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ORIGINAL PAPER

Modeling the Transport of Human Rotavirus and Norovirus in Standardized and in Natural Soil Matrix-Water Systems

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Abstract

We modeled Group A Rotavirus (RVA) and Norovirus genogroup II (GII NoV) transport experiments in standardized (crystal quartz sand and deionized water with adjusted pH and ionic strength) and natural soil matrix-water systems (MWS). On the one hand, in the standardized MWS, Rotavirus and Norovirus showed very similar breakthrough curves (BTCs), showing a removal rate of 2 and 1.7 $\log_{10}$, respectively. From the numerical modeling of the experiment, transport parameters of the same order of magnitude were obtained for both viruses. On the other hand, in the natural MWS, the two viruses show very different BTCs. The Norovirus transport model showed significant changes; BTC showed a removal rate of 4 $\log_{10}$, while Rotavirus showed a removal rate of 2.6 $\log_{10}$ similar to the 2 $\log_{10}$ observed on the standardized MWS. One possible explanation for this differential behavior is the difference in the isoelectric point value of these two viruses and the increase of the ionic strength on the natural MWS.

Keywords Rotavirus · Norovirus · Porous media · Transport · Filtration · Numerical modeling

Introduction

Viruses are an important biological contaminant of water sources being responsible for several illnesses including acute gastroenteritis and hepatitis among others (Estes and Greenberg 2013, WHO 2014, Xagoraraki et al. 2014). Enteric viruses present in sewage from septic tanks can be transported through the soil and eventually contaminate groundwater used for irrigation of crops or drinking water. A soil-based natural treatment process and viral reduction is observed during the passage of the virus through the soil. Understanding the factors which drive viral transportation and reduction in the soil is important in order to protect aquifers mainly used for drinking water and irrigation.

Although enteric viruses occur in low concentrations in the aquatic environment, especially groundwater, they have low infectious doses causing an important health problem (Aw 2018). For example, in some cases, it is necessary to take tens of liters of field sample in order to detect viruses (Haramoto et al. 2018). The numerical modeling of virus transport is a tool that can help deal with this problem. Numerical modeling can deal with the individual behavior of each virus, if the governing transport parameters are known, and simulate concentrations that might be impossible to measure: dose–response estimations made by Regli et al. (1991) indicate that in order to reduce the risk of infection to under 10^{-4}/person/year, the maximum allowable concentration in drinking water is 2.22×10^{-7} RoV/L. However, in order to develop these models, it is necessary to characterize not only the physical environment and the properties of the virus but also the particular interaction between them. This can be achieved through soil column experiments where viruses' breakthrough curves (BTC) can be obtained (Wong

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et al. 2014; Betancourt et al. 2019). As not all viruses can be quantified by plaque assay, an alternative is quantification by qPCR. One of the main issues associated with this method is that it does not quantify the amount of viable virus, but the amount of viable genetic material (Rose et al. 1997). Attachment to soil and soil organic matter can cause virus inactivation, which will not be measured by qPCR, since non-infectious virus particles are also counted. Therefore, the decay rates obtained with this methodology will be higher than the actual virus inactivation rates. That said, for many human viruses qPCR is the only alternative for quantification.

There are many experimental works in the literature focused on virus surrogates transport on porous media (Walshe et al. 2010; Frohnert et al. 2014; Hornstra et al. 2018), and in many of them, its numerical modeling is also addressed (Schijven et al. 2002; Sadeghi et al. 2011; Zhang et al. 2012; Mondal and Sleep 2013; Amin et al. 2014; Morales et al. 2014; Kvitsand et al. 2015; Mayotte et al. 2017; Sasidharan et al. 2018; Tesson et al. 2018). However, the number of papers modeling human virus transport through porous media, and reporting the governing parameters, is noticeably less (Schijven et al. 2003; Stevenson et al. 2015; Kokkinos et al. 2015; Syngouna et al. 2017; Betancourt et al. 2019). As pointed by Wong and Molina (2017), besides Human Adenovirus (HADV) and Rotavirus, no studies have applied qPCR to report BTC data of other enteric viruses.

