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ORIGINAL ARTICLE

The burden of norovirus disease in children: a multi-country study in Chile, Brazil, Thailand and the Philippines

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ABSTRACT

Background: Noroviruses (NoVs) cause acute gastroenteritis (AGE) worldwide, affecting children in particular. We aimed to estimate the burden of disease due to NoV among children aged <6 years in Brazil, Chile, Philippines and Thailand.

Methods: This was a prospective, hospital-based, observational study. Children were recruited over one year between 2014 and 2017. Four cohorts were analysed: community-acquired AGE outpatients and inpatients, nosocomial AGE inpatients, and asymptomatic outpatients. We collected demographic and clinical data, and a stool sample that was tested for NoV. Positive samples were tested for Rotavirus (RV) and NoV-genotyped. Disease severity was assessed by the Vesikari and modified Vesikari scores. Prevalence and incidence of NoV-AGE were estimated by cohort and country.

Results: 1637 participants yielded valid laboratory results. The proportion of NoV-positive cases was 23.8% (95% CI 20.8-27.2) in the outpatient cohort, 17.9% (15.0-21.3) in the hospital cohort, 21.4% (12.7-33.8) in the nosocomial cohort and 9.6% (6.9-13.2) in the asymptomatic cohort. Genotype GII.4 was predominant (58%). Less than 4% samples had RV coinfection. In general, NoV-positive subjects had more severe presentations than NoV-negative subjects.

Conclusions: NoV caused AGE with substantial burden throughout the studied settings, with higher relative frequency in Brazil where RV vaccination coverage is high.

Introduction

Acute gastroenteritis (AGE) is one of the leading causes of death in children under 5 years of age worldwide. Mortality due to AGE has decreased in the past decades, thanks to improved hygiene conditions and rotavirus (RV) vaccination among other interventions (GBD 2017 Diarrhoeal Disease Collaborators, 2020). However, AGE is responsible for significant morbidity and burden on health services and societies worldwide, including deaths in resource deprived countries (GBD 2017 Diarrhoeal Disease Collaborators, 2020).

Norovirus (NoV) has been identified as the major cause of AGE across all ages and the second most frequent cause, after rotavirus,

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of AGE in children under 5 years of age (Lopman et al., 2011, Operario et al., 2017, Pires et al., 2015). AGE due to NoV is estimated to cause 699 million illnesses and 219,000 deaths globally every year (Bartsch et al., 2016). Of these, 453 million illnesses and 95,000 deaths occur in children under 5 years of age. The prevalence of NoV infection has been estimated at 18% among all cases of AGE, 20% among AGE outpatient cases, and 17% among hospitalized AGE cases (Ahmed et al., 2014). NoVs have been classified into 10 genogroups, although not all infect humans (Chhabra et al., 2019). Although recombinant genotypes are continuously emerging, GII genotypes, and specifically the GII.4 Sydney capsid genotype, remain the most frequent cause of human illness (de Graaf et al., 2016, Mans, 2019, van Beek et al., 2018).

Evidence shows that NoV has become the leading cause of severe childhood AGE in some countries that have instituted widespread RV vaccination, like Finland, the United States, and Brazil (Hemming et al., 2013, Payne et al., 2013, Santos et al., 2017). In low-resource settings, single-site studies show a similar trend (Mans, 2019), although some multi-site analyses suggest that RV or other pathogens remain the primary source of AGE (Operario et al., 2017, Platts-Mills et al., 2018). Furthermore, existing studies use different case definitions and laboratory methods, and do not give a global picture of the disease burden.

In this multi-country study, including lower- and upper-middle income countries, we aimed to estimate the burden of AGE due to NoV infection in outpatient, hospitalized and nosocomial cohorts of children less than 6 years of age. Our primary objective was to estimate the proportion of AGE cases due to NoV in these cohorts. As secondary objectives, we assessed the incidence of NoV infection, clinical presentation of the disease, molecular epidemiology, proportion of coinfections with RV and proportion of asymptomatic NoV infections. We expect that these analyses will contribute to a better understanding of NoV disease burden in childhood and provide information to guide NoV vaccine development and introduction in paediatric populations.

Methods

Methods are summarized here and described in detail in Supplementary Text.

Ethical considerations

Ethical approval was obtained from the local ethical committees or institutional review boards. The legal guardians of all participants provided signed informed consent to enrol in the study.

Study design and site attributes

This study was a prospective, hospital-based observational study among children younger than 6 years of age, in nine sites in four countries (Brazil, Chile, the Philippines and Thailand). Sites were tertiary or community hospitals in predominantly urban settings. All sites were public or public-private institutions serving mostly middle- and low-socioeconomic strata of the population, except for Hospital Sabara (Brazil), which is a private hospital serving middle to middle-high income populations. RV vaccination was introduced in the National Immunization Program (NIP) in Brazil since March 2006. The Philippines were in limited rollout phase when the study was conducted, but not in the region where the study was carried out. In all countries RV vaccination was available in the private sector.

