



Norovirus in São Paulo city, Brazil, 2010-2016: a cross-sectional study of the leading cause of gastroenteritis in children*

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Abstract

Objective: to describe cases of acute diarrheal disease caused by norovirus in children under 5 years old in São Paulo city, Brazil. **Methods:** this was a cross-sectional study using data from Epidemiological Surveillance of Gastroenteritis due to Rotavirus; cases were defined as patients hospitalized in a sentinel unit because of acute diarrheal disease and laboratory identification of norovirus as the etiological agent between 2010 and 2016. **Results:** during the study period, the proportion of norovirus cases in children under 5 years old exceeded the proportion of Rotavirus, an agent considered predominant in childhood; norovirus was associated with 28.4% of total reported cases, occurring all year round, especially in warmer months. **Conclusion:** norovirus was the leading etiological agent identified in children under 5 years old with acute diarrheal disease in São Paulo city.

Keywords: Norovirus; Diarrhea; Epidemiological Monitoring; Epidemiology, Descriptive.

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Introduction

Norovirus is an RNA virus of the *Caliciviridae* family and is considered to be highly contagious. It is classified into seven genogroups and the large variety of its strains hinders acquired immunity when compared to other RNA virus family genera. Its incubation period varies from 24 to 48 hours¹⁻³ and its seasonality can be influenced by mode of transmission, host susceptibility and virus resistance in the environment.⁴⁻⁶

The diagnostic test for identifying norovirus as the etiological agent of viral gastroenteritis is performed on samples having a negative rotavirus result.

Considered to be the world's leading cause of gastroenteritis,^{1,7} studies have shown norovirus to be the etiological agent of outbreaks and sporadic cases of viral gastroenteritis in hospitals, child care centers, cruise ships and long-stay institutions.⁸⁻¹¹ In São Paulo city, although studies on norovirus infection are rare, it has stood out as an etiological agent of gastroenteritis.^{12,13}

Epidemiological Surveillance of Gastroenteritis due to Rotavirus has taken place in São Paulo city since 2009 with the aim of enhancing assessment of rotavirus vaccination in children under 5 years old and also in order to differentiate the etiology of acute diarrheal disease (ADD). As part of this surveillance, the diagnostic test for identifying norovirus as the etiological agent of viral gastroenteritis is performed on samples having a negative rotavirus result.¹⁴

Surveillance of Diarrheal Diseases caused by Rotavirus is conducted in three laboratories in Brazil: Instituto Adolfo Lutz (IAL) in São Paulo, SP, a branch of the São Paulo State Health Department; Fundação Instituto Oswaldo Cruz (Fiocruz) in Rio de Janeiro, RJ, a branch of the Health Ministry; and Instituto Evandro Chagas (IEC) in Belém, PA, a branch of the Health Ministry's Health Surveillance Secretariat. The three institutions are responsible for testing samples from all over Brazil.

Defined by Federal Ordinance No. 205, dated February 17th 2016,¹⁵ and ratified by Consolidation Ordinance No. 5 (Annex XLIII), dated September 28th 2017,¹⁶ Rotavirus Surveillance is conducted based on

notification and investigation of cases hospitalized in sentinel units. In São Paulo state, IAL is the referral laboratory for Public Health sample analysis. In São Paulo city there are two sentinel units: São Luiz Gonzaga Hospital, located in the northern region of the municipality; and Doutor Fernando Mauro Pires da Rocha Hospital in the city's southern region.¹⁷

Rotavirus Surveillance can contribute both to identifying norovirus and also to demonstrating its importance: disease caused by these two viruses has similar symptoms, mode of transmission and duration. They can be transmitted directly via the fecal-oral route, through intake of contaminated water and food, or through indirect exposure owing to contact with fomites or contaminated surfaces.⁴ Affected individuals may have fever, nausea, vomiting, gastrointestinal pain and diarrhea.^{1,7}

The objective of this study was to describe cases of acute diarrheal disease caused by norovirus in children under 5 years old identified by Epidemiological Surveillance of Gastroenteritis due to Rotavirus in São Paulo city.