Virus behavior in saturated porous media is complex and varies significantly from one virus to another. Wong et al. (2014) reported that the bacteriophage MS2, one of the most commonly used surrogates for human viruses, showed significant differences on porous media transport with respect to human adenovirus (HAdV2, strain 6), especially at high ionic strengths. Shi et al. (2012) also show that the mobility of MS2 on column filtration experiments can be much higher than human adenovirus (HAdV41). Pang et al. (2014) found that MS2 phage overpredicted concentrations of adenovirus (AdV41) and rotavirus (RoV, VR-2018), by 1 and 2 orders of magnitude, respectively. Kokkinos et al. (2015) found that adenovirus (HAdV35) average mass recovery values were lower than those reported for MS2 and ϕX174. Xu et al. (2017) reported that adenovirus behaves similarly in terms of transport and retention with bacteriophage ϕX174 in non-reactive porous media, but very differently in reactive porous media. Betancourt et al. (2019) observed differences in the removal rates among viral surrogates and viruses naturally present in treated wastewater effluents recharged during soil aquifer treatment. Therefore, there is no consensus about a universal viral indicator or surrogate (Hernroth et al. 2002; Diston et al. 2015).

Human Norovirus is recognized as the leading cause of acute gastroenteritis in all age groups (Hall et al. 2013). Rotavirus is an important cause of diarrheal morbidity and mortality in young children and the elderly worldwide (Widdowson et al. 2009). According to effluent and clinical studies, the levels of Norovirus and Rotavirus are similar in the population of Salto, Uruguay (Victoria et al. 2014). However, in a study conducted in the Salto aquifer, in which the presence of Rotavirus A, Norovirus GII, and human Adenovirus were evaluated, only the presence of Rotavirus A was systematically detected (Gamazo et al. 2018).

In this paper, we present the results of Group A Rotavirus (RVA) and Norovirus genogroup II (GII NoV) transport experiments in standardized (crystal quartz sand and deionized water with adjusted pH and ionic strength) and natural soil matrix-water systems (MWS) from the Salto Aquifer. To the author's knowledge, this is the first work reporting Norovirus breakthrough curves data and its transport parameters by applying qPCR. We evaluate different models to simulate virus transport and we report the parameters that best adjust the BTC. We also evaluated transport differences and similarities of these two viruses in both systems, and we discuss the implications of these results on the search for a common surrogate for field experiments.

Materials and Methods

Porous Medium and Circulating Water

For this work, we used two sets of materials to prepare the column experiments: one set with a clean crystal quartz sand and deionized water, which will be referred as standardized MWS, and another set with sand and water from the Salto aquifer, which will be referred as natural MWS.

For the standardized MWS, crystal quartz sand was acquired from Gebrüder Dorfner with a size ranging from 0.1 to 0.8 mm. To remove possible impurities, the sand was heated 4 h to 850 °C, soaked in hydrochloric acid 12 M for 48 h, and washed with deionized water until no changes in pH were observed. For the column experiments, deionized water 1 mM (NaCl) with a pH of 7.2 (NaOH) was used as pore water.

For the natural MWS, the aquifer sample used had a particle size distribution of 1.1% very coarse sand, 9.2% coarse sand, 47.8% medium sand, 40.5% fine sand, and 1.4% very fine sand. The pore water used for this set was extracted from the aquifer and was classified as a magnesium/calcium bicarbonate water, and had an ionic strength of 15.1 mM and a pH of 7.2.

Virus Samples

For this work, a 10% fecal suspension containing RVA and GII NoV were introduced in the water used for the column

experiments. These particular viruses were selected since they are the most common viral pathogens implicated in gastroenteritis cases in Salto Uruguay and worldwide (Tort et al. 2015; Victoria et al. 2016; Crawford et al. 2017; Atmar et al. 2018).

The organic matter of the fecal suspension was determined by the methodology described in Standard Methods for the Examination of Water and Wastewater (APHA 2017) at 105 °C and 550 °C weight loss.

Column Experiments

The experiments were performed in glass stratigraphy columns (Solventplus®) with an internal diameter of 50 mm and a length of 100 mm. Each column was wet packed in 0.5 cm increments with the corresponding water (deionized water for the quartz sand and aquifer water for the aquifer sand) up to 67 mm. Each column was flushed upward with approximately 10 pore volumes (PV) at a rate of 0.05 cm/min to remove air bubbles and to establish steady-state flow.

