Detection of the pandemic norovirus variant GII.4 Sydney 2012 in Rio Branco, state of Acre, northern Brazil

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Noroviruses (NoVs) are important cause of gastroenteritis in humans worldwide. Genotype GII.4 is responsible for the majority of outbreaks reported to date. This study describes, for the first time in Brazil, the circulation of NoV GII.4 variant Sydney 2012 in faecal samples collected from children aged less than or equal to eight years in Rio Branco, state of Acre, northern Brazil, during July-September 2012.

Key words: norovirus - gastroenteritis - GII.4 variant

Noroviruses (NoVs) are the major cause of epidemic viral gastroenteritis and the leading cause of foodborne outbreaks in diverse countries in the world. The disease in most cases occurs with diarrhoea and vomiting, affecting mainly children, the elderly and immunocompromised persons. These viruses are often associated with outbreaks in closed settings such as schools, hospitals, hotels, cruise ships and nursing homes (Green 2007).

The importance of NoV as agents of childhood gastroenteritis extends throughout the developing countries since the late 1970s, being considered the second causative agent of viral gastroenteritis in children under five years old (Rackoff et al. 2013). NoVs possess an icosahedral virion of 27-32 nm in diameter, single stranded, positive-sense, polyadenylated RNA genome of 7,400-7,700 nucleotides (Kapikian et al. 1972). The viral genome is organised into three open reading frames (ORFs). ORF1 encodes the non-structural proteins, including the RNA-dependent RNA polymerase (Clarke & Lambden 2000). The ORF2 encodes the structural capsid protein VP1, the main component of the viral capsid, divided into three major structural domains: S, P1 and P2. The P2 domain contains the most variable sequence and is located on the surface of the capsid, playing an important role in immune recognition and receptor interaction (Prasad et al. 1999, Glass et al. 2000).

NoVs exhibiting a large genetic diversity and are classified into five genogroups (GI-GV) based on VP1 amino acid sequence and these are further subdivided into multiple genotypes (Zheng et al. 2006). Human infection is associated with GI, GII and GIV genogroups, of which the GII.4 genotype has maintained importance over more than 30 years in both outbreaks and sporadic cases (Siebenga et al. 2009, Rackoff et al. 2013). Diverse GII.4 variants have been associated with global epidemics of acute gastroenteritis from 1996 to the present, including the US 1995/96 variant in 1996 (White et al. 2002), Farmington Hills variant in 2002 (Widdowson et al. 2004), Hunter variant in 2004 (Bull et al. 2006), 2006a and 2006b variant in 2007-2008 (Eden et al. 2010), New Orleans variant in 2009-2012 (Yen et al. 2011) and most recently Sydney 2012 (van Beek et al. 2013).

The first report of Sydney 2012 variant was in March 2012 in New South Wales, Australia (van Beek et al. 2013). Thereafter, it was detected in the United States of America (USA) in September 2012 (Barclay et al. 2013), in Belgium, September and December 2012, and in Denmark in November 2012 (Fonager et al. 2013). In this period, various countries, such as New Zealand, France, Scotland, Japan, Hong Kong and the USA, have reported a higher incidence of NoV outbreaks (Barclay et al. 2013, Bennett et al. 2013, Chan & Chan 2013). According to published data obtained by NoroNet, there are suggestive evidences that this increase is related to this new NoV GII.4 2012 variant (van Beek et al. 2013).

This study analysed 25 stool samples of sporadic cases of acute gastroenteritis among hospitalised children which were obtained within the National Surveillance Program of Rotavirus Gastroenteritis coordinated by the Brazilian Ministry of Health. This program comprises three officials Reference Centres and Evandro Chagas Institute is one of them.

The detection of NoV was first performed using a commercial enzyme immunoassay (EIA) (Ridascreen® Norovirus 3rd Generation, R-Biopharm AG, Darmstadt, Germany) following the manufacturer's instructions. Viral RNA was extracted from a 10% faecal suspension (the same used in the EIA test) using a guanidine isothiocyanate/silica method (Boom et al. 1990) followed by cDNA synthesis performed using a pd(N)6™ random primer (Amersham Biosciences, UK) and the Superscript™ II RNAse H Reverse Transcriptase (Invitrogen, USA).

