Published in final edited form as:
Trans R Soc Trop Med Hyg. 2011 December ; 105(12): 734–736. doi:10.1016/j.trstmh.2011.08.009.

Norovirus prevalence in 'pathogen negative' gastroenteritis in children from periurban areas in Lima, Peru

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Summary

Norovirus was detected in 17.4% of 224 diarrhoeal samples from children younger than 24 months of age in Lima, in whom all common pathogens had been excluded (pathogen negative). Norovirus was identified more frequently in children older than 12 months of age than in younger children (34% vs 8%, $P<0.001$). Among norovirus-positive samples, genogroup II was the predominant group (92%). Compared with rotavirus, norovirus episodes tended to be of shorter duration and less severe. The role of norovirus as a cause of diarrhoea and the ascertainment of its severity in developing countries needs further confirmation by future epidemiological studies.

Keywords

Norovirus; gastroenteritis; viral genogroup; diarrhoea; children; Peru

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Authors' contributions: All authors were responsible for the study design; LE was responsible for the database, and AIG was responsible for the preservation of stool samples; FPR, JR, AMM and EM were responsible for the execution of the laboratory work; FPR, TJO, JR, LE, AIG, LH, AMM, EM and CFL drafted the manuscript. All authors critically revised the manuscript for intellectual content, and read and approved the final version. FPR and TJO are guarantors of the paper.

Conflicts of interest: None.

Ethical approval: The study was approved by the Institutional Review Boards of the Universidad Peruana Cayetano Heredia, Instituto de Investigación Nutricional, and Instituto Nacional de Salud del Niño, all in Lima, Peru.

1. Introduction

Norovirus is now recognized as an important cause of endemic nonbacterial gastroenteritis worldwide, especially in children in developing countries.[1] The diversity among norovirus is high; genogroups GI and GII are the most prevalent.[1] The availability of more sensitive diagnostic techniques has changed the understanding of the epidemiology of norovirus disease;[1,2] however, these diagnostic methods are not widely available in clinical laboratories in developing countries. The aim of this study was to determine the prevalence of norovirus in Peruvian children with gastroenteritis in whom all common pathogens had been excluded ('pathogen negative').

2. Materials and methods

Specimens analyzed in this study were obtained as part of a prospective, passive surveillance cohort diarrhoea study in children 2–24 months of age in periurban communities of Lima, Peru.[3] One thousand thirty-four children aged 2 months of age were enrolled and followed up to 12 months of age. Parents were asked to bring their children to the study clinic every time the child developed diarrhoea that needed medical attention. The study was conducted between September 2006 to December 2007 (1034 children)[3] and from January to July 2008 (529 children randomly selected from the initial cohort were followed during this period). Diarrhoea was defined as ≥3 liquid or semiliquid stools passed in a 24-h period or ≥1 loose stool with blood. Persistent diarrhoea was defined as diarrhoea that lasted >14 days.[3] Clinical information of the diarrhoeal episodes was obtained from the medical records filled by study doctors. We used a modified Vesikari score to determine the severity of a norovirus-associated diarrhoea episode and to compare it with the clinical characteristics of rotavirus diarrhoea from infants of the same cohort study.[3] The score included duration of diarrhoea (0–3 points), maximum number of stools per day (1–3), number of days with vomiting (0–3), maximum number of emesis episodes per day (0–3), presence of fever (0–1), dehydration (0–3), and treatment (0–2).

A total of 1102 stool samples obtained from children with diarrhoea in the cohort study were analyzed for the presence of *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio* species, rotavirus and parasites by conventional methods and diarrhoeagenic *Escherichia coli* by molecular methods, as previously described[3,4]. A total of 739 (67%) stool samples were negative for all those pathogens and were considered pathogen negative. From these, 224 (30%) samples were available for the current study; 515 samples were not available (94 were stool diaper samples, 157 had insufficient stool and 264 were not stored).

Viral RNA was extracted from 10% fecal suspension by a spin column technique, according to the manufacturer's instructions (QIAamp Viral RNA Mini Kit, Qiagen, Washington, DC, USA); and was reverse transcribed using a commercial kit according to the manufacturer's instructions (SuperScript Reverse Transcriptase II, SS RTII; Invitrogen, Grand Island, NY, USA). Two sets of primers were used to amplify capsid region of norovirus as previously described.[5] G1SKF and G1SKR for norovirus GI, and G2SKR for norovirus GII. We used a new forward primer to detect norovirus GII, G2NOF: 5′-ATTCTCAGATCTGAGCACGTGGGA-3′ to improve the efficiency of the primer set. We used 5 μL of viral cDNA for the PCR reaction. Thermocycling conditions consisted of 10 minutes at 95 °C to activate the HotStart enzyme (AmpliTaq Gold, Applied Biosystems, Foster City, CA, USA), 45 cycles of 20 seconds at 95 °C, 30 seconds at 50 °C, and 20 seconds at 72 °C, with a final extension for 10 min at 72 °C. Amplified products were 330pb for GI and 361pb for GII.

