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Prevalence and genetic diversity of viral gastroenteritis viruses in children younger than 5 years of age in Guatemala, 2014–2015

Marta Diez-Valcarce[a], Maria Renee Lopez[b], Beatriz Lopez[b], Oneida Morales[b], Manuel Sagastume[c], Loren Cadena[d,e], Susan Kaydos-Daniels[f], Claudia Jarquin[b], John P. McCracken[b], Joe P. Bryan[d,e], Jan Vinjé[g,*]

[a] Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA
[b] Universidad del Valle de Guatemala, Guatemala City, Guatemala
[c] Department of Epidemiology, Ministry of Public Health and Social Welfare, Guatemala City, Guatemala
[d] Division of Global Health Protection, US Centers for Disease Control and Prevention, Atlanta, GA
[e] Central American Regional Office, Centers for Disease Control and Prevention, Guatemala City, Guatemala
[f] Influenza Program, US Centers for Disease Control and Prevention, Guatemala City, Guatemala
[g] Division of Viral Diseases, US Centers for Disease Control and Prevention, Atlanta, GA, USA

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ABSTRACT

Background: Acute diarrhea is an important cause of morbidity and mortality in children and is associated with approximately 500,000 deaths/year globally. Rotavirus and norovirus are leading causes of acute diarrhea accounting for more than half of this burden.

Objective/study design: To determine the prevalence and genotype distribution of acute diarrhea caused by rotavirus, norovirus, sapovirus and astrovirus among children < 5 years of age at two departments in Guatemala from January 2014 to December 2015, we tested 471 stool specimens (202 samples from hospitalized children and 269 samples from children in ambulatory clinics) by real-time reverse transcription-PCR and genotyped positive samples.

Results: Rotavirus was detected in 20.4%, norovirus in 18.5%, sapovirus in 7% and astrovirus in 4.2% of the samples. Co-infection of rotavirus and norovirus was found in 2.6% of the samples. Most norovirus (87.4%) and rotavirus (81.3%) infections were detected in children in the 6–12 months age group. The proportion of patients with rotavirus (34%) and norovirus (23%) was higher in hospitalized patients compared to ambulatory patients, whereas the prevalence of sapovirus and astrovirus was similar in both settings. Of the 40 genotyped norovirus strains, 62.5% were GII.4 and 15% GII.3. Sapovirus genotypes included GI.1 (15.4%), GII.2 (15.4%), GII.5 (38.5%) and GIV.1 (30.8%).

Conclusions: Our data demonstrate that in 2014–2015, gastroenteritis viruses account for 50% of acute diarrhea in children younger than 5 years of age in Guatemala, highlighting the importance of continuous surveillance to guide impact of the current rotavirus vaccine and formulation of future norovirus vaccines.

1. Background

Acute diarrhea is a leading cause of morbidity and mortality worldwide, accounting for over 1.8 million deaths annually in children aged < 5 years, the vast majority of which occur in developing countries [1]. The World Health Organization estimates that in Guatemala for each 1000 live births, roughly 2 deaths are caused by diarrheal diseases [2]. Globally, rotavirus and norovirus are major etiologic agents associated with mortality in children < 5 years of age, primarily in developing countries [3,4]. Recent data shows that norovirus accounts for approximately one-fifth of acute gastroenteritis (AGE) cases globally [4], and in countries that have introduced rotavirus vaccines, norovirus has become the leading cause of medically-attended AGE [5]. Since most studies in developing countries include results from hospitalized children with AGE [6], the prevalence of norovirus and other gastroenteritis viruses, such as sapovirus and astrovirus, in outpatient clinics is unknown. To date, the majority (68%) of published norovirus studies in Latin American countries report data from hospitalized patients [7].

Noroviruses and sapoviruses are non-enveloped genetically diverse

* Corresponding author at: Division of Viral Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, USA.
E-mail address: jvinje@cdc.gov (J. Vinjé).