Recruitment started on 24 November 2014 at the first site, and on 06 January 2016 at the last site (Figure 1).

Study cohorts, enrolment criteria and case definition

The study had four main cohorts designated as follows:

1 Outpatient: Community-acquired AGE outpatient cohort.
2 Hospital: Community-acquired AGE hospital cohort.
3 Nosocomial: Nosocomial AGE hospital cohort.
4 Asymptomatic: Non-AGE, outpatient cohort.

Subjects in the outpatient and asymptomatic cohorts were recruited in the emergency or outpatient department of participating hospitals, while those in the hospital and nosocomial cohorts were recruited in the paediatric wards.

Inclusion criteria were age $\geq$15 days and $<$6 years, and residence in the reference area of the hospital. For the community-acquired cohorts, subjects had to present with any watery or loose stools or vomiting in the 24 hours prior to consultation or admission. For the nosocomial cohorts, children had acute onset of loose stools or vomiting with onset 48 hours after admission. For nosocomial and asymptomatic cohorts, children had to present to the hospital for reasons other than AGE. Children with diagnosis of chronic diseases with symptoms of diarrhoea or vomiting, or with any obvious non-infectious cause for AGE symptoms, or with respiratory symptoms in the previous 3 days were excluded.

The main analysis included only children who complied with the following definition of AGE: children with at least three loose stools in 24 hours OR vomiting at least three times in 24 hours OR diarrhoea or vomiting with two or more additional symptoms (diarrhoea, vomiting, abdominal cramps, abdominal pain, fever, nausea, blood or mucus in the stool).

Case identification and data collection

Cases were identified by the treating physician or study nurse, and by screening admission registries. Per each case recruited to the outpatient cohort, an individual of the same age group presenting to the same facility for a reason other than AGE was invited to participate in the asymptomatic cohort. Asymptomatic subjects had to be recruited within 2 weeks of the index case. Participants were recruited to AGE and asymptomatic cohorts in parallel, except for one site in Chile where participants were recruited to the asymptomatic cohort only during the first of two years of the overall recruitment period.

Data were collected from information routinely recorded in the medical records and through direct report from site staff and the parent/guardian of patients using a patient diary. Data were collected for all patients on demographics, medical history (including RV vaccination status), clinical examination, and AGE symptoms. Data on AGE symptoms were used to calculate AGE severity using the original and modified Vesikari scoring systems (Freedman et al., 2010, Ruuska and Vesikari, 1990), as described in Supplementary text, pages 8-9.

Stool samples were collected for all subjects within 72 hours of enrolment, processed at the study sites, and shipped to a central analytical laboratory (Naval Health Research Center, San Diego, CA) to detect NoV by real time RT-PCR analysis. Briefly, viral RNA was extracted using QIAGEN QIAamp® Viral RNA Mini Kit and subjected to the Norovirus Duplex Real-time (TaqMan®) RT-PCR Assay, using the Applied Biosystems 7500 Fast DX Real-time PCR system (Thermo Fisher Scientific, USA). NoV-positive samples (cycle threshold $\leq$40) were tested for RV using the xTAG® Gastrointestinal Pathogen Panel (GPP) (luminexcorp.com), and genotyped targeting open reading frame 1 and 2 (regions B and C) if sufficient material was available (Supplementary text, pages 4-5). A follow up contact via phone call or visit was scheduled approximately two weeks after consultation or discharge (except for the asymptomatic cohort), to collect the patient diary with information on

