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Rotavirus infection among children under five years of age hospitalized with acute gastroenteritis in Myanmar during 2018–2020 — Multicentre surveillance before rotavirus vaccine introduction

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Abstract

Background: Rotavirus gastroenteritis (RVGE) is a leading cause of severe diarrhea in children under-five worldwide, with the majority of mortality in lower -income countries. This study aimed to provide baseline information on epidemiology of rotavirus and circulating strains before rotavirus vaccine introduction in Myanmar.

Methods: Hospital-based, prospective surveillance was conducted from May 2018 to January 2020 at four sentinel sites; two hospitals in Lower Myanmar, one hospital each in Middle Myanmar and East Myanmar. Children under five years of age hospitalized for acute gastroenteritis were enrolled; demographic and clinical data were collected. Stool samples were screened by ELISA (ProSpecT™ Rotavirus, OXOID-UK) for rotavirus antigen and a subset of ELISA positive samples were genotyped by reverse transcription polymerase chain reaction.

Results: Rotavirus was detected in 45.7% (799/1750) of cases enrolled at three sites in May 2018–April 2019 and 42.5% (521/1227) at four sites in May 2019–January 2020. RVGE cases were predominantly male (58.7%; 775/1320) and 92.6% (1223/1320) of RVGE cases occurred in <2 years old. Rotavirus detection was higher in cold and dry season (November–April). RVGE compared to non-RVGE cases had more frequent vomiting (78.3% Vs 68.1%, p<0.01), fever

Conflict of interest
None to declare.

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(65.8% Vs 61.3%, p=0.01), severe dehydration (3.6% Vs 2.1%, p<0.01) and requirement of treatment by IV fluid (58.3% Vs 53.1%, p<0.01). The most prevalent genotypes identified were G1P[6] (113/359, 31.5%), G1P[8] (94/359, 26.2%) and G2P[4] (33/359, 9.2%).

Conclusions: This study confirms the persistent high prevalence of RVGE among children under-five admitted to hospitals in different parts of Myanmar and diversity of rotavirus strains over time prior to vaccine introduction. The rotavirus vaccine was introduced nationwide in February 2020 in Myanmar and these data will be important baseline data for post-vaccination monitoring of vaccine impact and circulating strains.

Keywords

Rotavirus; Rotavirus genotypes; Rotavirus vaccine; Rotavirus Surveillance; Myanmar

1. Background

Rotaviruses are the most common cause of severe diarrheal disease in young children throughout the world and, according to World Health Organization (WHO) estimates in 2013, approximately 215,000 children under five years old die each year from rotavirus infections [1,2]. The WHO recommends introduction of rotavirus vaccines in all national immunization programs and this is considered a priority in countries in South and Southeast Asia and sub-Saharan Africa where the vast majority of deaths occur [1, 2].

In Myanmar, the under-five mortality rate in 2019 was 44.7 deaths per 1000 live births with diarrhea being a leading cause of childhood death [3]. According to the data from the Ministry of Health and Sports, Myanmar, the incidence rate of diarrheal disease deaths in 2015 was 0.3 per 100,000 population [4]. Approximately 42–56% of acute gastroenteritis (AGE) hospitalizations are attributable to rotavirus infection [5,6]. This high burden of rotavirus infection led to the consideration of rotavirus vaccine (RV) introduction and in February of 2020 the rotavirus vaccine Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium), a monovalent, live, oral rotavirus vaccine, was introduced in Myanmar.

We previously reported trends in rotavirus hospitalizations and genotype distribution from sentinel surveillance carried out continuously from 2009 through 2017 at a major referral hospital, Yangon Children Hospital (YCH). Here, we provide data from three additional sentinel sites from different geographical regions for the period 2018–2020. These data immediately precede the period of RV introduction in Myanmar and provide updated and important baseline data for post-vaccination monitoring of vaccine impact and circulating strains.

