

Occurrence of norovirus genogroups I and II in recreational water from four beaches in Belém city, Brazilian Amazon region

Danielle Rodrigues de Deus, Dielle Monteiro Teixeira, Jainara Cristina dos Santos Alves, Vanessa Cavaleiro Smith, Renato da Silva Bandeira, Jones Anderson Monteiro Siqueira, Lena Lillian Canto de Sá Morais, Hugo Reis Resque and Yvone Benchimol Gabbay

ABSTRACT

This study aimed to investigate the presence of norovirus (NoV) in recreational waters of four estuarine beaches located in Mosqueiro Island, Belém city, Brazilian Amazon, during two years of monitoring (2012 and 2013). NoV particles were concentrated on filtering membrane by the adsorption-elution method and detected by semi-nested RT-PCR (reverse transcription polymerase chain reaction) and sequencing. NoV positivity was observed in 37.5% (39/104) of the surface water samples, with genogroup GI (69.2%) occurring at a higher frequency than GII (25.7%), with a cocirculation of both genogroups in two samples (5.1%). This virus was detected in all sampling points analyzed, showing the highest detection rate at the Paraíso Beach (46.2%). Statistically, there was a dependence relationship between tide levels and positive detection, with a higher frequency at high tide (46.7%) than at low tide (25%) periods. Months with the highest detection rates (April 2012 and April/May 2013) were preceded by periods of higher precipitation (March 2012 and February/March 2013). Phylogenetic analysis showed the circulation of the old pandemic variant (GII.4-US_95-96) and GI.8. The NoV detection demonstrated viral contamination on the beaches and evidenced the health risk to bathers, mainly through recreational activities such as bathing, and highlighted the importance of including enteric viruses research in the recreational water quality monitoring.

Key words | Amazon region, beaches, norovirus, RT-PCR, semi-nested, surface water

Danielle Rodrigues de Deus

Postgraduate Program in Parasitology Biology in the Amazon,
State University of Pará,
Tv. Perebebui, 2623, Marco, Belém,
PA CEP 66087-662,
Brazil

Dielle Monteiro Teixeira

Jainara Cristina dos Santos Alves
Renato da Silva Bandeira
Jones Anderson Monteiro Siqueira
Hugo Reis Resque

Yvone Benchimol Gabbay (corresponding author)

Virology Section, Evandro Chagas Institute, Health Surveillance Secretariat,
Brazilian Ministry of Health,
Br. 316 Km 07 S/N, Levilandia, Ananindeua,
PA CEP 67030-000,
Brazil
E-mail: yvonegabbay@iec.gov.br

Vanessa Cavaleiro Smith

Postgraduate Program in Virology, Evandro Chagas Institute, Health Surveillance Secretariat,
Brazilian Ministry of Health,
Br. 316 Km 07 S/N, Levilandia, Ananindeua,
PA CEP 67030-000,
Brazil

Lena Lillian Canto de Sá Morais

Environment Section, Evandro Chagas Institute, Health Surveillance Secretariat,
Brazilian Ministry of Health,
Br. 316 Km 07 S/N, Levilandia, Ananindeua,
PA CEP 67030-000,
Brazil

INTRODUCTION

Untreated sewage discharge into water bodies is one of the main factors contributing to increased fecal contamination in aquatic environments used for recreation and represents a great risk to human health (Gibney *et al.* 2017; Sekwadi *et al.* 2018). Enteric viruses are transmitted by the

fecal-oral route and their presence in recreational waters has already been well reported (Vergara *et al.* 2016).

In recent years, unraveling of the worldwide distribution of norovirus (NoV) has warranted the importance of including them in epidemiological surveillance, since this

pathogen is considered as the main cause of viral acute gastroenteritis (AGE) outbreaks worldwide, accounting for 46% of outbreaks reported in areas with different types of waters intended for recreation (Patel *et al.* 2008; Sinclair *et al.* 2009).

In Brazil, the evaluation of recreational water quality does not include viral pathogens in its analysis. The conditions for bathing are regulated by CONAMA resolution No. 274/2000, which is based on the presence of thermotolerant coliforms, *Escherichia coli* (*E. coli*) and enterococci (BRASIL 2000). However, several studies demonstrated that enteric viruses are widely disseminated in different matrices from Brazilian regions, including surface water and untreated sewage from Belém (Teixeira *et al.* 2016) and recreational water from Manaus (Vieira *et al.* 2016), Florianópolis (Rigotto *et al.* 2010) and Rio de Janeiro (Vieira *et al.* 2012; Victoria *et al.* 2014a).

NoV belongs to genus *Norovirus*, family *Caliciviridae*. It is divided into seven genogroups (GI–GVII) and is transmitted by the fecal-oral route, mainly through contaminated food and water (Vinjé 2015). NoV GI, GII and GIV have been associated with human infections, with GII, especially the genotype 4 (GII.4), accounting for the largest share of AGE cases and outbreaks (Ramani *et al.* 2014; Vinjé 2015). In aquatic environments, the GI is widely described at a frequency greater than or equal to that of the GII (Kitajima *et al.* 2012; Lee *et al.* 2014; Zhu *et al.* 2018).