Methods

This was a cross-sectional study of norovirus infection responsible for sporadic cases in children aged under 5 hospitalized owing to ADD in sentinel units in São Paulo city between 2010 and 2016.

Data relating to clinical and epidemiological aspects were retrieved from records held on the Notifiable Diseases Information System (SINAN). Laboratory aspects of diagnosis were obtained from IAL's hospital management information system.

Notification and epidemiological investigation of suspected rotavirus cases were carried out following their identification in the Rotavirus Surveillance sentinel units. A suspected case of rotavirus was defined as any child aged under 5, diagnosed as having ADD, who had been given intravenous rehydration solution, regardless of rotavirus vaccination status.¹⁷

We used the rotavirus notification and investigation form available on SINAN to collect and record data relating to clinical and epidemiological aspects. Clinical samples were collected from patients at the unit where they were hospitalized and sent to the referral laboratory for identification of the etiological agent.

The ELISA immunoenzymatic method or the RT-PCR molecular method was used for laboratory diagnosis of norovirus.¹⁸

We calculated the proportion of individual norovirus ADD cases taking the number of laboratory confirmed norovirus cases as the numerator, while the denominator was the total number of Epidemiological Surveillance of Gastroenteritis due to Rotavirus notifications, per year, during the entire period studied.

The following variables were used for individual case description:

- a) month and year of notification, Administrative District, Health Surveillance Supervision (SUVIS), Regional Health Coordination Office (CRS) and municipality of residence (São Paulo or other municipality);
- b) signs and symptoms (diarrhea; vomiting; fever; blood in stools);
- c) disease duration (in days);
- d) sex (male; female);
- e) age (in months); and
- f) number of deaths.

We calculated the mean, median, standard deviation and range of variation for each continuous variable; and absolute and relative frequencies for the categorical variables. We used Microsoft Office Excel 2010 to input and systematize information in graph and table format, and Epi InfoTM version 3.5.4 for data description.

When comparing norovirus and rotavirus case distribution between the years 2010 and 2016, we used OpenEpi software to conduct a binomial proportion test with a 5% significance level. When evaluating the quality of SINAN data, we considered the percentage values for completion of fields on the rotavirus notification forms according to a performance scale for the quality of this database defined by the Ministry of Health: above 90%, excellent; 70%-89%, regular; below 70%, poor.¹⁹

The study was conducted in accordance with all ethical procedures required by National Health Council (CNS) Resolution No. 466, dated December 12th 2012. As we used secondary data without case identification, this study was authorized by the São Paulo City Health Department and approved by the University of São Paulo Public Health Faculty and the São Paulo City Research Ethics Committees on September 21st 2016: Certification of Submission for Ethical Appraisal (CAAE) Protocol No. 58621816.4.0000.5421.

Results

Between the years 2010 and 2016, we identified 1565 Epidemiological Surveillance of Gastroenteritis due to Rotavirus notifications that met the case definition. There were 444 confirmed norovirus cases (28.4%) and 368 confirmed rotavirus cases (23.5%); 753 cases (48.1%) were discarded because the etiological agent was not identified. Norovirus was the leading etiological agent identified in São Paulo city between 2011 and 2016 (Table 1).

Over the period 2011-2016, the ratio between the proportion of laboratory confirmed norovirus and rotavirus cases became inverse (Figure 1). In 2010 the ratio was 0.4; in 2015 it was 2.6. The increase in the ratio between norovirus and rotavirus cases was approximately 20.7% per year ($p < 0.001$) (Table 1).

Figure 2 shows the distribution of viral gastroenteritis caused by rotavirus and norovirus by month and year. Norovirus circulated all year round, with possible peaks in the hottest months of some years.

Norovirus cases resident in São Paulo city came mainly from its northern and southern regions, accounting for 29.5% (205/694) and 28.3% (196/693), respectively, of the samples tested in these regions, which are where the two Rotavirus Surveillance sentinel units are located. Cases resident in other regions of the city (west, east and southeast) accounted for 34.5% (10/29) of the samples; while cases from municipalities neighboring the regions where the sentinel units are located accounted for 22.1% (33/149) (Table 2).