2. Methods

2.1 Surveillance design and surveillance sites

Prospective, hospital-based surveillance was conducted at three hospitals from different geographical regions of Myanmar; Yangon Children's Hospital (YCH; 1300 bed) in Yangon Region (Lower Myanmar), Mandalay Children Hospital (550 bed) in Mandalay Region (Middle Myanmar) and Women and Children Hospital, Taunggyi (800 bed) in the southern

part of Shan State (East Myanmar) from May 2018 to April 2019 (Figure 1). An additional site, North Okkalapa General and Teaching Hospital (NOGTH; 200 bed) in the Yangon Region, conducted surveillance from May 2019 to January 2020. All hospitals are tertiary care referral hospitals within their respective regions.

2.2 Population under surveillance, case definition and selection criteria

The population under surveillance was children less than five years of age admitted to the sentinel hospitals for treatment of acute gastroenteritis between May 2018 and January 2020. Acute gastroenteritis was defined as passage of three or more looser-than-normal or liquid stools in a 24-hour period during the illness with onset of diarrhea ≤14 days at presentation. Children with bloody diarrhea and children transferred from another hospital were excluded from the study.

2.3 Surveillance methodology, case enrolment and sample collection

Surveillance was carried out according to the WHO generic protocol on hospital-based surveillance for rotavirus gastroenteritis [7]. Written informed consent was obtained from the parents or legal guardian of all eligible cases who met the selection criteria and then enrolled in the study. Upon enrollment, a case report form (provided from the WHO) was completed. Demographics, clinical history, physical examination, treatment and outcome were extracted from medical charts. A stool sample containing at least 3 ml was collected within 48 hours of admission. The sample bottles from hospitals from Yangon Region were transported on the same day, using coolers with ice packs to preserve cold chain, to the Virology Research Division at the Department of Medical Research (DMR). Bottles from other sites were stored at −20º C in sentinel hospitals and shipped to DMR biweekly frozen. At DMR, all samples were stored at −20º C until testing was performed. Upon hospital discharge, the date and outcome of patient admissions were recorded on the case report form.

2.4 Sample testing

All samples were tested for rotavirus antigen by ProSpecT™ Rotavirus ELISA kit, Oxoid, UK according to the manufacturer's instructions. A subset of rotavirus-positive stool samples (approximately 30% of ELISA positive samples from each month) was randomly chosen for G (VP7) and P (VP4) genotyping. RNA was extracted by using QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Germany) and the extracted dsRNA was amplified by QIAGEN one step RT-PCR kit using specific oliginucleotide primers (VP7F, VP7R, G1, G2, G3, G4, G8, G9, G10 and G12 for VP7 typing and CON 2, CON 3, P[4], P[6], P[8], P[9], P[10], P[11] for VP4 typing). The primers and RT-PCR protocol were provided by the WHO Rotavirus Reference Laboratory, CMC, Vellore, India [8].

2.5 Data entry and analysis

Data entry was done using Microsoft Excel. For descriptive analyses, the distributions of demographics, clinical characteristics, and mortality between RVGE and non-RVGE groups were compared using Chi-square test. P-value <0.05 was considered statistically significant.

2.6 Ethical consideration

This study was approved by the Institutional Review Board on Medical Research involving human subjects, Department of Medical Research, Ministry of Health and Sports, Myanmar (Approval Number: Ethics/DMR/2015/108AEA/2018E/2019).

3. Results

During the 21-month surveillance period from May 2018 to January 2020, a total of 3742 eligible children were identified from the four sentinel hospitals, and of these, 3226 (86.2%) were enrolled and stool samples were collected from 2977 patients (92.3%). Overall, rotavirus was detected in 44.3% (1320/2977) of cases. Between May 2018 and April 2019, rotavirus was detected in 45.7% (799/1750) of specimens from three surveillance sites and between May 2019 and January 2020 the rotavirus positivity was 42.5% (521/1227) from four surveillance sites (Table 1).

