Journal of Medical Virology 85:737–744 (2013)

Norovirus Infection in Children Admitted to Hospital for Acute Gastroenteritis in Belém, Pará, Northern Brazil

Jones Anderson Monteiro Siqueira,[1] Alexandre da Costa Linhares,[2] Thaís Cristina Nascimento de Carvalho,[3] Glicélia Cruz Aragão,[4] Darleise de Souza Oliveira,[2] Mirleide Cordeiro dos Santos,[2] Maisa Silva de Sousa,[5] Maria Cleonice Aguiar Justino,[2] Joana D'Arc Pereira Mascarenhas,[2] and Yvone Benchimol Gabbay[2]*

[1]*Postgraduate Program in Tropical Diseases, Tropical Medicine Center, Federal University of Pará State. Belém, Pará, Brazil*
[2]*Virology Section, Evandro Chagas Institute, Health Surveillance Secretariat, Brazilian Ministry of Health. Ananindeua, Pará, Brazil*
[3]*Institute of Health Sciences, Federal University of Pará State. Belém, Pará, Brazil*
[4]*Postgraduate Program in Parasite Biology in the Amazon, Center for Biological and Health Sciences, University of the Pará State. Belém, Pará, Brazil*
[5]*Tropical Medicine Center, Federal University of Pará State. Belém, Pará, Brazil*

Noroviruses are the leading cause of epidemic, non-bacterial outbreaks of acute gastroenteritis, and are also a major cause of sporadic acute gastroenteritis in infants. The aim of the present study was to identify norovirus infections in children not infected by rotavirus admitted to hospital for acute gastroenteritis in Belém. A total of 348 fecal specimens were obtained from children with diarrhea aged less than 5 years, all of whom had tested negative for rotavirus, between May 2008 and April 2010. Fecal samples were screened for norovirus antigen using enzyme-immunoassay (EIA). Specimens were subjected to reverse-transcription polymerase chain reaction (RT-PCR) using the primers Mon432/434–Mon431/433 for detection of the GI and GII norovirus strains, respectively. Based on both methods, the overall norovirus positivity rate was 36.5% (127/348). Of the 169 samples collected in the first year, 44.4% (n = 75) tested positive for norovirus using both methods, 35.5% (n = 60) by EIA and 40.8% (n = 69) by RT-PCR. Using RT-PCR as a reference standard, a sensitivity of 78.3%, specificity of 94%, and agreement of 87.6% were recorded. Genome sequencing was obtained for 22 (31.9%) of the 69 positive samples, of which 90.9% (20/22) were genotype GII.4d and 9.1% (2/22) were genotype GII.b. Norovirus infection was most frequent in children under 2 years of age (41.5%–115/277). The peak incidence (62.1%) of norovirus-related acute gastroenteritis in these patients (not infected by rotavirus) was observed in February 2010. These findings emphasize the importance of norovirus as a cause of severe acute gastroenteritis among children in Belém, Pará, Northern Brazil. ***J. Med. Virol. 85:737–744, 2013.***

© 2013 Wiley Periodicals, Inc.

KEY WORDS: norovirus; gastroenteritis; children admitted to hospital

INTRODUCTION

Acute gastroenteritis is a leading cause of morbidity and mortality worldwide, especially among children of up to 5 years of age. Globally, diarrhea causes 1.2 million deaths per year, with the highest numbers being recorded in developing countries [Black et al., 2010]. Acute gastroenteritis in children is associated with a variety of infectious agents, such as bacteria, parasites, and viruses, of which the latter are recognized as being the main cause of endemic and epidemic

Grant sponsor: Coordination of Improvement of Higher Education Personnel (CAPES); Grant sponsor: Evandro Chagas Institute, SVS, MS.

No conflicts of interest were identified.

*Correspondence to: Yvone Benchimol Gabbay, PhD, Seção de Virologia, Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde. Ananindeua, Pará, Brazil.
E-mail: yvonegabbay@iec.pa.gov.br

Accepted 22 November 2012

DOI 10.1002/jmv.23506
Published online 28 January 2013 in Wiley Online Library (wileyonlinelibrary.com).

© 2013 WILEY PERIODICALS, INC.

gastroenteritis [Glass et al., 2006]. Acute gastroenteritis in humans has been associated with a number of different viruses. Of these, rotaviruses (RV), noroviruses (NoV), sapoviruses (SaVs), human astroviruses (HAstVs), and enteric adenoviruses (EAds) are the most common causes of sporadic gastroenteritis requiring hospitalization, and together constitute a major burden for public health systems worldwide [Glass et al., 2000, 2006; Sasaki et al., 2006].

Human caliciviruses (HuCVs) are part of the Caliciviridae family which includes five genera: *Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*, and *Nebovirus*. While *Norovirus* and *Sapovirus* are known to infect humans, the other genera are mainly of veterinary interest [Clark et al., 2012].