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viruses with a singel-stranded RNA genome that belong to separate genera in the family *Caliciviridae*. Noroviruses are classified into at least seven genogroups (GI-GVII) [8], with the majority of infections in humans caused by GI and GII viruses, which are further divided into 9 and 22 genotypes, respectively [8]. Of these, GII.4 viruses have been associated with pandemics over the last two decades [9–11]. However, other genotypes such as GII.17, and more recently GII.2, have emerged as predominant viruses in China, Japan and South Korea and at least temporarily replaced GII.4 viruses in these countries [12–15]. Sapoviruses can be classified into up to 19 genogroups (GI–GXIX) [16], of which viruses from GI, GII, GIV and GV infect humans [17]. GI (7 genotypes) and GII (8 genotypes) are the most frequently detected sapoviruses worldwide. GIV (1 genotype) and GV (2 genotypes) viruses are both detected rarely [18].

2. Objective

To determine the prevalence of viral acute diarrhea and genotype distribution to provide the molecular basis for potential future vaccines against norovirus and sapovirus. We tested stool samples collected in 2014 and 2015 from children < 5 year with acute diarrhea in 2 hospitals and 2 clinics in the departments of Santa Rosa and Quetzaltenango in Guatemala, where the rotavirus vaccine was introduced in 2010, for rotavirus, norovirus, sapovirus and astrovirus.

3. Study design

3.1. Study, sites and population

This study was conducted as part of the integrated collaborative surveillance system (VICo) carried out by the Center for Health Studies of the Universidad del Valle de Guatemala (UVG), the Ministry of Public Health and Social Assistance (MOH) represented by the National Epidemiology Center, and the US Centers for Disease Control and Prevention (CDC). VICo surveillance was performed in Santa Rosa and Quetzaltenango departments in government hospitals and health centers. These sites represent the geographic, climatic and ethnic diversity in Guatemala. A more detailed description of the study sites and population covered has been described previously [19–21].

3.2. Recruitment and enrollment

All patients < 5 years of age in hospitalized or ambulatory settings presenting with acute diarrhea, defined as ≥3 loose/liquid stools in a 24-hr period and an onset of symptoms within the previous 14 days, were considered eligible for the study. Only eligible patients whose parents or guardians voluntarily provided their written informed consent were enrolled in the study. The study was approved by the Institutional Review Boards of the UVG, MOH and CDC.

3.3. Sample collection and processing

Rotavirus was detected in fecal specimens using a commercial qualitative enzyme immunoassay (IDEIA rotavirus test kit, Oxoid Ltd.). Testing of norovirus, sapovirus and astrovirus was performed by real-time reverse transcription PCR (RT-qPCR). Briefly, nucleic acid was extracted from 10% (wt/vol) clarified stool suspensions and amplified using previously published assays [9,22,23]. Based on availability of specimens for further testing, a subset of 46 norovirus and 25 sapovirus RT-qPCR positive samples were genotyped by conventional RT-PCR [9,24]. Amplified products were sequenced by the Sanger method and the genotypes were determined by comparison with reference sequences. Information on rotavirus genotype distribution will be described elsewhere (Lopez, manuscript in preparation).

3.4. Statistical analysis

The Fisher's exact test (GraphPad Prism v 7.04, San Diego, CA, USA) was used to compare the proportion of virus positive samples in hospitals versus ambulatory settings, and between the proportion of rotavirus or norovirus positive samples in children younger than 1 year and older children.

4. Results

Of 933 enrolled patients with acute diarrhea between January 2014 and December 2015 (534 and 399 in 2014 and 2015, respectively), whole stool samples of adequate volume from 202 hospitalized and 269 ambulatory children were available to test for rotavirus, norovirus, sapovirus and astrovirus. Four hundred specimens were submitted from Santa Rosa Department and 71 specimens were submitted from Quetzaltenango. The majority 259/471 (55%) of the children with acute diarrhea were 6–12 months old. In hospitals, 91.1% (184/202) of children were ≤1 year old compared to 64.3% (173/269) in ambulatory settings.

4.1. Seasonal distribution of enteric viruses infecting children in Guatemala

Overall, gastrointestinal viruses were detected in 236 samples (50.1%) throughout the year (Fig. 1). The highest numbers of rotavirus and norovirus were detected between January and March in both study years and during the cooler, dry season (November to April). In 2015, norovirus positive samples were detected from January until May (Fig. 1). The numbers for sapovirus and astrovirus were too low to identify seasonal trends.