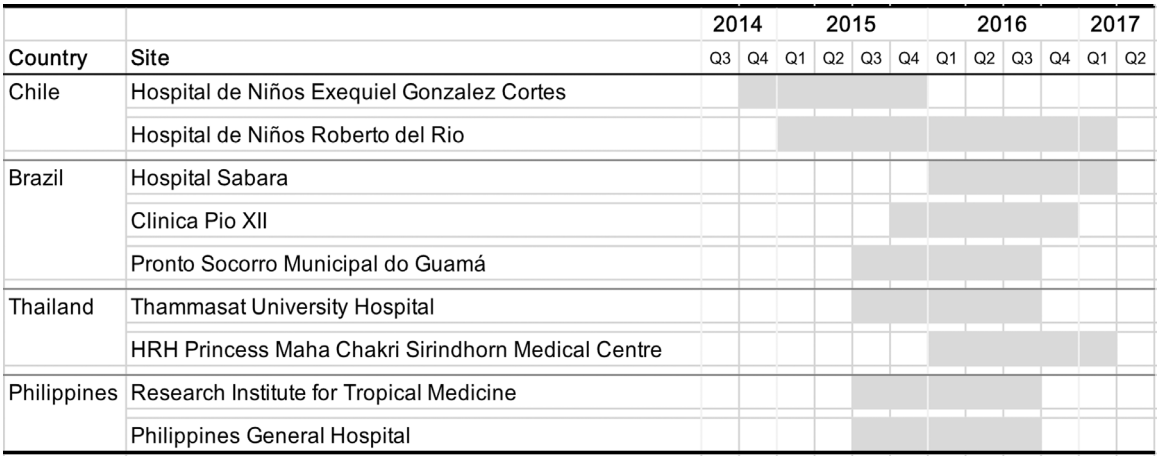

Figure 1. Recruitment sites and periods.

outcomes and illness duration. Numbers of children in the catchment areas were derived from official census/statistics sites (Supplementary text, pages 6-7).

Sample size and data analysis

Sample size was estimated aiming to achieve 95% confidence intervals with a half-width of 6% for certain anticipated frequencies. The anticipated frequencies were derived from the literature (see Supplementary text, page 7). The total sample sizes determined were: 800 children for each of the community-acquired cohorts (200 per country), 200 children for the nosocomial cohort (50 per country), and 400 children for the asymptomatic cohort (100 per country).

Data were entered directly into an electronic data capture (EDC) system. The number of cases in the study cohorts was derived from the clinical information in the EDC system. Analyses were performed for all countries pooled, for each country separately and stratified by case definition, age group and cohort.

Descriptive analyses (summary and frequency statistics) were done to explore the qualitative and quantitative nature of the data collected. Proportions for the study objectives were calculated and presented with a 95% confidence interval (CI), to allow comparisons between NoV-positive and NoV-negative AGE subjects (Supplementary text, page 8). Categorical variables were tested by means of Chisquare test or Fisher's exact test and continuous variables via Mann-Whitney U test (Wilcoxon test). Two-sided p-values were considered significant if <0.05. Incidence of AGE was calculated overall (not by age group) using the number of children in the catchment area as denominators (Supplementary text, page 6). Incidence of AGE caused by NoV was calculated by multiplying the estimate of AGE incidence by the estimate of the proportion of AGE caused by NoV (further details in Supplementary text, page 9).

To account for fluctuations due to seasonality, the primary analysis was repeated weighted by the number of cases of AGE seen per week at the recruitment site divided by the total number of cases of AGE seen during the study period. Statistical analyses were carried out using SAS© version 9.3 or higher (SAS Institute Inc., Cary, NC, USA.).

Results

Subject attrition

1800 children were screened to participate in the study, of whom 1702 were included in the analysis (Table 1).

Baseline characteristics

Mean/median ages across all countries were similar in the outpatient, hospital and asymptomatic cohorts, with younger subjects in the nosocomial cohort. Most subjects recruited were in the 12-23 months age group. A history of chronic disease was generally most frequent in the nosocomial cohorts. The proportion of RV vaccinated subjects was highest in Brazil, where RV vaccination has been part of the NIP since March 2006, compared with the other countries where RV vaccination was either not in the NIP (Chile, Thailand) or was in limited roll-out phase (Philippines). Globally, most parents/guardians had completed secondary school studies. Brazil and Thailand had the highest proportion of parents/guardians with university-level education (Table 2).

Prevalence and Incidence of NoV infection

1637 subjects had a valid laboratory result (Table 1). Overall, the proportion of AGE cases that tested positive for NoV was 23.8% in the outpatient cohort (95%CI 20.8-27.2; 27.7% when adjusted by seasonality), 17.9% in the hospital cohort (95%CI 15.0-21.3; 19.3% when adjusted by seasonality), 21.4% in the nosocomial cohort (95%CI 12.7-33.8; 19.7% when adjusted by seasonality), and 9.6% (95%CI 6.9-13.2) in the asymptomatic cohort.

Brazil had the highest proportion of NoV-positive cases in all symptomatic cohorts. Chile had a similar proportion as Brazil in the outpatient cohort, while Thailand and Philippines had lower proportions of NoV-positive cases in all cohorts. There were no cases of nosocomial AGE due to NoV collected in the Philippines and Thailand. The proportion of asymptomatic NoV infection was lowest in Brazil (6.6%; 95%CI 2.6-15.7) and around 10% in the other countries (Figure 2).

