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# Animal and human enteric viruses in water and sediment samples from dairy farms

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ABSTRACT

The detection of enteric viruses accompanied by a characterization of the viruses found in a given environmental matrix may inform about the sources of fecal contamination. In the present work, 55 water samples and 20 sediment samples were collected from 21 small farms in southern Brazil. Coliform counting was done as well as molecular detection of human enterovirus (EV), and human and animal adenoviruses. Viral detection was performed using real-time quantitative PCR (qPCR). Furthermore, the viral viability of human AdV (HAdV) by ICC-RT-qPCR in sediment and water samples was analyzed. Regarding to the coliforms, only 72.7% of the samples showed fecal contamination. HAdV was detected in 87.3% of water samples, followed by AvAdV (27.3%), CAV (20%), BAV (7.3%) and PoAdV or EV (1.8%). From the sediment samples, HAdV (80%) followed by CAV (20%), BAV (5%) and no positive results for PoAdV or EV. The viral loads ranged from $1.57 \times 10^2$ gc/L up to $6.68 \times 10^9$ gc/L (water), and from $1.97 \times 10^3$ gc/g to $2.18 \times 10^8$ gc/g (sediment). Most of these viral particles in water should be non-infectious, since after the ICC-PCR, HAdV was detected in only 4 samples (8.8%). On the other hand, it is noticeable that 5 sediment samples (25%) gave positive results for the presence of infectious viral particles.



## 1. Introduction

The overall quality of soils and sediments is highly impacted by fecal contamination. It is well known that these environmental matrices may harbor greater amounts of enteric viruses and other fecal microorganisms than water (Rao et al., 1986). The soil can act as an important reservoir of varied natural resources; however, it may also allow the permanence of various microorganisms that cause diseases (Nasser and Metcalf 1987; Santamaría and Toranzos, 2003). Sediment is the result of soil erosion, and it is suggested that the sediment from rivers, lakes and dams can act as a reservoir for pathogens (Alm et al., 2003). Viruses associated with particulate matter in suspension or in solid matrices tend to remain viable for a longer time than if they were dispersed in water (Lipson and Stotzky, 1984; Schenewski and Julich, 2001). Enteric viruses present in the soil as a result of the release of sewage, irrigation, and waste from agro-pastoral activities can migrate to the deepest layers of the soil, reaching groundwater as a result of the successive adsorption–desorption phenomena (Schwartzbrod, 1995). The shedding of viral particles depends on the virus studied: it has been suggested that differences in the surface charge of the virions have an important role in the association of viral particles to solids (Gerba and Bitton, 1984). Retention of viral particles in the soil also depends on the soil type, temperature, pH, moisture level (Gerba et al., 1988), isoelectric point and hydrophobicity (Williamson et al., 2005). Detection of these pathogens in the sediments is an alternative environmental impact assessment (Greening et al., 2002). In developing countries, ever-expanding areas for farming and intensive land use have led to rapid soil degradation, especially in tropical and subtropical areas (Arshad and Martin, 2002). According to Shepherd and Wyn-Jones (1997), the risk of transmission for waterborne diseases by consuming water sources from rural properties is 22 times greater than the consumption of water from a public supply system. Water contamination in rural areas may triggers considerable losses in milk production by the involvement of these pathogens in their animals, besides causing diseases in human beings (Jacintho et al., 2005).

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Enteric viruses are a heterogeneous group of viral agents associated with subclinical infections and diseases in humans and animals, such as the viruses studied here: human adenovirus (HAdV), bovine adenovirus (BAV), canine adenovirus (CAV), avian adenovirus (AvAdV), porcine adenovirus (PoAdV) and enterovirus (EV). The agents mentioned above are characterized by their stability both in the gastrointestinal tract as in the environment as well as the characteristic of being excreted through humans and animals feces and can resist environmental contaminants (soil and water) for long periods of time (Katayama et al., 2002). Moreover, it is suggested that such viruses are important indicators of fecal contamination (Jiang et al., 2001; Carducci et al., 2008; Katayama et al., 2008; Ley et al., 2002; Hundesa et al., 2006; De Oliveira et al., 2012). Most of the enteric viruses are host-specific, thus being able to track the primary source of fecal contamination in a given environment (Ahmed et al., 2010; Jiang et al., 2007). Enteric viruses are often found in groundwater, and being waterborne, are responsible for a significant proportion of cases of gastroenteritis related to drinking water (Abbaszadegan et al., 1999; Borchardt et al., 2003).

It is important to assess the viral viability for a proper evaluation of infection risks. ICC-RT-qPCR allows us to confirm the presence of infectious viruses by the analysis of the infection of cultured cells and subsequent transcription of viral mRNA, thus, viral detection of mRNA indicates the presence of infectious viral particles (Fongaro et al., 2013), the sensitivity is higher, since the cell culture prior to nucleic acid amplification increases the amount of infectious virus allowing viral detection before to produce observable cytopathic effect (Li et al., 2010).

The aims of this article are the detection of coliforms and various fecal-oral transmission of viral agents (HAdV, BAV, CAV, PoAdV, AvAdV and EV) in water and sediment samples from springs, wells, dams and streams in rural properties from the cities of Rolante and Riozinho, southern Brazil, to quantify viral loads from both matrices and assess the viability of HAdV detected in the water and sediment samples on farms. The main goals of the present study are: (a) to gather information about the presence and diversity of viral markers of fecal contamination in water samples collected inside small farms in a populated watershed; and (b) tracing the main sources of contamination, from domestic animals to human beings, for water bodies located in these farms.