4.2. Comparison of enteric virus detection in hospital and ambulatory settings

Of the 471 samples tested, norovirus was detected in 87 (18.5%) and rotavirus in 96 (20.4%) samples. In addition, 33 (7%) samples tested positive for sapovirus and 20 (4.2%) samples positive for astrovirus. Among the 202 hospitalized children admitted with acute diarrhea symptoms, norovirus was detected in 46 (22.8%), rotavirus in 69 (34.2%), sapovirus in 15 (7.4%) and astrovirus in 10 (5%) samples (Table 1). Among 269 samples from patients in clinics, norovirus was detected in 41 (15.2%), rotavirus in 27 (10%) sapovirus in 18 (6.7%) and astrovirus in 10 (3.7%) of the samples. Co-infections of viral pathogens were detected in 27 (5.7%) samples. No virus was found in 142 (30.2%) of the samples. The prevalence of norovirus and rotavirus was significantly higher in hospitalized children compared to children with AGE in clinics ($p = 0.0371$ and $p < 0.0001$ for norovirus and rotavirus, respectively). There was no statistical difference in the proportion of positive cases for sapovirus or astrovirus detected in hospitals compared to ambulatory settings.

4.3. Age distribution of children infected with norovirus and rotavirus

Of the norovirus positive samples, 76 (87%) were from children younger than 1 year of age. Among those, 45 (59.2%) were from hospitalized patients and 31 (40.8%) from ambulatory patients. (Table 1). Rotavirus was found in 96 (20.4%) of the samples of which 78 (81.3%) were from children one year old or younger. In this age category, 80.8% of samples were from hospitalized children and 19.2% were from ambulatory patients (Table 1). Overall, there was a statistically significant difference between the prevalence of norovirus in the < 1 year of age group (87%) compared to the older children (13%) ($p = 0.0009$). This difference, however, was not statistically significant for rotavirus ($p = 0.1621$).