3.1 Demographic characteristics

The AGE cases admitted to the sentinel hospitals during the surveillance period ranged from 5 days old to 59 months; however, the greatest number of cases (2712/2977, 91.1%) occurred in children under 2 years of age. Accordingly, 55.9% (738/1320) and 92.6% (1223/1320) of rotavirus positive cases occurred in children less than one year and children less than two years of age respectively ($p<0.01$). By age group, the highest proportion of rotavirus was found among the 12–23 month group (485/1013, 47.9%), followed by the 6–11 month group (549/1213, 45.3%), 0–5 month group (189/486, 38.9%), and 24–59 month group (97/265, 36.6%). Males comprised a slightly greater proportion of all AGE cases (1772/2977, 59.5%) than females, which was also reflected in the higher proportion of males among rotavirus positive cases (775/1320, 58.7%) (Table 1).

3.2 Clinical characteristics

Vomiting was more frequently observed in RVGE group (1034/1320, 78.3%) than non-RVGE group (1129/1657, 68.1%; $p <0.01$). Fever (defined as ≥38°C, or ≥100.4°F) was also more common in the RVGE group (868/1320, 65.8%) compared to non-RVGE group (1015/1657,61.3%) and this difference was statistically significant ($p=0.01$). Severe dehydration (RVGE: n=48, 3.6%; non-RVGE: n=35,2.1%; $p<0.01$) and IV fluid treatment (RVGE: n=770, 58.3%; non-RVGE: n=880, 53.1%; $p<0.01$) were more frequently observed in RVGE than non-RVGE (Table 1). Of enrolled children, two patients who tested rotavirus positive died; however, no mortality was found among non-RVGE cases.

3.3 Seasonality of rotavirus infection

Figure (2) illustrates the distribution of AGE hospitalization and proportion of rotavirus cases by month (NOGTH was excluded because of their later initiation of surveillance). AGE cases were seen at sentinel hospitals year round, with rotavirus detected, throughout the surveillance period although prevalence increased in the cold, dry season from November to April. The proportion of AGE hospitalizations attributed to rotavirus ranged from a high of 62.6% (107/171) in December 2019 to a low of 17.9% (14/78) in September 2018.

3.4 Genotype distribution

From 1335 rotavirus positive specimens detected, 359 (26.9%) were selected randomly for genotyping. Overall, G1 was the most prevalent G genotype (227/359, 63.2%) followed by G3 (55/359, 15.3%) and G2 (41/359, 11.4%). The P[6] genotype was predominant (144/359, 40.1%), followed by P[8] (134/359, 37.3%) and P[4] (34/359, 9.5%). The most common rotavirus strains detected were G1P[6] (11/359, 31.5%) followed by G1P[8] (94/359, 26.2%) and G2P[4] (33/359, 9.2%) (Figure 3, Table 2).

3.5 Geographical distribution of rotavirus diarrhea cases

We analyzed the YCH and NOGTH as combined data in Lower Myanmar Region. The proportion of rotavirus detected by region was similar, ranging from 45.9% (572/1247) in Lower Myanmar, to 44.0% (580/1318) in Middle Myanmar and 40.8% (168/412) in Eastern Myanmar. The seasonal peak of RVGE in Lower and Middle Myanmar was December 2019 while in Eastern Myanmar the peak occurred in January 2020. Rotavirus G1P[8] was predominant in Lower Myanmar (59/182, 32.6%) and East Myanmar (18/59, 30.5%) while G1P[6] was predominant in Middle Myanmar (58/118, 49.2%) (Table 2).

Discussion

This hospital surveillance in four tertiary pediatric hospitals across different geographical regions in Myanmar demonstrates high prevalence of rotavirus infection in children under-five years hospitalized with AGE. The rotavirus prevalence (44.3%) in this study confirms the persistent high disease burden of RVGE throughout a decade period when compared with the previous data showing rotavirus prevalence of 49.9% between 2009 and 2014 and 48.8% in 2015 to 2017 [5,6]. This detection rate is also comparable to other neighboring Asian countries like Cambodia (50%; 2010–2016), Vietnam (46.6%; 2012–2015) and Indonesia (47.5%; 2010–2015) [9,10,11]. However, rotavirus prevalence was lower than the 64% reported in Bangladesh from 2012 to 2017 and higher than the 27.5% reported in Thailand in 2014–2016 [12,13].