A number of outbreaks of non-bacterial gastroenteritis in a variety of environments have been attributed to NoV, due primarily to its marked potential for transmission and the low dose required to establish an infection. These environments include hospitals, day care centers, nursing homes, hotels, cruise ships, and schools [Borges et al., 2006; Bull et al., 2006; Sasaki et al., 2006]. Since the 1990s, the use of molecular methods has also highlighted the role of NoV as a common agent of sporadic, self-limiting episodes of acute gastroenteritis, which are normally caused by the consumption of contaminated food or water [Bull et al., 2006]. Person-to-person transmission of NoV has occurred mainly through the fecal–oral route. Airborne transmission following contact with contaminated surfaces also appears to be common [CDC, 2011]. Individuals of all age groups are known to be susceptible to NoV infections [Ribeiro et al., 2008]. NoV infection is normally characterized by mild and self-limiting acute gastroenteritis. Its most common symptoms are diarrhea, vomiting, nausea and abdominal pain, which last for about 48 hr after an incubation period of 8–72 hr [Bull et al., 2006; Verhoef et al., 2008].

The NoV are classified in five distinct genogroups. Two of these—genogroup I (GI) and genogroup II (GII)—are the most frequently detected in humans. Each of these groups encompasses a variety of genetic clusters. A recent study [CDC, 2011] found that the GI and GII strains contain at least 8 and 21 genotypes, respectively. During the past few years, the GII.4 genotype has predominated in both outbreaks and sporadic cases worldwide [Sasaki et al., 2006].

The great genetic diversity of the NoV hampers the development of a universal detection system capable of identifying all the different strains. The reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR have been used extensively for the detection and characterization of NoV through the use of primers designed to target different regions of the genome, mainly that represented by the RNA-polymerase, which appears to be conserved across all genetic groups. It is important to have a rapid and conclusive diagnosis of the virus during outbreaks in order to implement strategies for containment within the shortest possible time, thus minimizing the dispersal of the virus within the population [Jiang et al., 1999; Koopmans et al., 2002; Borges et al., 2006].

Data from Brazil have shown an increase in the prevalence of NoV in infants and young children admitted to hospital with acute gastroenteritis. A high prevalence of NoV infection has been reported from Rio de Janeiro and other regions of Brazil, with frequencies ranging from 41.6% to 49.8% [Ferreira et al., 2010; Morillo et al., 2011]. The aim of the present study was to seek for NoV infection among RV-negative children admitted to hospital for treatment for acute gastroenteritis in Belém, in northern Brazil, between 2008 and 2010. In addition, the different NoV strains detected during the study were characterized using molecular methods.

MATERIALS AND METHODS

Study Area

The present study was based on the monitoring of cases of community acquired acute gastroenteritis at a large pediatric hospital in the city of Belém, in the Northern Brazilian state of Pará, in the Amazon region. An estimated 40% of the cases of children admitted to hospital with acute gastroenteritis in Belém are referred to this facility. Belém has 1,437,600 inhabitants, and is located close to the equator line, and thus has a relatively hot and high humid climate (relative humidity generally over 80%). Median monthly temperatures vary from 25.7 to 27.8°C throughout the year [PMB, 2010; INMET, 2012].

Clinical Samples

Diarrhea is defined as the passage of at least three looser than normal stools over a 24 hr period. The hospital was monitored between May 2008 and April 2010, focusing on the children of less than 5 years of age who were admitted to hospital for acute gastroenteritis. Fecal samples collected within 48 hr of admission were collected and sent to the Evandro Chagas Institute where they were stored at −20°C until testing for the presence of RV and NoV. A total of 2,095 samples were obtained and first screened for group A-RV antigens using a Ridascreen® Rotavirus enzyme-immunoassay (EIA; R-Biopharm, Darmstadt, Germany). A representative sample of 348 of the 1,649 RV negative specimens was selected for NoV testing, considering a 95% confidence level and a sampling error of 4%. The samples were selected randomly each month, using the random (non-replacement) statistic tool of the BioEstat 5.0 software package [Ayres et al., 2007]. The study was approved by the Research Ethics Committee of the Evandro Chagas Institute (CEP/IEC—No. 0023/10–CAAE: 0024.0.072.000-10).

Norovirus Detection

The 348 RV-negative samples were screened for the presence of NoV antigens using a third generation

commercial Ridascreen® Norovirus EIA (R-Biopharm), according to the manufacturer's instructions. Microplates sensitized with monoclonal antibodies (Mab) were provided by the manufacturer. Biotin-conjugated antibodies and streptavidin–peroxidase conjugate were used in successive steps, followed by the addition of chromogenic substrate and blocking by sulfuric acid. Samples that yielded an absorbance value equal to or greater than the cut-off value ([absorbance value of the negative control + 0.150] + 10%) were considered positive.

