Epidemiology and Genetic Diversity of Rotavirus in Kunming, China, in 2015

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Keywords

Rotavirus · Diarrhea · G and P types · Epidemiology

Abstract

Group A rotavirus (RVA) is a serious public problem in China, with a prevalence of 29.7% in diarrhea cases under 5 years of age. A total of 1,224 fecal specimens were collected in 2015 from children under 5 years of age with acute gastroenteritis in Kunming, China, for detection and characterization of rotavirus. The prevalence of RVA was 28.5%. The frequency of RVA detection was greatest (52%) among children aged 7–12 months. The following strains were the most common types: G9P(8) (58.2%), G3P(8) (14.9%), and G1P(8) (6.9%). The following strains were uncommon types: G3P(4), G8P(9), and G9P(4) (1.1%); G1P(4), G2P(8), and G4P(8) (0.9%); G2P(6), G3P(9), G4P(6), and G9P(6) (0.6%); and G4P(4) (0.3%). G3G1P(8) (5.2%), G1G2P(4) (0.9%), G1G9P(8) (0.6%), and G2P(4)P(8) (0.3%) were mixed types. This study documents the molecular epidemiology, genetics, and diversity of rotavirus strains in children under 5 years of age in Kunming, China, and suggests that it may be important to offer RVA vaccination and scientific evidence to stop and control RVA-related diarrhea.

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Introduction

Acute gastroenteritis is a significant cause of morbidity and mortality in infants and young children under 5 years of age worldwide. It has caused 1.7 billion diarrhea episodes and over 700,000 childhood deaths [1]. In a variety of investigations, attempts were made to estimate the burden of bacterial, parasitic, and viral agents responsible for episodes of diarrhea in a group of children [2]. It was determined that, globally, rotavirus remains a major cause of morbidity and mortality in developing countries [3]. Rotaviruses belong to the family Reoviridae and are classified into 7 antigenic groups (A to G), of which groups A, B, and C are known to infect humans [4]. Group A rotaviruses (RVA) infect all children in their first few years of life and account for the vast majority of rotavirus infections worldwide, while B and C rotaviruses are often found to be associated with outbreaks and sporadic cases [4]. RVA-related diarrhea was ubiquitous; RVA infects almost all children, leading to 9.8 million severe diarrhea episodes and 193,000 child deaths worldwide every year.

Both authors contributed equally to this work.

It has been estimated that, worldwide, each year, RVA-associated diarrhea results in 9.8 thousand deaths in children aged less than 5 years, which account for 27% of all diarrhea-associated deaths and 3.0% of all childhood deaths [1]. The importance of the pathogen has prompted extensive research on the structure and replication of the virus as well as the pathogenesis and the immune responses [5, 6]. Rotavirus infections range from subclinical infection to severe gastroenteritis with diarrhea, vomiting, and lethal dehydration [3]. The major protective immune response against rotavirus is directed against the 2 outer capsid proteins VP7, a glycoprotein (G protein), and VP4, a protease-sensitive protein (P protein). Both proteins are used to type strains, which have been shown to vary over time within geographic locations and from region to region [3]. Molecular epidemiological studies have distinguished 27 G genotypes and 35 P genotypes. Of major worldwide epidemiological importance are the genotypes G1P(8), G2P(4), G3P(8), G4P(8), and G9P(8) and, to a lesser extent, G12P(8) [7].

The prevalence of RVA in children with gastroenteritis is 29.7% in China, and it is the most commonly identified enteric causative agent in acute gastroenteritis patients [8]. At present, data of RVA-related diarrhea is very limited in developing region such Yunnan Province, China. Furthermore, the molecular epidemiological characteristics of RVA are not clear. The aim of this study was to investigate the RVA disease burden and RVA epidemiological characteristics and genotypes in children aged <5 years from Kunming, China. The results of this study might be a pivotal reference for introduction of RVA vaccination to decrease the RVA-related disease burden.