In Belém, stilt houses (*palafitas*) are commonly observed along the water courses that drain and surround the region, where human sewage is usually discharged *in natura* (Ribeiro 2004). In addition, the precariousness of the sanitation sector, with extremely low rates of collection and treatment of sewage, reveals that more than 90% of the sewage generated in this municipality is released without any previous treatment into the water bodies that are a part of the drainage network that drains in the Guajará Bay (Trata Brasil 2016). In the present study, the presence of NoV was investigated in samples collected during a two-year period of monitoring recreational waters along four estuarine beaches on Mosqueiro Island, an important balneary located in the Metropolitan Region of Belém.

METHODS

Study area

Mosqueiro Island has approximately 33,232 inhabitants and is an administrative district of Belém city, capital of the state of Pará, Northern Brazil, within the Amazon region (IBGE 2013). The city is located on the margin of the Guajará Bay that is formed by the confluence of the Guamá, Acará and Moju Rivers. Mosqueiro Island has 17 km of estuarine beaches and is located 80 km from Belém, in a highly dynamic environment in front of Marajó Bay, with strong tidal currents and high turbidity, making the water look muddy. This Island is surrounded by numerous small water bodies known regionally as *furos* and *igarapés*. In addition, Marajó Bay is confluent with Guajará Bay and the Atlantic Ocean, forming a typical estuarine environment on the Amazon coast of Brazil (Bastos *et al.* 2002; Gregório & Mendes 2009; Sousa *et al.* 2017). The climate in this region is hot and humid, with an average annual temperature of 26 °C, an average rainfall of 2.834 mm/year and two distinct seasonal periods: high (December to June) and low (July to November) rainy season (PMB 2012).

The beaches of Farol (FR), Murubira (MU), Areião (AR) and Paraíso (PA) were selected to investigate the presence of NoV along the Mosqueiro Island (Figure 1). All these beaches are much visited; they receive rainwater drainage galleries and untreated sewage from residences and bars located nearby, mainly during holidays and vacations. Paraíso Beach is the closest to the Atlantic Ocean and farthest from an urban center. Farol and Murubira beaches are the most frequented during vacations and holidays. Areião Beach is located in the urban center of the Island, in the region closest to Guajará Bay.

Sample collection and viral concentration

A total of 104 surface water samples were collected monthly from January 2012 to December 2013, mainly after the weekends and holidays, with fortnightly collections in July, representing a period of great increase in the number of bathers due to school holidays.

Viral particles were concentrated from 2 L of water samples by the adsorption-elution method on filtering the

