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ORIGINAL PAPER

Mineral Waste Containing High Levels of Iron from an Environmental Disaster (Bento Rodrigues, Mariana, Brazil) is Associated with Higher Titers of Enteric Viruses

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Abstract

Although the effects of heavy metals on the behavior, including infectivity, of bacteria have been studied, little information is available about their effects on enteric viruses. We report an investigation of effects on the biosynthesis of human adenoviruses (HAdV) and hepatitis A (HAV) of waters contaminated with mineral waste following an environmental disaster in Mariana City, Minas Gerais State, Brazil. The study area was affected on November 5, 2015, by 60 million m^3 of mud (containing very high concentrations of iron salts) from a mining reservoir (Fundão), reaching the Gualaxo do Norte River (sites evaluated in this study), the "Rio Doce" River and finally the Atlantic Ocean. We found substantial counts of infectious HAdV and HAV (by qPCR) in all sampled sites from Gualaxo do Norte River, indicating poor basic sanitation in this area. The effects of iron on viral infection processes were evaluated using HAdV-2 and HAV-175, as DNA and RNA enteric virus models, respectively, propagated in the laboratory and exposed to this contaminated water. Experiments in field and laboratory scales found that the numbers of plaque forming units (PFU) of HAdV and HAV were significantly higher in contaminated water with high iron concentrations than in waters with low iron concentration (< 20 µg/L of iron). These findings indicate that iron can potentiate enteric virus infectivity, posing a potential risk to human and animal health, particularly during pollution disasters such as that described here in Mariana, Brazil.

Keywords Mariana disaster · Enteric viruses · Heavy metals · Iron infectivity · Public health

Introduction

Mariana city, in the Bento Rodrigues rural area of Minas Gerais State, Brazil, is situated in the "ferriferous quadrangle" (FQ), an economically important area in the center-east of Minas Gerais. Minerals, including gold, manganese and iron are exploited by mining companies, and iron extraction is the main economic activity (Ruyters et al. 2011).

In November 2015, Mariana City was the scene of one of the largest mineral disasters in the world, when an iron mine dam—the Fundão (Mariana)—spilled 60 million m^3 of mud into the "Gualaxo do Norte", a river in the Rio Doce Basin; the mud spill reached the Atlantic Ocean. Iron was the main contaminant of the mud and resulted from iron mining activities including extraction (soil excavation), rock transportation by trucks to the crusher, transport by conveyor belt to the plant, milling, mud removal by washing with water, flotation (involving the use of maize starch, alkyl

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amines and, surfactants), pulp thickening and transportation through pipes (Segura et al. 2016).

The social impact of the Fundão disruption and the cytotoxicity of the mud has been extensively studied (Segura et al. 2016, Fernandes et al. 2016). However, the impact on public health, in particular as concerns the spread and survival of microbial pathogens, has not been studied. This is important because the sanitation in the affected area is poor, facilitating the contamination of rivers and supply waters with enteric pathogens (bacteria, viruses and protozoa).

Interaction between metals and microbial pathogens in environmental systems is an important field of research especially in areas contaminated by mine activity such as the Mariana area or more generally areas with high natural metal concentrations. Numerous articles describe the actions of metals on microorganisms, although most address bacteria; there have been few studies of the effects on enteric or other viruses (Chaturvedi et al. 2004; Otth et al. 2005). Enteric viruses are pathogens of health importance causing for example diarrhea and hepatitis. They have advantages as bioindicators of environmental contamination by human activities over bacteria, including that they are more resistant to destruction in the environment (Langlet et al. 2008; Boudaudn et al. 2012). Enteric viruses have a coat of proteins that protect their genetic material and mediate attachment of the *virion* to the host. Metal ions may act as mediators of interactions between proteins and their ligands (Deerfield et al. 2001). Therefore, iron may influence the susceptibility to and course of a variety of viral infections. Also iron can alter the genome of viruses and consequently may contribute to the emergence of new infectious strains (Chaturvedi et al. 2004; Shrivastava et al. 2002).

We report testing for human adenoviruses (HAdV) and Hepatitis A virus (HAV), as models of enteric DNA and RNA viruses, respectively, in the contaminated waters affected by the mud from Fundão. We also investigated the effects of iron (the main contaminant of this mud) on the infectivity of the model enteric viruses.

Materials and Methods

Site Sampling and Water Characterization

Five 1-L water samples were collected at each of five different sites on the Gualaxo do Norte River, 6 months after the Fundão spill (May 2016). Sites 1 and 2 were about 2 km upstream from the mine dam and were not affected by the mud. Sites 3, 4 and 5 were 2 km, 5 km and 10 km downstream from dam, respectively, and were contaminated (Fig. 1). Sampling was performed in triplicate, such that 45 water samples were collected.