Materials and Methods

Specimens

From January 2015 to December 2015, stool specimens were collected from children aged <5 years hospitalized for acute diarrhea in 3 different hospitals in Kunming, China (i.e., First People's Hospital of Yunnan Province, Children's Hospital of Kunming City, and Kunming City Maternal and Child Health Hospital). In the present study, acute gastroenteritis was defined as the passage of ≥ 3 abnormal stools (e.g., watery, liquid, mucous, or bloody stool) a day with or without associated symptoms such as vomiting, fever, and abdominal pain. Stool specimens ($n = 1,224$) were obtained from acute gastroenteritis patients and then each stool sample was kept frozen at -80°C , transported to the laboratory on wet ice, and stored at -80°C until further analysis. Epidemiologic data including age, dates of diarrhea onset, and specimen collection were available for the majority of the patients. The method employed for specimen collection was approved by the institutional human ethics committee.

Rotavirus Antigen Detection

Suspensions (10%, w/v) of each stool specimen prepared in 0.01 M phosphate-buffered saline (pH 7.2) were tested for RVA antigen by enzyme-linked immunosorbent assay (ELISA) using an Oxoid ProSpecT Rotavirus Microplate Assay (Oxoid Ltd., Hampshire, UK) as per the manufacturer's instructions.

RNA Extraction

Rotavirus dsRNA was extracted from 10% (w/v) suspensions of all ELISA-positive stool specimens using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's protocol.

G and P Genotyping

The VP7 and VP4 genes were genotyped by multiplex reverse transcription (RT)-PCR according to the method described earlier with minor modifications in the thermal cycling program [4]. The viral RNA was subjected to 1-step RT-PCR (Qiagen, Hilden, Germany) using the outer primers 9Con1-L/VP7-R deg [9] and Con 3/Con 2 [10] and oligonucleotide primers that could amplify the VP7 genotypes G1 to G4, G8 to G10, and G12 and the VP4 genotypes P(4), P(6), P(8), P(9), P(10), and P(11). Briefly, 4 μL of dsRNA was denatured at 95°C for 5 min and then chilled in ice for 2 min. A reaction mix of 46 μL containing 5 $\times$ buffer, dNTPs, RNase-free water, the primers 9Con1-L/Con3 and VP7-Rdeg/Con2, and 2 μL of enzyme mix was added to make a final volume of 50 μL . The reaction tubes were placed in a thermocycler for 30 min at 45 and 95°C for 15 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, and 70°C for 2.5 min, with a final extension at 70°C for 7 min. All PCR products were analyzed by electrophoresis using Tris acetate EDTA buffer (pH 8.3) on 2% agarose gel containing ethidium bromide (0.5 $\mu\text{g}/\text{mL}$) and visualized under UV illumination.

Statistical Analysis

Data analysis was performed with SPSS 19.0 (IBM, USA); OR and 95% CI for categorical variables were calculated with the χ^2 test, and medians and means of quantitative variables were analyzed using the t test. Statistical methods was applied according to the characteristics of the data. $p < 0.05$ was considered statistically significant.

Results

Prevalence of RVA in Diarrhea Cases

We collected a total of 1,224 stool specimens from children hospitalized for acute gastroenteritis during January 2015 to December 2015 in Kunming, China. Of these, 349 (28.5%) were positive for rotavirus antigen by ELISA. The mean age ($\pm\text{SD}$) of the children with diarrhea was 15.2 ± 12.5 months. The mean age of the rotavirus-infected children was 11.6 ± 7 months, which was significantly lower ($p < 0.01$) than that of the rotavirus-uninfected children (i.e., 16.6 ± 14.2 months).

Diarrhea cases were further classified into the following 4 age groups: 0–6 months ($n = 438$; 35.8%), 7–12 months ($n = 479$; 38.1%), 13–24 months ($n = 165$; 13.5%), and 25–60 months ($n = 142$; 11.6%). The prevalence of RVA was

Table 1. Age distribution of rotavirus positivity in children with acute gastroenteritis in Kunming, China

	Age group				χ^2	<i>p</i> value
	0–6 months (<i>n</i> = 438)	7–12 months (<i>n</i> = 479)	13–24 months (<i>n</i> = 165)	25–60 months (<i>n</i> = 142)		
Children infected with RVA, <i>n</i> (%)	105 (24.0)	182 (38.0)	35 (21.2)	27 (19.0)	35.96	0.000

significantly different among these 4 groups ($\chi^2 = 35.96$, $p = 0.000$); the age group with the highest RVA detection rate was 7–12 months (38.0%, 182/479), followed by 0–6 months (24.0%, 105/438), 13–24 months (21.2%, 35/165), and 25–60 months (19.0%, 27/142) (Table 1).

