schijven and Hassanizadeh, 2000), which may be identified by qPCR either as an infectious or non-infectious particle.

Therefore, the present study sought to (i) assess the recovery of infectious HAdV-5 and genome copies from simulated solutions containing tropical solids under controlled pH values in the presence or absence of organic matter; and (ii) define a mathematical equation to assess the recovery rate from clay soils under simulated conditions.

2. Material and methods

The U.S. Environmental Protection Agency (USEPA, 2011) defines the acceptable level of total dissolved solids (TDS) in drinking water as 500 and 1000 $mg \cdot L^{-1}$ and at pH ranges of 6.0–9.5 (Brazil, 2005) and 6.5–8.5 (USEPA, 2011). Thus, the experiments were conducted to simulate maximum levels of solids in water contaminated with infectious HAdV-5, using the reference pH values of 6.0 and 8.0.

2.1. Soil characterisation and preparation

Gleysoil (hydromorphic soil) is a typical soil of riverbanks in tropical environments (Rosolen et al., 2014). This type of soil is associated with poor land management (use and occupation) and intense precipitation runoff from the landscape. In these conditions, organic matter and minerals from these soils can reach the rivers (FAO, 2006; Reatto et al., 1998) and can be found during the water treatment process. Thus, gleysoil samples were collected at a depth of up to 15 cm from a native palm swamp area in the municipality of Bela Vista de Goiás, the central portion of the State of Goiás, Brazil in the Cerrado ecoregion (17° 00′S 48° 47′W). This type of soil is typically found at river and lake borders and wetlands (Rosolen et al., 2014) and is the most common sediment carried inside rivers and lakes (FAO, 2006; Reatto et al., 1998). The samples were air-dried, passed through a 2-mm mesh sieve, and any excess plant debris were removed with the aid of tweezers and a magnifier to yield the fine earth fraction described by Gee and Bauder (1986).

The soil was subdivided into two portions: (i) soil with organic matter (WOM) and (ii) soil without organic matter (OM consumed by H_2O_2 – LOM – less organic matter/OM-free). The latter was treated with hydrogen peroxide (Whittig and Allardice, 1986) and autoclaved at 121 °C and 0.105 MPa for 3 h, three times, with 24-h intervals between each treatment (Zhao et al., 2008), to intentionally remove the soil organic matter. To ensure maximal homogeneity, the soil samples were crushed and stored at room temperature. The results of physicochemical and instrumental analyses of WOM and LOM soil samples are shown in Table 1.

The clay fraction was determined by X-ray diffraction (XRD). The silt, clay (iron-free fraction and saturated with K, Mg, Mg + glycerol, K + 350 °C, and K + 550 °C), and sand fractions were also separated. Preparation for XRD and XRD itself was performed as described by Whittig and Allardice (1986) and Resende et al. (2005). Fig. 1 shows the X-ray diffractogram obtained.