Water turbidity and pH were analyzed according to APHA methods (2012). Metal concentrations (iron,

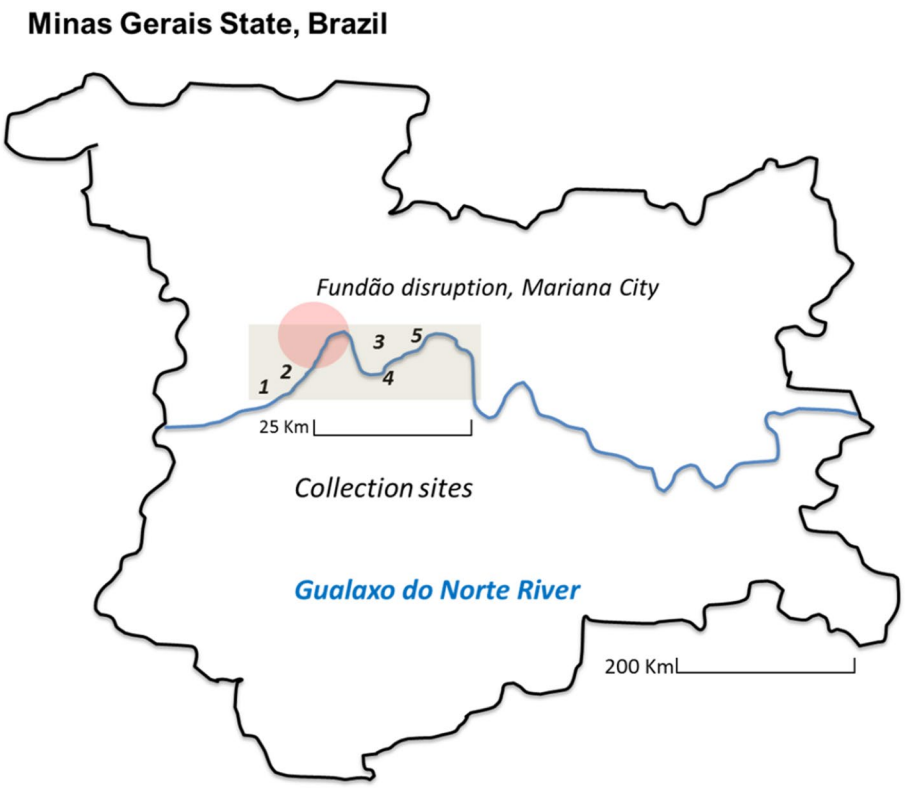

Fig. 1 Water collection sites on the Gualaxo do Norte River, in Mariana, Minas Gerais State—Brazil. Sites 1 and 2: upstream from the Fundão spill; Sites 3, 4 and 5 downstream from the Fundão spill

manganese, zinc and aluminum) were determined with a Inductively Coupled Optical Emission Spectrometer with radial vision (SPECTRO, Ciros CCD model—Geochemistry Laboratory, LGqA/UFOP).

Enteric Virus Surveillance in Water Samples

Two 1-L water samples were clarified and concentrated (final volume of 25 mL) for HAdV and HAV analyses using the glycine buffer method coupled with polyethylene glycol precipitation. Viral particles were eluted using glycine buffer (pH 9.5) and re-concentrated with PEG 6000. After centrifugation (8000 rpm for 90 min), the supernatant was discarded, and the pellet was suspended in 5.0 mL of phosphate buffer (0.1 mol/L, pH 7.2), as described by Viancelli et al. (2011).

To assay HAdV, concentrated samples at a non-cytotoxic dilution (1:32) were evaluated by plaque assay using A-549 cells (HAdV-permissive cells, derived from human lung carcinoma, European Collection of Cell Cultures), cultivated in essential basic medium at 37 °C under a 5% CO_2 atmosphere, as described by Cromeans et al. (2008). After 7 days of incubation, the total number of plaque-forming units (PFUs) was counted and plaque size was assessed by inverted light microscopy and with a digital caliper.

Quantitative PCR (qPCR) was used as described by Jothikumar et al. (2005) to assay HAV, as cell culture is not appropriate for wild HAV. Briefly, viral nucleic acid was extracted using the QIAmp MinElute Virus Spin Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and DNA produced by reverse transcription (RT) using the Sensiscript RT Kit (Qiagen). A StepOne Plus Real-Time PCR System (Applied Biosystems) was used for qPCR. Each sample was analyzed in duplicate, and genome copies (GC) were calculated. Ultrapure water was used as non-template control.