To estimate column porosity and dispersion coefficient, a sodium nitrate solution ($NaNO_3$ 1 M) was injected into the columns and then washed out with 10 PV of the corresponding water, as done by Stevenson et al. (2015). 30 samples of the columns effluent were analyzed and a BTC was constructed. Porosity and dispersion coefficient values were obtained solving the advection dispersion equation using the Hydrus-1D program (see the Mathematical model section) (Pang et al. 2014; Betancourt et al. 2019). For the standardized MWS, a porosity value of 0.41 and a dispersion coefficient of 0.02 cm were obtained; and for the natural MWS, the values obtained were 0.25 and 0.04 cm, respectively.

The injection water for each column was prepared by inoculating a known amount of Rotavirus and Norovirus into a sample of pore water. Two PV of virus-loaded water was injected at a constant flow rate into the columns, followed by, the incoming water that was replaced by virus free water.

For the standardized MWS, the flow rate used was 0.058 cm/min, containing 1.30E+9 genome copies per milliliter (gc/ml) of Rotavirus and 3.60E+8 gc/ml of Norovirus. For the natural MWS, the flow rate used was 0.054 cm/min, and the viral concentration of the injection water was 6.80E+8 gc/ml for Rotavirus and 2.46E+8 gc/ml for Norovirus.

Samples (32) of the columns' outgoing water were frozen at − 15 °C for subsequent virus quantification.

During each experiment, a sample of the water with viruses was maintained in the same conditions as the column in order to evaluate the natural decay of the viruses in the water. All the experiments were carried out at a temperature of 21 °C which coincides with the average annual temperature value of the aquifer (Gamazo et al. 2018).

Viral Molecular Analysis

Nucleic acids were extracted using QIAmp viral RNA mini kit (QIAGEN®, Hilden, Germany) according to the manufacturer's instructions. cDNA synthesis was performed using the SuperScript® II Reverse Transcriptase and Random Hexamer Primers, following the recommendations of the manufacturers.

The concentration of RVA and GII NoV was determined using qPCR with TaqMan® technology, SensiMix™ II Probe Kit (Bioline Reagents Ltd.), and Rotor-Gene Q instrument (Qiagen®) following the manufacturer's recommendations. Standard curves were performed using two plasmids kindly provided by Dr. T. Fumian from the Laboratory of Comparative and Environmental Virology, Oswaldo Cruz Foundation, Brazil, which contain NSP3 and OFR1-ORF2 junction genomic region for RVA and NoV GII, respectively (Kageyama et al. 2003; Zeng et al. 2008).

Genomic quantification of RVA by qPCR was performed with primers towards the NSP3 gene, as described by Zeng et al. (2008), with a standard curve performed with nine points of serial dilutions of plasmid [10^8 to 10^0 genomic copies/reaction (gc/r)] that yielded a slope of − 3.59 and a reaction efficiency of 0.90.

Quantification of NoV genogroup II (GII) was achieved using the ORF1-ORF2 junction region with a standard curve performed with the same serial dilutions as the RVA quantification that yielded a slope of − 3.43 and a PCR efficiency of 0.96 (Kageyama et al. 2003).

Data Analysis

Mathematical Model

Most studies consider the advection diffusion–dispersion equation to model virus transport through porous media. The majority of these studies may consider a two-site attachment–detachment kinetic model, with a limit in the maximum amount of viruses that can be attached to the solid phase, and inactivation in the solid and in the aqueous phase (Schijven et al. 2002; Pang et al. 2014; Hornstra et al. 2018). By considering all these processes, the governing equations are:

$$\frac{\partial \theta c}{\partial t} + \rho \frac{\partial s_1}{\partial t} + \rho \frac{\partial s_2}{\partial t} = \frac{\partial}{\partial x}\left(\theta \alpha_L v \frac{\partial c}{\partial x}\right) - \frac{\partial \theta v c}{\partial x} - \mu_l \theta c - \mu_s \rho (s_1 + s_2) \quad (1)$$

$$\rho \frac{\partial s_i}{\partial t} = \theta k_{a,i}\left(1 - \frac{s_i}{s_{i,\max}}\right) c - k_{d,i} \rho s_i \ (i = 1, 2) \quad (2)$$

where θ is the porous media porosity, c is the virus concentration, ρ is the dry bulk density, s_i is the concentration of the adsorbed viruses at the kinetic sorption site i, α_L is the longitudinal dispersivity coefficient, v is the aqueous phase flow, μ_l is the inactivation rate coefficient of viruses on the liquid

phase, μ_s is the inactivation rate coefficient of adsorbed viruses, $k_{a,i}$ and $k_{d,i}$ are respectively the attachment and detachment rate coefficients for the kinetic sorption site i, and $s_{i,\max}$ is the maximum concentration for attached viruses for the kinetic sorption site i (blocking). There are other works in the literature that model the transport of viruses considering fewer processes (Anders and Chrysikopoulos 2009, Pang et al 2014).

