Group A rotavirus and norovirus display sharply distinct seasonal profiles in Belém, northern Brazil

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Several viruses have been associated with acute gastroenteritis (AGE), and group A rotavirus (RVA) and norovirus (NoV) are the most prevalent. This study aimed to assess their prevalence among children hospitalised for diarrhoea during a three-year surveillance study. From May 2008-April 2011, overall positivity rates of 21.6% (628/2904) and 35.4% (171/483) were observed for RVA and NoV, respectively. The seasonality observed indicated distinct patterns when both viruses were compared. This finding may explain why hospitalisation for AGE remains constant throughout the year. Continuous AGE monitoring is needed to better assess the patterns of infection.

Key words: rotavirus - norovirus - seasonality

Several viruses have been associated with acute gastroenteritis (AGE) in humans and the most prevalent are group A rotavirus (RVA) and norovirus (NoV). RVA and NoV often cause disease that may require hospitalisation and this translates into a significant public health burden (Clark & McKendrick 2004).

According to the International Committee on Taxonomy of Viruses, rotaviruses are members of the Reoviridae family and are subdivided into five serological species (A-E) and two tentative species (F-G) based on the antigenic properties of the VP6 protein (Attoui et al. 2012) and VP6 sequence-based cut-off values. Groups A, B and C are associated with infection in humans and a variety of animals (Matthijnssens et al. 2012).

RVA is the most common cause of AGE and requires medical attention or hospitalisation for young children worldwide, accounting for approximately 2.4 million hospitalisations, and more than half a million deaths annually among children less than five years of age (Tate et al. 2012).

RVA tends to be more common in cooler, drier months in most settings, but seasonal peaks have been noted year round in different areas and can differ over time in the same location (Levy et al. 2009). Attempts to relate these patterns to climatic variables, such as temperature, humidity and rainfall, have led to conflicting results (D'Souza et al. 2008). Many developing countries are located in the tropics, where traditionally RVA activity has been thought to lack seasonality, leading to high levels of year-round disease transmission (Cook et al. 1990). However, a recent study conducted in Belém, state of Pará, northern Brazil, Amazon Region, on children hospitalised in the same clinic studied in this study reinforced a possible seasonality for this virus (Justino et al. 2011).

NoV belongs to the Caliciviridae family, which also includes four other genera: Sapovirus, Vesivirus, Lagovirus and Nebovirus. While Norovirus and Sapovirus are known to infect humans, the remainder are mainly of veterinary interest (Clark et al. 2012).

Studies estimate that every year NoV is responsible for 900,000 episodes of AGE, requiring clinic visits and 64,000 hospitalisations of children less than five years of age in economically developed countries. In developing countries, more than 1.1 million hospitalisations and 128,000 deaths per year are believed to be associated with NoV (Patel et al. 2008).

Seasonal variation in NoV infection is a recognised, albeit poorly understood, phenomenon (Rohayem 2009). NoV epidemic characteristics and timing are markedly consistent from year to year, with a peak incidence during the wintertime (from October-April) and specific peaks in February and March. However, outbreaks of NoV do occur during summer (Verhoef et al. 2008). The temporal distribution is most likely based on biological, environmental and behavioural factors that regulate the transmission, virulence and persistence of the virions in host populations (Rohayem 2009).

In view of the importance of RVA and NoV as causes of AGE that may require hospitalisation, this cross-sectional study aimed to assess the prevalence of RVA and NoV among children hospitalised for AGE during a three-year surveillance study.

The surveillance of community-acquired AGE was conducted in a large paediatric hospital located in Belém from May 2008-April 2011. Approximately 40% of all paediatric hospitalisations for AGE in Belém occur in this hospital.

Eligible cases for inclusion were children aged less than five years who were hospitalised for AGE and presented diarrhoea, which was defined as the presence of

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≥ 3 liquid or semi-liquid stools in a 24-h period. Only faecal samples collected up to 48 h following admission were tested for the presence of RVA and NoV.

A total of 2,904 samples were obtained during the study period and screened for RVA antigen using a commercial Ridascreen® Rotavirus enzyme-immunoassay (EIA) (R-Biopharm, Darmstadt, Germany). Reverse transcription polymerase chain reaction (RT-PCR) was used to determine the RVA G and P types (Gentsch et al. 1992).

Of 2276 RVA-negative samples with sufficient material, a representative number of 483 samples (≅ 20%) was randomly selected on a monthly basis for NoV testing. NoV was detected using a third-generation commercial Ridascreen® Norovirus EIA (R-Biopharm, Darmstadt, Germany) and RT-PCR using a pool of primers (Mon 432/434-431/433) specific for the polymerase region, which detected GI and GII, respectively (Anderson et al. 2001). The samples were tested with EIA and RT-PCR using the same faecal suspension and those with positive results with at least one method were considered positive. Subsequently, only amplicons of positive samples that displayed clear bands were selected for genotyping by nucleotide sequencing using the same primers used for PCR.

Statistical analysis was performed using BioEstat 5.0 software (Ayres et al. 2007). The variation between the RVA and NoV prevalence rates was analysed using the Mann-Whitney *U* test in the months in which higher differences in positivity were observed. p-values ≤ 0.05 were regarded as statistically significant. The study was approved by the Ethical Research Committee in Humans of the Evandro Chagas Institute (0003.0.072.000-08 and 0024.0.072.000-10).

From May 2008-April 2011, an overall positivity of 21.6% (628/2904) was found for RVA using EIA. NoV was detected in 35.4% (171/483) of samples using both EIA and RT-PCR.