We found that children less than two years of age bore the highest burden of rotavirus disease with detection peaking in the 6–23 month age group, similar to previous studies conducted prior to vaccine introduction elsewhere [11, 14, 15]. This highlights the need for the introduction of rotavirus vaccines early in the first year of life to prevent the greatest numbers of AGE cases. The occurrence of a higher proportion of males in both RVGE and non-RVGE has remained consistent with previous reports of Myanmar as well as other countries [5,6,15,16], perhaps because of higher likelihood of healthcare seeking among parents for boys. We cannot discount potential misclassification of rotavirus gastroenteritis which may over or underestimate rotavirus prevalence, however studies have shown that rotavirus diagnosis by ELISA is highly correlated with rotavirus disease [17], thus misclassification is likely minimal. Assessment of clinical characteristics revealed that vomiting was a prominent feature of rotavirus infection, observed more frequently in RVGE cases than non-RVGE cases, similar to other studies [11,14,18]. RVGE induced vomiting itself may lead to dehydration as well as inability to take sufficient amounts of oral rehydration solution (ORS). Severe dehydration and IV fluid treatment were observed

more in RVGE than non-RVGE cases. These findings are also in accordance with other studies [11,12,14]. The mortality among RVGE cases was 0.2% and although morbidity is high, rotavirus associated mortality is low in this study. This may be due to the availability of prompt treatment at these tertiary care hospitals with improvement in management of AGE cases by pediatricians. However, there may be some RVGE-associated deaths occurring outside of the hospital that are not captured by this surveillance system. Rotavirus infection in this study demonstrated a strong seasonal pattern in colder, drier months from November through April as in other Asian countries like Cambodia, Vietnam, Bangladesh, and Thailand [9,10,12,19].

The predominant genotypes identified during this study period 2018–2020 were G1P[6], G1P[8] and G2P[4] and these three strains together accounted for 66.9%. In the previous study (2015–2017), the predominant genotype in 2015–2016 was G9P[8] and in 2016–2017 was G3P[8] [6]. The changing pattern of rotavirus strains year by year was recognized throughout rotavirus surveillance in pre-vaccination period of 2009–2020 in Myanmar. Although rotavirus vaccines are effective against rotavirus vaccine strains as well as other non-vaccine strains, monitoring of circulating rotavirus strains is recommended to detect any new or sustained changes after vaccine introduction as well as for evaluation of strain specific vaccine effectiveness.

Conclusion

This study confirms the persistent high prevalence of RVGE among children under-five admitted for AGE to hospitals in different parts of Myanmar and the diversity of rotavirus strains. Given this high burden of rotavirus hospitalizations prior to vaccine introduction in Myanmar, a rotavirus vaccine effectiveness of even 49–64% (which has been reported from other high to medium child mortality Asian countries) could have a dramatic impact on reductions in rotavirus hospitalizations [20].The rotavirus vaccine Rotarix was introduced nationwide in Myanmar in February 2020. These data immediately precede vaccine introduction and will be useful as important baseline data for post-vaccination monitoring of vaccine impact and circulating strains.

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Highlights

- This study describes rotavirus epidemiology before vaccine introduction in Myanmar.
- Rotavirus detection was 45.7% in May 2018–Apr 2019 and 42.5% in May 2019–Jan 2020.
- The highest proportion of positivity was among 6–11 month old children (41.6%).
- Diversity of rotavirus strains over time before vaccine introduction was found.
- This study will be important baseline data for monitoring of vaccine impact.