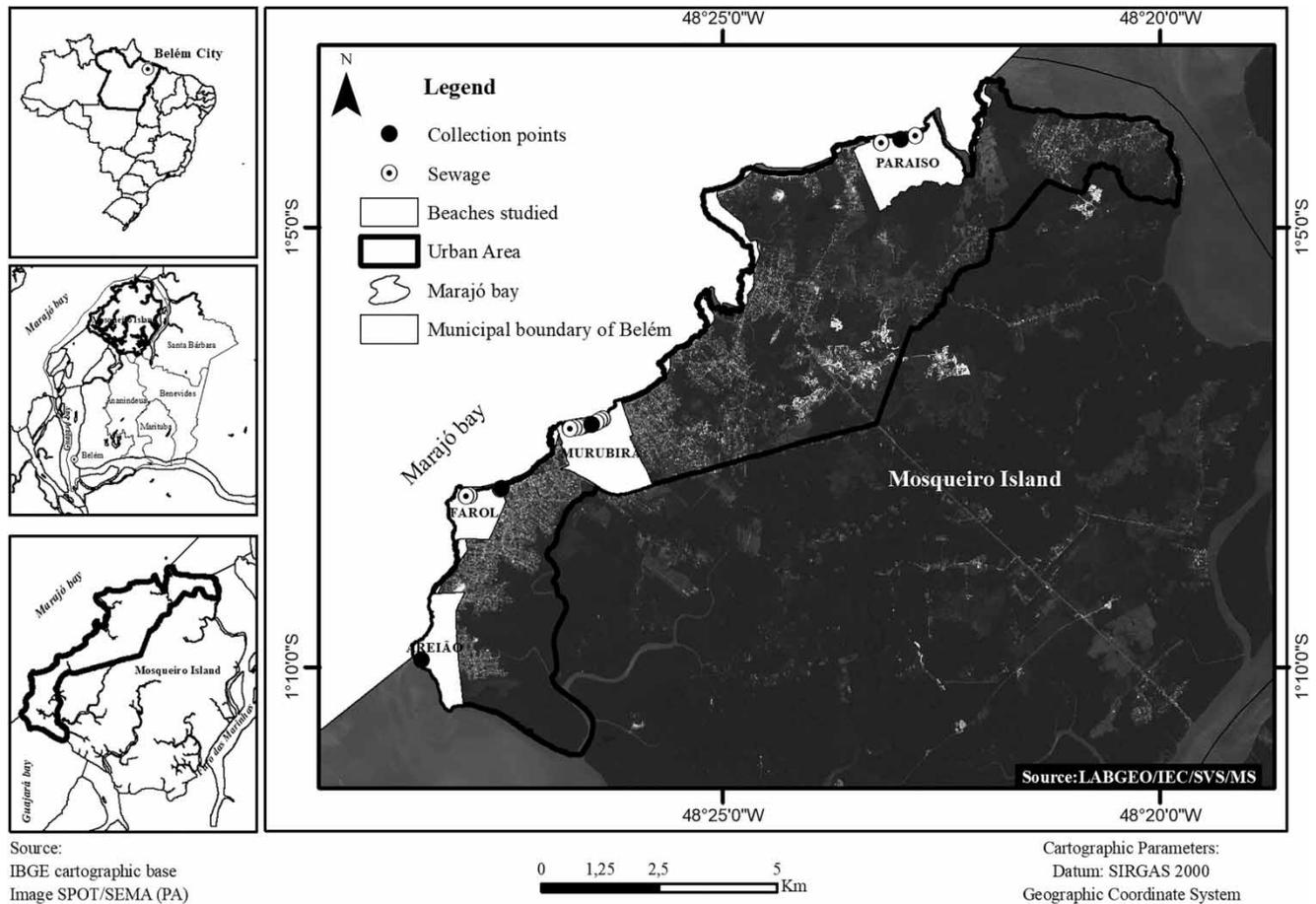


Figure 1 | Map of four beaches of Mosqueiro Island, Metropolitan Region of Belém, Pará. Source: Geoprocessing Laboratory (LABGEO) Evandro Chagas Institute, Secretariat of Health Surveillance, Brazilian Ministry of Health.

membrane (Katayama *et al.* 2002), obtaining a final volume of 2 ml after a second filtration using an Amicon Ultra-15 Centrifugal Filter Unit (Merck Millipore, Ireland).

NoV molecular detection

Viral RNA was extracted from 400 μ l of the concentrated sample using isothiocyanate guanidine (silica method) (Boom *et al.* 1990). Semi-nested RT-PCR (reverse transcription polymerase chain reaction) was performed in two steps with specific primers for NoV detection (Boxman *et al.* 2006). In the first step, RT-PCR was performed using the primers JV13I and JV12Y (327 bp), targeting the RNA-dependent RNA polymerase (RdRp) gene in ORF1 (region A). All the RT-PCR mixtures had a total volume of 10 μ l containing 2 μ l of RNA, 40 mM of deoxynucleotides

(0.5 μ l), 10 \times of PCR buffer (1.0 μ l), 50 mM of MgCl₂ (0.3 μ l), 20 μ M of each primer (0.375 μ l), 20 U/ μ l of SuperScript II Reverse Transcriptase (0.1 μ l) (Invitrogen, USA) and 5 U/ μ l of Taq DNA polymerase (0.2 μ l) (Invitrogen, USA). In the second step, this RT-PCR product was subjected to separate semi-nested reactions, using primers pairs JV13I/GI and JV12Y/NoroII-R for specific detection of GI and GII, respectively. Briefly, 2 μ l of RT-PCR products was added to a final volume of 25 μ l containing 40 mM of deoxynucleotides (1.0 μ l), 10 \times PCR buffer (2.5 μ l), 50 mM of MgCl₂ (1.0 μ l), 20 μ M of each primer (0.65 μ l) and 5 U/ μ l of Taq DNA polymerase (0.2 μ l) (Invitrogen, USA). Nucleic acids were tested undiluted and 10-fold dilution.

The products were analyzed in 1.5% agarose gel electrophoresis and stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA). The amplicons of 187

and 236 bp visualized under UV light were taken as indicative of NoV GI and GII, respectively. In all procedures, positive (positive NoV stool sample previously sequenced) and negative (nucleic acid-free water) controls were used. To avoid cross-contamination, all tests were performed in separated rooms.

NoV genotyping

After the semi-nested RT-PCR, RNA from the positive samples was analyzed by the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), using in the first round the primers Mon431/Mon432/G2SKR (Anderson *et al.* 2001; Fankhauser *et al.* 2002) targeting the ORF1–ORF2 junction region of the genome, with an expected amplicon of 550 bp. In the second round, primer pairs COG1F/G1SKR (nested) and COG2F/G2SKR (semi-nested), targeting the 5' ORF2 region, were used for specific detection of GI (380 bp) and GII (390 bp), respectively (Kojima *et al.* 2002; Kageyama *et al.* 2003). Reactions were performed following the manufacturer's instructions.

Positive samples analyzed in the 5' ORF2 region that yielded good quality of amplicons were purified with the commercial QIAquick® PCR Purification or QIAquick® Gel Extraction kits (QIAGEN, Valencia, CA, USA) following the manufacturer's instructions. Sequences were obtained using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Assembly of NoV sequences was performed using Geneious v.10.0.8 (Kearse *et al.* 2012) program through the reference mapping method using prototypes of NoV from the NCBI database. The alignment was obtained using the Aliview v.3.0 program (Larsson 2014) with the MAFFT program strategy (v.7.221) (Katoh & Standley 2013) and compared to other sequences stored in GenBank. A phylogenetic tree was constructed with the IQTree v.1.3.0 program (Nguyen *et al.* 2015), using maximum likelihood with 1,000 bootstrap replicates, disregarding the statistical values with supports less than 70%, and the graphical tree was edited using the MEGA 6.0 program (Tamura *et al.* 2013).