Globally, the 12-23 months age group had the highest proportions of NoV-positive cases (31.4 [95%CI 25.6-37.8] in the outpatient cohort and 28.7% [95%CI 22.3-36.0] in the hospital cohort). Since the younger age groups had very small sample sizes and yielded large confidence intervals (see Global table – Supplementary material), these were combined into a single age group (15 days – 11 months) for the final analysis. The proportion of NoV-positive samples in this age group was 18.0% [95%CI 13.1; 24.3] in the outpatient cohort and 15.4% [95%CI 11.0; 21.2] in the hospital cohort. The proportion of NoV-positive cases in the asymptomatic cohort was similar across age groups, ranging from (5.9% [95%CI 2.5; 13.0] in the 3-5 years age group to (12.3% [95%CI 6.1; 23.2] in the 2 years age group (Figure 2). The small sample size of

Table 1
Study flow of participants per cohort and country

	Brazil	Chile	Philippines	Thailand	All
Outpatient					
Screened	176	208	184	200	768
Enrolled (case definition 1)	175	208	184	200	767
Included in the analysis (case definition 2)	154	205	171	175	705
Provided stool sample	140	197	171	175	683
Valid laboratory result	139	195	171	175	680
Hospital					
Screened	190	129	84	199	602
Enrolled (case definition 1)	189	129	84	199	601
Included in the analysis (case definition 2)	183	126	82	189	580
Provided stool sample	165	124	82	189	560
Valid laboratory result	164	124	82	188	558
Nosocomial					
Screened	25	22	4	17	68
Enrolled (case definition 1)	25	22	4	17	68
Included in the analysis (case definition 2)	19	18	4	15	56
Provided stool sample	19	18	4	15	56
Valid laboratory result	19	18	4	15	56
Asymptomatic					
Screened	73	102	87	100	362
Enrolled	73	102	87	100	362
Included in the analysis	73	101	87	100	361
Provided stool sample	61	101	87	100	349
Valid laboratory result	61	97	87	98	343

Table 2
Demographic characteristics of the study population, per cohort and country.

	Brazil	Chile	Philippines	Thailand	All
Outpatient					
All patients, N	154	205	171	175	705
Age at informed consent, mean (median), months	28.7 (24.5)	22.2 (17)	25.8 (21)	23.8 (17)	24.9 (19)
Gender, % male	48.7	54.1	52.6	56	53
Parental education, % complete university level	46.1	9.3	9.9	41.7	25.5
History of chronic disease, %	0.6	6.8	0	14.9	5.8
RV vaccination received, %	89.0	0.5	1.2	16.6	24.0
Hospital					
All patients, N	183	126	82	189	580
Age at informed consent, mean (median), months	23.2 (19)	18.3 (14)	22.5 (14.5)	24.2 (19)	22.3 (17)
Gender, % male	59.6	64.3	54.9	49.7	56.7
Parental education, % complete university level	38.8	15.1	11.0	26.5	25.7
History of chronic disease, %	9.3	14.3	0	13.2	10.3
RV vaccination received, %	89.6	0.8	0	16.4	33.8
Nosocomial					
All patients, N	19	18	4	15	56
Age at informed consent, mean (median), months	17.3 (15)	8.7 (5.5)	7.5 (4.5)	15.1 (13)	13.3 (11)
Gender, % male	52.6	55.6	100	66.7	60.7
Parental education, % complete university level	31.6	16.7	0	20	21.4
History of chronic disease, %	5.3	44.4	0	26.7	23.2
RV vaccination received, %	89.5	0	25.0	13.3	35.7
Asymptomatic					
All patients, N	73	101	87	100	361
Age at informed consent, mean (median), months	32.2 (30)	23.3 (19)	23.1 (20)	22.8 (18.5)	24.9 (20)
Gender, % male	46.6	55.4	51.7	46.0	50.1
Parental education, % complete university level	46.6	11.9	12.6	21	21.6
History of chronic disease, %	6.8	21.8	0	37	17.7
RV vaccination received, %	91.8	3	3.4	12.0	23.5

RV = Rotavirus

the nosocomial cohort did not allow an analysis by age group and country in this cohort (Global table – Supplementary material).

Incidences were estimated for the different cohorts by country and collectively. The values obtained differed between countries by up to one order of magnitude, with the highest values in Brazil and the lowest values in the Philippines. The overall incidence of AGE associated with NoV infection leading to a medical visit was estimated at 0.9/100 person-years (1.0/100 person-years when adjusted by seasonality) and it was highest in Brazil and Chile. The overall incidence of AGE hospitalizations associated with NoV was estimated at 0.10/100 person-years (0.11/100 person-years when adjusted by seasonality) and it was highest in Brazil. The overall incidence of nosocomial AGE associated with NoV was estimated at 0.14/100 person-years (0.13/100 person-years when adjusted by seasonality), and it was higher in Chile than in Brazil.