Numerical Code and Parameter Estimation

For this work, virus BTCs were modeled using the Hydrus-1D program. Hydrus-1D can solve Eqs. 1 and 2 by the finite element method and implements a Marquardt–Levenberg type of parameter estimation technique for inverse estimation of virus transport parameters (Šimůnek et al. 2012). The program can identify the value of the parameters that best match the simulated concentrations with those observed for a given model. However, the tool is not capable of automatically evaluating different models, for example, compare a model with one kinetic sorption site to the one with two kinetic sorption sites. Therefore, different models have to be manually set on Hydrus and parameter estimation for each is performed automatically.

Model Evaluation

Different models were systematically evaluated, gradually increasing the number of processes involved. Models were run with one or two kinetic sorption sites, with or without upper limits in the concentration of attached viruses, and with or without inactivation in the solid phase. The performance of the models was evaluated by comparing the R squared (R^2) of the predicted versus observed log value and an Akaike Information Criterion (AIC). This last criterion penalizes the adding of fitting parameters and allows identifying the model that best reproduce observations with a smaller number of parameters, following the principle of parsimony (Mashayekhi et al. 2016) (Fig. 1).

Results

Standardized Matrix-Water System

For the experiments performed on clean crystal quartz sand and deionized water, the model that shows best performance in simulating the Rotavirus and Norovirus transport was the one that considers two kinetic sorption sites, no blocking, and inactivation in the liquid and the solid phase. Parameter

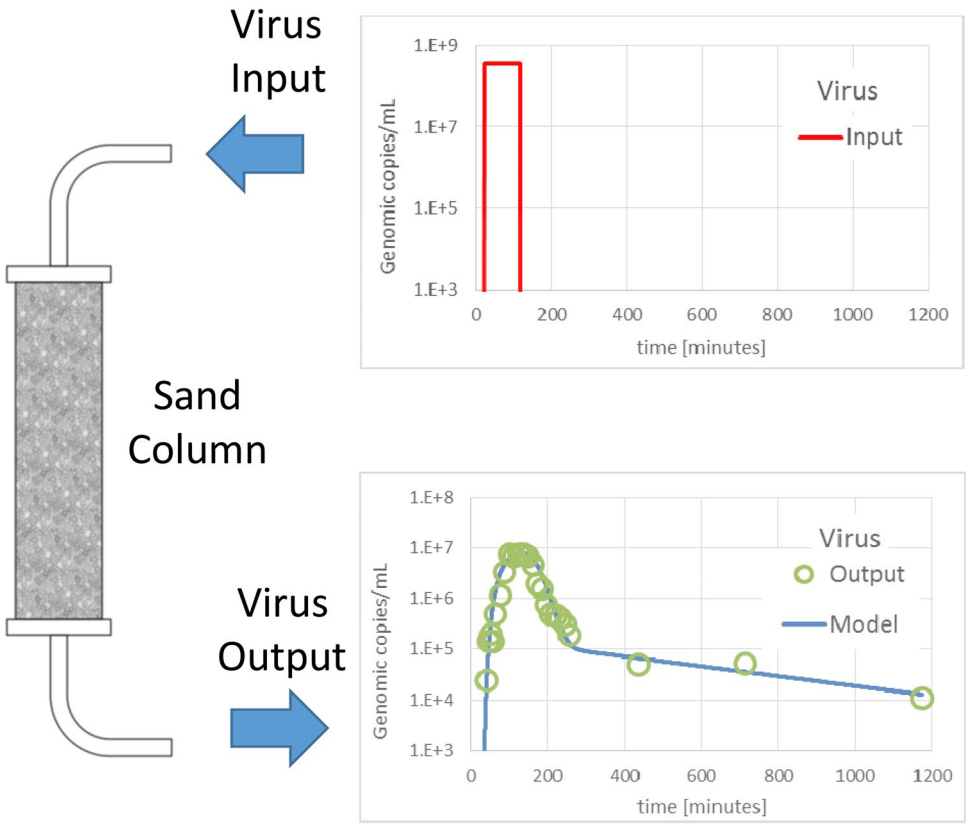

Fig. 1 Experiment setup graphical description

values are shown in Table 1. Values for R^2 were 0.953 for the Rotavirus experiment, and 0.972 for Norovirus.