Molecular Characterization

Viral RNA was extracted from the samples collected during the first year of the study (May 2008 to April 2009) using the isothiocyanate guanidine (silica method) [Boom et al., 1990]. A random hexamer (9 A_{260} units/μl, 3 mM Tris–HCl (pH 7.0), and 0.2 mM EDTA—Invitrogen, Eugene, OR) were used in the reverse transcription reaction to obtain the complementary DNA (cDNA), and PCR amplifications were conducted with a pool of Mon 431/433 and 432/434 primers in order to detect GI and GII, respectively [Anderson et al., 2001]. Amplicons of the positive samples that presented clear bands were selected and purified using either the QIAquick® PCR purification kit or the QIAquick® gel extraction kit (QIAgen, Science, MD). Sequencing was conducted using capillary electrophoresis in an ABI Prism 3130XL DNA Sequencer (Applied Biosystems, Foster City, CA) using the same primers as the RT-PCR reaction and a Big Dye kit (v. 3.1; Applied Biosystems). The nucleotide sequences obtained here were aligned and edited using the BioEdit Sequence Alignment Editor (v. 7.0.9.1) software and compared with other sequences obtained from the GenBank database (National Center for Biotechnology Information, US-[www.ncbi.nlm.nih.gov]), including reference sequences of each genotype. The dendogram was constructed by the neighbor-joining method using the MEGA 5 software [Tamura et al., 2011], supported by bootstrap procedures based on 2,000 replicates. The sequences determined in this study were submitted to the GenBank under accession numbers JQ683744–JQ683765.

Statistical Analysis

Statistical analyses were run in EpiInfo 3.3.2. (http://www.cdc.gov/epiinfo) and BioEstat 5.0 [Ayres et al., 2007]. The screening test was performed to assess the sensitivity and specificity of the results obtained by EIA in comparison with those of the RT-PCR. The kappa test was used to assess the reproducibility of the EIA and the odds ratio (OR) test was applied to evaluate a possible difference in NoV infection rates between younger (≤2 years old) and older (>2 years) children. The association between gender and the proportion of positives results for NoV was calculated by chi-square test. The variation between the first and second years of monitoring on the NoV positivity, as measured by EIA, was analyzed using the Mann–Whitney test. The Fisher exact test was used to analyze the positivity rate of the months in which there was not a similar pattern of monthly fluctuation. A multiple linear regression was used to assess the relationship between the proportion of NoV-positivity rates and climate parameters. P-values ≤ 0.05 were considered to be statistically significant.

RESULTS

Between May 2008 and April 2010, an overall NoV positivity of 36.5% (127/348) was confirmed using both EIA and RT-PCR. The age distribution of these positive cases is shown in Table I. The observed NoV-positivity was significantly higher in children of 2 years old or less (41.5%; 115/277; 95% CI [1.66–6.05]; OR = 3.1671; $P = 0.0005$) in comparison with other age groups. No relationship was found between the NoV-positivity rates and gender ($X^2 = 1.539$; $P = 0.259$). Similarly, no clear relationship was found between NoV-positivity and climate variables (Fig. 1), that is, temperature ($P = 0.53$), rainfall ($P = 0.82$), or relative humidity ($P = 0.96$).

Based on the results of the EIA, NoV-positivity was 35.5% in the first year of the study, and 29.0% in the second year ($P = 0.0304$). A similar pattern of monthly fluctuation was observed in both years of the study period (Fig. 2), except for February—18.7% in 2009 versus 62.1% in 2010 ($P = 0.0287$)—and October, with 27.3% in 2008 and no cases being recorded in 2009

TABLE I. Frequency by Age Group of the Positive for NoV Cases Detected by PCR and/or EIA Methods, in Fecal Samples of Children Admitted to Hospital With Acute Gastroenteritis in Belém, Pará, Brazil, from May 2008 to April 2010

Age group (months)	Positive/total tested (%)	Odds ratio	95% confidence interval
0–6	5/22 (22.7)	0.4854	0.1747–1.3488
6–12	46/102 (45.1)	1.6429	1.0252–2.6327
12–24[a]	64/153 (41.8)	1.4719	0.9491–2.2827
24–36	8/46 (17.4)	0.3193	0.1440–0.7081
≥36	3/17 (17.6)	0.3531	0.0995–1.2532
Not informed[b]	2/8 (25.0)	—	—
Total	128/348 (36.8)	—	—

[a]Norovirus infection was more frequent in children aged <2 years (41.5%; 115/277; OR = 3.1671; 95% CI [1.66–6.05]).
[b]Patients whose information about the date of birth or date of collection of fecal samples was not provided.

