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Acute norovirus gastroenteritis in children in a highly rotavirus-vaccinated population in Northeast Brazil

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ABSTRACT

Background: Gastroenteritis is one of the most important causes of morbidity and mortality in children and an important etiological agent is norovirus.
Objective: We describe the occurrence and characteristics of norovirus diarrhoea in children from Sergipe, Northeast-Brazil, over two consecutive periods of three years following rotavirus vaccine introduction.
Study design: A cross sectional hospital-based survey conducted from October-2006 to September-2009 and from July-2011 to January-2013. Acute diarrhoea cases had a stool sample collected and tested for norovirus by RT-PCR and positive samples were sequenced.
Results: In total 280 (19.6%) of 1432 samples were norovirus positive, including 204 (18.3%) of 1113 samples collected during the first period and 76 (23.9%) of 318 collected during the second period. The proportion of children with norovirus infection increased significantly through the second study period (χ^2 for trend = 6.7; p = 0.009), was more frequent in rotavirus vaccinated and in younger children (p < 0.001). Of 280 norovirus-positive specimens, 188 (67.1%) were sequenced. Of these, 12 were genogroup I and 176 genogroup II. The main genotype was GII.4 (149/188, 79.3%), followed by GII.2 (6, 3.2%) and GII.6 (5, 2.6%).
Conclusion: Norovirus annual detection rates increased over the study period. The detection of norovirus was higher among young children.

1. Background

Gastroenteritis is one of the most important causes of morbidity and mortality in children [1] and an important etiological agent is norovirus (NoV). This single-stranded RNA virus is a common cause of both sporadic and epidemic acute diarrhoea [1,2] and its importance is second only to rotavirus in areas where rotavirus vaccines have not been introduced [2]. Rotavirus vaccines are rapidly being introduced across the world and the relative importance of NoV may increase with the successful reduction of the rotavirus burden. However the epidemiology and burden of NoV has been poorly documented in low and middle income countries introducing rotavirus vaccines [2,3] and it is unclear whether its incidence and severity will remain the same or increase to fill the environmental niche of rotavirus [4]. Although NoV has become the most common cause of severe diarrhoea in industrialized countries with high rotavirus immunization [1,2], few studies have described NoV epidemiology since the introduction of rotavirus vaccines in other settings [5–8]. Brazil was one of the first countries to introduce a monovalent rotavirus vaccine (Rotarix) on a large scale in March 2006 [9], reaching high vaccine coverage levels (>80%) within a year of vaccine introduction and rapidly reducing the incidence of rotavirus-related hospitalizations [10,11].

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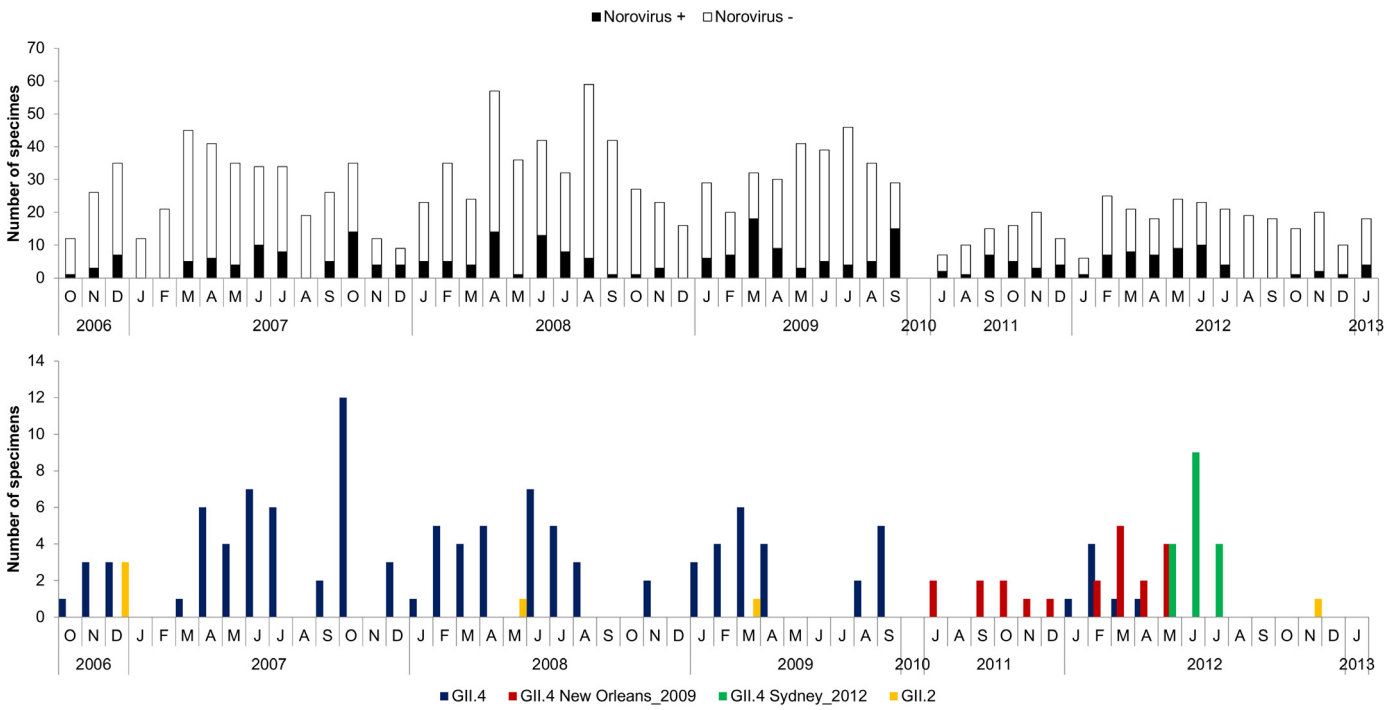