As shown in Fig. 2, Rotavirus was removed at a rate of approximately 2 $\log_{10}$ on the standardized MWS and the rate of removal was 1.7 for Norovirus (the viral load on the injection water was 1.30E+9 gc/ml for Rotavirus and 3.60E+8 gc/ml for Norovirus, both with a quantification error of less than 1%.). The experimental removal efficiency based on the particle breakthrough, calculated according to Tufenkji and Elimelech (2004), was 2.02E−02 for Rotavirus and 1.65E−02 for Norovirus.

Table 1 Transport parameter values and standard deviation for the standardized matrix-water system

Parameter	Rotavirus		Norovirus	
	Value	σ	Value	σ
μ_l [min^{-1}]	2.95E−03	–	1.23E−03	–
μ_s [min^{-1}]	5.09E−03	1.80E−03	2.27E−03	2.34E−04
$k_{a,1}$ [min^{-1}]	2.91E−01	5.25E−02	1.76E−01	3.92E−02
$k_{d,1}$ [min^{-1}]	4.58E−01	1.12E−01	1.81E−01	4.67E−02
$k_{a,2}$ [min^{-1}]	9.24E−02	1.56E−03	7.85E−02	2.80E−03
$k_{d,2}$ [min^{-1}]	7.12E−05	3.51E−05	4.82E−05	9.82E−06

Natural Matrix-Water System

For the experiments in which sand and water from the Salto aquifer were used, different transport processes had to be considered in order to best describe the BTC of each virus. The model that fitted the Rotavirus BTC best includes the inactivation in the liquid and in the solid phase and two kinetic sorption sites: one with blocking and no detachment, and another with attachment and detachment. The values for the R^2 for this model was 0.905. Removal of Norovirus in the natural MWS was much higher than in the standardized MWS and the model that fitted the BTC best was one kinetic site with blocking, no detachment, and inactivation in the liquid phase. The value of the R^2 for this model was 0.348. Calibration was done manually, since the Hydrus1D calibration algorithm failed to converge to acceptable solutions. Parameter values for both models are shown in Table 2. The