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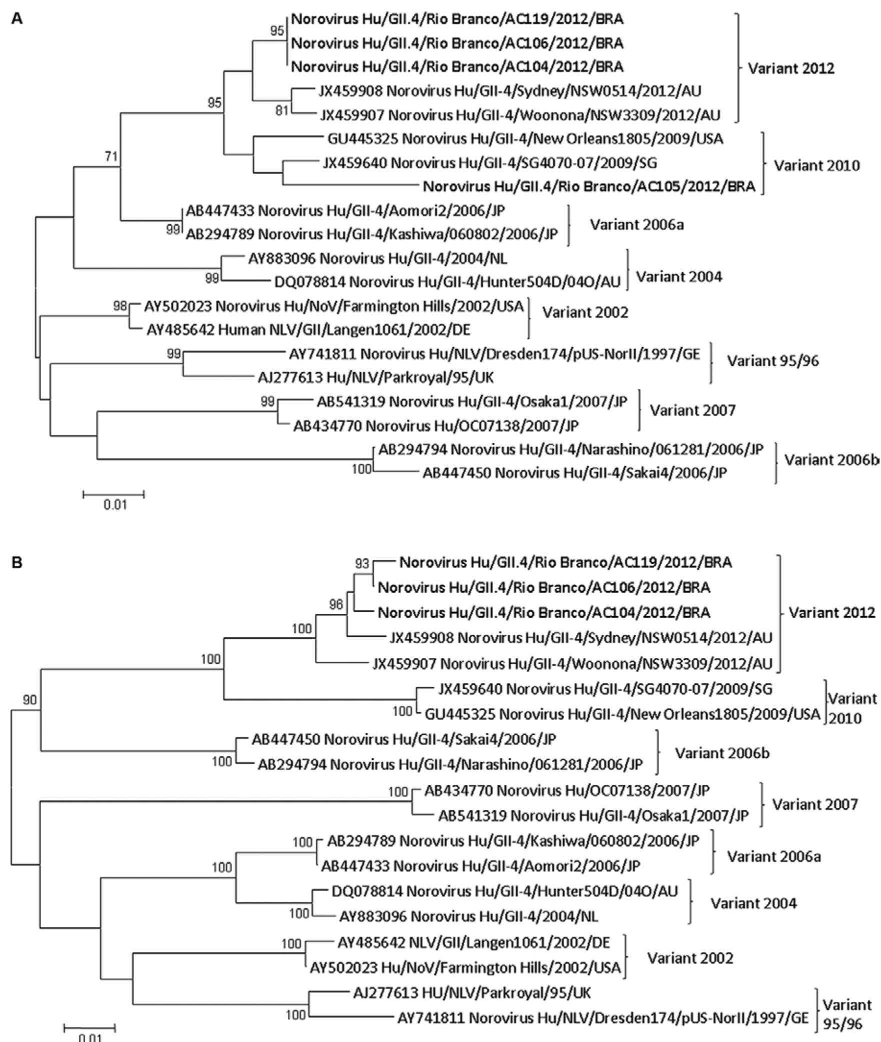
Initially, the viral genome was amplified by reverse transcription polymerase chain reaction (RT-PCR) using primers for B region of the polymerase gene (ORF1) (Anderson et al. 2001, Fankhauser et al. 2002). For genetic characterisation were used primers for region D (ORF2) of the viral capsid of NoV GII with primers Cap C, Cap D1 and Cap D3 (Vinjé et al. 2004). In addition, these samples were tested with primers for P2 region (EVP2F and EVP2R) described to define GII.4 variants (Vega et al. 2011). The amplicons obtained were purified with a QIAquick® PCR Purification Kit and QIAquick® gel Purification Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's recommendations. DNA sequencing was performed using the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were edited using the BioEdit Sequence Alignment Editor (v.7.0.9.1) software. The evolutionary history was inferred using the neighbour-joining method, bootstrap 2,000 replicates and model using MEGA 5.1 (Saitou & Nei 1987, Tamura et al. 2011).

From July-September 2012, 25 samples were collected by the Surveillance Program from children with acute gastroenteritis the city of Rio Branco, state of Acre. Patient's ages ranged from four month-eight years (40%, 0-12 months; 24%, 1-2 years old; 28% > 2 years old; 8% no information). These samples were analysed by EIA and RT-PCR using region B with a positivity of 48% (12/25) in both, but one sample was positive only by EIA and another only by RT-PCR. All positive cases were shown to have diarrhoea as the major symptom, with duration of four days on average; there were no other symptom recorded on medical histories sent to us.

NoVs genotypes were characterised in four samples using primers for the capsid region (region D). The New Orleans 2009 was detected in one sample and Sydney 2012 GII.4 variants in three samples. The analyses of P2 region confirmed the presence of the Sydney 2012 GII.4 variant and the sequences obtained had a high nucleotide identity among them (range 98.8-99.5%) and differed in the range of 1.2-2.6% when compared with the prototype



Dendograms constructed using region D (A) and P2 region (B) amplified from strains from diarrhoeic children of the city of Rio Branco, state of Acre, Brazil. Prototype strains are presented. Study samples were marked in bold. The number above each branch corresponds to the bootstrap value (2,000 replicates). The scale bar is proportional to the genetic distance.

strain (Figure). These sequences were submitted to GenBank under accessions KF360222-KF360228.

Briefly, this study demonstrated by phylogenetic analysis the circulation of the two most recently identified GII.4 variants, New Orleans 2009 and Sydney 2012 in Rio Branco and, to our knowledge, this represents the first detection of the recently emerged GII.4 Sydney 2012 variant in Brazil.

Several studies demonstrate that Sydney 2012 variant has the potential for strain replacement and can rapidly diversify within the population, a phenomenon driven by evolutionary forces and leading to persistence of the GII.4 variants in a community (Eden et al. 2010, Fonager et al. 2013). As a consequence, a rapid worldwide dispersion of the GII.4 variants may occur; nevertheless, further studies are needed in order to better assess this issue, including its association with gastroenteritis outbreaks.

Our results warrant additional studies in other settings in Brazil to see whether this variant is spreading across the country.

In addition, further local studies are needed, including a larger sample size to better evaluate epidemiological and molecular aspects related to the emergence of this new GII.4 2012 variant.

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