The nucleotide sequences of NoV GI and GII obtained in this study were submitted to the GenBank database under nucleotide accession numbers MH165841–MH165844.

Tide data and precipitation

The tide data were obtained from the Navy Hydrography Center website (<https://www.marinha.mil.br/chm/tabuas-de-mare>) according to the available tide tables and considering the days and times of the collections conducted in the years of 2012 and 2013, using the four main periods of the day available on the website as a reference, with the respective maximum (high tide) and minimum (low tide) heights of tidal levels measured in meters (m).

Rainfall information was downloaded from the National Institute of Meteorology website (http://www.inmet.gov.br/portal/index.php?r=home/page&page=rede_estacoes_auto_graf).

Statistical analysis

Descriptive and analytical analyses of data were performed with BioEstat 5.3 software (Ayres *et al.* 2007). The association between positivity, tide levels, and the mean rainfall of the collection day in relation to the previous one was evaluated by the Simple Logistic Regression test. The Chi-square test was performed to compare the positivity obtained in the first and second semester. Results were considered statistically significant when $p \leq 0.05$.

RESULTS

A total of 104 surface water samples were analyzed for amplification of the partial RdRp gene region of the NoV genome by semi-nested RT-PCR. NoV was detected in 37.5% (39/104) of the samples. The NoV GI 69.2% (27/39) presented a higher frequency than GII 25.7% (10/39), and the cocirculation of both genogroups was also verified in two samples (5.1% – 2/39) (Table 1), detected on the beaches of Paraíso and Murubira in the second fortnight of July and in November of 2012, respectively.

This virus was detected at all sampling points. The highest occurrence for NoV was observed on Paraíso Beach (46.2% – 12/26) followed by the beaches of Farol (42.3% – 11/26), Areião (34.6% – 9/26) and Murubira (26.9% – 7/26) (Table 1).

Table 1 | Detection of NoV GI and GII according to tidal cycles in 104 recreational water samples from four beaches of Mosqueiro Island, Metropolitan Region of Belém, Pará

Collection point	High tide % (no. of positive/total)				Low tide % (no. of positive/total)				Total positivity
	GI	GII	GI + GII	Total	GI	GII	GI + GII	Total	
Farol	60 (9/15)	0 (0/15)	0 (0/15)	60 (9/15)	9.1 (1/11)	9.1 (1/11)	0 (0/11)	18.2 (2/11)	42.3 (11/26)
Murubira	33.3 (5/15)	0 (0/15)	6.7 (1/15)	40 (6/15)	9.1 (1/11)	0 (0/11)	0 (0/11)	9.1 (1/11)	26.9 (7/26)
Areião	26.7 (4/15)	13.3 (2/15)	0 (0/15)	40 (6/15)	9.1 (1/11)	18.2 (2/11)	0 (0/11)	27.3 (3/11)	34.6 (9/26)
Paraíso	26.7 (4/15)	13.3 (2/15)	6.7 (1/15)	46.7 (7/15)	18.2 (2/11)	27.3 (3/11)	0 (0/11)	45.5 (5/11)	46.2 (12/26)
Total	36.7 (22/60)	6.7 (4/60)	3.3 (2/60)	46.7 (28/60)	11.4 (5/44)	13.6 (6/44)	0 (0/44)	25 (11/44)	37.5 (39/104)

In the two years of study, 57.7% (60/104) of the samples were collected at high tide and 42.3% (44/104) at low tide. NoV was detected with higher frequency during high tide (46.7% – 28/60) than during low tide (25% – 11/44) (Table 1). Statistical analysis showed a dependence relationship between tide levels and positivity ($p = 0.0386$). The distribution of genogroups was different between tidal cycles. At high tide, most of the positive samples were classified as GI (36.7%) and a small percentage as GII

(6.7%) and GI + GII (3.3%) (Table 1), while during low tide the frequency was similar between genogroups, with 11.4% for GI and 13.6% for GII (Table 1).

During the study period, NoV was detected on all beaches in April 2012 and April/May 2013, followed by the months of March (PA, MU and FR), the first fortnight of July 2012 (PA, FR and AR) and December (MU, FR and AR) 2013 with the detection at three beaches (Figure 2). Although there was no statistical relationship ($p = 0.0877$)