Common Genotypes among Diarrhea Cases

Among the 349 rotavirus-infected children identified by RT-PCR from the diarrhea cases, 16 (4.6%) were partially typed. In 7 samples the P genotype was identified but the G genotype was still not distinguished, and in 9 RVA specimens the G genotype was defined but P genotype remained negative; 333 (95.4%) samples were identified to have both G and P genotypes. G9P(8) strains had the highest score (58.2%), followed by: G3P(8) (14.9%); G1P(8) (6.9%); G3G1P(8) (5.2%); G3P(4), G8P(9), and G9P(4) (1.1%); G1P(4), G2P(8), G4P(8), and G1G2P(4) (0.9%); G2P(6), G3P(9), G4P(6), G9P(6), and G1G9P(8) (0.6%); and G4P(4) and G2P(4)P(8) (0.3%) (Table 2). These data suggested that G9P(8) strains are prevalent among children infected with RVA in Kunming, China.

RVA and Common Genotype Distribution by Age in Diarrhea Cases

Among the rotavirus-infected children, 105 (30%) were in the age group of 0–6 months, 182 (52%) were in the age group of 7–12 months, 35 (10%) were in the age group of 13–24 months, and 27 (8%) were in the age group of 25–60 months. The proportion of each genotype was not very different in the four age groups, except for G1P(4), which was more detected in diarrhea cases in the age group 13–24 months (Table 3).

Discussion

RVA is a serious public health problem in China, with a prevalence of 29.7% in diarrhea cases under 5 years of age [8]. The aim of this study was to describe the epide-

Table 2. Distribution of G and P type rotavirus strains in children with acute gastroenteritis in Kunming, China

G and P types	Age group 0–60 months (<i>n</i> = 349)
Common types (total)	279 (79.9)
G1P(8)	24 (6.9)
G3P(8)	52 (14.9)
G9P(8)	203 (58.2)
Uncommon types (total)	30 (8.6)
G1P(4)	3 (0.9)
G2P(6)	2 (0.6)
G2P(8)	3 (0.9)
G3P(4)	4 (1.1)
G3P(9)	2 (0.6)
G4P(4)	1 (0.3)
G4P(6)	2 (0.6)
G4P(8)	3 (0.9)
G8P(9)	4 (1.1)
G9P(4)	4 (1.1)
G9P(6)	2 (0.6)
Mixed types (total)	24 (6.9)
G1G2P(4)	3 (0.9)
G1G9P(8)	2 (0.6)
G2P(4)P(8)	1 (0.3)
G3G1P(8)	18 (5.2)
G/P untyped	16 (4.6)

Values are presented as *n* (%).

miologic feature of RVA and the RVA-related disease burden among acute gastroenteritis patients under 5 years of age in Kunming, China, in 2015. The prevalence of RVA (28.5%) in this study was approximately equal to that in a 5-year surveillance study conducted in China (29.7%) [8], but the prevalence of RVA in this study was higher than those in other studies conducted in developed countries such as Japan, Belgium, and Italy (11.9, 6.4, and 2.6%, respectively), where the RVA vaccine has been introduced in national immunization programs [11–13]. The prevalence of RVA is still as high as 34.0% in diarrhea

Table 3. Age distribution of G and P type rotavirus strains in children with acute gastroenteritis in Kunming, China

