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Epidemiology and strain characterization of rotavirus diarrhea in Malaysia

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KEYWORDS
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Summary
Objectives: The objectives of the study were to describe the epidemiology and strain characterization of rotavirus (RV), to determine the proportion of hospitalizations for diarrhea attributable to RV among children under 5 years of age, and to estimate the disease burden of RV diarrhea in Malaysia.
Methods: All children 0—59 months of age admitted for acute gastroenteritis to Kuala Lumpur Hospital (KLH) or Hospital Umum Sarawak (HUS) were surveyed. The periods of surveillance were from February 1, 2001 to April 30, 2003 in KLH and April 1, 2001 to March 31, 2003 for HUS.
Results: The highest rate of RV-associated diarrhea was among children aged 6—17 months, accounting for 55% of RV-associated diarrhea. There was no seasonality observed in either hospital. P[8]G9 strains were predominant, accounting for 73% of all strains in both hospitals, 80% from KLH and 61% from HUS. There was no mortality.
Conclusions: RV was responsible for 38% of hospitalizations for diarrhea. It was most common in the 6—17 months age group. There was no seasonality observed for RV-associated diarrhea. The most prevalent strain of RV was P[8]G9. The estimated incidence of RV-associated diarrhea was 27 per 10 000 population under the age of 5 years per year.

Introduction

Rotavirus (RV) infection is the leading cause of severe gastroenteritis among infants and young children worldwide.[1] Each year, RV causes approximately 111 million episodes of gastroenteritis that require home care only, 25 million clinic visits, and 2 million hospitalizations in children under 5 years of age worldwide.[2] RVs form the genus *Rotavirus* of the Reoviridae family and possess a genome consisting of 11 segments of double-stranded RNA. The genome and several enzymes necessary for viral replication (VP1, VP3) are enclosed in a

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triple layer of proteins: the core layer (VP2), the middle layer (VP6), and the outer layer (VP7 and VP4). VP6 determines the group specificity (A—G), VP7 the G type and VP4 the P type. For G types, a complete concordance of serotypes and genotypes has been achieved, while for P types, it is not the case. Accordingly, the P serotype is indicated by a free Arabic number (e.g., P1, P2), and the P genotype is indicated by numbers in brackets (e.g., P[8], P[12]).

In developed countries, acute gastroenteritis in infants and young children is still an important cause of morbidity with significant health costs. It remains a leading cause of death in developing countries. In Malaysia, diarrhea was one of the top five leading causes of death in children aged 1—19 years in the 1970s—1980s. Over the last decade, sepsis has overtaken diarrhea as one of the top five leading causes of death. However, diarrheal diseases remain one of the leading causes of childhood morbidity, and rotavirus diarrhea is the most important cause of childhood acute gastroenteritis in this country.[3–5]

The objectives of the study were to describe the epidemiology and strain characterization of RV in two large cities in Malaysia, to determine the proportion of hospitalizations for diarrhea attributable to RV among children under 5 years of age, and to use these data to estimate the disease burden of RV diarrhea in Malaysia.

Methods

Study area and population

This was a prospective hospital-based study in collaboration with the Centers for Disease Control and Prevention (CDC), Atlanta, USA. The World Health Organization (WHO) Generic Protocol for hospital-based surveillance to estimate the burden of RV gastroenteritis was used. Two government hospitals were selected for the study; the Kuala Lumpur Hospital (KLH) Institute of Paediatrics is located in Peninsular Malaysia while Hospital Umum Sarawak (HUS) is located in Kuching, East Malaysia. All children aged 0—59 months admitted for treatment of acute gastroenteritis to KLH or HUS were enrolled in the study. The periods of surveillance were from February 1, 2001 to April 30, 2003 in KLH and from April 1, 2001 to March 31, 2003 for HUS.

Specimen collection

For children who were eligible for the study, demographic data were extracted from the medical records and a whole stool sample was obtained within the first 48 hours of admission. The first stool specimen collected from the patient was sent to the laboratory in KLH, daily for KLH specimens and weekly for HUS specimens. Stool specimens were tested for RV antigen using enzyme immunoassay (EIA) (Rotaclone, Meridian Diagnostics, Cincinnati, OH, USA) at KLH laboratory. A child was considered to have RV-associated diarrhea if the stool tested positive for RV antigen using the EIA (Rotaclone) test.