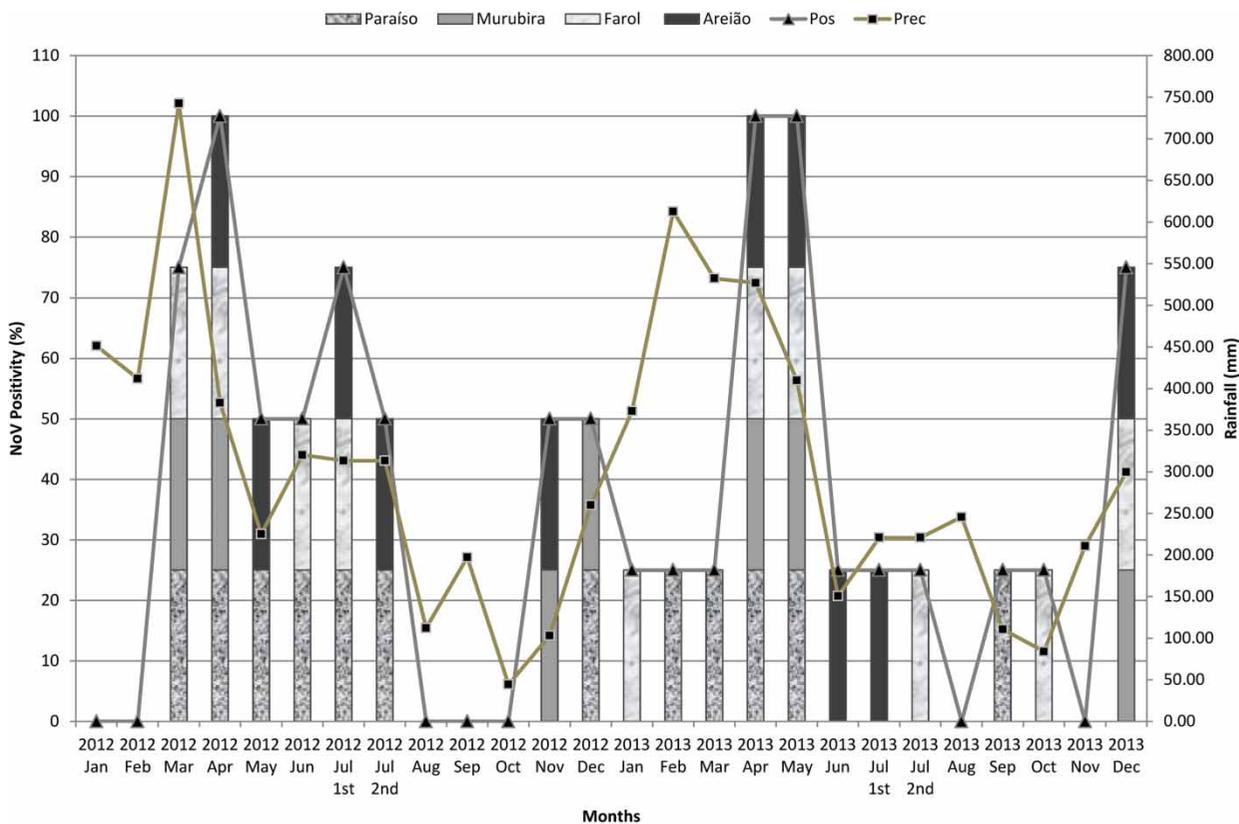
**Figure 2** | Monthly distribution of NoV in recreational water from four beaches of Mosqueiro Island, Metropolitan Region of Belém, Pará. Pos, positivity; Prec, precipitation.

Table 2 | Detection and genotyping of NoV by 5'-end ORF2 region (capsid)

Collection point	Semi-nested (GII) and nested (GI) RT-PCR N Positive	Partial capsid sequencing		
		GI Genotype (N)	GII Genotype (N)	GI + GII Genotype (N)
Areião	3	GI.8 (1) NG (1)	NG (1)	–
Farol	2	NG (2)	NG (1)	–
Murubira	2	NG (1)	–	GII.4/NG (1)
Paraíso	5	NG (2)	GII.4 (2) NG (1)	–
Total	13	53.8% (7/13)	38.5% (5/13)	7.7% (1/13)

NG, non-genotyped.

between NoV positivity and average rainfall, the months with greater positivity (April/2012 and April/May 2013) were preceded by periods of higher precipitation (March/2012 and February/March 2013) (Figure 2). Despite the significant presence of NoV in periods with higher precipitation levels in Belém, the analysis to verify the positivity observed between the first and second semesters showed no statistically significant relation ($p = 0.067$).

In the semi-nested/nested RT-PCR, a total of 33.3% (13/39) samples were positive for the ORF1–ORF2 junction region of the NoV genome, and these samples were genogrouped as GI (53.8% – 7/13), GII (38.5% – 5/13) and GI + GII (7.7% – 1/13) (Table 2). Among these, phylogenetic analysis of the partial sequencing of the capsid region was done for four samples, of which three were classified as GII.4, demonstrating similarity to the variant US-95-96, and one as GI.8; the others did not present reliable sequences and therefore could not be genotyped (Figure 3).

Among the NoV positive samples, the highest percentage was found in undiluted RNA samples with 53.8% (21/39) compared to diluted RNA samples (15.4%, 6/39).

DISCUSSION

According to the sanitation ranking, the city of Belém occupies the third position among the 10 worst municipalities in the country, with very low rates of collection (7.1%) and treatment (1.9%) of sewage (Trata Brasil 2016). These data highlight the public health risks associated with

fecal contamination and NoV circulation in recreational waters. Thus, the presence of this virus around Mosqueiro Island could be related to the discharge of sewage, directly or indirectly, by rainwater drainage on the studied beaches.