G and P types	Age group				χ^2	<i>p</i> value
	0–6 months (<i>n</i> = 105)	7–12 months (<i>n</i> = 182)	13–24 months (<i>n</i> = 35)	25–60 months (<i>n</i> = 27)		
Common types (total)	88 (83.8)	149 (81.9)	21 (60.0)	21 (77.8)	8.82	0.032
G1P(8)	11 (10.5)	7 (3.8)	2 (5.7)	4 (14.8)	7.04	0.071
G3P(8)	19 (18.1)	25 (13.7)	4 (11.4)	4 (14.8)	1.37	0.716
G9P(8)	58 (55.2)	117 (64.3)	15 (42.9)	13 (48.1)	7.62	0.054
Uncommon types (total)	7 (6.7)	13 (7.1)	7 (20.0)	3 (11.1)	5.61	0.132
G1P(4)	1 (1.0)	0 (0.0)	2 (5.7)	0 (0.0)	7.88	0.049
G2P(6)	0 (0.0)	1 (0.5)	1 (2.9)	0 (0.0)	5.61	0.132
G2P(8)	2 (1.9)	1 (0.5)	0 (0.0)	0 (0.0)	2.31	0.511
G3P(4)	0 (0.0)	3 (1.6)	0 (0.0)	1 (3.7)	4.57	0.206
G3P(9)	0 (0.0)	2 (1.1)	0 (0.0)	0 (0.0)	2.62	0.455
G4P(4)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	2.41	0.492
G4P(6)	1 (1.0)	1 (0.5)	0 (0.0)	0 (0.0)	0.935	0.817
G4P(8)	0 (0.0)	1 (0.5)	2 (5.7)	0 (0.0)	6.78	0.079
G8P(9)	2 (1.9)	0 (0.0)	1 (2.9)	1 (3.7)	6.26	0.099
G9P(4)	0 (0.0)	3 (1.6)	0 (0.0)	1 (3.7)	4.57	0.206
G9P(6)	0 (0.0)	1 (0.5)	1 (2.9)	0 (0.0)	3.15	0.369
Mixed types (total)	6 (5.7)	14 (7.7)	3 (8.6)	1 (3.7)	1.07	0.785
G1G2P(4)	1 (1.0)	2 (1.1)	0 (0.0)	0 (0.0)	1.193	0.755
G1G9P(8)	0 (0.0)	1 (0.5)	0 (0.0)	1 (3.7)	3.68	0.298
G2P(4)P(8)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	1.31	0.728
G3G1P(8)	5 (4.8)	10 (5.5)	3 (8.6)	0 (0.0)	3.64	0.303
G/P untyped	4 (3.8)	6 (3.3)	4 (11.4)	2 (7.4)	4.03	0.259

Values are presented as *n* (%).

cases in low-income and middle-income countries, where RVA vaccination is not obtained. Hence, these data suggest that safe, effective, and affordable RVA vaccination is an effective way to reduce the numbers of RVA infections and RVA related deaths. Two rotavirus vaccines, i.e., Rotarix™ and RotaTeq®, have been licensed in many countries, except for China, for use against rotavirus diarrhea. Both vaccines are recommended by the World Health Organization (WHO) in childhood immunization programs conducted globally [14]. Studies have reported difference in the efficacies of these vaccines against severe rotavirus diarrhea in high- and middle-income (85–98%) and low-income (39–72%) countries [15]. Although RVA vaccination has a low influence on the reduction of RVA-related diarrhea in developing countries, RVA vaccine can reduce obviously severe RVA-associated diarrhea and death worldwide. Rotavirus viral antigens VP7 and VP4 are considered important targets for vaccine development because they are immunogenic and elicit protective neutralizing antibodies [16]. However, considering RVA genotypes varied in terms of their spa-

tial-temporal distribution, surveillance of G and P combination genotypes was an important reference factor for choosing and introducing RVA vaccine in the prevaccine era. We found that the rate of diarrhea cases among the 4 age groups was significantly different; the prevalence of RVA was 24.0% (105/438) in the age group of 0–6 months, but it increased sharply to 38.0% (182/479) in the age group of 7–12 months, and then the detection rate of RVA declined continuously with increasing age. This conclusion was in keeping with previous studies [17–19]. We speculated that children under 6 months of age may obtain protective immunity against RVA. There are 2 hypotheses that can explain the phenomenon. One hypothesis is that children under 6 months of age can stop RVA infection by IgG acquired from the mother [20]. Another hypothesis is that breast milk has substantial IgA and other nonspecific immune factors such as lactoferrin that can prevent RVA infection [20, 21]. In addition, we observed that both G and P genotypes were identified in 333 (95.4%) samples. G9P(8) (58.2%) had predominantly a G and P combination, and the total proportion of G9P(8),

G3P(8), and G1P(8) being as high as 79.9% in all RVA strains in this study. A similar conclusion was obtained in other study [17]. In addition, the proportion of each genotype was not very different in several age groups, except for G1P(4), which was more detected in diarrhea cases in the age group of 13–24 months.

In conclusion, our study demonstrated the epidemiological characteristics and genetic (G/P genotypes) diversity of RVA in Kunming, China, before introduction of the RVA vaccine. These data are significant, suggest the need to introduce the RVA vaccine, and provide scientific evidence to stop and control RVA-related diarrhea.

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Disclosure Statement

The authors have no conflict of interests to declare.

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