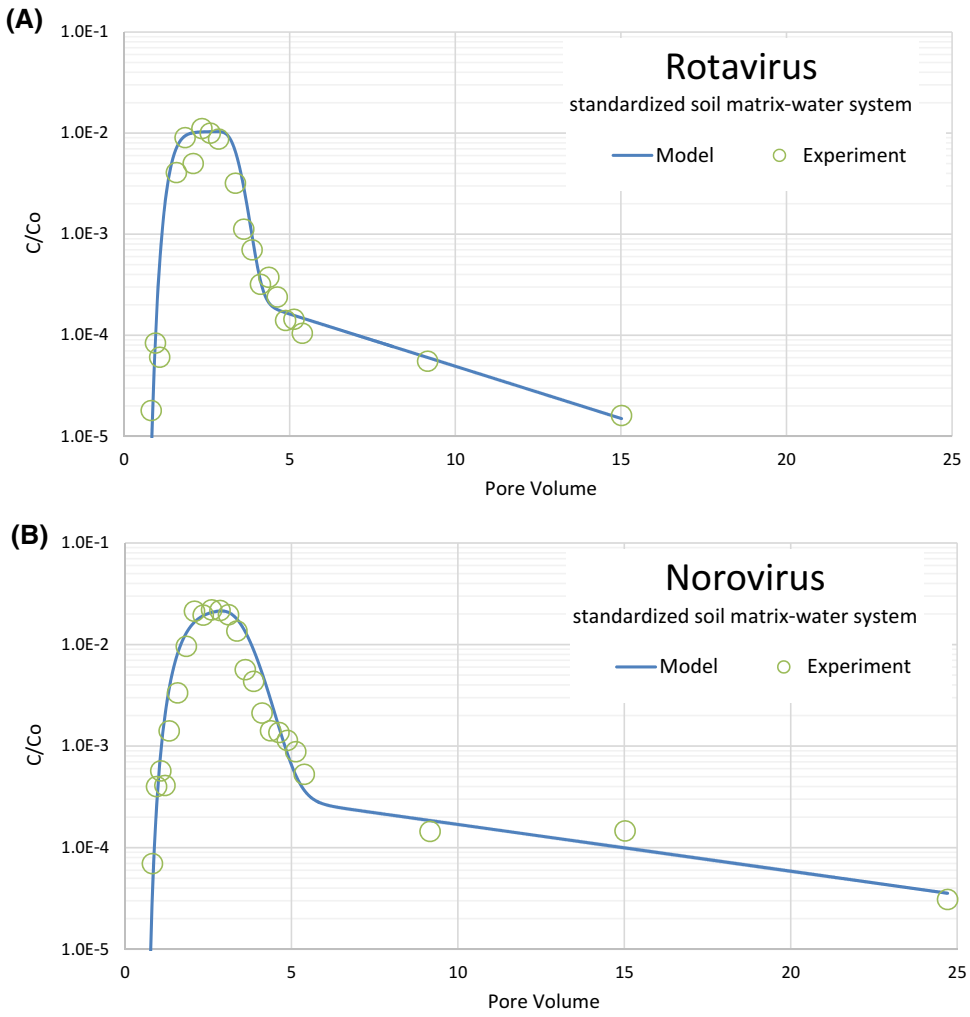

Fig. 2 Breakthrough curves for the standardized matrix-water system: **a** Rotavirus and **b** Norovirus

Table 2 Transport parameter values and standard deviation for the natural matrix-water system

Parameter	Rotavirus		Norovirus	
	Value	σ	Value	σ
μ_l [min^{-1}]	2.28E−03	–	1.72E−3	–
μ_s [min^{-1}]	2.81E−03	1.44E−03	–	–
$k_{a,1}$ [min^{-1}]	1.72E−01	3.71E+08	3.71E−1	–
$s_{1,max}$	9.76E+07	7.58E−02	3.30E+08	–
$k_{a,2}$ [min^{-1}]	1.11E−01	9.04E−02	–	–
$k_{d,2}$ [min^{-1}]	1.37E−04	1.35E−04	–	–

Norovirus BTC in the natural MWS and its modeling should be considered qualitatively. It is very likely that the removal rate was so large that the concentrations dropped below the detection limit of the method.

As shown in Fig. 3, Rotavirus that was removed was 2.6 $\log_{10}$ in the natural MWS and Norovirus was about 4 $\log_{10}$ (the viral load on the injection water was 6.80E+8 gc/ml for Rotavirus and 2.46E+8 gc/ml for Norovirus, both with a quantification error of less than 1%). The experimental removal efficiency based on the particle breakthrough was 1.96E−02 for Rotavirus and 2.89E−02 for Norovirus.

Discussion

Due to the expense and the health risk involved in working with pathogenic viruses, the understanding of their transport and retention in saturated porous media (especially in groundwater aquifers) is limited (Pang et al. 2014). That is why most studies focus on the transport of bacteriophages as human virus surrogates in porous media. Nevertheless, as many authors have reported, a particular surrogate can behave similarly to a certain virus in one MWS, but can have a very different response in another system (Stevenson et al. 2015; Betancourt et al. 2019). Therefore, the applicability of surrogates in the field is case-dependent, and specific experiments involving human viruses are necessary.

From a bibliographic review, it can be noted that while most of the papers report parameters associated to the

