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Short Communication

Rotavirus G2P[4] and G2P[4]+[6] infections during norovirus gastroenteritis outbreak: summer season 2010, Brazil

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ABSTRACT

Introduction: This study aimed to monitor the seasonality of rotavirus infection, and gain insight into the variability of Brazilian strains. **Methods**: A total of 28 stool samples were analyzed from 698 revised cases of gastroenteritis during a norovirus outbreak in the summer of 2010 in Guarujá, Brazil. Diagnosis was performed using enzyme-linked immunosorbent assay (ELISA), reverse transcription polymerase chain reaction (RT-PCR), and sequencing. **Results:** Rotavirus infection was detected in 17.9% (5/28) of samples; 4 samples were G2P[4] genotype, and one G2P[4]+P[6] genotype. G2 and P[4] sequences showed a genetic relationship to strains from India and Russia, respectively. **Conclusions**: The seasonal pattern of rotavirus may be a consequence of human activity apart from climate factors.

Keywords: Rotavirus. Summer. Seasonality.

A key characteristic on rotavirus (RV) epidemiology is its distinct seasonal pattern[1]; however, the mechanism responsible for this seasonality is not clear[2]. In temperate climates, RV disease occurs in the cooler, dryer months of the years, and far less often in tropical areas[2]. In Brazil, the seasonality is distinct, with positive RV specimens appearing to peak during the winter or dry season in central and southern states that exhibit a temperate-like climate, but not in north and northern areas, where RV infections occur equally over the year[3].

During summer 2010, the State of São Paulo (SP), located in southern area of the country, experienced a large gastroenteritis outbreak due to norovirus (NoV)[4], a common viral pathogen associated with diarrhea, which has a marked seasonality during summer. The coastline area of SP was most greatly affected[4], likely due to the increase of population density during the summer vacations and flooding in conjunction with the waterborne and seafood components of NoV transmission[5].

In the popular seashore City of Guarujá, atypical RV infections were observed at the time of a large NoV outbreak. The aims of this study were to contribute to the knowledge of seasonality of RV infection in tropical countries, and to perform sequence analyses to gain insight into the variability of Brazilian strains.

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The increase in acute gastroenteritis cases from Guarujá was detected by the Epidemiological Surveillance Center of São Paulo (CVE)[5], which receives weekly reports from sentinel sites of the Acute Diarrhea Disease Monitoring Program. An epidemiological retrospective investigation was conducted by CVE, and a total of 698 cases were analyzed[5]. The Enteric Diseases Laboratory tested 28 stool specimens from the 698 Guarujá patients.

Rotavirus was detected using a commercial immunoenzymatic assay (RIDASCREEN® Rotavirus, R-Biopharm AG, Darmstadt, Germany), performed according to the manufacturer's instructions. RV-positive stool samples were typed after reverse transcription (RT) followed by semi-nested polymerase chain reaction (PCR)[6] and sequencing on an ABI 3100 thermocycler (Applied Biosystems, Foster City, CA, USA). Sequence data was aligned and edited with the BioEdit Sequence Alignment Editor (version 7.0.5.2) software (Ibis Therapeutics, Carlsbad, CA). A genetic tree was constructed using molecular evolutionary genetics analysis (MEGA) software version 4.0 by the neighbor joining (NJ) method[7].

Rotavirus infection was detected in 17.9% (5/28) of samples. The median age of the patients was 14.9 years; twenty percent of patients were female and 80% were male. According to the Enteric Diseases Laboratory records, NoV infection were detected in 10 (35.7%) of the 28 fecal samples, confirming the large NoV outbreak occurring during the same period[4]. Mixed NoV and RV infections were not observed.

In State of São Paulo surveillance data available on the CVE website (http://www.cve.saude.sp.gov.br/htm/hidrica/hidri_estat.html), there were no RV outbreak reports in the

metropolitan area of Santos during the summer periods. In addition, no RV cases from Santos were identified in the Enteric Diseases Laboratory records during the rainy seasons. Taken together, these findings suggest that a minor RV outbreak occurred in Guarujá alongside the major NoV outbreak. RV outbreaks during the summer season are very uncommon[8]. The seasonal pattern of RV diarrhea cases may be a consequence of unmeasured factors apart from climate factors, including human activity and environmental factors other than the weather[9].

The RT-PCR assay identified the G2P[4] genotype in 4 samples. Recently, a high prevalence of G2P[4] has been reported in Brazil and linked with the inclusion of the G1P[8] live oral RV vaccine in the Brazilian vaccination program[10], suggesting that this monovalent vaccine possibly created conditions in which G2P[4] could acquire selective advantage over P[8] genotypes. Alternatively, a temporal periodicity, within an approximately 10-year cyclic pattern of G2P[4] have been observed in Brazil[11], and should be considered as an alternative explanation to the increased detection of this genotype since 2006.