HAdV and Hepatitis A Virus Virulence Tests

Changes in viral infectivity/virulence as a consequence of the iron concentration (iron as the main metal contaminant) were tested using (i) waters collected from site 3 and (ii) solutions of various concentrations of iron prepared in the laboratory.

Collected Water Samples

Plaque assays were used as previously described to determine HAdV PFU counts in water samples. PFU counts and plaque sizes (mm) were measured macroscopically using a digital caliper (pachymeter).

Laboratory Model

Human adenovirus type 2 (HAdV-2) and hepatitis A virus type 175 (HAV-175) were used to inoculate water samples containing various concentrations of Fe^{2+} and virus replication and virulence tested. Aliquots of 1 L of water were inoculated with 2.5×10^8 PFU/L of HAdV-2 or 4.5×10^6 PFU/L of HAV-175. The water samples used included: (i) uncontaminated water samples ($n = 2$) containing 10 µg/L or 100 µg/L of Fe^{2+} and (ii) samples of water contaminated ($n = 3$) by Fundão mud containing 1500 µg/L, 2000 µg/L, or 3500 µg/L of Fe^{2+}. Non-spiked water was used as negative control, and water samples containing 4000 µg/L [Fe^{2+}] and 20 µg/L [Fe^{2+}] were used as positive controls. After inoculation of the virus into the test water, the samples were homogenized by rotation at 100 RPM for 1 h, at ambient temperature (20 ± 4 °C) and in ambient light. Virus infectivity was evaluated by plaque assay according to Cromeans et al. (2008) for HAdV-2 and to Su and D'Souza (2011) for HAV-175.

Virus Re-infection Test

We studied transmission of infectivity to the second generation of HAdV-2 and HAV-175 viral particles. Briefly, virions were collected from PFUs (first infection) into pipette tips by suction and directly used to re-inoculate A-549 and FRhK4 cells for HAdV-2 and HAV-175, respectively. The re-infection was conducted without addition of iron. Plaque assays were used and results compared with those from the first viral infection.

Statistics

Analysis of variance (ANOVA) and correlations tests were performed using GraphPad Prism 5.01 (GraphPad Software, CA, USA). Differences between means were considered statistically different when $p < 0.05$ in Tukey's posttest.

Results and Discussion

Water Turbidity and Metal Concentration

Iron concentrations in samples collected upstream from the mine dam (sites 1 and 2, not affected by the mud of Fundão) were consistent with class 1 water according to the Brazilian legislation for water quality and classification (< 0.3 mg/L). Samples from downstream from the dam (sites 3, 4 and 5, affected) showed higher iron concentrations and turbidity (Table 1). Water column movements in the river may disturb

Table 1 Water turbidity and iron, manganese, zinc and aluminum concentrations in samples collected from the Gualaxo do Norte River, at sites upstream and downstream from the mine dam, the site of the Fundao spill ($n = 15$/site)

Site	Turbidly ± SD (NTU)*	Iron ± SD (µg/L)*	Manganese ± SD (µg/L)	Zinc ± SD (µg/L)	Aluminum ± SD (µg/L)
1[a]	10 ± 2	12.3 ± 2.3	34.4 ± 4.2	8.4 ± 1.2	33.2 ± 1.2
2[a]	12 ± 2	10.4 ± 4.2	46.2 ± 4.8	9.6 ± 0.9	23.4 ± 2.1
3[b]	3200 ± 130	2700 ± 120	37.3 ± 2.8	8.3 ± 0.7	25.8 ± 2.3
4[b]	2400 ± 120	1800 ± 160	45.6 ± 4.1	11.6 ± 1.1	48.7 ± 3.2
5[b]	1100 ± 80	1200 ± 110	59.8 ± 3.9	9.8 ± 0.9	12.9 ± 1.2

[a]Upstream from the dam

[b]Downstream from the dam

*Significant difference ($p < 0.05$) between upstream and downstream from the dam