Strain characterization

From August 2001 to July 2002, stool specimens of children tested positive for RV antigen using the EIA were sent for molecular characterization of RV G and P types. Stool samples were transported from KLH to Universiti Putra Malaysia on ice and stored at −80 °C. The RV RNAs were extracted from the diluted stools using protocols from the manufacturer PUREScript (Gentra Systems Inc. Minneapolis, MN, USA). cDNA synthesis was performed on the RNA extracts according to the protocols from RETROscript (Ambion Inc. Austin, TX, USA). Subsequently, the synthesized cDNAs were further characterized for VP4 (P genotypes) and VP7 (G serotypes) using RT-PCR methods. The multiplex PCR uses specific primers for G and P genotypes provided by CDC, Atlanta. The PCR products were then analyzed on 2% agarose gel electrophoresis and run at 75 V for 30 minutes.

Results

A total of 3317 patients were admitted for diarrhea during the study period. There were 2353 (70.9%) patients from KLH and 964 (29.1%) from HUS. Stool samples were collected from 2668 (80.4%) patients; 1756 (74.6%) from KLH and 912 (94.6%) from HUS. Overall, 1265 (38.1%) of the diarrhea patients were positive for RV with a yield of 820 (34.8%) from KLH and 445 (46.2%) from HUS (Table 1). Of the 1265 RV-associated diarrhea patients, 784 were male and 481 female, giving a male to female ratio of 1.63:1. Of all the diarrhea patients, 57.8% were Malay, 10.8% were Chinese, 14.1% were Indians, and 17.3% were of other ethnic groups including the Iban and Dayak.

In the year 2002, there was a total of 27 011 admissions to KLH Institute of Paediatrics, and 19 884 of these were general

Table 1 Number of diarrhea hospitalizations enrolled in Malaysian study centers from February 2001 to April 2003

	KLH	HUS	Total
No. of diarrhea cases	2353 (70.9%)	964 (29.1%)	3317 (100.0%)
No. of stools screened	1756 (74.6%)	912 (94.6%)	2668 (80.4%)
No. of RV positive stools	820 (34.8%)	445 (46.2%)	1265 (38.1%)

KLH, Kuala Lumpur Hospital; HUS, Hospital Umum Sarawak; RV, rotavirus.

Table 2 Age distribution of diarrhea patients and rotavirus positive stool for children aged 0—59 months in Malaysia from February 2001 to April 2003

Age group (months)	No. of patients	No. (%) RV positive	Cumulative (%) RV positive
0—2	108	14 (13.0)	14 (1.1)
3—5	268	59 (22.0)	73 (5.8)
6—8	470	178 (37.9)	251 (19.8)
9—11	466	187 (40.1)	438 (34.6)
12—17	765	336 (43.9)	774 (61.2)
18—23	334	141 (42.2)	915 (72.3)
24—35	478	186 (38.9)	1101 (87.0)
36—47	263	114 (43.3)	1215 (96.0)
48—59	165	50 (30.3)	1265 (100.0)
Total	3317	1265 (38.1)	1265 (100.0)

RV, rotavirus.