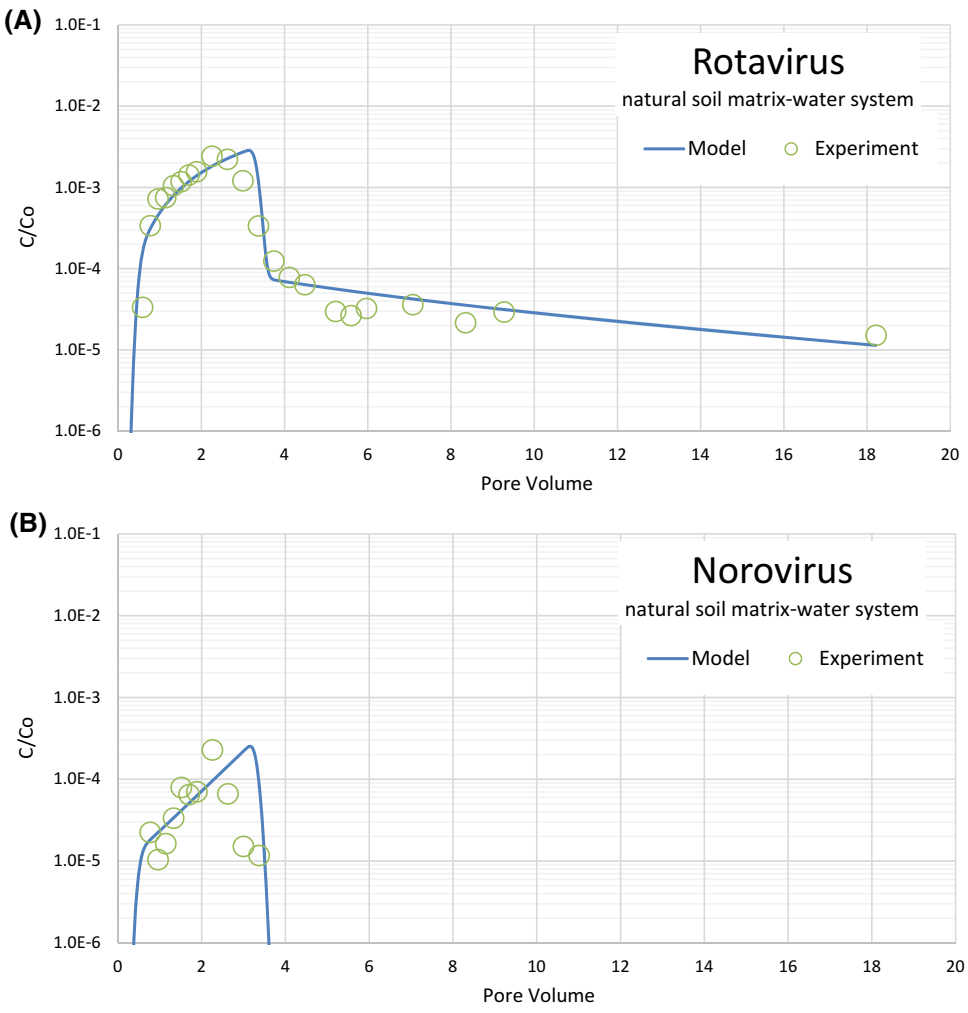

Fig. 3 Breakthrough curves for the standardized matrix-water system: **a** Rotavirus and **b** Norovirus

transport of human virus surrogates in porous media (Anders and Chrysikopoulos 2009; Frohnert et al. 2014; Hornstra et al. 2018; Betancourt et al. 2019), only a few report the actual human virus transport parameters. The majority of reported human virus parameters are obtained in standardized MWS, while very few do so for natural MWS (Stevenson et al. 2015). In this work, we report actual Rotavirus and Norovirus transport parameters for a particular natural MWS. To the author's knowledge, this is the first work in which human Norovirus parameters are reported. We also show experimentally that the similarity of the transport observed in standardized MWS for these two viruses might not be observed on natural systems.

In the standardized MWS. Rotavirus and Norovirus showed very similar BTC. The models that best fit the observations of both viruses considered inactivation in the aqueous and the adsorbed phase, and two kinetic exchange sites without blocking.

Pang et al. (2014) conducted similar experiments with Rotavirus but for the modeling, they considered two kinetic exchange sites, one reversible and the other one irreversible, and they did not consider inactivation. For the reversible kinetic exchange site, they obtained average attachment and detachment coefficient values in the same order of magnitude of the ones obtained in this work on the standardized MWS (Pang: $k_{a,1}=4.70\text{E}{-}02\ \text{min}^{-1}$, $k_{d,1}$ $6.51\text{E}{-}02\ \text{min}^{-1}$; this work: $k_{a,1}=2.91\text{E}{-}01\ \text{min}^{-1}$, $k_{d,1}$ $4.58\text{E}{-}01\ \text{min}^{-1}$). For the irreversible kinetic exchange site, they obtained an average attachment coefficient value of the same order, but slightly lower than the one obtained in this work (Pang: $k_{a,2}=2.45\text{E}{-}02\ \text{min}^{-1}$, this work: $k_{a,2}=9.24\text{E}{-}02\ \text{min}^{-1}$, $k_{d,2}$ $7.12\text{E}{-}05\ \text{min}^{-1}$). The value of the second detachment coefficient obtained in this work for Rotavirus was 3 orders of magnitude smaller than the attachment value.