Clinical Presentation

In all cohorts, vomiting and nausea were more frequent in NoV-positive than NoV-negative children. This difference was significant for vomiting in the outpatient (88% vs 57%, $p<0.0001$) and hospital cohorts (95% vs 74%, $p<0.0001$). As expected due to inclusion criteria, diarrhoea was very frequent in the NoV-positive and

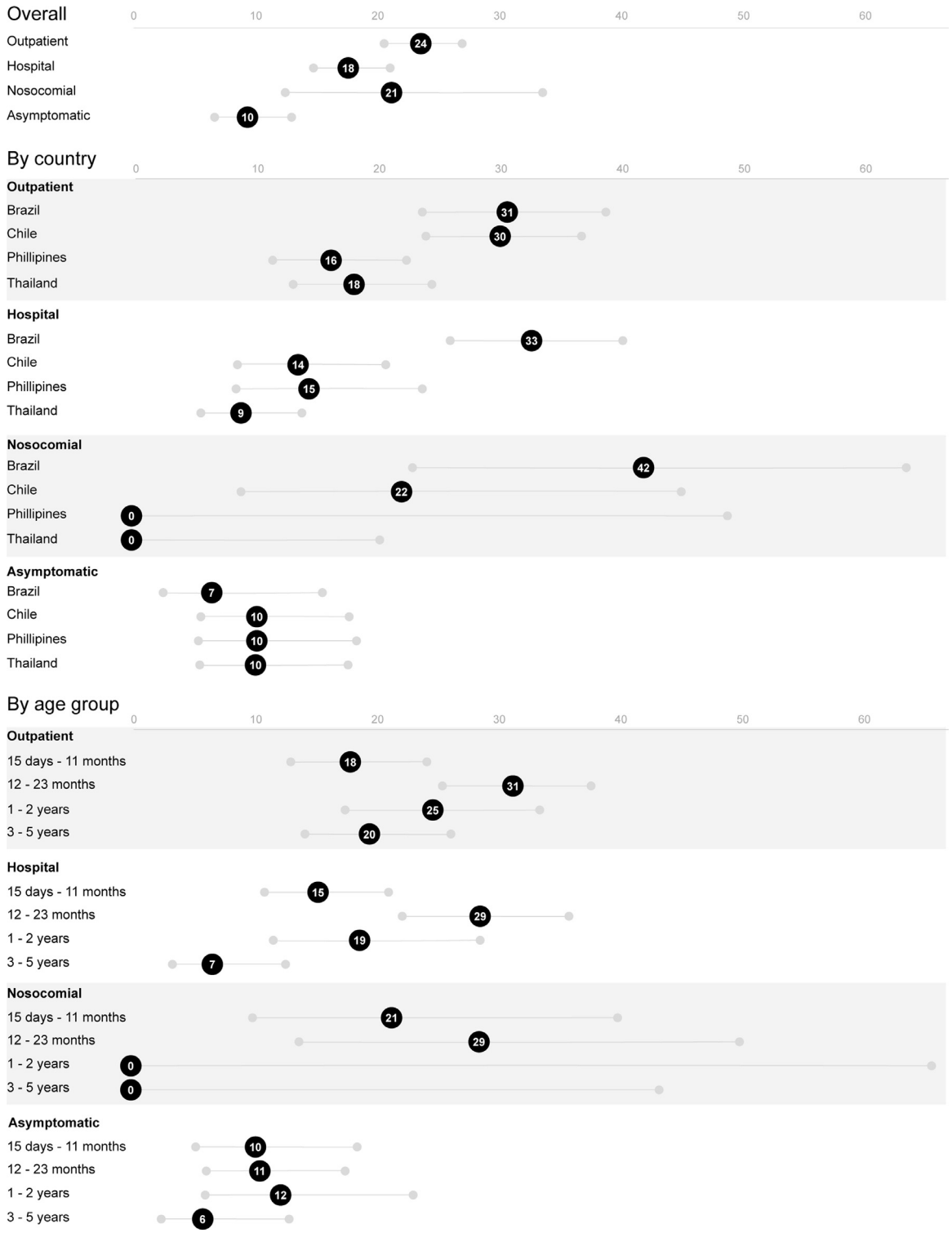

Figure 2. Proportion of NoV positive AGE cases in the different cohorts, overall, by country and by age group. Black dots are the observed percentages, grey lines and dots show the 95% CI estimate.

negative children in all cohorts, but less NoV-positive than NoV-negative children had diarrhoea in the outpatient cohort (83% vs 94%, $p<0.0001$). In the hospital and nosocomial cohorts, abdominal pain or cramps were more frequent in NoV-positive children than in NoV-negative children. Conversely, fever was less frequent in NoV-positive children than in NoV-negative children in the outpatient and hospital cohorts. However, not all these comparisons were significant (Table 3).

