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Surveillance of Rotavirus Gastroenteritis (2015-2017): Epidemiology and Circulating Rotavirus Genotypes in Pre-rotavirus Vaccine Introduction Period in Myanmar

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Abstract

A hospital-based prospective active surveillance for rotavirus gastroenteritis (RVGE) was conducted among hospitalised acute gastroenteritis (AGE) patients to identify the infecting rotavirus strains and to provide epidemiological information on RVGE in Myanmar. Stool samples were collected from children less than 5 years old admitted to Yangon Children's Hospital (YCH) for AGE during January 2015 to September 2017. Collected stool samples were screened for rotavirus antigen by ELISA and genotyped by reverse transcription polymerase chain reaction (RT-PCR). Overall, 48.8% (1,167/2,393) of samples were ELISA positive for rotavirus and the most affected were children aged 6-23 months, 81.9% (956/1,167). RVGE occurred in a seasonal cycle with peak detection in the cold and dry months (November to February). As compared with non-RVGE, RVGE cases had significant higher percentage of vomiting (84.5% versus 73.0%; P<0.05), fever (80.1% versus 71.8%; P<0.05) and severe clinical scoring (79.4% versus 67.5%; P<0.05). Genotyping revealed that G9P[8] was predominant in the year 2015 (53.3%) and 2016 (30.9%), but it was replaced in 2017 by G3P[8] (58.2%). Information from this surveillance not only highlights facts for consideration of rotavirus vaccine introduction plan in pre-vaccination era, but also provides vital baseline data for post-vaccination monitoring of vaccine impact and effectiveness.

Keywords: rotavirus, gastroenteritis, pre-vaccination, Myanmar

Introduction

Acute gastroenteritis (AGE) is among the top causes of childhood mortality worldwide with rotavirus as the leading cause of severe AGE in children less than 5 years of age.[1] The annual global burden of rotavirus deaths in 2013 in this age group is estimated at 215,000 (range 197,000 – 233,000 deaths) and approximately half (49%) of these deaths occurred in India, Nigeria, Pakistan and the Democratic Republic of Congo.[2]

In order to reduce the burden and mortality of rotavirus gastroenteritis (RVGE), prevention by vaccination is considered to be critical, because it cannot be prevented with just improvements in sanitation and hygiene practices.[3] The World Health Organization (WHO) recommended that rotavirus vaccine (RV) be incorporated in the childhood immunisation programme and particularly in those with high child mortality due to diarrhea such as Southeast Asia and Sub-Saharan Africa. Two currently available vaccines with demonstrated efficacy against severe rotavirus disease are Rotarix (GSK Bio, a monovalent vaccine containing a single G1P[8] strain) and RotaTeq (Merck, containing 4 common G types (G1–G4) and 1 common P type P[8]).[4] As of August 2018, 96 countries around the world including 46 low-income countries have introduced rotavirus vaccines in their universal immunisation programs which have demonstrated dramatic impact. According to the WHO report, there was 40% decrease in the prevalence of rotavirus in countries that introduced the vaccine and rotavirus-related child deaths was reduced from 800,000 in 1985 to 215,000 in 2016.[5]

In Myanmar, the National Health Plan (2011-2016) declared diarrhea as a high-priority childhood

disease.[6] Also Myanmar is a member of the Global Rotavirus Surveillance Network (GRSN) and has been conducting hospital-based rotavirus surveillance since 2009. This surveillance is funded by WHO. Every year, more than 80% of eligible AGE cases were enrolled and stool samples were collected and tested for rotavirus. The surveillance data revealed that the proportion of RVGE among hospitalised less than 5 years old children with diarrhea at Yangon Children's Hospital (YCH) ranged from 42% to 56% during 2009-2014 which demonstrated a high burden of the disease and called for introduction of rotavirus vaccine.[7] Before introducing vaccines into target populations, the baseline data of the epidemiology of rotavirus infection must be established. Therefore, this study was conducted to provide information on the epidemiology of rotavirus infection and circulating rotavirus genotypes to establish vital baseline, as well as determine key factors to decision makers for vaccination plan, including target population, dose scheduling and selection of appropriate vaccines in pre-vaccination era.