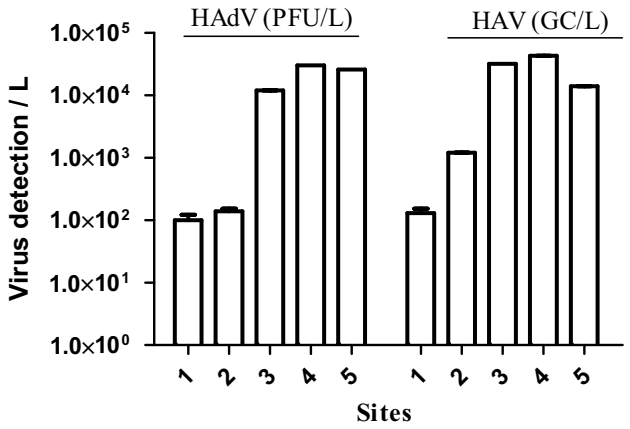

Fig. 2 HAdV and HAV titers in water collected from uncontaminated (sites 1 and 2) and contaminated (sites 3, 4 and 5) sites on the Gualaxo do Norte River

sediments, and this may have caused the high turbidity and Fe concentration at the downstream sites even 6 months after the Fundão mud spill.

Testing for Enteric Viruses Upstream and Downstream from the Dam

Mean HAdV (PFU/L) and HAV (GC/L) assay results at the five sites are presented in Fig. 2. HAdV and HAV were found in water samples from all sampling sites; 40 and 39 of the 45 water samples were positive for HAdV and HAV, respectively. The titers were significantly higher in sites affected by the mud than in those unaffected (ANOVA, $p < 0.05$). The spill flowed through houses, septic tanks, animal farms and previously still waters in deactivated mines, and presumably the mud flushed the viruses into the river. It should be emphasized that both HAdV and HAV exclusively originated from human feces (Hamza et al. 2011).

The high enteric virus titers at the sites affected by the mud may have been due in part to increased viral persistence: the stability of these pathogenic viruses is increased by attachment to electropositive solid particles. Iron can bind to solid particles, aggregating viral particles (Shrivastava et al. 2002). Aggregation can facilitate viral stability, protecting enteric viruses from inactivating factors such as UVA and UVB radiations, which are prevalent in open ecosystems (Gassilloud and Gantzer 2005; Dika et al. 2011). Iron can also potentiate the precipitation of solid particles and consequently the precipitation of viruses that are aggregated to them (Sendner et al. 2009; Fongaro et al. 2013). Thus, the presence of the mud and the high iron concentration may have increased viral persistence in the affected areas.

Effect of Mineral Waste and Iron on Viral Viability

Natural Conditions

One hundred HAdV plaques obtained using untreated water from each water sample were studied (Fig. 3). The mean diameter of plaques from unaffected sites (sites 1 and 2) was 2.3 mm, and that of plaques from affected sites was 5.3 mm; the difference was significant (ANOVA, $p < 0.05$).

The larger plaques suggest higher viral infectivity/virulence. Therefore, the mud spill and associated increased iron concentration appear to have increased viral virulence. Metal ions can alter adsorption capacity and viral penetration into host cells; it can also increase cellular protein synthesis, elevating the replicative capacity of viruses in this cells, leading to a greater cytopathic effect (Cromeans et al. 2008; Goh et al. 2016).

Laboratory Model

Plaque size was also studied in laboratory conditions (Fig. 4). Mean plaque size with uncontaminated water samples (mean Fe^{2+} concentration of 10 µg/L and 100 µg/L) was 2.1 mm for HAdV-2 and 1.8 mm for HAV-175. Mean plaque sizes with contaminated water were 5.4 mm for HAdV-2 and 3.2 mm for HAV-175. In some cases, plaque diameter in high iron concentrations was three time greater

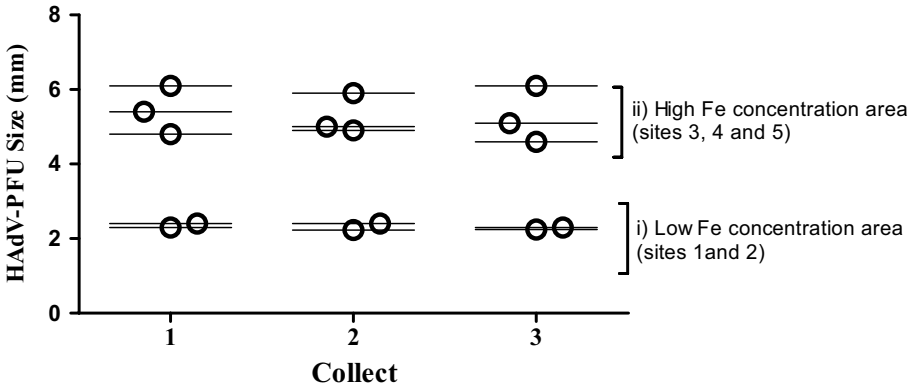

Fig. 3 HAdV plaque size (mm). The samples can be classified into two groups on the basis of plaque size: Group i is made up of waters with low iron concentration (10–100 µg/L) and Group ii those with high iron concentration (< 1000 µg/L)