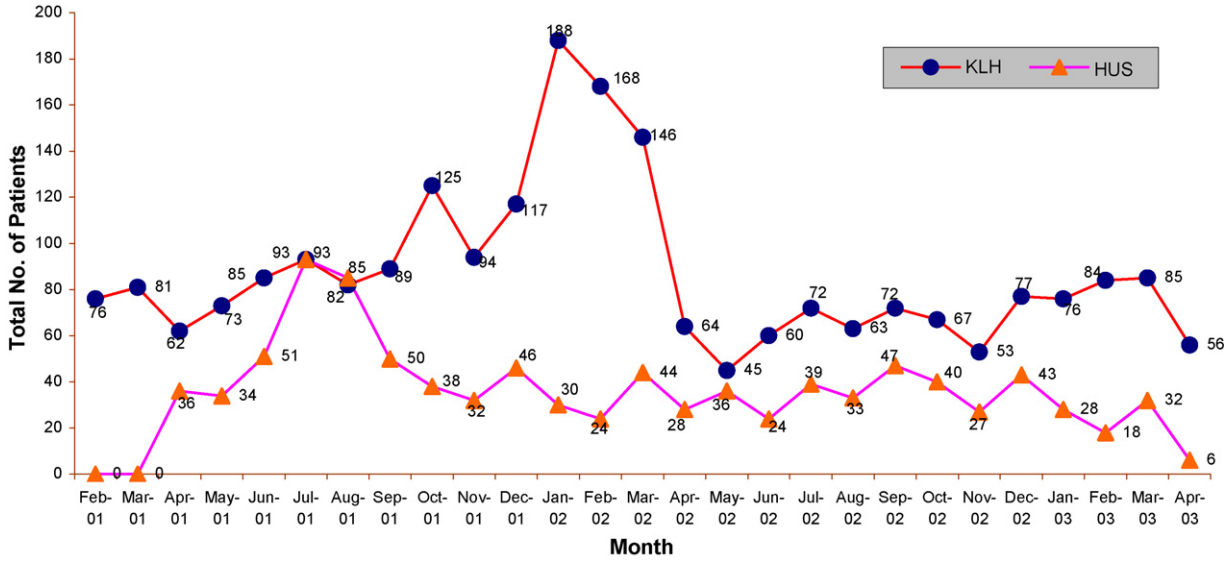

Figure 1 Total number of hospitalizations for diarrhea in Kuala Lumpur Hospital (KLH) and Hospital Umum Sarawak (HUS) from February 2001 to April 2003.

pediatric admissions. Diarrhea and gastroenteritis hospitalizations constituted 4.0% (1075/27 011) of total admissions and 5.4% (1075/19 884) of general pediatric admissions to KLH. In HUS, there was a total of 6792 pediatric admissions, and 6385 of these were general pediatric admissions. Diarrhea and gastroenteritis hospitalizations constituted 6.1% (415/6792) of total pediatric admissions and 6.5% (415/6385) of general pediatric admissions in HUS. Therefore, acute diarrhea and gastroenteritis hospitalizations constituted 5.6% of the total general pediatric admissions to the two hospitals.

RV was found to be a cause of hospitalization among children of all age groups in the study population. The highest rate of RV-associated diarrhea was among children aged 6—17 months, who accounted for 55.4% of all cases. About a quarter (26.6%) of children with RV-associated diarrhea were aged 12—17 months, 14.8% were infants aged 9—11 months, and 14.1% were infants aged 6—8 months. While only 5.8% of all RV-associated diarrhea occurred amongst infants less than 6 months old, still 22% of infants aged 3—5 months and 13% of infants less than 2 months old with diarrhea had RV in their stools. Seventy-two percent of the RV-associated diarrhea occurred in children under 2 years old (Table 2). There was no mortality from rotavirus diarrhea in this study.

Over the two-year study period, there was no seasonality observed in either site. The number of diarrhea or gastro-