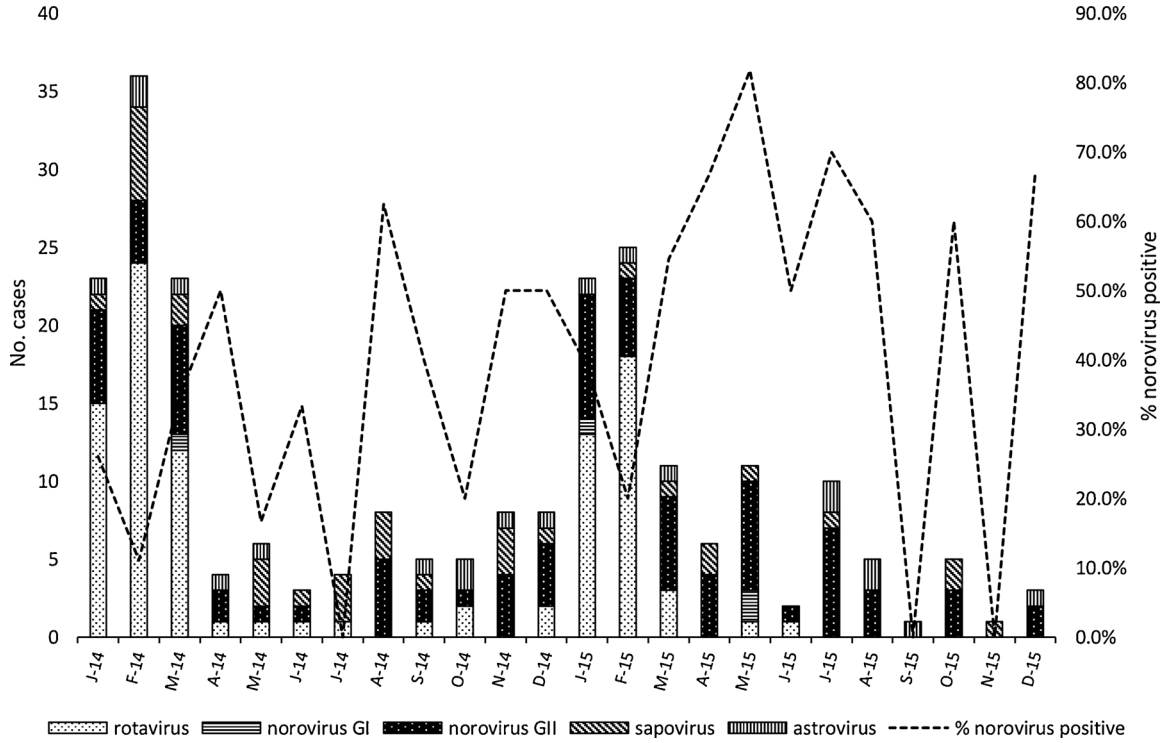

Fig. 1. Monthly distribution of rotavirus, norovirus GI and GII, sapovirus and astrovirus infections in hospitalized children and children visiting ambulatory clinics with acute diarrhea in Santa Rosa and Quetzaltenango, Guatemala (January 2014-December 2015). Primary y-axis shows number of cases; secondary y-axis (and dotted line) shows percentage of monthly acute diarrheal cases positive for norovirus (GI or GII).

4.4. Co-infections

A total of 27 (5.7%) samples tested positive for at least 2 viruses (Table 2) including 14 (52%) positive for both norovirus and rotavirus. The majority (86%) of coinfections with norovirus and rotavirus occurred in children 1 year or younger. The second most frequent co-detection was rotavirus and sapovirus (5 (18%)) of which 4 (80%) were detected in children 1 year or younger. Combinations of norovirus and sapovirus were detected in 3 (11%) samples and norovirus and astrovirus in 2 (8%) samples followed by 1 (4%) sample positive for rotavirus and astrovirus and 1 (4%) positive for sapovirus and astrovirus. Finally, one sample tested positive for norovirus, rotavirus and astrovirus.

Table 2
Coinfections of gastroenteritis viruses in fecal specimens from children < 5 years of age in Guatemala, 2014–2015.

Coinfections	No. samples
norovirus & rotavirus	14
rotavirus & sapovirus	5
norovirus & sapovirus	3
norovirus & astrovirus	2
rotavirus & astrovirus	1
sapovirus & astrovirus	1
norovirus & rotavirus & astrovirus	1
TOTAL	27

4.5. Norovirus and sapovirus genotype distribution

Genotyping was successful on 40 (87%) of the 46 norovirus real-time RT-PCR positive samples and 13 (52%) of the 25 sapovirus real-time RT-PCR positive samples. Eighteen of the 40 norovirus samples were from ambulatory settings and 22 from hospitalized children.

Table 1
Age distribution of children < 5 years of age with acute diarrhea due to rotavirus, norovirus, sapovirus and astrovirus in Guatemala, 2014–2015.

			Total	Rotavirus	Norovirus	Sapovirus	Astrovirus
Hospital	Santa Rosa n = 153	0–5 months	40	9	6	2	1
		6–12 months	37	43	25	9	7
		1–5 years	76	5	1	2	1
	Quetzaltenango n = 49	0–5 months	15	2	4	0	1
		6–12 months	18	9	10	2	0
		1–5 years	16	1	0	0	0
		SUBTOTAL	202	69 (34.2%)	46 (22.8%)	15 (7.4%)	10 (5%)
Ambulatory	Santa Rosa n = 247	0–5 months	29	2	3	0	1
		6–12 months	61	13	26	10	4
		1–5 years	157	11	10	3	2
	Quetzaltenango n = 22	0–5 months	6	0	2	1	1
		6–12 months	4	0	0	4	2
		1–5 years	12	1	0	0	0
		SUBTOTAL	269	27 (10%)	41 (15.2%)	18 (6.7%)	10 (3.7%)
		TOTAL	471	96 (20.4%)	87 (18.5%)	33 (7%)	20 (4.2%)

Table 3a
Norovirus genotype distribution among children < 5 years of age with acute diarrhea in Guatemala, 2014–2015.