All sporadic cases having positive laboratory confirmation of norovirus in the study period had ADD, whereby diarrhea is a notification criterion. Other symptoms recorded on the individual records of these patients and present among positive cases were vomiting (77.9%), fever (52.5%) and blood in stools (13.1%). Symptoms in cases with negative norovirus test results were vomiting (60.1%), fever (56.4%) and blood in stools (15.8%).

Symptoms in sporadic norovirus cases lasted for between 1 and 14 days, whereby median duration was 3 days and mean duration was 4.5 days (standard deviation: 2.6). There was no disparity between the sexes, although cases were predominant among children aged 12-23 months (35.1%; 150/427) (Table 3). No deaths were identified. All variables studied were 100% complete, except for 'disease duration' which was 90.3% complete and therefore also considered to be excellent.

Discussion

Between the years 2011 and 2016, norovirus was the leading etiological agent of sporadic cases detected by Epidemiological Surveillance of Gastroenteritis due to Rotavirus. Norovirus did not show seasonality. Case symptoms were acute diarrhea, vomiting and fever, predominantly among the 1 to 2 year age group. No norovirus deaths were identified.

The epidemiology of childhood viral gastroenteritis changed once laboratory testing for norovirus began. Prior to this, rotavirus was considered to be its main agent.^{9,20} This may have occurred following the introduction of rotavirus vaccination on the national immunization calendar in 2006, in addition to “herd immunity” in years with high vaccination coverage.^{6,21} Implementation of norovirus diagnosis may also have contributed to the increased proportion of cases detected in hospital units in children under 5 years old hospitalized due to norovirus.¹⁴

The identification of norovirus as the leading etiological agent is in keeping with the literature: namely, reports of increased sporadic cases with specific epidemiological and laboratory investigation of the virus and the disease burden owing to the large variety of circulating norovirus strains in recent years.^{9,10,18,20}

Studies conducted in different regions of Brazil have characterized infection in the same way as the findings of our study: norovirus had been identified as the etiological agent of viral gastroenteritis in childhood, with incidence in different periods of the year, having diarrhea and vomiting as the main symptoms.^{8,9,11,22,23}

Ribeiro et al.⁸ studied hospitalized children in a care center in Vitória, Espírito Santo, and noted the occurrence of norovirus cases at different times of the year. In the study conducted by Andrade et al.,¹¹ the authors state that norovirus infection mainly occurred in winter and spring months, contrary to our findings, which may indicate the influence of climate on norovirus seasonality.

In São Paulo city, norovirus infection showed no defined seasonality and cases occurred all year round. However, in some years a peak can be seen in the hottest months, this being a pattern of seasonality in keeping with studies conducted in the Southern Hemisphere in regions characterized by a humid subtropical climate with dry winters and very rainy summers.^{5,18,24} Rotavirus cases had well-defined seasonality, with an annual peak in winter months. This finding is in agreement with the findings of studies conducted in other regions of the world.²⁵ Understanding the relationship between climate and norovirus may help identification of new cases, and how climate and environmental changes can influence norovirus distribution and dissemination.