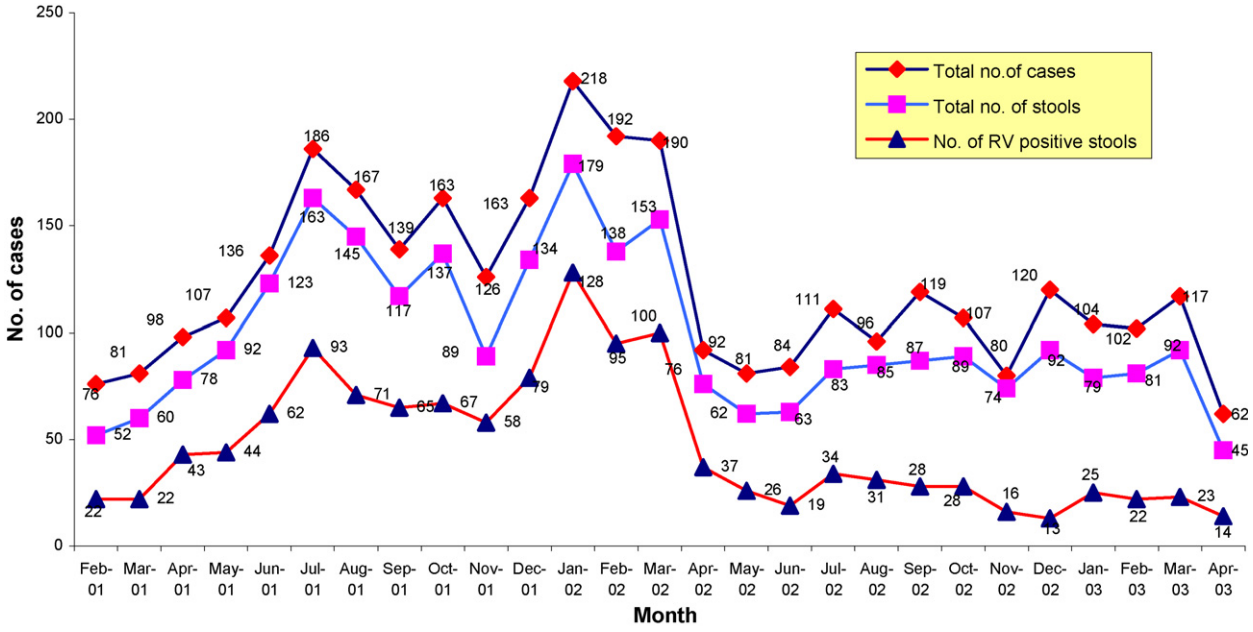

Figure 2 Total number of hospitalizations for diarrhea and rotavirus-associated diarrhea in both Kuala Lumpur Hospital and Hospital Umum Sarawak from February 2001 to April 2003.

Table 3 The primers used in G and P typing and the number of RV detected

Product name	Sequence (5′ to 3′)	Strain	Total number of RV (%)
G type			
9con1	tag ctc ctt tta atg tat gg	Wa/G1	7 (4.5)
9T-1	tct tgt caa agc aaa taa tg	Wa/G1	
9T-2	gtt aga aat gat tct cca ct	S2/G2	8 (5.1)
9T-3P	gtc cag ttg cag tgt agc	107e1B/G3	0
9T-4	ggg tcg atg gaa aat tct	ST3/G4	0
9T-9B	tat aaa gtc cat tgc ac	116E/G9	120 (76.4)
Untypeable	—	—	22 (14.0)
Total			157 (100)
P type			
con3	tgg ctt cgc tca ttt ata gac a	KU/P[8]	126 (80.3)
1T-1	tct act tgg ata acg tgc	KU/P[8]	
2T-1	cta ttg tta gag gtt aga gtc	RV-5/P[4]	8 (5.1)
3T-1	tgt tga tta gtt gga ttc aa	1076/P[6]	0
4T-1	tga gac atg caa ttg gac	K8/P[9]	0
5T-1	atc ata gtt agt agt cgg	69M/p[10]	0
ND2(JRG237)	agc gaa ctc acc aat ctg	116E/P[11]	0
Untypeable	—	—	23 (14.6)
Total			157 (100)

RV, rotavirus.

enteritis patients admitted to the two hospitals was not associated with wet or dry seasons. There were two peaks, July 2001 and January—February 2002, but there were no similar peaks observed in July 2002 and January—February 2003 (Figure 1). The trend in the number of RV-associated diarrhea cases was similar to the number of diarrhea and gastroenteritis admissions (Figure 2). There was an outbreak of acute gastroenteritis in KLH in January—February 2002; this was not observed in HUS Kuching. There was also no similar outbreak in January—February 2003. Similarly, there was an increase in the total number of diarrhea patients in Kuching in July—August 2001 with no similar increase the following year (Figure 1).

From August 2001 to July 2002, there was a total of 779 RV positive stools. Out of these, 157 isolates were selected for G and P characterization by RT-PCR. The index of G genotyping was 86.0% with the following proportion: G9 (76.4%), G2 (5.1%), and G1 (4.5%). There were no G3 and G4 genotypes or mixed infections detected. Twenty-two (14.0%) of the specimens were untypeable for G type. For P genotype, P[8] was predominant with 126 (80.3%) of tested specimens positive (Table 3).

The predominant G- and P-type combinations were P[8]G9 (73.3%) and P[4]G2 (4.5%). P[8]G9 strains were the most common strain in both sites; 80% for children admitted to KLH and 61% for those admitted to HUS. A total of 76.5% were G9 strain either in combination or in isolation. A total of 84.1% were of P[8] genotype, of which 73.3% were P[8]G9, 3.8% P[8]G1, and 7.0% P[8] alone. Overall, 6.4% of the strains were untypeable, 1% from KLH and 15.7% from HUS (Table 4). The reasons for these strains being untypeable could be due to storage degradation, the probes used were not sensitive enough, or the existence of untypeable strains with the present available probes.