Most studies used viruses produced in the cell culture for porous media transport experiments. In this work, we used viruses from fecal suspensions, which implies the presence of organic matter in the virus-loaded water. The interactions of organic matter with viruses during porous media transport are complex and are probably responsible for part of the uncertainty in predictions. There are examples in the literature that show different behaviors depending on the virus type and the MWS (Schijven and Hassanizadeh 2000). Organic matter affects the interaction between viruses and soil attachment sites and can produce virus aggregation, which can have a significant impact on quantification and behavior of viruses during transport (Gerba and Betancourt 2017). The fecal suspension material used in this work (1 ml) results in a virus-loaded injection water with a relatively low amount of organic matter (6 mg/l). As the results for the Rotavirus transport in the standardized MWS were similar to the ones obtained by Pang et al. (2014), it could be considered that the effect of organic matter is not significant for this case.

For the natural MWS, the two viruses show very different BTC. The Rotavirus transport model showed small changes with respect to the standardized MWS: on one site blocking had to be incorporated and detachment dismissed, but the values of the rest of the parameters maintained the same order of magnitude. Probably the presence of organic matter in the water and the soil is responsible for the appearance of blocking for Rotavirus (Wong et al. (2013) showed that the presence of soil and dissolved organic carbon reduce the attachment of human adenovirus). The Norovirus transport model showed significant changes, since BTC showed a removal rate of 4 logs, while Rotavirus showed a removal rate of 2.6 logs, similar to the 2 logs observed on the standardized MWS. One possible explanation for this differential behavior is the difference in the isoelectric point value (IPV) of these two viruses and the increase of the ionic strength (IS) on the natural MWS. Several studies show that an increase in IS may increase the attachment rate of viruses to solid particles (Sadeghi et al. 2011; Wong et al. 2014). According to the DLVO theory, attachment rates of viruses to solid particles may increase with IS due to the compression of double layers around soil grains (Schijven and Hassanizadeh 2000). One possible explanation for this difference is the one proposed by Dowd et al. (1998). These authors suggest that more negatively charged viruses attach more than less negatively charged viruses in the case of high concentrations of multivalent cations. This is due to the formation of a Stern double layer (Bohn et al. 1979) which consist of two layers of cations associated with negatively charged soil particles. These cation layers neutralize the negative charge of the soil mineral and create a cation excess in a diffuse layer, which attracts anions closer to the soil particles (Jury et al. 1991). As reported by Michen and Graule (2010), Rotavirus has a IPV of 8.0, while Norovirus IPV is 5.5–6.0. As the pH of the natural MWS is 7.2, Norovirus will have a negative charge, while Rotavirus a positive. However, as several authors have pointed out, the dynamics of virus adsorption in porous media is complex and the traditionally adopted virus isoelectric point as a relevant physicochemical descriptor for virus adhesion might not be adequate, since its value might be affected by the ionic strength, flow permeation degree, and the chemical and structural details of viruses (Langlet et al. 2008; Dika et al. 2015). Therefore, more detailed studies should be carried out to determine the factors (such as ionic strength, presence of different cations, organic matter, the mineral composition of the porous medium and the molecular characteristic of the binding interactions) that affect differently the transport of Rotavirus and Norovirus.

The results of this work show that removal of Norovirus in the natural MWS is much greater than that of the

Rotavirus. This does not prove that the same phenomenon observed in the column can be observed on the field. Nevertheless, this pattern is consistent with observations made in the Salto aquifer. According to effluent and clinical studies, the levels of Norovirus and Rotavirus are similar in the population of Salto (Victoria et al. 2014). However, in a study conducted in the aquifer where the presence of Rotavirus A, Norovirus GII, and human Adenovirus were evaluated, only the presence of Rotavirus A was systematically detected (Gamazo et al. 2018). Since the removal rate of Norovirus in the natural MWS was so high, it was not possible to observe tailing in the BTC. Tailing characterization is important because it can result in leaching low doses of the virus, which can be relevant when viruses are present at high concentrations. Therefore, further experiments involving higher Norovirus concentrations are necessary to evaluate if, despite the high initial removal, its tailing is more extensive than Rotavirus, as was observed in the standardized MWS.

There are conflicting opinions on whether it is possible to find a universal indicator for viral contamination. However, surrogates might be found for specific viruses in particular MWS. To do so, specific validation experiments should be carried out to compare the behavior of viruses and their subrogates in conditions similar to those found in the field. These experiments could also help to understand how the particularities of each system affect the parameters associated with the modeling of virus transport through porous media. With the proper parameter, numerical model could be used to predict the transport of human viruses in natural systems and to assess the effectiveness of corrective measures.

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