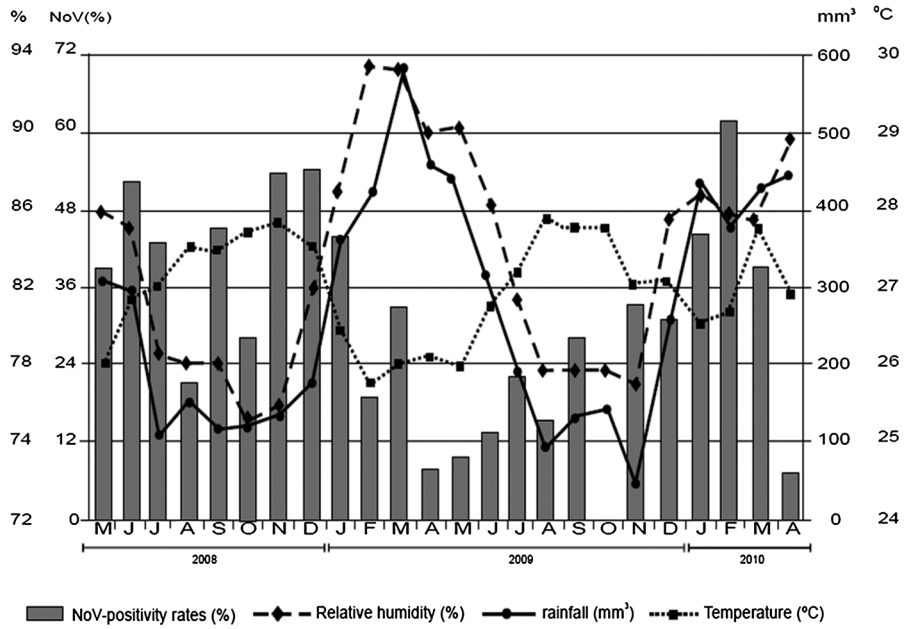

Fig. 1. Correlation between monthly NoV-positivity rates and humidity, precipitation and temperatures recorded in Belém, Pará, Brazil, from May 2008 to April 2010.

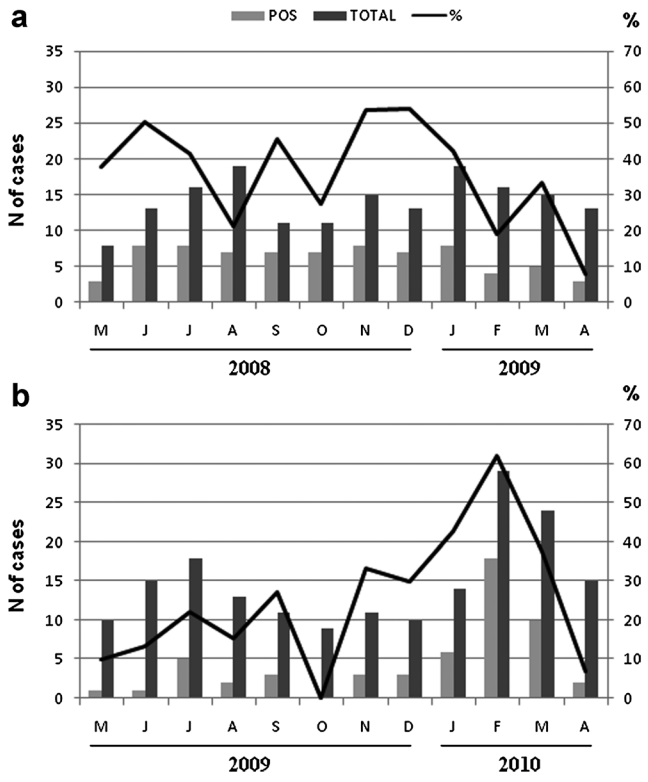

Fig. 2. Monthly distribution of NoV infection detected by EIA in 348 fecal specimens collected from children admitted to hospital with acute gastroenteritis in Belém, Pará, Brazil: (**a**) May 2008 to April 2009; (**b**) May 2009 to April 2010.

($P = 0.0047$). The highest monthly value was that of February 2010 (62.1%).

Of the 169 samples collected during the first year of the study, 44.4% (75/169) were positive for NoV, with 40.8% (69/169) being confirmed by RT-PCR and 35.5% (60/169) by EIA. There was an 87.6% (148/169) agreement between the two approaches, indicating excellent reproducibility (Kappa = 0.7, $P < 0.0001$). If RT-PCR is considered to be the "gold standard," EIA yielded a sensitivity of 78.3% and specificity of 94%, with a positive predictive value (PPV) of 90% and a negative predictive value (NPV) of 86.2% (Table II).

Twenty-two (31.9%) of the 69 positive samples for NoV were sequenced using the same pair of PCR primers that target the RNA polymerase region. Of these, 20 (90.9%) were classified as GII.4d and 2 (9.1%) as GII.b (Fig. 3).

DISCUSSION

In the present study, the rate of NoV positivity recorded by both techniques (36.5%) was similar to those recorded in other regions of Brazil, such as Espírito Santo state (39.7% in 2004–2006), and in Germany 49% in 2002–2008 [Ribeiro et al., 2008; Spackova et al., 2010]. However, the positivity-rate recorded in Belém was much higher than those reported from hospitals and emergency departments in this city in previous years, 14.6% in 1992–1994, 8.9% in 1998–2000, and 12.5% in 2003 [Nakamura et al., 2006; Aragão et al., 2010, unpublished data].

TABLE II. Comparison of the Results Obtained by EIA and RT-PCR for 169 Fecal Specimens Collected From Children With Diarrhea Admitted to Hospital in Belém, Pará, Brazil, From May 2008 to April 2009

	RT-PCR		
EIA	Positive	Negative	Total
Positive	54	6	60 (35.5%)
Negative	15	94	109
Total	69 (40.8%)	100	169

Children 2 years old and younger who were not infected with RV were three times more likely to develop acute gastroenteritis caused by NoV than those of other age groups, with a positivity-rate of 41.5%. Similar results were obtained previously in Belém and also in Vitória, the capital of Espírito Santo, in Southeast Brazil [Ribeiro et al., 2008; Aragão et al., 2010]. However, in a study involving children less than 3 years of age in the Brazilian Midwest, Borges et al. [2006] found no significant difference between age groups, nor any difference in relation to the gender, which is consistent with the results of the present study in Belém.