Although there have been no reports of recreational outbreaks caused by water in Brazil, numerous studies conducted in other regions of the world have highlighted outbreaks caused by viral pathogens after recreational contact with surface waters contaminated by feces (Sinclair *et al.* 2009; Di Bartolo *et al.* 2015; Sekwadi *et al.* 2018).

In Brazil, the presence of NoV and other enteric viruses has been described in different types of recreational water (sea, lagoon, and river), revealing the risk of bathers' exposure to waterborne diseases (Miagostovich *et al.* 2014; Vieira *et al.* 2016). The present analysis, carried out in estuarine beaches, obtained a NoV-positivity rate of 37.5% by semi-nested RT-PCR, a value higher than those previously described in this country in investigations related to the spread of these viruses in aquatic environments by quantitative real-time polymerase chain reaction (qPCR) and nested PCR (Vieira *et al.* 2012; Victoria *et al.* 2014a; Teixeira *et al.* 2016).

NoV was investigated in recreational water at Rio de Janeiro by two different studies: one in the sea and the other in freshwater. In the first study, NoV was detected in 14% of the samples from three urban saltwater beaches (Arpoador, Ipanema and Leblon) (Victoria *et al.* 2014a). Moreover, a positivity of 17.5% was observed in freshwater of an urban lagoon (Rodrigo de Freitas), which is interconnected with other ecosystems (Macacos River and Leblon Beach) (Vieira *et al.* 2012). The result obtained in the

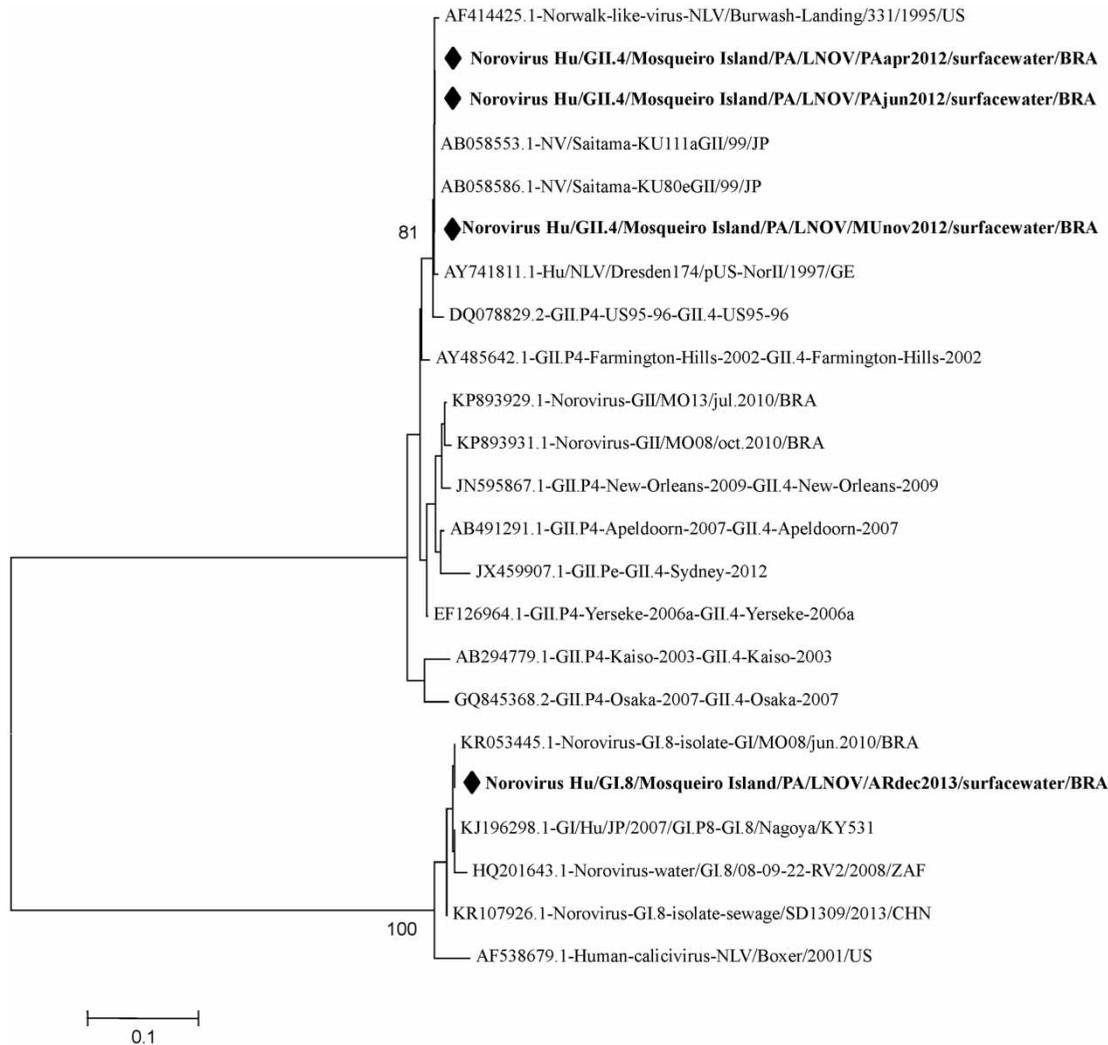


Figure 3 | Phylogenetic tree of NoV GI and GII genotypes found in recreational waters of four beaches of Mosqueiro Island, Metropolitan Region of Belém, Pará. Built using maximum likelihood analysis and 1,000 bootstrap replicates. Study samples of the present study are highlighted in bold and filled diamonds.