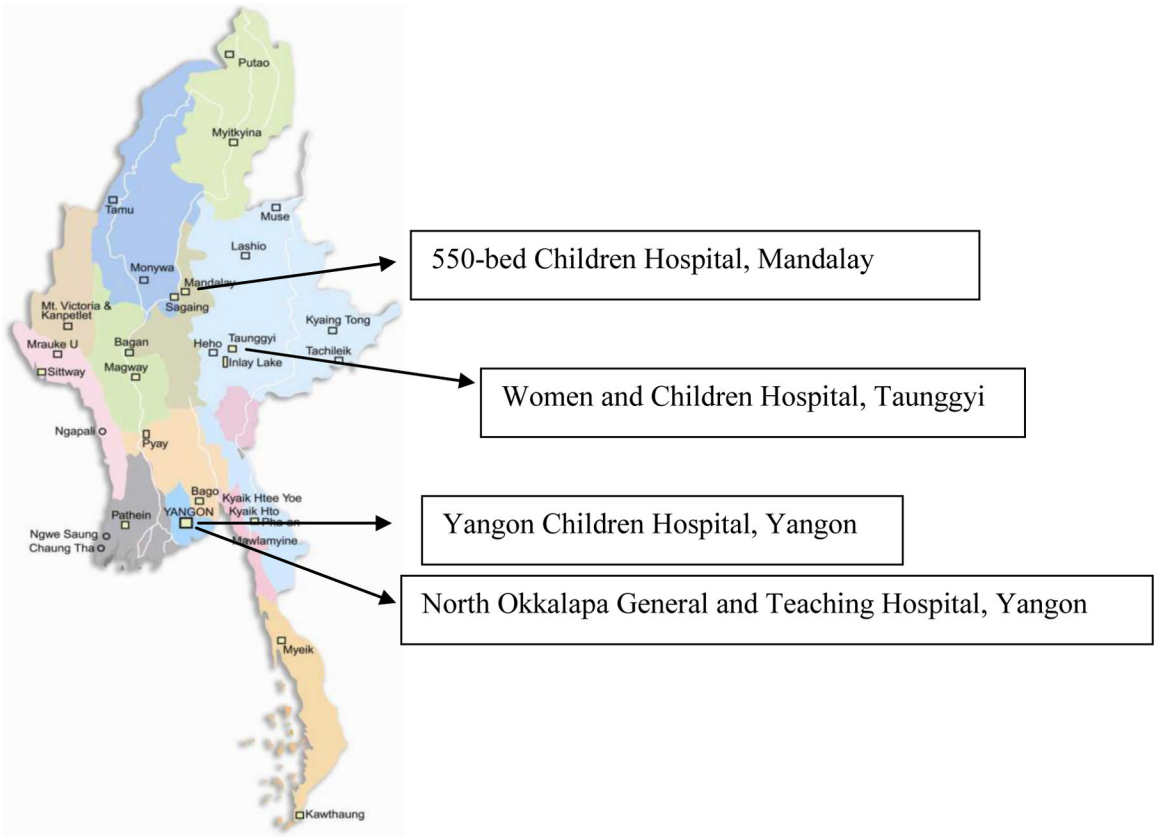

Figure (1).
Sentinel surveillance sites in Myanmar

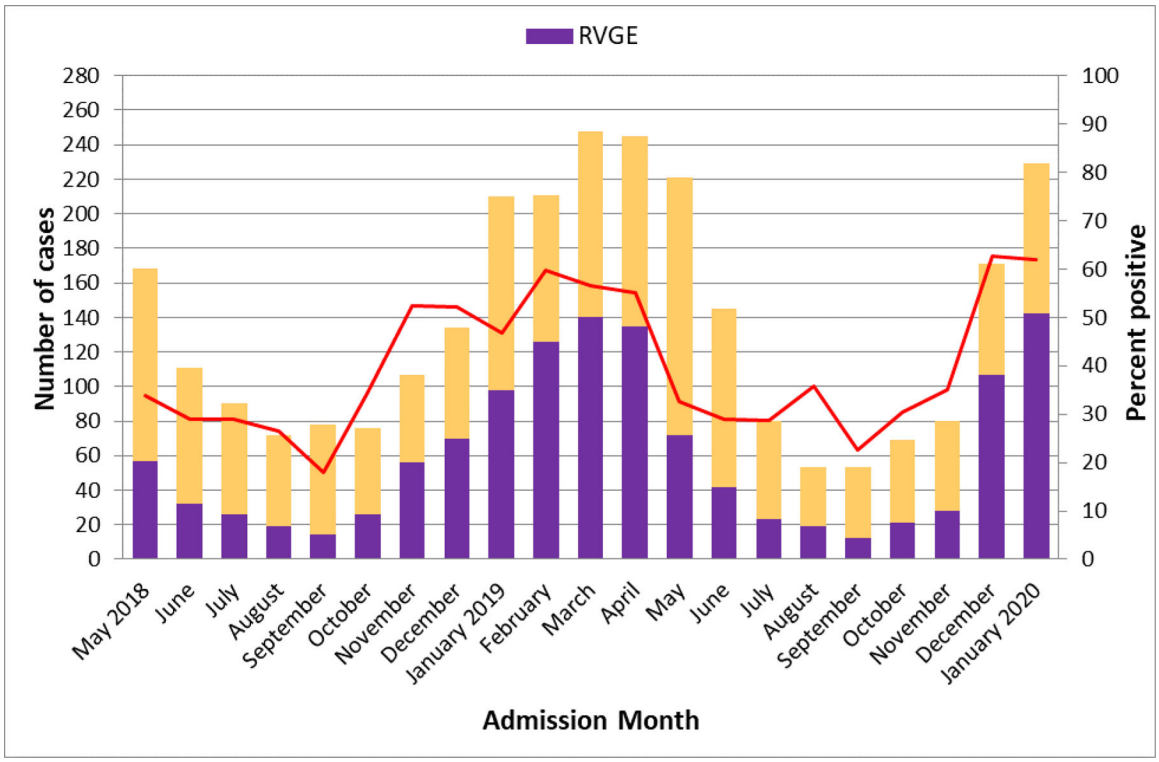

Figure (2).

Number and positivity of rotavirus stool specimens among children <5 years old by month, Myanmar May 2018- January 2020.