Most studies of seasonal patterns have described winter peaks of NoV infection (both outbreaks and sporadic cases) in temperate countries [Fretz et al., 2005]. In tropical regions, however, NoV tends to be detected year-round, with no clear seasonal pattern of occurrence. In Southeastern Brazil, for example, Soares et al. [2007] found no marked seasonal variation over an 8-year period (1998–2007). In the subtropical Brazilian Midwest, however, while Borges et al. [2006] also recorded NoV infections throughout the year, a peak was observed during the more humid rainy season, from September to March. This is consistent with the results of EIA in the present study, in which a peak of NoV infection was recorded in February 2010, the middle of the local rainy season. Victoria et al. [2007] recorded biphasic peaks in Rio de Janeiro in 2004, with the highest rates of NoV gastroenteritis being observed between March and May, and in September and October. Overall, these results indicate the need for a long-term and more systematic analysis of possible seasonal patterns in NoV infection.

While overall positivity rates were relatively high, during the first year of the present study, a similar pattern of variation was observed in both years, except for February and October. No clear relationship was found between positivity rates and any of the climatic variables analyzed (temperature, rainfall, or relative humidity). It is important to note that, as Belém is located close to the equator line, climatic variables, especially temperature, tend to be relatively constant throughout the year [PMB, 2010; INMET, 2012].

The EIA presented an agreement of 87.6% when compared to the NoV RT-PCR, which was similar to the values of 85.9% and 90.7% reported for children with diarrhea from Venezuela and Japan, respectively [Okitsu-Negishi et al., 2004; González et al., 2006].

In the present study, GII.4 was predominant, accounting for over 90% of the strains identified. This genotype has been circulating in Belém since 1998 (unpublished data). In a review of the available long-term data (1990–2008) for a number of different regions, including Latin America, Patel et al. [2008] concluded that this genotype was responsible for 75–100% of all cases of NoV-related gastroenteritis. This study also recorded a marked diversity of GII.4 variants, highlighting the potential for genetic variability in the strains of this virus worldwide [Okada et al., 2007; Johansen et al., 2008]. Fioretti et al. [2011] recorded a number of different GII NoV genotypes in a Brazil-wide survey of NoV-related gastroenteritis, but found a predominance of GII.4 (78%). Future studies are being planned, targeting at the capsid region (ORF 2) which may yield a more accurate genotyping of these samples.

The primers used in the present study targeted the RNA polymerase (ORF1), which is known to be a highly conserved region of the NoV genome. Two samples were classified as genotype GII.b. Bull et al. [2007] assign the isolated clusters GII.a, GII.b, GII.c, and GII.d, to unclassified polymerases, suggesting possible recombinations. These types of recombinants, especially those of GII.b (polymerase region) and GII.3 (capsid region), have recently been identified as the cause of outbreaks in Europe, Australia, and Asia [Chhabra et al., 2010]. The ORF1 amino acid sequence of the two GII.b samples detected in the present study was 100% identical to that of the Paris Island prototype strain (AY652979). Confirmation of the presence of a recombination would nevertheless require the molecular characterization of the ORF 2 in order to differentiate this strain from the more common GII.3 strains [Buesa et al., 2002; Chhabra et al., 2010].

While it was only possible to test by EIA and RT-PCR 21% of the 1,649 specimens collected during the present study, they appear to constitute a representative sample of the study population. As mixed infections involving RV are relatively rare, the omission of samples that were positive for RV may have influenced the rates of NoV-positivity recorded in the present study, leading to a possible overestimation. This sampling criterion is standard practice in Brazil and other countries [Soares et al., 2007; Andreasi et al., 2008; Le et al., 2010; Mahar and Kirkwood, 2011].

The testing of samples by EIA only in the second year of the current study may have underestimated overall prevalence. The chi-square test (data not shown) indicated a highly significant difference ($P = 0.004$) between the positivity rates obtained for first and second years: 44% (75/169) and 29% (52/179), respectively. This can be considered to be a weakness of the present study.

In the post-RV vaccine era, it may be worth reinforcing the monitoring of other potential agents of gastroenteritis, such as NoV, in particular, to assess a possible increase in the incidence of these agents