Methods

Study Design and Setting

A cross-sectional, active hospital-based sentinel surveillance for RVGE among children under 5 years of age was conducted at YCH from January 2015 through September 2017 according to the World Health Organization (WHO) generic protocol.[8] YCH is the largest and main tertiary care referral pediatric hospital in Yangon with a pediatric inpatient unit consisting of three wards and 1,300 beds. Approximately 10,000 to 13,000 children under 5 years of age are admitted year-round to these wards, with admissions to each ward occurring on a rotating basis.

Inclusion and Exclusion Criteria for Participants

Inclusion criteria for patients eligible for enrollment were children less than 5 years of age, who presented with diarrhea (≥3 looser-than-normal stools in a 24-hour period during the illness, with onset of diarrhea ≤14 days at presentation) and treated in one of the three pediatric-medical wards of YCH.[8] Exclusion criteria was presence of either blood or mucus in the stool or failure to obtain an informed consent from parents or legal guardians of patient.

Surveillance Methods

Active case finding was conducted from every Monday to Friday during the study period in the pediatric-medical wards. Only the patients on the first post-admission day were approached. As such, children admitted on late Friday or Saturday were not recruited, and children admitted on Sunday were approached for enrollment on Monday. All hospitalized AGE patients of less than 5 years old, who met the selection criteria were enrolled in the study.

Upon enrollment, a case report form containing information on demographics, clinical history, physical examination, treatment and outcome was completed. A stool sample containing not less than 3 ml was collected within 48 hours of admission, using wide-mouth screw capped bottles. The bottles were labeled, kept below 4°C, and transported in the same day to the Virology Research Division laboratory at the Department of Medical Research, and subsequently stored at -20°C until testing was performed. Upon discharge, the date and outcome of the cases were recorded on the case report form.

Laboratory Analysis

All samples were tested for rotavirus antigen by ProSpecTTM Rotavirus ELISA kit, Oxoid, UK, according to the manufacturer's instructions. Patients with a positive test result were defined as RVGE cases. 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 using QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Germany) and the extracted dsRNA was amplified by RT-PCR using specific oligonucleotide primers provided by the WHO Rotavirus Reference Laboratory, CMC, Vellore, India.[9]

Statistical Analysis

Data were entered into Microsoft Excel. For descriptive analyses, number and percentage were calculated and Chi-square test was performed to determine statistically significant differences between RVGE and non-RVGE groups regarding characteristics of patients, clinical presentations and outcomes. A *p*-value less than 0.05 was considered significant.

Ethical Consideration

This study was approved by the Ethics Review Committee, Department of Medical Research, Myanmar. Written informed consent was obtained from the parents or guardians of children prior to enrolment.

Working Definitions

Acute gastroenteritis is the sudden onset of diarrhea and/or vomiting, usually with three or more bouts of diarrhea or vomiting and diarrhea.[10] Also, the severity of the disease is assessed using Vesikari Clinical Severity Scoring System.[11]

Results

From January 2015 to September 2017, 2,939 eligible children were identified by the surveillance system. Of these, 84.8% (2,494/2,939) were enrolled and stool samples were collected from patients 95.9% (2,393/2,494). Among them, 48.8% (1,167/2,393) were

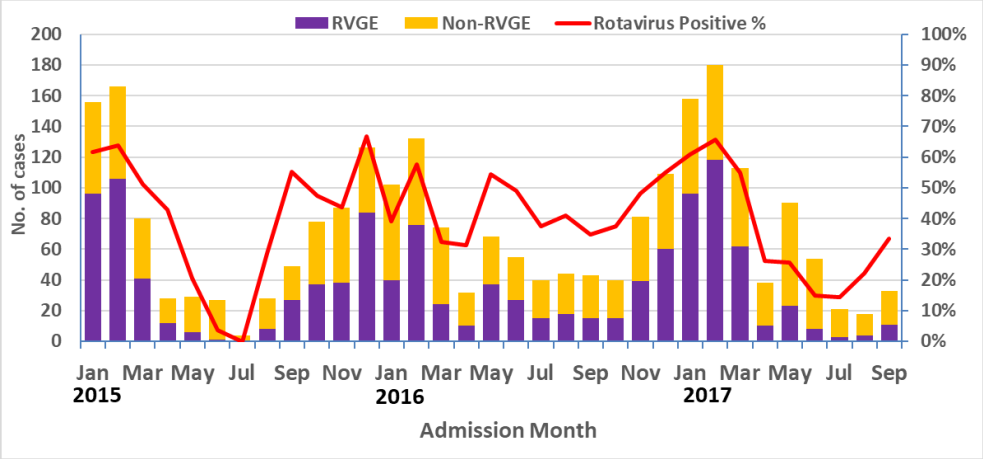

Figure 1. Number of RVGE, non-RVGE cases and percentage of rotavirus positive among hospitalized <5 years old children by month, YCH, September 2015-2017