## 2. Materials and methods

### 2.1. Sampling

The municipalities of Rolante and Riozinho, located in Vale do Paranhana, Rio Grande do Sul, have most of their populations living on small farms, and the economy is based on dairy production. In addition, some have cattle, poultry, swine or fish. The three main rivers in the region are Rolante, Areia and Riozinho Rivers. Fifty-five water samples and 20 sediment samples from springs, wells, dams and streams from 21 farms located in the municipalities neighboring Rolante (14 farms) and Riozinho (7 farms) (Table 1). Samples were obtained from a single collection in the sites mentioned above (on 03/15/2011 and 03/22/2011, respectively). Each collection point had its location demarcated by Global Positioning System and its UTM coordinates annotated and plotted. Water samples (500 mL each) and sediment samples (100 g each) were collected aseptically from each point in sterilized glass bottles. The samples were transported to the laboratory under refrigeration, and were kept at 4 °C until sample concentration. Water abstracted from wells and springs on farms from Rolante and Riozinho are used for both human and animal consumption, washing utensils (including those used for milk storage), personal hygiene, and crop irrigation. Five properties have dams for fish farming, and some of these are alongside a dunghill. Several present streams that originate in their own dams, springs and household sewage, are contaminated by the direct discharge of human and animal feces in water bodies, in all properties, cattle has direct access to ponds and rivers, and excreta from bovine and other species runs into water by superficial runoff. These are the most likely sources of contamination, and the same situation was found in all farms.

**Table 1**
Geographic coordinates (Sirgas Datum 69) of the farms chosen for the present study, municipalities of Rolante (1) and Riozinho (2), Brazil.

| Farms | Geographic coordinates | |
|---|---|---|
| P1[1] | S29°38′26.0″ | W050°35′18.9″ |
| P2[1] | S29°38′19.5″ | W050°34′31.3″ |
| P3[1] | S29°38′30.4″ | W050°34′37.9″ |
| P4[1] | S29°38′29.1″ | W050°34′43.1″ |
| P5[1] | S29°39′42.9″ | W050°35′13.7″ |
| P6[1] | S29°39′55.8″ | W050°34′46.0″ |
| P7[1] | S29°38′50.9″ | W050°32′09.5″ |
| P8[1] | S29°38′52.1″ | W050°32′07.2″ |
| P9[1] | S29°39′14.1″ | W050°32′53.9″ |
| P10[1] | S29°38′18.0″ | W050°32′08.1″ |
| P11[1] | S29°36′23.7″ | W050°31′43.3″ |
| P12[1] | S29°35′11.0″ | W050°34′03.6″ |
| P13[1] | S29°37′17.5″ | W050°34′17.2″ |
| P14[1] | S29°39′02.6″ | W050°34′21.5″ |
| P1[2] | S29°37′58.7″ | W050°28′27.9″ |
| P2[2] | S29°38′03.2″ | W050°28′48.1″ |
| P3[2] | S29°37′05.4″ | W050°26′55.0″ |
| P4[2] | S29°36′48.6″ | W050°26′06.5″ |
| P5[2] | S29°35′54.0″ | W050°27′27.1″ |
| P6[2] | S29°37′31.5″ | W050°25′49.1″ |
| P7[2] | S29°37′28.7″ | W050°24′55.8″ |

### 2.2. Coliform detection

Fecal coliforms were detected by a Colilert® test kit following the manufacturer's methodology within 24 h after collection. The specific nutrient indicators that make up the Colilert® are the substrate ONPG (ortho-nitrophenol-β-galactopiranoside) and MUG (4-methyl-umbeliferil-β-D-glucuronic). The test was considered positive for fecal coliforms when staining showed blue fluorescence when exposed to UV light. The test was considered negative in the absence of staining. The results were expressed in MPN (most probable number in 100 mL of water) according to the table provided by the manufacturer.

### 2.3. Sediment samples

In order to detect viruses from soil samples, 1 g of the solid (sediment) was diluted 1 mL of Eagle's minimum essential medium (E-MEM, Nutricell; pH 11.5). The solution was homogenized by vortexing it for 1 min and then it was centrifuged at 14,000 rpm for 10 min (Staggemeier et al., 2015). The supernatant was used for the DNA/RNA extraction.

### 2.4. Virus concentration

Water samples were concentrated using an adsorption–elution method previously described by Katayama et al. (2002) with minor modifications. All procedures were conducted in biosafety cabinets to avoid sample contamination. Briefly, 0.6 g of $MgCl_2{\cdot}6H_2O$ was mixed with 500 mL of each water sample and the pH was adjusted to 5.0 using a solution of 10% HCl. Subsequently, the resulting mixture was vacuum filtered through a negatively sterile membrane (type HA, 0.45 m pore size; 47 mm diameter). The membrane was rinsed with 87.5 mL of a 0.5 mM $H_2SO_4$ (pH 3.0) solution followed by elution of viral particles adsorbed by the membrane with 2.5 mL of 1 mM NaOH (pH 10.5). The filtrate was then neutralized with