The genetic relationships between the VP7 sequences of 4 G2 RV strains: R2047 (HQ844990), R2050 (HQ844988), R2053 (HQ844989), and R2057 (HQ844987), and representative strains of the G2 genotype: DS1 (AB118023), Thailand (EF199723), Australia (HRU73955), India (GQ229046), Italy (DQ172854), KO-2 (AF401754), and Brazil (FJ492764), are shown as a distance tree in **Figure 1**. The SP G2 RV sequences showed 93%-97.5% similarity when compared to the representative strains, and 100% similarity between them. The analysis of the VP7 gene showed a genetic relationship with the RV strain from India, and also allowed classification of these strains into lineage II using the Page and Steele[12] classification. In a previous study conducted in the State of Pará, Brazil[13], the phylogenetic analysis performed in G2 RV strains also grouped to lineage II.

The genetic relationships between the VP4 sequences of 3 P[4] RV strains: R2047 (HQ844984), R2050 (HQ844985), and R2053 (HQ844986), and representative strains of the P[4] genotype: Brazil (DQ857927), Italy (DQ172842), DS1 (AB118025), Brazil (FJ492780), Bangladesh (EU839950), and Russia (GU356610), are shown as a distance tree in **Figure 2**. The SP P[4] RV sequences showed 94.6%-99.6% similarity when compared to the representative strains, and 99.9%-100% similarity between them. The SP P[4] RV strains analyzed in this study showed a genetic relationship with the RV strain from Russia.

A P-mixed unusual infection G2P[4]+P[6] was detected in 1 sample from a 10-year-old unvaccinated male. The dual infection was confirmed by RT-PCR using specific set of primers; however, although several attempts were made to obtain the VP4 sequences from this sample, they were unsuccessful. Thus, no data was obtained that could identify the potential origin of this strain.

The isolation of a Brazilian G2 RV strain exhibiting double P[4] and P[6] specificity was not unexpected, suggesting that this was not the same strain circulating during this period and that there was not a common source of RV contamination. In Nigeria, a G2 RV strain displaying dual P[4] and P[6] specificity was identified in 2000[12], and in Guinea-Bissau this unusual strain was the most frequent cause of RV infections in hospitalized children in 2002[14]. These findings may reflect environmental characteristics related to tropical areas, as Brazil, Nigeria, and Guinea-Bissau share the hot and humid tropical climate characteristic of the region between the tropics of Cancer and Capricorn.

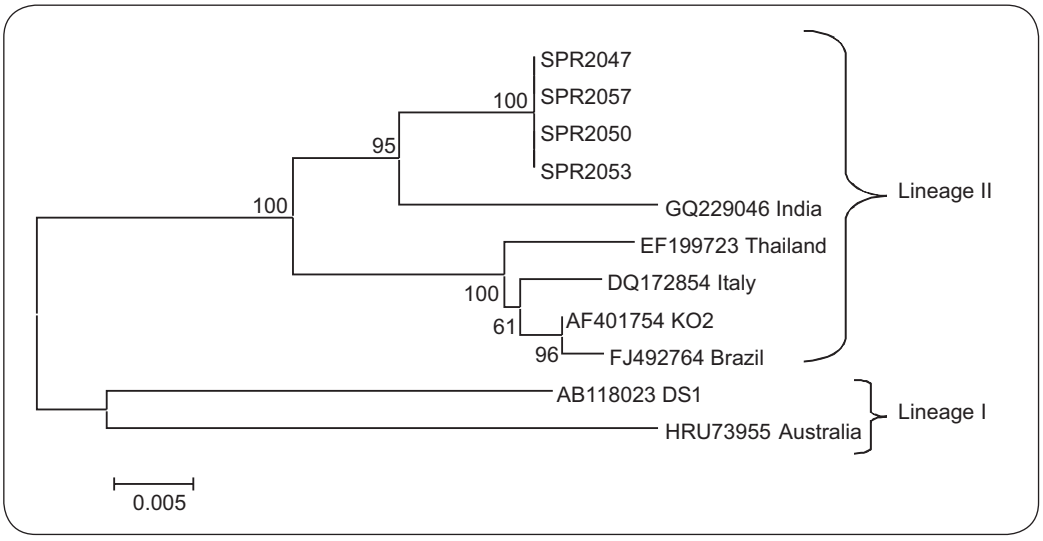

FIGURE 1 - Dendrogram of the VP7 gene nucleotide sequences of human rotavirus G2 strains (SP R2047, SP R2057, SP R2050, and SP R2053) from Guarujá, São Paulo, Brazil. The tree was constructed using the neighbor joining method with the molecular evolutionary genetics analysis (MEGA) 4.0 software package. The numbers on each branch indicate the bootstrap values.