rotavirus positive and the proportion of rotavirus positivity was 52.5% (456/868), 45.9% (376/820) and 47.5% (335/705) in 2015, 2016 and 2017 respectively. AGE cases are admitted to YCH year round, and rotavirus positivity has also been detected year round except July 2015. High numbers of AGE cases due to rotavirus were observed from November through February, which comprised 58-66% positivity of each year, and often peaked in February (Figure 1).

Table 1 compared the demographic characteristics, clinical presentations and outcome of RVGE and non-RVGE cases. Male to female ratio was 1.6:1 among both RVGE and non-RVGE cases.

The 6-23 months age group accounted for the majority (81.9%) of rotavirus cases, followed by the 0-5 months age group (9.1%) and the 24-59 months age group (9.0%). Additionally, the 6-23 months age group also had a significantly higher percentage of rotavirus positive (52.1%) compared to 0-5 months age group (34.9%) and 24-59 months age group (41.3%). Both overall and annually, nearly 60% of the RVGE cases were found among children aged less than 12 months, and over 90% among children aged less than 24 months (Table 2).

Hospitalised AGE cases commonly presented with vomiting, fever and dehydration. Among them, vomiting (84.5% versus 73.0%; P<0.01), fever (80.1% versus 71.8%; P<0.01) and severe clinical score (79.4% versus 67.5%) were more frequently observed in RVGE compared with non-RVGE patients. Furthermore, dehydration (76.0% versus 73.4%) and hospital stay of more than 5 days (3.0% versus 2.0%) were also more frequently observed in RVGE although not statistically significant (Table 1).

Distribution of rotavirus genotypes by seasonal year (from July to June of the following year) is shown in Table 2 and Table 3. G9 was the most prevalent G genotype in 2014-2015 and 2015-2016 and accounted for 79.3% and 50.6% respectively of the genotypes. However, G3 was the most common genotype in 2016-2017 accounting for 60.0%. Regarding P genotype distribution, P[8] was consistently predominant throughout the study period accounting for 66.7%, 69.1% and 83.6% in 2014-2015, 2015-2016 and 2016-2017 respectively.

From July 2014 to June 2015, G9P[8] was predominant, and accounted for 53.3% (72/135) followed by partially typed strain 39.4% (41/135). The G9P[8] strain was still predominant in 2015-2016 and partially typed strain accounted up to 41.9% (34/81). However, the most prevalent strain changed to be G3P[8] in 2016-2017, and accounted for 58.2% (32/55).

Discussion

The percentage of rotavirus positivity ranged from 46% to 53% in the present study during 2015-2017, highlighting that RVGE burden is persistent in Myanmar when compared to the previous data of 42-56% during 2009-2014.[7] This proportion was also similar to that of the neighboring countries; 53% in Kolkata in 2011-2013, and 42% in Bangladesh in 2008-2012.[10,13] According to the WHO's rotavirus surveillance network data, the median rotavirus detection in the Southeast Asian Region was 35% in 2012-2013 with Myanmar having the highest proportion at 47%.[14] This study additionally demonstrated that rotavirus infection has a strong seasonal peak in colder, drier months as seen in other Asian countries.[15]

Table 1. Characteristics, clinical presentations and outcome of hospitalised children with rotavirus gastroenteritis and non-rotavirus gastroenteritis, September 2015-2017 (n=2,393)

Characteristics	RVGE (%) n=1,167 (48.8%)	Non RVGE (%) n=1,226 (51.2%)	*P* value
Sex			
Male	721 (61.8%)	753 (61.4%)	0.86
Female	446 (38.2%)	473 (38.6%)	
Age in months			
0-5 months	106 (9.1%)	198 (16.2%)	
6-23 months	956 (81.9%)	879 (71.6%)	<0.01
24-59 months	105 (9.0%)	149 (12.2%)	
Clinical Symptoms			
Vomiting	986 (84.5%)	895 (73.0%)	<0.01
Fever	935 (80.1%)	880 (71.8%)	<0.01
Dehydration	887 (76.0%)	900 (73.4%)	0.14
Vesikari Clinical Score			
Mild (<7)	24 (2.1%)	26 (2.1%)	
Moderate (7-10)	216 (18.5%)	373 (30.4%)	<0.01
Severe (≥11)	927 (79.4%)	827 (67.5%)	
Hospital Stay			
<2 days	93 (8.0%)	123 (10.0%)	
2-5 days	1,039 (89.0%)	1,079 (88.0%)	0.06
>5 days	35 (3.0%)	24 (2.0%)	
Outcome			
Recovery	1,167 (100.0%)	1,225 (99.9%)	0.49
Expired	0	1 (0.1%)	