Table 4 Strain characterization of rotavirus in Malaysia by centers from August 2001 to July 2002

RV Strain	KLH (%)	HUS (%)	Total (%)
P[8]G9	80 (80.0)	35 (61.4)	115 (73.3)
P[8]G1	2 (2.0)	4 (7.0)	6 (3.8)
P[4]G2	5 (5.0)	2 (3.5)	7 (4.5)
P[4]	1 (1.0)	0 (0.0)	1 (0.6)
P[8]	7 (7.0)	4 (7.0)	11 (7.0)
G1	0 (0.0)	1 (1.8)	1 (0.6)
G2	0 (0.0)	1 (1.8)	1 (0.6)
G9	4 (4.0)	1 (1.8)	5 (3.2)
Untypeable	1 (1.0)	9 (15.7)	10 (6.4)
Total	100 (100)	57 (100)	157 (100)

Discussion

Acute diarrhea and gastroenteritis hospitalizations constituted 5.6% of the total general pediatric admissions to the two hospitals. This was lower than the 8.2% of pediatric admissions per year in a teaching hospital in Kuala Lumpur in 1996—1999.[6] In our study, 72% of the RV-associated diarrhea occurred in children under 2 years of age, similar to a study done in 1996—1999.[6] As in other studies, RV-associated diarrhea was more common in males, with a male to female ratio of about 1.5:1.[6–8] However, a study carried out in KLH in 1981—1982 showed no significant difference in the number of RV-associated diarrhea cases between the sexes.[9]

Rotavirus was responsible for about 38% of hospitalizations for diarrhea in children under 5 years of age in our study population. In 1988—1989, a study done in Peninsular Malaysia, where stool specimens were tested for RV by an ELISA using Rotazyme (Abbott Laboratories, Chicago, USA) or by electron

microscopy, showed that only 28% of diarrheal stool specimens tested positive for RV. By contrast, a study done in KLH in 1981—1982 showed that 46% of hospitalizations for diarrhea were for RV-associated diarrhea. This wide variation in the proportion of RV-associated diarrhea could be due to various factors such as differences in methodology of study, improvements in the storage, transport and processing of stool specimens, an increase in the sensitivity of the tests used to detect RV, or an actual increase in the proportion of RV-associated diarrhea.

In temperate countries, seasonal variation has been observed with a peak incidence occurring during the winter months.[10] Studies from some tropical countries where there are distinct cooler months have also shown that the incidence is higher during the cooler months,[11] while others have shown no significant correlation.[12] In tropical countries where the main seasonal variation is the amount of rainfall, there was no significant correlation observed between the incidence of RV-associated diarrhea and the amount of rainfall.[3,9] Over our two-year study period, there was no seasonality observed in either site. There was an outbreak of acute gastroenteritis in Peninsular Malaysia with a proportional increase in the number of RV-associated diarrhea cases in January—February 2002.[13] This outbreak did not occur in East Malaysia.

In Malaysia, a study done in 1992 on the distribution of RV antigenic types on 268 RV positive stool samples showed that all isolates were found to be group A rotavirus.[14] From 1977—1988, serotype analysis on the distribution of rotavirus G showed that 71% were serotype G4, and there was no serotype G9 detected.[15] In our series, serotype analysis on the distribution of rotavirus G showed that 73% were P[8]G9, 80% in KLH and 61% in HUS. The results show that there has been a change in the predominant strain of RV from serotype G4 to serotype G9 in children with diarrhea over the last two decades. This G9 serotype change has implications for vaccine development strategies and recommendations where protection against serotype G9 may be required for a successful vaccination program in this country. This would also mean that continuous surveillance is important to detect any change in the predominant strain over time.

From the Malaysian National population census for the year 2000, the catchment population of children under 5 years old for KLH was 129 641 and 69 515 for HUS, Sarawak. Using our hospital-based data and assuming that all diarrhea patients were admitted to these two hospitals and all diarrhea patients admitted were from the catchment areas only, the estimated incidence of RV-associated diarrhea was 30 per 10 000 population under 5 years old per year for Kuala Lumpur and 24 per 10 000 population under 5 years old per year for Kuching.

In summary, the proportion of hospitalization for diarrhea in children under 5 years of age was 5.6% of the total general pediatric admission in the two hospitals. Rotavirus was responsible for about 38% of diarrhea hospitalizations in children under 5 years old in Malaysia. The most commonly affected age group was 6—17 months, accounting for 55% of all rotavirus-associated diarrhea in children under 5 years old. There was no seasonality noted for severe acute gastroenteritis and rotavirus-associated diarrhea.

The most prevalent strain of rotavirus in the population under surveillance was P[8]G9. The estimated incidence of rotavirus-associated diarrhea was 27 per 10 000 population under 5 years old per year.

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Conflict of interest: No conflict of interest to declare.

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