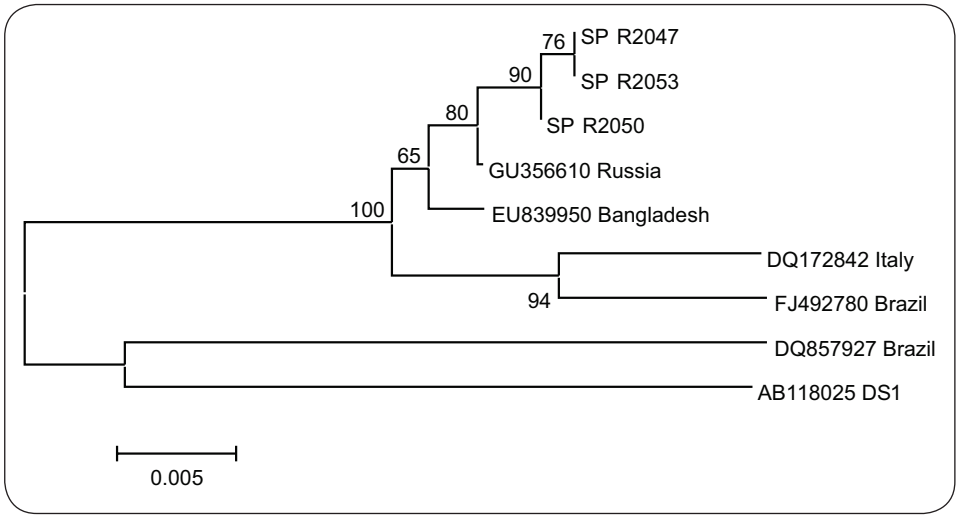

FIGURE 2 - Dendrogram of the VP4 gene nucleotide sequences of human rotavirus P[4] strains (SP R2047, SP R2050, and SP R2053) from Guarujá, São Paulo, Brazil. The tree was constructed using the neighbor joining method with the molecular evolutionary genetics analysis (MEGA) 4.0 software package. The numbers on each branch indicate the bootstrap values.

The inclusion of alternative P types in serotype G2 strain gene constellations may provide a means of escaping protective immunity and generating increased RV diversity[12]. Uncommon associations could also be related to reassortment between co-circulating RV strains[15].

The outbreak investigation also attempted to investigate the bacterial and parasitological etiology. These enteropathogenic investigations were conducted through the Adolfo Lutz Institute online Integrated Hospital Management system (*Sistema Integrado de Gestão Hospitalar*-SIGH), and the findings are summarized in **Table 1**. Overall, this study recognized pathogenic agents in 75% of the examined stool samples, and this elevated rate of isolation could be an indicative of a high rate of oral-fecal exposure in this population.

TABLE 1 - Detection of enteric pathogens in stool samples from patients with acute gastroenteritis during a norovirus outbreak in the summer of 2010, Guarujá, São Paulo, Brazil

	Positive	
Enteric pathogens	Number	Percentage
RV	4	14.3
NoV	8	28.6
RV + *E. coli* (EPEC)	1	3.6
NoV + *E. coli* (EPEC)	2	7.1
E. coli (ETEC)	1	3.6
E. coli (EHEC)	1	3.6
E. coli (EPEC) + *Ascaris lumbricoides*	1	3.6
Giardia lamblia	2	7.1
Giardia lamblia + *Entamoeba coli*	1	3.6
Negative	7	24.9
Total	28	100.0

RV: rotavirus, NoV: norovirus, EPEC: enteropathogenic *Escherichia coli,* ETEC: enterotoxigenic *Escherichia coli*, EHEC: enterohemorrhagic *Escherichi.*

In conclusion, RV was recognized as the etiological agent of a minor gastroenteritis outbreak, affecting children and adults, in the city of Guarujá during the summer of 2010. These results could improve the understanding of RV transmission in tropics, paying close attention to human and climatological variables, and its relationship to other viral gastroenteritis pathogens. A major finding in the present study was the detection of the G2 P[4]+P[6] genotype, reflecting the importance of continued monitoring of RV strains circulating throughout the world.

Ethical considerations

Ethics Committee approval was granted by the Adolfo Lutz Institute, São Paulo, Brazil (Ref. 14/2005). This was an anonymous, unlinked study and informed consent was not required according to the resolution 196/96 concerning research involving human beings (Conselho Nacional de Saúde/ Ministério da Saúde, Brasília, 1996).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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