river (33.3%) that flows into the lagoon was consistent with the one obtained at the Areião Beach (34.6%), while on Leblon beach, the presence of NoV was lower (16.7%) than that observed in all the beaches of the present study.

In the Northern region, NoV GI and GII (33.9%) was found in surface waters and sewage of Belém (Teixeira *et al.* 2017) and NoV GII in surface waters (5.8%) and water samples from the Negro River (7.4%) in Manaus (Miagostovich *et al.* 2008; Vieira *et al.* 2016).

The greater circulation of GI (74.3%) compared to GII (30.7%) agrees with the frequency detected in environmental samples (rivers and sewage) (Kitajima *et al.* 2012;

Lee *et al.* 2014; Farkas *et al.* 2018). Nevertheless, in clinical samples from Belém, GII has predominated in studies of hospitalized and outpatient children treated for symptoms of gastroenteritis (Aragão *et al.* 2010; Siqueira *et al.* 2013).

The detection of genogroups related to infections in humans suggests that the possible contamination of visitors on the beaches of Mosqueiro Island with the spread of NoV GI in this environment could be more related to the occurrence of asymptomatic or sporadic infection, which may not require medical attention (Kitajima *et al.* 2012; Wade *et al.* 2018). This hypothesis is reinforced by the prevalence of

GI compared to GII in asymptomatic cases described in children from Belém city by [Siqueira *et al.* \(2017a\)](#).

The small differences in NoV positivity observed between each beach may be related to the specific characteristics of each area, climate conditions, influence of environmental factors (tidal and rainfall), and anthropogenic impacts on water quality resulting from urbanization.

The greater positivity observed at the Paraíso Beach can be linked to several factors that favor the reduction of pollution, due to the distance of the beach from the center of the Island and the few bars and hotels adjacent to the beach. In addition, there is a cove area, which favors the sedimentation of organic matter and as these viruses are found in low concentration in environmental samples, the probable accumulation of viral particles at this site increased their availability for molecular detection ([Bosch *et al.* 2008](#); [Farkas *et al.* 2018](#)).

Farol and Murubira are considered the most popular beaches of Mosqueiro Island and are used for recreational activities, such as kitesurfing. In addition, during holidays and vacations, they receive a large discharge of sewage from the neighboring houses that directly enter the beaches and water bodies surrounding the region. Located in the urban center of Mosqueiro Island (*Vila* neighborhood), Areião Beach receives intense urban influence through residences, bars, restaurants and hotels with excessive discharge of effluents that arrive at this beach without any type of treatment. The washing of small fishing boats that dock at Areião Beach possibly contributes to the contamination of the water due to the discharge of solid and liquid waste ([Viana 2017](#)).

Molecular methods, such as RT-PCR, are widely used by most studies for the detection and subsequent genotyping of NoV in aquatic environments worldwide ([Victoria *et al.* 2010](#); [Lee *et al.* 2014](#); [Zhu *et al.* 2018](#)). Semi-nested RT-PCR was also applied in Uruguay, employing the same primers used in this research and obtaining a greater occurrence (51%) in the wastewater samples directly discharged from the Uruguay River, which delineates the triple border between Brazil, Argentina, and Uruguay ([Victoria *et al.* 2014b](#)). A recent study carried out on three beaches in Argentina presented a similar result (31.8%) to that found in the present analysis (37.5%), employing the semi-nested RT-PCR approach ([Masachessi *et al.* 2018](#)).

According to [Abdelzaher *et al.* \(2010\)](#), the movement of water caused by high tide allows microbial indicators to reach the water column after submersion of the intertidal zone. Similarly, the high NoV frequency at high tide suggests that the washing zone exposed to the movement of water between low and high tide could have facilitated the transport of viral particles to the aquatic surface ([Vandieken *et al.* 2017](#)).

The relationship between rainfall and NoV frequency did not show seasonality during the analyzed period ($p = 0.0877$). This may be associated with the fact that in the Amazon region, there are no defined seasons of the year, with predominance of more or less rainy periods. However, the marked presence of this agent was observed at all the beaches studied in the months of April 2012 and April/May 2013, and at 75% of beaches studied in March and December 2012, corresponding to the period of higher levels of rainfall in Belém (December to June). During this period, the dissemination of these agents in surface water may be associated with the increased urban runoff (rainy galleries and untreated sewage) ([Haramoto *et al.* 2006](#)). These data corroborate a study carried out in Rio de Janeiro, where the concentration of NoV was higher after rainfall events ([Victoria *et al.* 2014a](#)).