Fig. 1. A. Number of samples positive and negative for norovirus by month. B. The bars represent the norovirus specimens most frequently genotyped during the study period.

2. Objective

We have examined the patterns of NoV diarrhoea in children residing in Aracaju, Northeast Brazil over two consecutive periods from October 2006 to September 2009 and from July 2011 to January 2013.

3. Study design

3.1. Study population and sample collection

This was a cross sectional survey conducted from October 2006 to January 2013 aiming to establish the proportion and severity of acute diarrhoea episodes due to rotavirus [11] and NoV. Children <12 years old presenting with acute diarrhoea attending the paediatric emergency service of Sergipe Emergency Hospital (Hospital de Urgência de Sergipe – HUSE) were enrolled consecutively at the time of presentation. HUSE provides 24-h free medical services and is a reference hospital for Sergipe State (~2 million population). For logistical reasons, only children attending between 8 am and 4 pm from Monday to Friday were included. Due to funding constraints, data for NoV was analysed from October 2006 to September 2009 and from July 2011 to January 2013, but data collection used the same methods and protocols over the study period [10]. After obtaining written parental consent, children were assessed to establish the medical history and clinical presentation and parents were asked for the child vaccination cards. Vaccination cards are routinely brought by parents when they visit health facilities and all vaccinations are recorded. A child was classified as vaccinated for rotavirus if the card had the 2 documented doses of the vaccine. Children with one or no rotavirus vaccines were classified as unvaccinated. Children without vaccination cards were classified as having an unknown vaccination status. Parents were asked to collect one stool specimen from the child before leaving the service and about 60% of parents managed to collect specimens and were included in the study. Stools specimens were stored at 4 °C for a maximum of 24-h and stored in a −80 °C freezer until processed.

3.2. Detection of norovirus

RNA was extracted from 140 μl of 10% stool suspensions using the QIAamp Viral RNA extraction kit (QIAGEN, CA, USA), and immediately stored at −80 °C prior NoV detection. Real time RT-PCR was performed on an ABI 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using primers and probes previously described [12] and the SuperScript III Platinum One-Step Quantitative RT-PCR System (Invitrogen, CA, USA). Briefly, the assay was carried out in a 25 μl final reaction mixture containing 5 μl of purified RNA with final concentrations of primers and probes of 600 and 300 nM, respectively. The thermal cycling conditions were carried out as follows: a RT step at 55 °C for 30 min, an initial denaturation step at 95 °C for 10 min, 45 cycles of PCR amplification at 95 °C for 15 s, and at 60 °C for 1 min. A 10-fold serial dilution of a plasmid containing the ORF1/2 junction was used to generate standard curves for virus quantification. Forty cycles were used in the reaction and samples with a cycle threshold <40 were regarded as positive.

NoV-positive samples were genotyped by sequencing the partial 5′-end of ORF2 region (320 nt in length, corresponding to the region C of the NoV genome), as previously described [12,13]. Amplicons were sequenced in both directions using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit® (v. 3.1) and an ABI Prism 3130xl DNA model sequencer (Applied Biosystems).

3.3. Phylogenetic analysis

Phylogenetic analysis was performed only on NoV-positive samples detected between July 2011 and January 2013 at the Rio de Janeiro Oswaldo Cruz Institute. Consensual sequences obtained were aligned and edited using the BioEdit Sequence Alignment Editor (version 7.0.5.3) program, and compared to the GenBank database using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The Geno-