The Vesikari and modified Vesikari AGE severity scores (Freedman et al., 2010, Ruuska and Vesikari, 1990) had higher mean and median values in the NoV-positive subjects in all cohorts. Both scales yielded similar results, with a tendency for slightly lower scores in the modified Vesikari score (1 point out of a 20-point scale). NoV-positive cases had significantly higher Vesikari scores for both the outpatient and hospital cohorts. Higher proportions of NoV-positive subjects than NoV-negative were clas-

Table 3
Frequency of symptoms by cohort and NoV infection status (positive or negative).

	Outpatient					Hospital					Nosocomial				
	NoV positive		NoV negative		p-value*	NoV positive		NoV negative		p-value*	NoV positive		NoV negative		p-value*
N	162		518			100		458			12		44		
	n	%	n	%		n	%	n	%		n	%	n	%	
Diarrhoea	134	83	487	94	<0.0001	98	98	444	97	0.749	12	100	44	100	1.000
Vomiting	142	88	294	57	<0.0001	95	95	338	74	<0.0001	8	67	12	27	0.018
Fever	61	38	241	47	0.058	61	61	348	76	0.002	3	25	10	23	1.000
Mucous in stools	8	5	84	16	0.0004	20	20	95	21	0.868	5	42	7	16	0.105
Abdominal pain	31	19	110	21	0.642	31	31	92	20	0.017	2	17	4	9	0.599
Nausea	19	12	34	7	0.049	21	21	48	10	0.004	2	17	2	5	0.198
Abdominal cramps	10	6	22	4	0.425	11	11	35	8	0.269	3	25	3	7	0.105
Blood in stools	2	1	18	3	0.186	6	6	49	11	0.153	0	0	1	2	1.000
Other symptoms	18	11	83	16	0.159	27	27	124	27	0.988	0	0	5	11	0.574

Percentages are based on N in each column. *P-value for the comparison between NoV positive and NoV negative in each cohort. Statistical test used was Fisher's exact test if cells had n≤5 or Chisquare test otherwise.

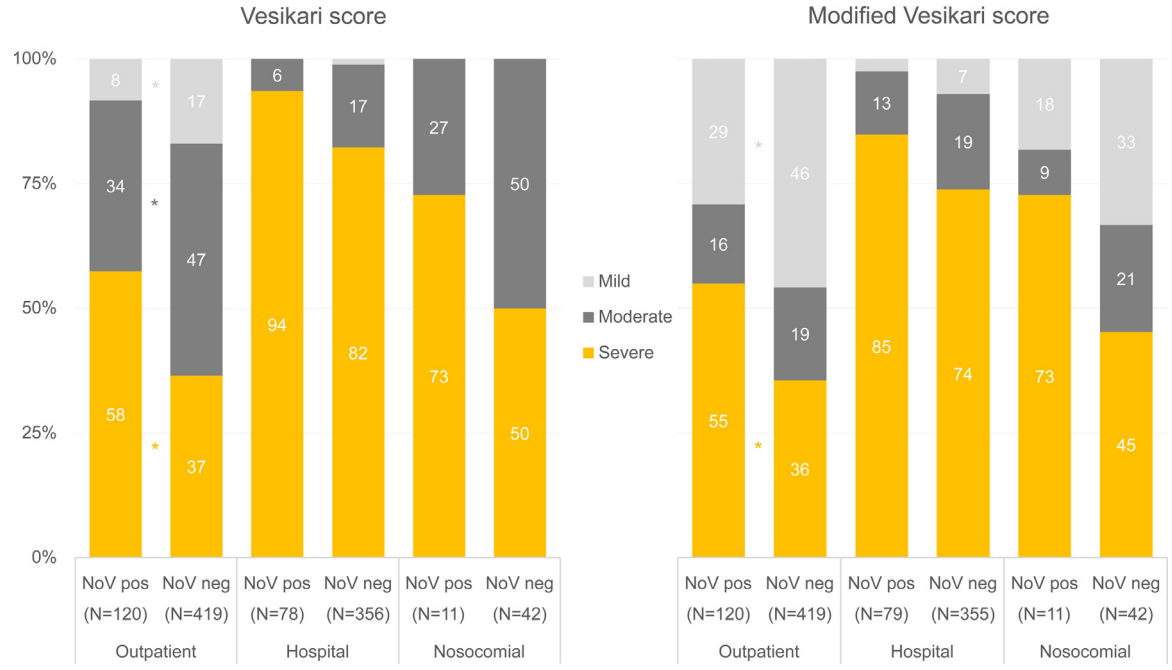

Figure 3. AGE disease severity in each cohort as assessed by the Vesikari and Modified Vesikari scores. Values are % of N in each column. Values lower than 5% are not labelled. Asterisks (*) denote significant difference between NoV positive and NoV negative cases (p<0.05).

sified as severe in all cohorts, but this difference was statistically significant only in the outpatient cohort (p=0.0028). In the outpatient cohort, over 50% of NoV-positive subjects were classified as severe (Figure 3, Supplementary table 3). No deaths were registered among the study participants.