In July, there is a decrease in rainfall, but the Island often receives many visitors/tourists due to school holidays, especially on weekends. The large agglomeration of people associated with the increase in residual water produced by bathers may have directly influenced the propagation of these viruses in water and favored their detection in samples collected shortly after the most visited days ([WHO 2003](#)).

In this study, one-step RT-PCR for NoV genotyping using primers targeting the 5' ORF2 region made it possible to detect two different genotypes (GI.8 and GII.4) on the beaches of Mosqueiro. The genotype GI.8 was also identified in sewage samples collected in Uruguay ([Victoria *et al.* 2016](#)) and in AGE cases among hospitalized and outpatients in Guatemala ([Estévez *et al.* 2013](#)). In Belém, GI.8 was previously detected in river, impacted stream, and untreated sewage in the year 2010, with high nucleotide similarity to that found on Areião Beach ([Teixeira *et al.* 2016](#)).

Several variants of the GII.4 pandemic have been associated with global epidemics of AGE, yielding great

impact on public health (Kroneman *et al.* 2013; van Beek *et al.* 2018). Among the genotypes circulating in Brazil, GII.4 remains the most prevalent (Fioretti *et al.* 2014), accounting for 72% of outbreaks caused by NoV in Rio Grande do Sul (Andrade *et al.* 2014). The previous study (2008–2010) showed the circulation of other GII.4 variants similar to Den Haag 2006b, New Orleans 2009 and Sydney 2012 in surface water and untreated sewage from the city of Belém (Teixeira *et al.* 2017). However, in the present study, the variant US_95/96 was found for the first time in recreational water samples from this city.

Recently, a study described the involvement of this variant in intragenotype recombination events (US_95-96/Kaiso_2003) in fecal specimens of children hospitalized in Belém in the year 2003 (Siqueira *et al.* 2016). This variant predominated among hospitalized and outpatient children of the city of Belém between 1998 and 2000, with cases in the population until 2002 (Siqueira *et al.* 2017b). The older pandemic variant detection shows the need to monitor strains after epidemic episodes due to NoV evolving, possibly through mutations and recombination which could initiate new epidemic cases of AGE caused by this new strains. The first detection of variant US_95/96 in the environmental samples of Mosqueiro Island suggests the circulation of this strain in asymptomatic or milder cases in the population of Belém, because although it is a pandemic variant, it has not currently been associated with outbreaks or clinical cases described in this town.

The presence of inhibitory substances, such as fine sediment particles, has not been evaluated and can be regarded as a limitation of this research due to the possibility of interference in the characterization of NoV detected around Mosqueiro's beaches. However, we emphasize that the use of filter membranes, silica and dilution of nucleic acids were applied in this study, which are commonly used as a means of removing possible inhibitors of RT and/or DNA polymerase enzyme (Boom *et al.* 1990; Queiroz *et al.* 2001; Farkas *et al.* 2017). It is extremely important to use markers (internal controls) to assess the inhibition level mainly in environmental virology studies and provides a more secure interpretation of the detection procedure (Haramoto *et al.* 2018).

It was not possible to evaluate viral recovery and this may be a limitation of this study, as the efficiency of the

method used can be influenced by intrinsic variables of each aquatic environment and viral presence often occurs at low levels in the environment (Bosch *et al.* 2008). Although not indicative of viral particle infectivity, PCR variations, such as nested and semi-nested RT-PCR, are satisfactorily used for NoV detection in environmental samples because of their high specificity and sensitivity (Kitajima *et al.* 2010; Victoria *et al.* 2010).

According to the value of *E. coli* used by Brazilian legislation (BRASIL 2000), 92.3% (96/104) of the samples collected in this study were considered adequate for bathing; however, the presence of NoV was identified in 37.5% (36/96) of these (*E. coli* data were kindly provided by the Environment Laboratory of the Evandro Chagas Institute – unpublished data). The relationship between the presence of NoV and *E. coli* values observed in this study concurs with those observed on Rio de Janeiro beaches considered suitable for bathing according to the maximum concentrations of *E. coli* (Victoria *et al.* 2014a). This reinforces the worldwide urgency of establishing viral parameters associated with bacteriological ones to ensure a more complete assessment of the quality of water used for recreation, since there is no correlation between the bacterial concentrations and the presence of viruses (Wyn-Jones & Sellwood 2001; Updyke *et al.* 2015).

CONCLUSION

This study verified the circulation of the NoV in beaches usually frequented by residents of Belém and tourists from other Brazilian states. This emphasizes that the health risk to bathers should not be neglected due to the worldwide distribution of outbreaks and sporadic cases of gastroenteritis caused by various enteric viruses in recreational waters such as beaches, lagoons, and rivers. In addition, the environmental circulation of GII.4 genotype corroborates clinical data from the same region which relate it mainly with viral gastroenteritis cases. The GI.8 detection showed that strains related to GI genogroup are circulating on the population, but its real burden may be underestimated by factors such as asymptomatic or mild infections. The genotype identification is extremely important since it provides

data on the circulation of common or emerging strains in the community.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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