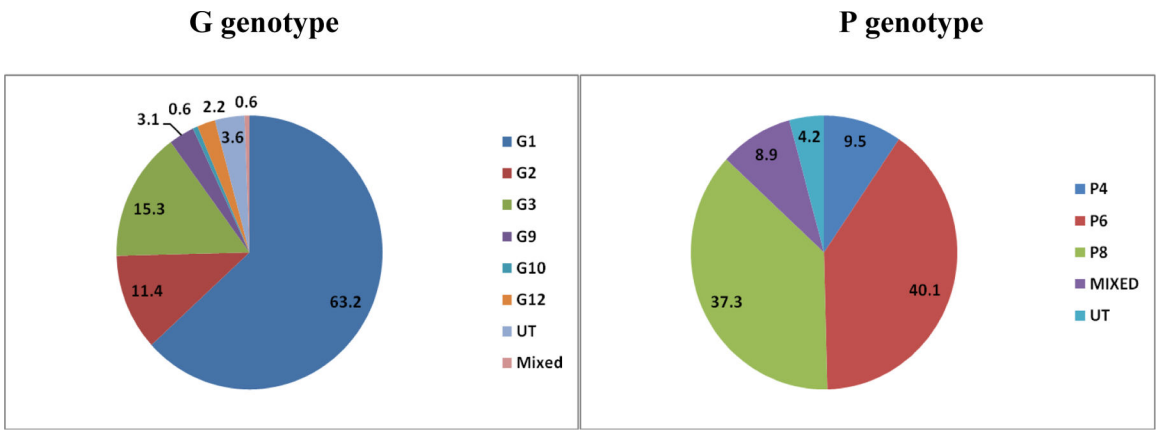

Figure (3). Distribution of rotavirus G genotypes and P genotypes

Mixed = concurrent infection of more than one G/P genotypes

UT = Untypable genotype

Table (1)