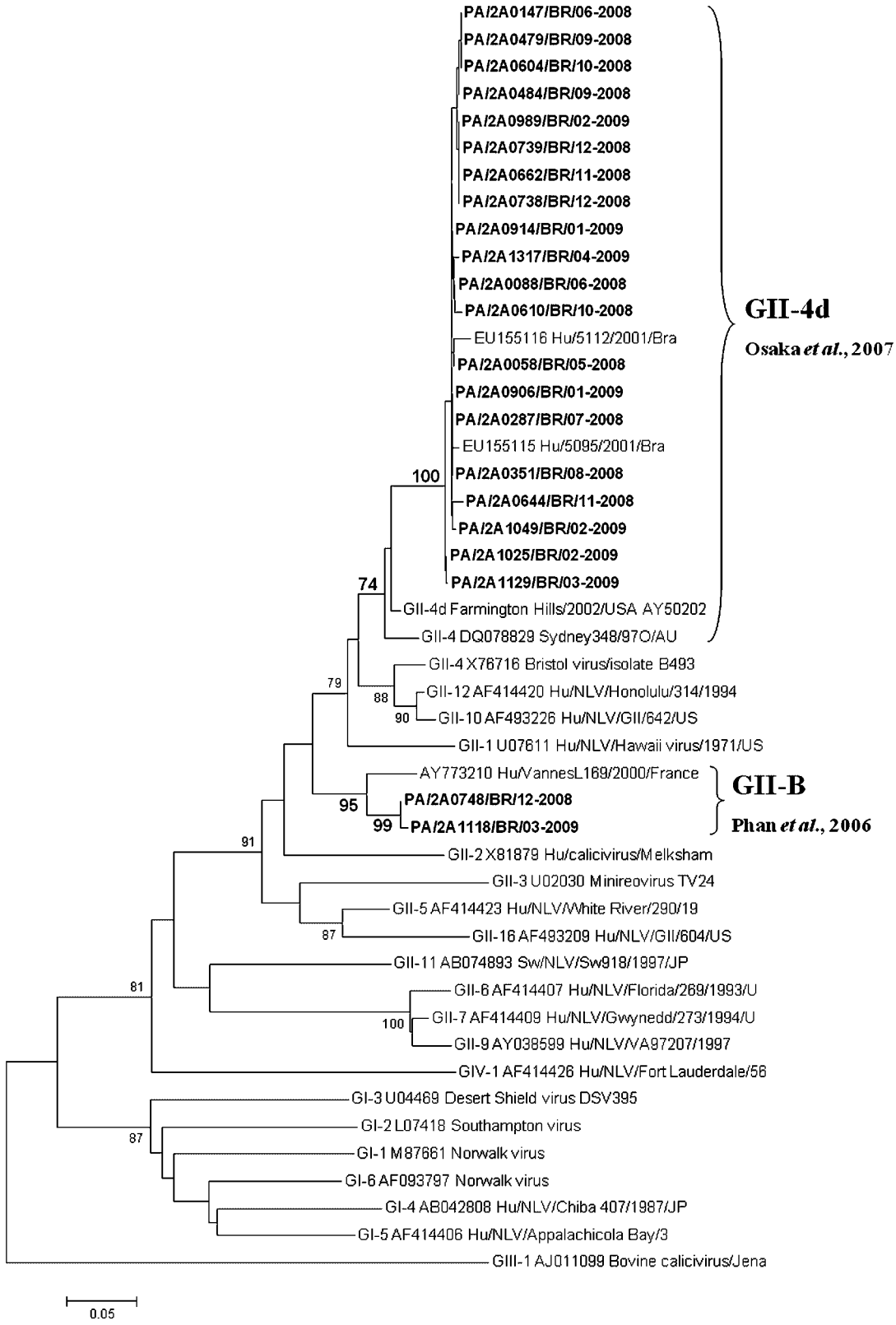

Fig. 3. Dendrogram showing the genetic classification of the samples that tested positive for NoV obtained from children with diarrhea admitted to hospital in Belém, Pará, Brazil, from May 2008 to April 2009. The codes representing the positive samples are organized as follows: study area (Pará)/ sample number/country of collection (Brazil)/month–year of collection.

following the introduction of the RV vaccine in Brazil's National Immunization Program in 2006. In the present study, the incidence of NoV-related acute gastroenteritis in RV-negative patients appears to be higher than that recorded in previous studies in Belém, prior to the implementation of the RV vaccination program [Nakamura et al., 2006; Aragão et al., 2010, unpublished data]. A similar trend appears to have occurred in Rio de Janeiro (49.8%) and other Brazilian states (41.6%) following the introduction of universal RV vaccination [Ferreira et al., 2010; Morillo et al., 2011]. Similar results were obtained in the city of León, Nicaragua, where a reduced incidence of RV and high prevalence of NoV were found in the local wastewater following the introduction of the pentavalent RotaTeq™ vaccine (RV5-Merck®) in October 2006 [Bucardo et al., 2011].

Finally, it is planned to expand this study to cover a third year of surveillance, and perform the molecular analysis of ORF 1 and ORF 2 regions, in order to achieve a broader molecular and epidemiological characterization of these data. The gathering of further robust data on the epidemiology of NoV infection in Northern Brazil may provide an important baseline for future studies into potential NoV vaccines.