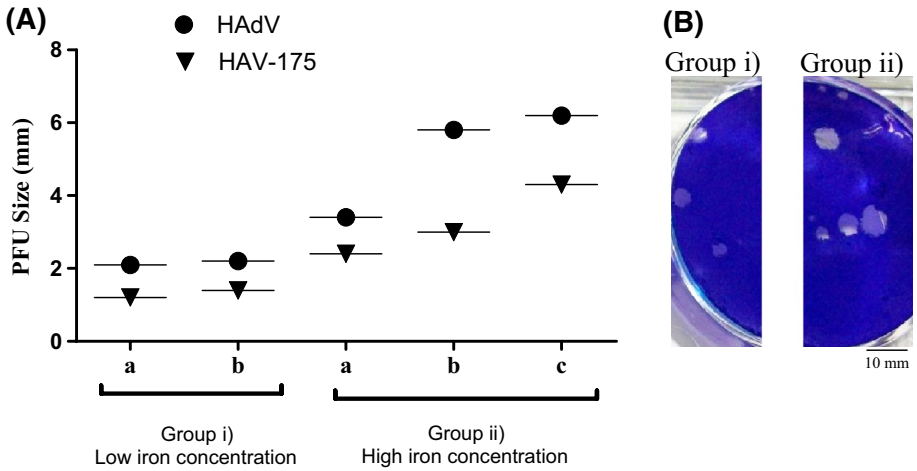

Fig. 4 HAdV-2 and HAV-175 plaque sizes (mm) for group i (water with 10 and 100 µg/L iron) and Group ii (1500, 2000 and 3500 µg/L iron). **a** Virus plaque sizes; **b** representative image of plaques for the two groups, on plates with the same viral concentration ($3\log_{10}$—PFU/mL) at same sample dilution (1:62), illustrating the different plaque sizes

than in low iron concentrations (Fig. 4). Viruses were re-inoculated to assess the infectivity on the second passage. No significant differences in plaque size were observed (the mean value for HAdV-2 was 2.1 ± 0.3 mm and for HAV-175 it was 1.4 ± 0.2).

There was a significant positive linear correlation ($p < 0.05$) between plaque size and iron concentration ($r^2 = 0.98$ for HAdV-2 and $r^2 = 0.97$ for HAV-175, respectively) (Fig. 5). Possibly, iron potentiates viral replication and does so by promoting interactions between the virus and the cell surface, increasing the rate of adsorption and cellular penetration. This would be consistent with Deerfield et al.'s (2001) findings that zinc and other metals increase virus-cell interaction. Some metals, including chromium and zinc, may alter the viral genome, increasing its replicative rate, for example in rotavirus and dengue virus (Shrivastava et al. 2002). In addition, metal ions may cause rearrangements of viral protein and therefore affect virulence (Aguilera et al. 2017).

In conclusion, we found a significantly high prevalence of the enteric viruses HAdV and HAV in water sampled from sites on the Gualaxo do Norte River affected by the spill of 60 million m^3 of mud from an iron mine dam (Fundão,

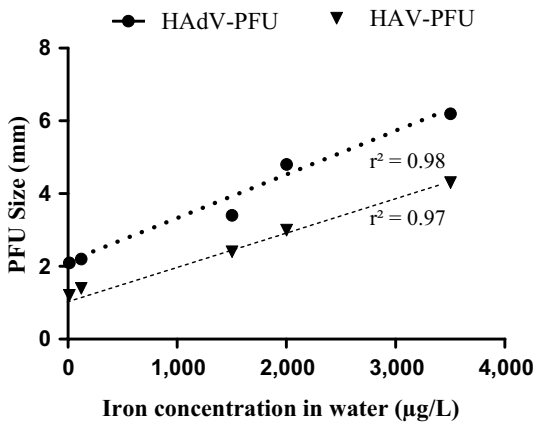

Fig. 5 Linear correlation between iron concentration in water and virus plaque size ($p < 0.05$)

Mariana, Brazil). This spill was an environmental disaster and in addition to mud contamination, the poor basic sanitation in the area favored fecal contamination of water resources. Our results demonstrate that iron in environmental water can enhance the titers and virulence of human enteric viruses. The spread of water contaminated with mud,

particularly mud rich in iron, thus appears to increase risks to human and animal health.

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