Norovirus genogroup	Genotype[1]	No. of samples	% of all genotypes	2014 (%)	2015 (%)
GI	GI.P1-GI.1	1	2.5	1 (8.3)	–
	GI.P7-GI.7	1	2.5	–	1 (3.6)
GII	GII.P16-GII.3	6	15.0	–	6 (21.4)
	GII.P17-GII.17	3	7.5	2 (16.7)	1 (3.6)
	GII.P4 Den Haag-GII.4 Den Haag	3	7.5	1 (8.3)	2 (7.1)
	GII.P7-GII.7	2	5.0	2 (16.7)	–
	GII.P7-GII.14	1	2.5	1 (8.3)	–
	GII.Pe-GII.4 Sydney	22	55.0	4 (33.3)	18 (64.3)
	GII.Pe-GII.17	1	2.5	1 (8.3)	–
Total		40	100.0	12 (100.0)	28 (100.0)

[1] norovirus dual genotype classification according to [9].

Table 3b
Sapovirus genotype distribution among children < 5 years of age with acute diarrhea in Guatemala, 2014–2015.

Sapovirus genogroup	Genotype	No. of samples	% of all genotypes	2014 (%)	2015 (%)
GI	GI.1	2	15.4	2 (22.2)	–
GII	GII.2	2	15.4	1 (11.1)	1 (25.0)
	GII.5	5	38.5	4 (44.4)	1 (25.0)
GIV	GIV.1	4	30.8	2 (22.2)	2 (50.0)
Total		13	100.0	9 (100.0)	4 (100.0)

Interestingly, the majority of sapovirus-positive samples were from children in ambulatory settings (n = 12) compared to one from a hospitalized child (genotyped as a GII.5). The distribution of genotypes in the 13 sapovirus-positive samples was as follows: GI.1 (n = 2), GII.2 (n = 2), GII.5 (n = 5) and GIV.1 (n = 4) (Table 3b). GII.Pe-GII.4 Sydney was the most commonly (n = 22; 55%) detected norovirus genotype followed by GII.P16-GII.3 (n = 6; 15%) (Table 3a). Seventeen (77%) of the GII.Pe-GII.4 Sydney patients were hospitalized with AGE. All 53 genotyped samples (40 norovirus and 13 sapovirus) were collected from children ≤2 years of age.

5. Discussion

This study highlights the importance of viral diarrhea in Guatemala 4 years after the introduction of rotavirus vaccination. We found that 50.1% of the fecal specimens from hospitalized and ambulatory patients (children < 5 years of age) in two departments in Guatemala tested positive for one or more viral pathogens. Norovirus and rotavirus were detected in 18.5% and 20.4% of samples, respectively. Additionally, 11.4% of the samples tested positive for sapovirus (7%) or astrovirus (4.2%). The prevalence was higher in Santa Rosa than in Quetzaltenango, but also more specimens were submitted from Santa Rosa Department (n = 400), a warm tropical climate compared with the cool, dryer climate of Quetzaltenango (n = 71). The majority (68.2%) of the positive detections were from samples collected during the cool dry months (November-March). Genotype data provided important information about strains a future norovirus vaccine should prevent.

The highest number of samples that tested positive for norovirus and rotavirus was observed during November through March in both study years. Although in countries in the Northern hemisphere norovirus has a winter seasonality, in Latin American countries norovirus seasonality is less clear [25]. For example, in a study of 2007–2010 in Guatemala at the same study sites [26], the highest number of norovirus cases occurred from November to January, the cooler months just after the rainy season, whereas in Nicaragua, norovirus peak activity was reported during June-July, corresponding to the early rainy season [27,28]. A similar discordant pattern has been observed for influenza in Nicaragua and Guatemala [29].