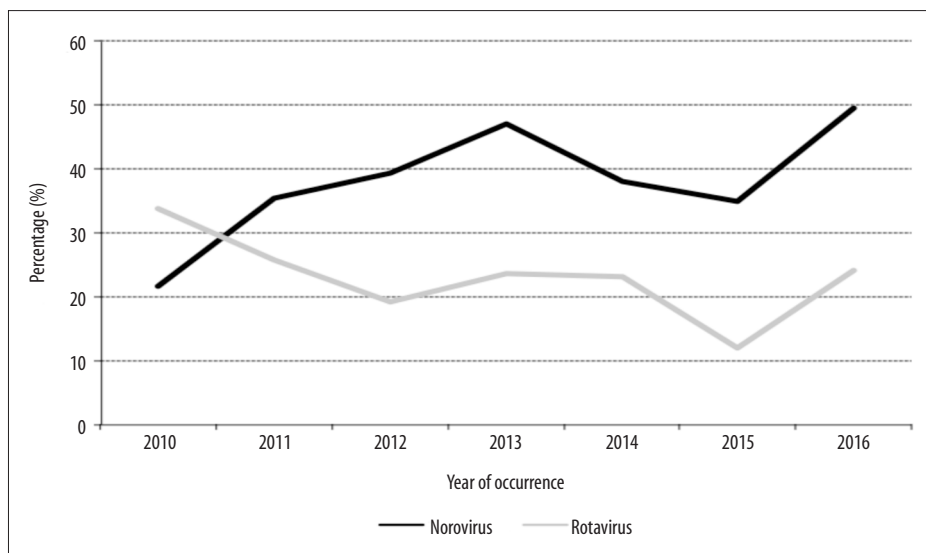


Figure 1 – Distribution of positive norovirus and rotavirus cases identified in 1565 cases of diarrhea collected during Epidemiological Surveillance of Gastroenteritis due to Rotavirus, São Paulo, SP, 2010-2016

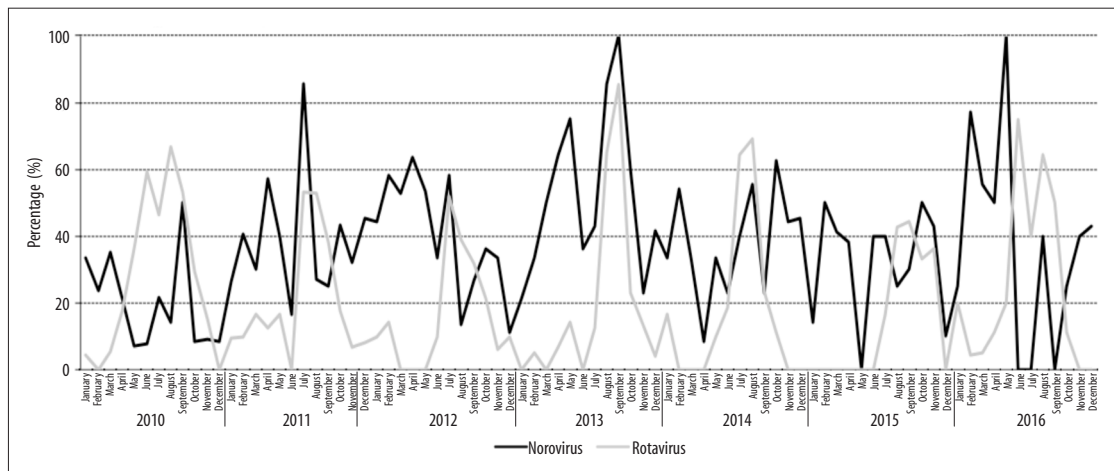


Figure 2 – Positive norovirus and rotavirus cases identified in 1565 cases of diarrhea collected during Epidemiological Surveillance of Gastroenteritis due to Rotavirus, by month and year of occurrence, São Paulo, SP, 2010-2016

Table 1 – Distribution of positive norovirus and rotavirus cases identified during Epidemiological Surveillance of Gastroenteritis due to Rotavirus, São Paulo, SP, 2010-2016

Notified cases	Year							Total
	2010	2011	2012	2013	2014	2015	2016	
Norovirus	38	80	105	73	52	51	45	444
% of positive cases	15.0	26.8	32.8	33.6	28.4	29.5	37.8	28.4
95%CI ^a	11.0;19.7	22.1;32.0	27.9;38.1	27.7;40.2	22.4;35.3	23.2;36.7	29.6;46.8	26.2;30.6
Rotavirus	89	78	63	48	41	20	29	368
% of positive cases	35.0	26.1	19.7	22.1	22.4	11.6	24.4	23.5
95%CI ^a	29.4;41.1	21.4;31.3	15.7;24.4	17.1;28.1	17.0;29.0	7.6;17.2	17.5;32.8	21.5;25.7
Total cases	254	299	320	217	183	173	119	1.565
Norovirus/rotavirus ratio	0.4	1.0	1.7	1.5	1.3	2.6	1.6	1.2
P value^b	<0.001	0.796	<0.001	<0.001	0.072	<0.001	0.002	<0.001

a) 95%CI: 95% confidence interval of the proportions of positive norovirus and rotavirus cases.
 b) Binomial test for a proportion between norovirus and rotavirus case distributions.