Seasonality evaluation was limited to the one-year length of the study, and no clear patterns were visible in any of the countries (data not shown).

Co-infection with RV

The proportion of NoV-positive cases who also tested positive for RV was 4% (7 out of 162) in the outpatient cohort and 3% (3 out of 100) in the hospital cohort. All cases of co-infection were observed in Chile, except one hospitalized case that occurred in the Philippines.

Molecular epidemiology

Of 307 NoV-positive samples, typing failed in 108, mostly because the sample was insufficient or the cycle threshold value was over 30. The 199 samples typed belonged to the 4 cohorts: 109/162 (67%) in the outpatient cohort, 68/100 (68%) in the hospital cohort, 9/12 (75%) in the nosocomial cohort and 13/33 (39%) in the asymptomatic cohort.

The predominant genotype in all AGE cohorts was GII.4 (58%), followed by GII.17B (11%), GII.2 (5%) and GII.3 (5%). GII.4 was more frequent in hospital (68%) and nosocomial (89%) cohorts than in outpatient (50%) and asymptomatic (23%) cohorts. The asymptomatic cohort was more heterogeneous, with a low proportion of GII.4 samples and higher proportions of other genotypes (Figure 4).

The proportions of the predominant genotypes were similar across age groups, except for the 3 to 5 years age group, which had less GII.4 (32% vs over 60% in other groups) and no cases of GII.17B. Given the small sample size, we do not present the analysis of NoV genotype distribution per age group for each country.

GII.4 was the predominant genotype in all countries, although more frequent in Brazil (62%) and Chile (65%) than in the Philippines (45%) and Thailand (46%). GII.17B was more frequent in Brazil (18%) than in the other countries (6-10%). GII.3 was frequent in the Philippines (10%) and Thailand (16%), but not in Chile (2%) or in Brazil (0%). GII.6 was the second most frequent genotype in the Philippines (20%) while it only represented 1 to 3% of the isolates

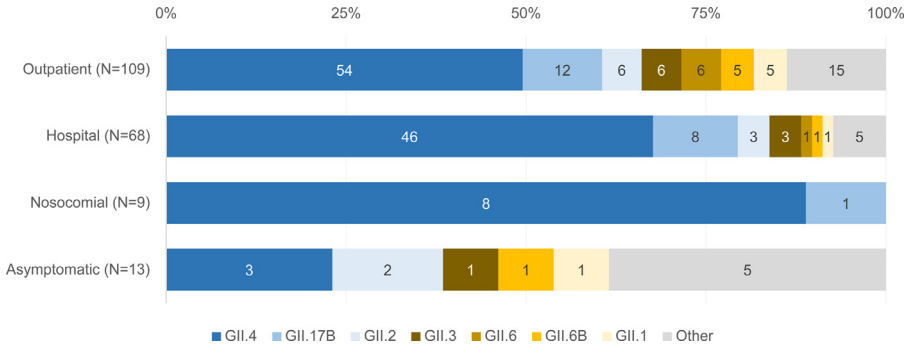

Figure 4. Distribution of NoV strains across cohorts. Values are the absolute number of cases observed in each category.

in the other countries (Genotypes global table – Supplementary material).

Discussion

We carried out a multi-country study on two continents to estimate the proportion of AGE caused by NoV in children younger than 6 years of age in four cohorts: community-acquired outpatient and hospital AGE, nosocomial AGE and asymptomatic children. Between November 2014 and March 2017, 1798 children were enrolled, of whom 1637 yielded valid laboratory results.

NoV was frequently associated with AGE in all cohorts and settings: 23.8% (95%CI 20.8-27.2) of all AGE presenting to an outpatient facility and 17.9% (95%CI 15.0-21.3) of all AGE hospitalizations were NoV-positive. 9.6% (95%CI 6.9-13.2) of asymptomatic children tested positive for NoV. Recent meta-analyses in Latin America and in developing countries have estimated similar values across the different cohorts (Nguyen et al., 2017, O'Ryan et al., 2017), and our values are in line with those reported previously in a global review (Ahmed et al., 2014). In the nosocomial cohort, 21.4% (95%CI 12.7-33.8) of AGE cases were NoV-positive. Literature on nosocomial NoV infections in children is scarce, and the sample size was small, but this proportion is in the same range as that observed in the hospital cohort.