ACKNOWLEDGMENTS

We thank the valuable technical support provided by Maryelle Gonçalves, Ian Lima, Maria Silvia Lucena, Paula Spada, Samya Costa, Dielle Teixeira, Juliana Hernandez, João Felipe, and our field staff. We are also grateful to the Coordination of Improvement of Higher Education Personnel (CAPES) for the scholarship for the authors Jones Siqueira and Glicélia Aragão; to the National Meteorological Institute (INMET) for providing climatic data and Mrs. Graça Santos for assistance with the plotting of these data; to the staff of the Evandro Chagas Institute library; to Mr. Stephen Ferrari for reviewing the manuscript and to Foundation of Support for Research Development (FADESP) by payment of this review; to all the employees at the Serzedelo Corrêa Hospital (formerly the Clínica Pediátrica do Pará) who were involved in this study. Special thanks to all the children who participated in the study and to their parents/guardians.

REFERENCES

Anderson AD, Garrett VD, Sobel J, Monroe AS, Fankhauser RL, Schwab KJ, Bresee JS, Mead PS, Higgins C, Campana J, Glass RI. 2001. Multistate outbreak of Norwalk-like virus gastroenteritis associated with a common caterer. Am J Epidemiol 11:1013–1019.

Andreasi MSA, Cardoso DDP, Fernandes SM, Tozetti IA, Borges AMT, Fiaccadori FS, Santos RAT, Souza M. 2008. Adenovirus, calicivirus and astrovirus detection in fecal samples of hospitalized children with acute astroenteritis from Campo Grande, MS, Brazil. Mem Inst Oswaldo Cruz 103:741–744.

Aragão GC, Oliveira DS, Santos MC, Marenhas JDP, Oliveira CS, Linhares AC, Gabbay YB. 2010. Molecular characterization of norovirus, sapovirus and astrovirus in children with acute gastroenteritis from Belém, Pará, Brazil. Rev Pan Amaz Saúde 1:149–157.

Ayres MMA, Jr., Ayres DL, dos Santos AS. 2007. BioEstat 5.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas. 5th edition. Belém-PA: Mamirauá.

Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T, Liu L, Mathers C. 2010. Global, regional, and national causes of child mortality in 2008: A systematic analysis. Lancet 375:1969–1987.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-Van Dillen PM, Van Der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503.

Borges AM, Teixeira JM, Costa PS, Giugliano LG, Fiaccadori FS, Franco RC, Brito WM, Leite JP, Cardoso DD. 2006. Detection of calicivirus from fecal samples from children with acute gastroenteritis in the West Central region of Brazil. Mem Inst Oswaldo Cruz 101:721–724.

Bucardo F, Lindgren PE, Svensson L, Nordgren J. 2011. Low prevalence of rotavirus and high prevalence of norovirus in hospital and community wastewater after introduction of rotavirus vaccine in Nicaragua. PLoS ONE 10:e25962.

Buesa J, Collado B, López-Andújar P, Abu-Mallouh R, Rodríguez Díaz J, García Díaz A, Prat J, Guix S, Llovet T, Prats G, Bosch A. 2002. Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. J Clin Microbiol 40:2854–2859.

Bull RA, Tu ET, McIver CJ, Rawlinson WD, White PA. 2006. Emergence of a new norovirus genotype II.4 variant associated with global outbreaks of gastroenteritis. J Clin Microbiol 44:327–333.

Bull RA, Tanaka MM, White PA. 2007. Norovirus recombination. J Gen Virol 88:3347–3359.

CDC. 2011. Updated norovirus outbreak management and disease prevention guidelines. MMWR 3:1–20.

Chhabra P, Walimbe AM, Chitambar SD. 2010. Complete genome characterization of Genogroup II norovirus strains from India: Evidence of recombination in ORF2/3 overlap. Infect Genet Evol 10:1101–1109.

Clark IN, Estes MK, Green KY, Hansman GS, Knowles NJ, Koopmans MK, Matson DO, Meyers G, Neill JD, Radford A, Smith AW, Studdert MJ, Thiel H-J, Vinjé J. 2012. Family caliciviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. London: Elsevier/Academic Press. p 977–986.

Ferreira MS, Victoria M, Carvalho-Costa FA, Vieira CB, Xavier MP, Fioretti JM, Andrade J, Volotão EM, Rocha M, Leite JP, Miagostovich MP. 2010. Surveillance of norovirus infections in the state of Rio de Janeiro, Brazil 2005–2008. J Med Virol 82:1442–1448.

Fioretti JM, Ferreira MSR, Victoria M, Vieira CB, Xavier MPTP, Leite JPG, Miagostovich MP. 2011. Genetic diversity of noroviruses in Brazil. Mem Inst Oswaldo Cruz 106:942–947.

Fretz R, Herrmann L, Christen A, Svoboda P, Dubuis O, Viollier EH, Tanner M. 2005. Frequency of Norovirus in stool samples from patients with gastrointestinal symptoms in Switzerland. Eur J Clin Microbiol Infect Dis 24:214–216.

Glass PJ, White LJ, Ball JM, Lepare-Goffart I, Hardy ME, Estes MK. 2000. Norwalk virus open reading frame 3 encodes a minor structural protein. J Virol 74:6581–6591.

Glass RI, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, Jiang B, Gentsch JR. 2006. Rotavirus vaccines: Current prospects and future challenges. Lancet 368:323–332.

González GG, Liprandi F, Ludert JE. 2006. Evaluation of a commercial enzyme immunoassay for the detection of norovirus antigen in fecal samples from children with sporadic acute gastroenteritis. J Virol Methods 136:289–291.