In countries with a temperate climate, such as in the United States and in Europe, norovirus and rotavirus cases are frequent in the winter.^{5,17}

We also found that in the majority of months, when the number of positive norovirus cases increased, the number of rotavirus cases decreased, and vice versa. This phenomenon, which has already been described in other studies, is known as the “see-saw effect”.²³

The diarrhea, vomiting and fever symptoms found in the cases analyzed are in agreement with the main clinical aspects of norovirus infection described by Abugalia et al.²⁵ and Sai et al.²⁶

The duration time of sporadic case symptoms found by us is in agreement with the data obtained by Lopman et al.² and Robilotti et al.²⁷ according to whom mean symptom duration was two to four days.

Norovirus clinical characteristics similar to those of rotavirus show the importance of laboratory diagnosis for differentiating the etiological agent.^{17,28} In our study, the majority of norovirus cases we found were children aged between 1 and 2 years old. The literature reports that norovirus occurs above all in childhood, although the age range of hospitalized patients differs depending on the study: Sai et al.²⁶ found

Table 2 – Distribution of positive norovirus cases identified by Epidemiological Surveillance of Gastroenteritis due to Rotavirus, by municipality, CRS a and SUVIS b of residence, São Paulo, SP, 2010-2016

São Paulo city	SUVIS ^b	No. of positive cases	No. of tested cases	%
North	Total	205	694	29.5
Jaçanã		169	561	30.1
Vila Maria		27	95	28.4
Santana		7	35	20.0
Casa Verde		1	2	50.0
Freguesia do Ó		1	1	100.0
South	Total	196	693	28.3
Campo Limpo		144	525	27.4
M'Boi Mirim		52	168	31.0
West	Total	7	23	30.4
Butantã		7	23	30.4
Southeast	Total	2	5	40.0
Vila Mariana		1	2	50.0
Penha		1	3	33.3
East	Total	1	1	100.0
São Mateus		1	1	100.0
Total – São Paulo city		411	1,416	29.0
Other municipalities in São Paulo state		33	149	22.1
Total – São Paulo state		444	1,565	28.4

a) CRS: Regional Health Coordination Office.

b) SUVIS: Health Surveillance Supervision.

case predominance in children under three years old; Chhabra et al.⁶ highlighted the one to two year age range in hospitalized children; while Abugalia et al.²⁵ found most cases in children under 1 year old.

Borges et al.²² assessed inpatients in public hospitals in Goiás, Goiânia and Brasília and found no differences between the sexes. Ferreira et al.⁹ detected higher incidence in children under two years old attending a daycare center in Rio de Janeiro. Siqueira et al.²³ reported lack of vaccine against the virus, the impossibility of diagnosing coinfection, since the test for norovirus is done when the rotavirus test result is negative, as well as reporting undefined norovirus seasonality.

In the literature we only found registered cases of norovirus deaths among the elderly or hospitalized patients.¹⁸ We did not find any norovirus deaths in our study, bearing in mind that the population we studied was under 5 years old.

High-income countries have specific surveillance systems for some of the main etiological agents of foodborne diseases. Worthy of note is the Rotavirus

Sentinel Surveillance done by the Pan American Health Organization (PAHO), which is based on data from health institutions selected according to their geographic location, clinical specialty, diagnosis capacity and information quality. The Rotavirus Sentinel Surveillance coordinated by PAHO comprises 18 countries of the Americas: Anguilla, Bolivia, Brazil, Chile, Colombia, Dominica, Ecuador, El Salvador, Guatemala, Guiana, Honduras, Nicaragua, Panama, Paraguay, Peru, Saint Vincent and the Grenadines, Surinam and Venezuela.²⁹