RVA infections were more prevalent in August 2008 (48%) and April 2009 (46.6%). For NoV, three prevalence peaks were observed throughout the study period: September and October 2008 (63.6% each month) and February 2010 (62.1%). Figure shows the monthly RVA and NoV positivity rates during the three-year surveillance period.

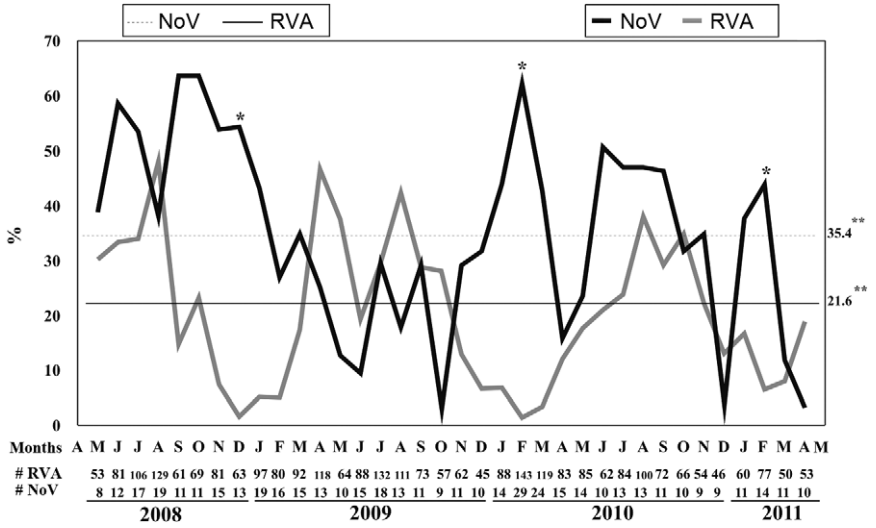
Statistical analyses were performed for the months in which higher prevalence differences between RVA and NoV were found. Significant differences were observed in December 2008 and February 2010 and 2011.

With regard to G2P[4] during the first year (May 2008-May 2009), the findings essentially reflected those of a previous vaccine effectiveness study, in which this serotype accounted for 82% (441/538) of isolates (Justino et al. 2011). During this same period, the RNA polymerase region was sequenced in 22 (31.9%) of the 69 NoV-positive samples, of which 90.9% (20/22) were genotype GII.4d and 9.1% (2/22) were genotype GII.b (Siqueira et al. 2013).

RVA tends to be more common in cooler, drier months in most settings, but seasonal peaks have been noted in different countries and can vary over time in the same country (Levy et al. 2009). Many studies have described an increase in the rates of NoV infection in the winter months in temperate countries. In contrast, in tropical countries, NoV infection is observed year round and does not show a clear seasonal pattern (Fretz et al. 2005).

The seasonality observed for both pathogens suggests sharply distinct seasonal profiles and a "seesaw effect" was observed between the two viruses. This finding may explain why hospitalisations for AGE occur throughout the year. Similar results were observed in 2003 by González et al. (2011) in Venezuela, a region geographically close to northern Brazil, where marked and opposite seasonal patterns were observed.

Although an effective RVA vaccine is available, no vaccine exists for NoV, making it difficult to control the



Monthly distribution of group A rotavirus (RVA) and norovirus (NoV) detected in faecal specimens from children hospitalised with acute gastroenteritis from May 2008-April 2011 in Belém, state of Pará, northern Brazil, Amazon Region. #: number of samples tested by month; *: statistically significant differences between RAV and NoV prevalence [December 2008 (p = 0.0034), February 2010 (p < 0.0001) and 2011 (p = 0.0311)]; **: horizontal dashed lines represent the triennial means of percentages for both viruses.

infection. When sample collection began in this study (2008), the RVA vaccination coverage was 89.9% for the first dose and 76.4% for the second dose (Leite et al. 2008). These rates did not increase significantly during the following years of the study.

NoV occurred at apparently higher rates than RVA. However, it should be noted that only RVA-negative samples were tested for NoV and this may not reflect the true NoV incidence rate, but rather the proportion of NoV-positive cases among RVA-negative patients. A weakness of this study was that possible mixed RVA and NoV infection was not identified, leading to possible overestimation. However, it is noteworthy that this sampling criterion is often used in Brazil and some other countries (Soares et al. 2007 Andreasi et al. 2008, Le et al. 2010, Mahar & Kirkwood 2011).

The RVA G2P[4] detected in this study also showed natural re-emergence in Latin America and many other parts of the world (Antunes et al. 2009, Munford et al. 2009). A review conducted by Patel et al. (2008) in different localities, including Latin America, over 18 years (1990-2008) concluded that NoV GII.4 was responsible for 75-100% of cases of AGE, including both outbreaks and sporadic cases.

Previous publications have suggested a limited sensitivity of the NoV Ridascreen EIA kit, except for the GII.4 type (Okitsu-Negishi et al. 2006, Kirby et al. 2010). However, all samples tested by EIA were also tested by RT-PCR, which is a more sensitive method for detecting any NoV genotype. For this reason, the results may not have been significantly influenced by a possibly lower EIA sensitivity.

This study underscores the importance of NoV as a cause of endemic disease that may require hospitalisation. In fact, the continuous monitoring of RVA and NoV-related AGE is needed to better assess the pattern of these viral infections in Belém; in addition, a group of RVA-positive samples should be tested for the presence of NoV to detect mixed infections.

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