INMET. Climatologia. Available in: http://www.inmet.gov.br/html/clima.php?lnk=http://www.inmet.gov.br/html/clima/graficos/index4.html. Accessed: January 15, 2012.

Jiang X, Espul C, Zhong WM, Cuello H, Matson DO. 1999. Characterization of a novel human calicivirus that may be a naturally occurring recombinant. Arch Virol 144:2377–2387.

Johansen K, Mannerqvist K, Allard A, Andersson Y, Burman LG, Dillner L, Hedlund K, Jönsson K, Kumlin U, Leitner T, Lysén M, Thorhagen M, Tiveljung-Lindell A, Wahlströmg C, Zweygberg-Wirgart B, Widell A. 2008. Norovirus strains belonging to the GII.4 genotype dominate as a cause of nosocomial outbreaks of viral gastroenteritis in Sweden 1997–2005. Arrival of new

variants is associated with large nation-wide epidemics. J Clin Virol 42:129–134.

Koopmans M, von Bonsdorff CH, Vinjé J, Médici D, Monroe S. 2002. Foodborne viruses. FEMS Microbiol Rev 26:187–205.

Le VP, Jung YC, Kang KS, Lim I, Myung SC, Kim W. 2010. Genetic characterization of II. Norovirus G4 2006b variants from Jeju Island, South Korea. J Med Virol 82:1065–1070.

Mahar JE, Kirkwood CD. 2011. Characterization of Norovirus strains in Australian children from 2006 to 2008: Prevalence of recombinant strains. J Med Virol 83:2213–2219.

Morillo SG, Luchs A, Cilli A, Ribeiro CD, Calux SJ, Carmona RCC, Timenetsky MCST. 2011. Norovirus 3rd generation kit: An improvement for rapid diagnosis of sporadic gastroenteritis cases and valuable for outbreak detection. J Virol Methods 173:13–16.

Nakamura LS, Oliveira DS, Silva PF, Lucena MS, Mascarenhas JDP, Gusmão RHP, Linhares AC, Gabbay YB. 2006. Molecular characterization of calicivirus in feces of children with acute diarrhea, attending a public hospital, in Belém-Pará. Virus Reviews & Research, XVII National Meeting of Virology 11: 95.

Okada M, Ogawa T, Yoshizumi H, Kubonoya H, Shinozaki K. 2007. Genetic variation of the norovirus GII.4 genotype associated with a large number of outbreaks in Chiba prefecture, Japan. Arch Virol 152:2249–2252.

Okitsu-Negishi S, Nguyen TA, Phan TG, Ushima H. 2004. Molecular epidemiology of viral gastroenteritis in Asia. Pediatr Int 46:245–252.

Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinjé J, Parashar UD. 2008. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 14:1224–1231.

PMB Prefeitura Municipal de Belém. Anuário Estatístico do município de Belém 2010: Demografia e caracterização do território. Available in: http://www.belem.pa.gov.br/app/c2ms/v/?id=1&conteudo=2995. Accessed: January 15, 2012.

Ribeiro LR, Giuberti RSO, Barreira DMPG, Saick KW, Leite JPG, Miagostovich MP, Spano LC. 2008. Hospitalization due to norovirus and genotypes of rotavirus in pediatric patients, state of Espírito Santo. Mem Inst Oswaldo Cruz 103:201–206.

Sasaki Y, Kai A, Hayashi Y, Shinkai T, Noguchi Y, Hasegawa M, Sadamasu K, Mori K, Tabei Y, Nagashima M, Morozumi S, Yamamoto T. 2006. Multiple viral infections and genomic divergence among noroviruses during an outbreak of acute gastroenteritis. J Clin Microbiol 44:790–797.

Soares CC, Santos N, Beard RS, Albuquerque MCM, Maranhão AG, Rocha LN, Ramírez ML, Monroe SS, Glass RI, Gentsch J. 2007. Norovirus detection and genotyping for children with gastroenteritis, Brazil. Emerg Infect Dis 13:1244–1246.

Spackova M, Altmann D, Eckmanns T, Koch J, Krause G. 2010. High level of gastrointestinal nosocomial infections in the german surveillance system, 2002–2008. Infect Control Hosp Epidemiol 31:1273–1278.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739.

Verhoef L, Depoortere E, Boxman I, Duizer E, van Duynhoven Y, Harris J, Johnsen C, Kroneman A, Le Guyader S, Lim W, Maunula L, Meldal H, Ratcliff R, Reuter G, Schreier E, Siebenga J, Vainio K, Varela C, Vennema H, Koopmans M. 2008. Emergence of new norovirus variants on spring cruise ships and prediction of winter epidemics. Emerg Infect Dis 14:238–243.

Victoria M, Carvalho-Costa FA, Heinemann MB, Leite JP, Miagostovich M. 2007. Prevalence and Molecular epidemiology of noroviruses in hospitalized children with acute gastroenteritis in Rio de Janeiro, Brazil, 2004. Pediatr Infect Dis J 26:602–606.