The National Respiratory and Enteric Virus Surveillance System is a laboratory-based system run by the United States Centers for Disease, Control and Prevention (CDC), which monitors different viruses on a weekly basis, including rotavirus: data is gathered from laboratories, hospitals and public health systems in order to monitor vaccine effectiveness in reducing morbidity and mortality, as well as gathering knowledge about different strains of the agent, risk groups and the seriousness of the disease.²⁸

Based on these examples, it is possible to assess the relevance of establishing specific surveillance of

Table 3 – Distribution of positive norovirus cases identified by Epidemiological Surveillance of Gastroenteritis due to Rotavirus, by sex and age range, São Paulo, SP, 2010-2016

Age range (in months)	Sex				Total	
	Male		Female		No. positive cases/ No. tested cases	%
	No. positive cases/ No. tested cases	%	No. positive cases / No. tested cases	%		
<12	94/387	24.3	85/301	28.2	179/688	26.0
12-23	96/256	37.5	54/171	31.6	150/427	35.1
24-59	62/227	27.3	53/223	23.8	115/450	25.6
Total	252/870	29.0	192/695	27.6	444/1,565	28.4

norovirus and developing research for the molecular characterization of the virus in sporadic cases.

Strain GII.4 is the leading genotype identified in sporadic norovirus cases worldwide. Brazilian studies have also detected the emerging GII.2, GII.6 and GII.17 strains. Hence the importance of monitoring circulating strains in order to identify the different genetic types of the virus.^{11,30}

The results of this study were strongly influenced by the characteristics of Rotavirus Surveillance. Notifications took place where surveillance was active. As such, interpretation of the results needs to take into consideration that we only analyzed norovirus cases notified in São Paulo city's two sentinel units. In order to minimize this limitation, we ensured that notifications met the case definition and that the laboratory test results only came from the Instituto Adolfo Lutz, which is São Paulo city's Public Health laboratory and is the referral laboratory for all of São Paulo state.

Another important limitation of this study that needs to be mentioned is the fact of samples found to be positive for rotavirus not having been tested for norovirus, with the aim of determining possible cases of coinfection, which is a common event in studies like this.

As to its design, cross-sectional studies do not enable identification of temporal association between the cause and effect of a disease. Descriptive studies in turn do not relate disease risk and duration in order to establish causal inferences. Notwithstanding, advantages such as easy access to data, low cost and high descriptive potential of the distribution and magnitude of health problems in the population, characteristic of these study models, enable the

formulation of etiological hypotheses for carrying out analytical studies.

Analysis of the results found enabled us to conclude that norovirus was the leading epidemiological agent identified in sporadic ADD cases in children under 5 years old in São Paulo city's sentinel units, exceeding rotavirus cases even though rotavirus is considered to be the predominant agent in childhood. In view of this scenario, we emphasize the importance of norovirus prevention and control measures, implementation of public epidemiological surveillance policies, provision of health care for the population, protection of vulnerable individuals and reduction of the number of cases.

The fact that only two laboratories are qualified to perform diagnostic tests and the absence of active surveillance of norovirus diagnosis contribute to the scarcity of research into the norovirus endemic in São Paulo city. Studying this infection based on data provided by an epidemiological surveillance and notification system is essential for establishing an integrated network extending to other states and countries and which enables knowledge of the disease, its genetic variety, seasonality and geographic distribution. When establishing Norovirus Surveillance, the presence of the disease in all age groups, the restriction of laboratories that perform the diagnostic test and the completeness of notification form fields need to be taken into consideration. Overcoming the limitations to producing and accessing the full set of information will enable it to be analyzed and used as a source of data. Improvements to surveillance and health worker training will also be necessary in epidemiological investigation and in collecting samples to identify the etiological agent.

Authors' contributions

Kamioka GA, Sato APS and Madalosso G contributed to conceiving and designing the study as well as data analysis and interpretation. All

the authors contributed to writing and critically revising the manuscript and approved its final version. They are responsible for all aspects of the work, including the guarantee of its accuracy and integrity.


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