Demographic and clinical characteristics of 2,977 children <5 years of age hospitalized with acute gastroenteritis, Myanmar (2018–2020)

Characteristics	RVGE N (%)	Non-RVGE N (%)	p-value
Total	**1320 (44.3)**	**1657 (55.7)**	
Age in months			
0–5	189 (14.3)	297 (17.9)	**<0.01**
6–11	549 (41.6)	664 (40.1)	
12–23	485 (36.7)	528 (31.9)	
24–59	97 (7.3)	168 (10.1)	
Gender			
Male	775 (58.7)	997 (60.2)	0.42
Female	545 (41.3)	660 (39.8)	
Symptoms			
Vomiting	1034 (78.3)	1129 (68.1)	**<0.01**
Fever	868 (65.8)	1015 (61.3)	**0.01**
Dehydration			
Severe	48 (3.6)	35 (2.1)	**<0.01**
Some	1189 (90.1)	1458 (88.0)	
None	83 (6.3)	164 (9.9)	
Rehydration therapy			
ORS	548 (41.5)	761 (45.9)	**<0.01**
IV	770 (58.3)	880 (53.1)	
Others	2 (0.2)	16 (1.0)	
Outcome			
Died	2 (0.2)	0 (0.0)	
Surveillance Period [1]			
May 2018— April 2019	799 (45.7)	951 (54.3)	0.08
May 2019—January 2020	521 (42.5)	706 (57.5)	

[1]Three sites (Yangon Children's Hospital, Mandalay Children Hospital, and Women and Children Hospital in Taunggyi) with surveillance data from May 2018 to April 2019. A fourth site (North Okkalapa General and Teaching Hospital) conducted surveillance from May 2019 to January 2020.

RVGE = rotavirus gastroenteritis; ORS = oral rehydration solution; IV = intravenous

Table (2)

Distribution of a subset of rotavirus genotypes by region in Myanmar (N=359). The predominant genotype in each region has been indicated in bold type.

	Lower Myanmar N (%)	**Middle Myanmar N (%)**	**Eastern Myanmar N (%)**	**Total**
G1P[6]	41 (22.7)	**58 (49.2)**	14 (23.7)	**113 (31.5)**
G1P[8]	**59 (32.6)**	17 (14.4)	**18 (30.5)**	94 (26.2)
G2P[4]	26 (14.4)	2 (1.7)	5 (8.5)	33 (9.2)
G2P[6]	0	3 (2.5)	0	3 (0.8)
G2P[8]	0	2 (1.7)	0	2 (0.6)
G3P[4]	1 (0.6)	0	0	1 ((0.3)
G3P[6]	9 (5.0)	9 (7.6)	5 (8.5)	23 (6.4)
G3P[8]	15 (8.3)	0	2 (3.4)	17 (4.7)
G9P[6]	0	1 (0.8)	0	1 (0.3)
G9P[8]	1 (0.6)	4 (3.4)	0	5 (1.4)
G10P[6]	0	1 (0.8)	1 (1.7)	2 (0.6)
G12P[8]	8 (5.0)	0	2 (3.4)	11 (3.1)
Mixed	13 (7.2)	11 (9.3)	5 (8.5)	29 (8.1)
Partially typed	7 (3.9)	10 (8.5)	6 (11.8)	24 (6.7)
Untypable	1 (0.6)	0	0	1 (0.3)
Total	**182 (100)**	**118 (100)**	**59 (100)**	**359 (100)**

Mixed = concurrent infection of more than one G/P genotypes

Partially typed = Either G or P genotype is identified

UT = Untypable genotype