This study showed male predominance in both RVGE and non-RVGE groups which is in accordance with the findings of other studies, such as in Lahore, where 60% of enrolled children were male, and Uganda, where 61% of children were male.[16,17] The factors underlying this difference are poorly understood and further study is warranted. Regarding the age distribution of RVGE patients, the highest proportion of rotavirus positive was among children 6-23 months of age and more than 90% of the cases had rotavirus infection by their second birthday. These results are in line with previous studies conducted prior to vaccine introduction in other countries.[18] Thus, the WHO's recommended dose schedule is applicable in Myanmar.[19]

Regarding the clinical presentations, vomiting, fever and severe clinical score were found to be significantly associated with rotavirus positivity. This finding is also in line with the findings of other studies where RVGE cases presented with more severe clinical manifestations compared to rotavirus negative cases.[20] Of enrolled children, only one patient, a 7-month-old male admitted in April 2016 who presented with high fever, severe dehydration and shock, and tested rotavirus negative, expired.

This study identified a different profile of genotype distribution by seasonal years. G9P[8] was predominant in 2014-2015 and continued its predominance in the following year 2015-2016. However in 2016-2017, G9P[8] disappeared and G3P[8] replaced it as the most predominant strain. In the previous study during 2009-2014, G12P[8] predominated consecutively for 3 years from 2009-2012 , and then the predominant strain changed to

Table 2. Distribution of rotavirus G genotypes by seasonal year, September 2015-2017

Genotype	G1 (%)	G2 (%)	G3 (%)	G9 (%)	G12 (%)	Mixed (%)	UT (%)
2014-2015 (n=135)	2.2	3.0	0	79.3	0	2.2	13.3
2015-2016 (n=81)	13.6	2.5	4.9	50.6	2.5	4.9	21.0
2016-2017 (n=55)	7.3	12.7	60.0	0	0	18.2	1.8

G2P[4] in 2012-2013 and again to G1P[8] in 2013-2014. This changing pattern is in agreement with the WHO's genotype distribution reported both globally and regionally with higher resemblance to regionally reported strains.[21] From 2009-2011, the globally dominant genotype was G1P[8], although the G12P[8] strain emerged in Southeast Asian Region starting from 2010. The dominant strain globally as well as regionally in 2013 and 2014 was G1P[8] and G9P[8] respectively.[15,20,22,23] Diversity of circulating rotavirus strains is reported regardless of vaccine use.[24] Enhanced surveillance is needed from the perspective of devising future vaccine strategies and monitoring strain specific vaccine effectiveness.

Limitations

This analysis was subject to limitations. This was a hospital based surveillance and conducted at one site only up to 2017, although YCH is the largest children's hospital and the major pediatric referral centre and its patients are drawn not only from urban and rural areas of Yangon Region, but also from other States and Regions especially from the lower part of Myanmar, and likely captures the epidemiological profile of a large portion of our population of interest.

Table 3. Distribution of rotavirus P genotypes by seasonal year, September 2015-2017

Genotype	P[4] (%)	P[6] (%)	P[8] (%)	Mixed (%)	UT (%)
2014-2015 (n=135)	1.5	3.7	66.7	4.5	23.6
2015-2016 (n=81)	0	1.2	69.2	0	29.6
2016-2017 (n=55)	0	9.1	83.7	3.6	3.6

Conclusions

The RVGE burden in Myanmar urges consideration of introducing rotavirus vaccine. The high proportion of RVGE is in children less than 5 years hospitalized with AGE, and the diversity of circulating genotypes and epidemiological patterns reported in this study provide vital inputs for vaccine programmers in planning vaccine introduction and monitoring vaccine impact and effectiveness in post-vaccine introduction period. As the GRSN recommendation, surveillance should be carried out continuously to assess disease trends over time, to monitor circulating genotypes after vaccine introduction and to serve as a platform for vaccine effectiveness and safety evaluation.

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