The impact of NoV infection was highest in Brazil for both symptomatic cohorts and in Chile for the outpatient cohort. The proportion of NoV-positive children was similar across countries for the asymptomatic cohorts. The magnitude of the difference in prevalence of NoV infection between outpatient and asymptomatic cohorts was larger in Brazil (30.9 vs 6.6%) and Chile (30.3 vs 10.3%) than in the Philippines (16.4 vs 10.3%) or Thailand (18.3 vs 10.2%). High prevalence of NoV infection in asymptomatic cohorts has been associated with NoV hyper-endemic settings, where the burden is higher (Lopman et al., 2016). However, the high NoV frequency observed in Brazil for the symptomatic cohorts likely reflects the impact of the RV vaccination program implemented in 2006 with over 90% coverage (Santos et al., 2017).

We observed the highest proportion of NoV-positive AGE in the 12-23-month age group, and only one case in the under 1 month age group (Thailand hospital cohort). This falls within the peak age-range for norovirus infections identified in a global systematic review, although the authors highlight that lower-income countries and inpatient settings have younger age distributions (Shioda et al., 2015). However, we did not see any difference in age distribution between the countries in the study. Some authors suggest breast milk may confer protection against NoV by blocking NoV binding to human antigens, or by providing maternal antibodies to infants aged under 6 months (Jiang et al., 2004, Saito et al., 2014). However, information on breastfeeding was not collected in this study.

The overall incidence of AGE caused by NoV was 0.9/100 person-years in the outpatient cohort. This value is slightly lower than that reported in outpatient populations under 5 years of age in the U.K. and U.S. (Grytdal et al., 2016, O'Brien et al., 2016, Payne et al., 2013), but our study also includes children between 5 and 6 years of age, although they represented less than 7% of the sample size in the outpatient cohort. Furthermore, the estimated incidences differed to up to one order of magnitude across countries. These discrepancies may be partially explained by the difficulty in obtaining fully reliable denominators in some countries (Brazil, the Philippines) for the incidence calculations.

In line with previous reports, GII.4 was the most frequent genotype detected (Hoa Tran et al., 2013, Mans, 2019, van Beek et al., 2018). GII.4 genotypes were more frequent in hospital and nosocomial settings, and less frequent in the outpatient and asymptomatic cohorts. This distribution supports reported associations of GII.4 strains with more severe AGE (Harris et al., 2019, Huhti et al., 2011, Mathew et al., 2019). Furthermore, the observed higher proportion of GII.4 genotypes in Brazil and Chile is consistent with a reported higher prevalence of histo-blood antigen secretors in Meso-American populations (Nordgren and Svensson, 2019). The globally emerging genotype GII.17B was also more frequent in Brazil and Chile while GII.3 was more frequent in Thailand and the Philippines. The GI genotype was nearly absent from the cohorts in this study.

NoV-positive children had higher mean and median Vesikari and modified Vesikari scores than NoV-negative subjects, and a higher proportion of subjects classified as severe. Although the difference in scores was small (1-2 points in a 20-point scale), this contradicts the traditional perception that NoV could cause less severe infections than other enteric pathogens, and is in line with recent studies suggesting that NoV may be as severe as RV (Operario et al., 2017, Riera-Montes et al., 2018, Rouhani et al., 2016). Furthermore, NoV disease was severe in an important proportion of cases requiring hospitalization (94%), and more than half (54%) of NoV positive children from the outpatient cohort were also classified as severe.

The present study has the strength and uniqueness of including a parallel evaluation, with equal epidemiological and laboratory methods across nine sites, in four countries and two continents. The main limitation of this study is that the sample size achieved was lower than that proposed in the protocol, affecting the precision of our estimates. This is particularly relevant for the nosocomial cohort and for specific age groups, for which the results should be interpreted with caution. Furthermore, the samples achieved in each country differ from the overall population in the proportion of parents with university-level education (The World Bank Group, 2020). Other limitations were the difficulty in delimiting the catchment area to calculate the incidence, and the short time span of the project in each site, which limits the evaluation of seasonality. We also did not assess co-infection with pathogens

other than RV, which may limit the usefulness of our data in estimating the potential number of cases averted when evaluating the introduction of NoV vaccines. Finally, NoV epidemiology may be influenced by regional factors, and generalization should be done cautiously, particularly for rural settings or countries other than the ones included in this study.

To conclude on the relevance of this study, we should note that young children are considered one potential priority target group for NoV vaccination. The results of this prospective study estimating the burden of NoV disease among children less than 6 years of age from four middle-income countries in two continents will be important to inform on public health policies related to the development and introduction of norovirus vaccines.

Conflicts of Interest

MRM and TV have received consulting fees from Takeda related to this work. MAS, ACL and MO have received funding from Takeda for ongoing norovirus epidemiological studies and have been consultants for norovirus vaccine development without receiving funding. LB, AT, RL, UT, RC and OP have no conflicts of interest to disclose.

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Preliminary results of this study have been presented at the Congress of the European Society of Pediatric Infectious Diseases in 2018, the Asian Vaccine Conference in 2019, and the International Calicivirus Conference in 2019.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.06.037.

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