Norovirus was more common among hospitalized children with acute diarrhea compared to ambulatory clinic patients (23% vs 15%, respectively). This is slightly higher compared to what was reported in a previous study in the same departments in Guatemala from 2007 to 2010 [26] in which the prevalence for both norovirus and rotavirus was 14%. A recent systematic review estimated that 1 out of 6 AGE hospitalizations of children < 5 years of age in Latin America can be attributed to norovirus [7]. The majority (62.5%) of norovirus infections in this study were typed as GII.4, similar to findings from another study in the region [27]. The highest prevalence was observed in children under 1 year of age. Our data demonstrate that 4 years after the introduction of rotavirus vaccine in Guatemala in 2010, rotavirus remains one of the most frequent causes of pediatric acute diarrhea. The likely explanation is the low rotavirus vaccine coverage (54% in 2014 and 79% in 2015) [30]. However, in Colombia, which reports a high rotavirus vaccine coverage (89–91%), rotavirus continues to be associated with acute diarrhea in young children [31]. In Nicaragua, which introduced a rotavirus vaccine in 2006 and reported a coverage of 98% in 2010 [32], rotavirus prevalence was down to 8% while norovirus is now the most frequently detected (24%) viral etiology of acute diarrhea followed by sapovirus (17%) [27].

We also tested the stool samples for sapovirus and astrovirus and found that 23.4% of the samples had co-infections with a combination of the four different viruses. Interestingly, although no dehydration was reported in our study, all patients that had co-infections reported they did vomit the week prior to presenting in the clinic or hospital. Previous data has shown that coinfections have been associated with more severe dehydration and clinical course in children irrespective of age and other demographic factors [33]

The norovirus genotype distribution found in this study is similar to a previous study in Guatemala [26] that showed that the majority of norovirus sampless were typed as GII.4 (84%). We also detected other genotypes, such as GII.3 and GII.17, of which in recent years GII.17 has been frequently associated with AGE outbreaks in Latin America [25]. Among sapoviruses, our data showed that GII viruses were detected more frequently than GI viruses (28% vs 8%) in contrast to other studies in the Latin America region where GI sapoviruses were more predominant (46% GI in Nicaragua in 2010 [27], 47% GI in Peru in 2007–2010 [34]). Overall 4.1% of the samples tested positive for astrovirus, slightly lower than reported in a previous study from Guatemala [35]. Interestingly, these levels are higher than recent data from South America and the Caribbean where astrovirus prevalence ranged from 2%–3.6% [36–38].

This study has several limitations. First, since stool sample volume was insufficient to test for viruses in half of the cases, the genotype distribution of norovirus and sapovirus may not be completely representative. In addition, approximately 30% of the sapovirus samples had a low viral load (Ct values ≥30), which may explain the low (52%) genotyping success rate. Since rotavirus was detected by enzyme immunoassay, its prevalence is likely underestimated. Finally, we did not test for enteric adenovirus which in some studies in children in the US

has been associated with up to 11.8% of AGE cases [39] and in China between 2.0–13.4% [40].

In summary, we report the prevalence of four gastroenteritis viruses in 2014–2015 in Guatemala four years after the introduction of rotavirus vaccine. Half of the stool specimens from hospitalized and ambulatory patients (children < 5 years of age) tested positive for one or more viruses. More than half of norovirus infections were caused by GII.4 viruses, and at least 4 other genotypes co-circulated during the same time period. Sapovirus GII followed by GI viruses were the most detected genogroups. In summary, our findings provide a foundation based on which public health strategies, including future vaccines, may help to decrease the burden of acute viral diarrhea in young children in Guatemala. Continued surveillance of acute diarrhea including laboratory testing is important to measure the impact of the current rotavirus vaccine and the burden and strain distribution of norovirus and other gastroenteritis viruses in Guatemala.

Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Conflict of interest

None.

CRediT authorship contribution statement

Marta Diez-Valcarce: Data curation, Methodology, Writing - original draft, Writing - review & editing. **Maria Renee Lopez:** Data curation, Writing - review & editing. **Beatriz Lopez:** Project administration, Writing - review & editing. **Oneida Morales:** Methodology, Writing - review & editing. **Manuel Sagastume:** Writing - review & editing. **Loren Cadena:** Writing - review & editing. **Susan Kaydos-Daniels:** Writing - review & editing. **Claudia Jarquin:** Writing - review & editing. **John P. McCracken:** Funding acquisition, Project administration, Writing - review & editing. **Joe P. Bryan:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing. **Jan Vinjé:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing - original draft, Writing - review & editing.

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