3. Results and Discussion

Norovirus was detected in 39 (17.4%) of 224 pathogen negative diarrhoeal samples. This prevalence was similar to other studies in Peruvian children (17–21%)[2,6] and similar to studies in other developing countries.[1] Norovirus prevalence in each age group was 8% in children 2–5 months of age (5/64 samples), 9% in children 6–11 months of age (7/80 samples), 33% in children 12–17 months of age (12/36 samples); and 34% in children 18–24 months of age (15/44 samples). The age distribution of norovirus is different from a previous study in children from the Peruvian Amazom.[6] Norovirus was identified more frequently in samples from children older than 12 months of age than in younger children (34% vs 8%, $P<0.001$), suggesting that age should be always considered in the interpretation of results.

Among norovirus-positive samples, norovirus genogroup II was identified more frequently (92%, 36/39) than norovirus genogroup I (8%, 1/39). Norovirus GII was the most frequent genogroup in each age group. Norovirus was present during the whole year without a clear seasonality. All episodes associated with norovirus were acute (<14 days). When comparing the clinical characteristics of norovirus with those of rotavirus, the former was more common in older children, while the latter was more common in younger children (mean 14.1 vs 8.3 months, $P<0.001$). Patients with norovirus had significantly more blood in stools but less fever and less emesis episodes per day than patients with rotavirus. The presence of blood may represent an unknown mixed infection with an invasive pathogen, such as *Shigella*, that was culture negative. The presence of fever and severe emesis are a hallmark of rotavirus infection in small children. Norovirus episodes tended to be of shorter duration and less severe than rotavirus episodes, however those tendencies were not significant (Table 1). The most common pathogens in diarrhoeal samples in the first part of the cohort study were *Campylobacter* (18.6%), rotavirus (17.2%), enteroaggregative *E. coli* (EAEC) (15.1%) and enteropathogenic *E. coli* (EPEC) (7.6%).[3] While the most common pathogens in the second part of the study were EPEC (15.6%), enterotoxigenic *E. coli* (ETEC) (14.6%) and EAEC (8.3%), however in this second cohort only diarrhoeagenic *E. coli* were searched.

There are some methodological limitations in our study. Some patients may have been ill with viral enterocolitis other than norovirus and rotavirus, leading to a potential underestimation of the number of mixed norovirus infections. Similarly, in this study we have only included pathogen negative cases, and thus we are likely underestimating the total norovirus prevalence, since potential mixed infection with bacterial pathogens was not studied. However, since this was a passive surveillance study, we may have overlooked information on milder illnesses not requiring medical attention thus underestimating the total norovirus diarrhoea prevalence in this population. However, the main limitation of the study is the large number of samples unavailable for analysis; therefore the overall prevalence of norovirus is unclear.

In summary, norovirus is a frequent cause of acute diarrhoea in older Peruvian children living in periurban areas. The role of norovirus as a cause of diarrhoea and severe diarrhoea needs further confirmation in future epidemiological studies in developing countries.

Acknowledgments

We would like to thank Dr. Luz Carbajal for her suggestions in data analysis.

Funding: This work was partially funded by: Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Spain, Programa de Cooperación Interuniversitaria e Investigación Científica con Iberoamérica (D/019499/08 and D/024648/09); Institutional Research Funds from Universidad Peruana Cayetano Heredia, Peru (TJO), from Instituto Nacional de Salud del Niño, Peru (L.H.), and from Instituto de Investigación Nutricional, Peru

(CFL); National Institutes of Health, USA, Public Health Service awards 1K01TW007405 (TJO); Fondo de Investigaciones Sanitarias, Spain, CP05/0130 (J.R.); and from Sanofipasteur.

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TABLE 1

Comparison of clinical characteristics of diarrhoea episodes due to norovirus and rotavirus in children.

	Norovirus (*n*=39)	Rotavirus[a] (*n*=85)
Age (months), mean ± DS	14.1 ± 5.7	8.3 ± 3.0[c]
Gender, % male	59	54
Duration in days, median (range)	3 (1–12)	4 (1–14)
Blood in the stools, %	15	2[b]
Fever, %	33	55[b]
Maximum number of stools per day, mean ± DS	5.6 ± 2.8	6.3 ± 2.6
Vomiting, %	46	38
Maximum number of emesis episodes per day[d], mean ± DS	1.6 ± 2.6	2.9 ± 3.1[b]
Received oral rehydration solution, %	38	54
Moderate dehydration, %	5	6
Severity score[e], mean ± SD	8.1 ± 3.2	9.2 ± 3.9

[a] Data from the same cohort study;

[b] $P<0.05$ and

[c] $P<0.001$ for the comparison between diarrhoea due norovirus and rotavirus;

[d] For this variable we have chosen only the day with the maximum number of emesis per day during the entire diarrhea episode;

[e] Modified Vesikari score.