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Emergence of G9 serotype rotavirus as a major cause of infectious gastroenteritis in southern Taiwan

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Infectious gastroenteritis is a common illness in children. This study investigated the etiology and clinical manifestations of hospitalized children with symptoms of infectious gastroenteritis in southern Taiwan. We studied 467 consecutive patients with infectious gastroenteritis aged from 2 days to 10 years hospitalized from April 2001 to March 2002. Rotavirus was the most common etiology (57%) of infectious gastroenteritis in these patients. Bacterial infection was noted in 57 cases (12%). Rotavirus was found in 92% of nosocomial infectious gastroenteritis ($p \leq 0.001$). Bloody stool was a presentation of bacterial infection in 74% of cases and rotavirus gastroenteritis in 8% of cases ($p \leq 0.001$). The G serotype of rotavirus was identified in 87 patients. Serotype G1 was the most common (51%), followed by G9 (31%). The emergence of serotype G9 strains in rotavirus infection has not been previously reported from Taiwan. Incorporation of G9 rotavirus into vaccines should be considered.

Key words: Gastroenteritis, rotavirus, serotyping

Acute infectious gastroenteritis is among the most common diseases in pediatric patients worldwide. It is characterized by diarrhea, and always accompanied by nausea, vomiting, fever and abdominal pain [1-3]. In developed countries, viruses are the most important causes of diarrhea, and cause approximately 70-80% of cases [4,5]. Other etiologies include bacteria and protozoal pathogens, estimated to cause 10-20% of cases. The most common viral pathogen in children is rotavirus. Rotavirus infection is common in winter months, and most mild to moderate infections are self-limited [6]. Although bacterial infection is not the most common pathogen of acute infectious gastroenteritis in children, it plays an important role in pediatric patients because of high mortality and morbidity rates if not treated properly. The aim of this study was to investigate the etiology and clinical manifestations of infectious gastroenteritis in hospitalized children in southern Taiwan.

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Materials and Methods

All children younger than 14 years hospitalized due to acute gastroenteritis at a 1200-bed medical center in southern Taiwan during the period April 2001 to March 2002 were included. All children with persistent symptoms for more than 2 weeks were excluded. Acute gastroenteritis was defined as a condition in which a patient developed vomiting and/or diarrhea. Diarrhea was defined as unformed stool passages more than twice a day [4,7,8]. Fever was defined as a rectal temperature higher than 38°C. Bloody stool was defined as positive for occult blood. A case was defined as nosocomial infection if the child had been hospitalized for 72 hours or more before the onset of diarrhea and/or vomiting. Data on age, gender, symptoms and signs, and stool characteristics were collected. One fecal specimen was collected from each patient and examined for rotavirus and adenovirus by enzyme immunoassay (EIA) [Premier Rotaclone & Premier Adenoclone; Meridian Diagnostics, Cincinnati, OH, USA]. Samples were also cultured for enteric bacterial pathogens, and screened for protozoa or parasite ova by microscopy. Samples

Table 1. Etiology of infectious gastroenteritis in 467 children hospitalized in a medical center in southern Taiwan, April 2001-March 2002

Etiology	Number of cases (%)		*p*
	Community-acquired	Nosocomial	
Rotavirus	213 (48)	24 (92)	≤0.001
Adenovirus	18 (4)	2 (8)	0.377
Bacteria	43 (10)	0 (0)	
Parasite	0 (0)	0 (0)	
Rotavirus and adenovirus	17 (4)	0 (0)	
Rotavirus and bacteria	13 (3)	0 (0)	
Rotavirus and adenovirus and bacteria	1 (0.2)	0 (0)	
Unknown	136 (31)	0 (0)	
Total	441 (100)	26 (100)	

with positive EIA results for rotavirus were sent to the Center for Disease Control in Taiwan to be further analyzed for VP7 (G) serotypes by reverse transcription-polymerase chain reaction (RT-PCR) as described previously [9]. Double-stranded RNA extracted from stool samples was used as the template for reverse transcription, which was amplified using Taq polymerase. The concentrations of $MgCl_2$, dimethyl sulfoxide, and template RNA were critical [9]. The choice of primer pairs allowed amplification of the entire segment or specific portions. The gene was identified in agarose gels. Categorical variables were compared using the chi-squared test.

Results

A total of 467 patients with acute gastroenteritis, aged from 2 days to 10 years, with a mean of 23.9 months, were hospitalized during the study period. No patient had symptoms for more than 2 weeks. No patient admitted for gastroenteritis was older than 10 years during the study period. Of the 467 patients included in this study, 299 (64%) were less than 2 years old and 431 (92%) were less than 5 years old. No mortality occurred during hospitalization. The male-to-female ratio was 1.5 (279/188). The etiologies of infectious gastroenteritis are shown in Table 1. Infectious gastroenteritis was community acquired in 441 cases (94%). Rotavirus infection was detected by EIA in 244 cases (55%), adenovirus infection in 36 (8%) and mixed infections in 31 (7%). Pathogenic bacteria were isolated in 57 cases, including *Salmonella* spp. (32 specimens, 7%), *Shigella* spp. (1 specimen, 0.2%), *Campylobacter* spp. (15 specimens, 3%), *Aeromonas* spp. (7 specimens, 1.5%) and *Staphylococcus aureus* (6 specimens, 1.3%). Four patients had more than 2 bacterial isolates. No *Clostridium difficile* was found. Nosocomial infection occurred in 26 patients (6%) aged from 2 months to 49 months old; the male-to-female ratio of these patients was 1.9 (17/9). Twenty four cases (92%) were positive for rotavirus and 2 cases (8%) were positive for adenovirus. No bacterial pathogen was found in patients with nosocomial infection. No parasite was found on microscopic examination of fecal specimens.

The clinical manifestations of patients with community-acquired infectious gastroenteritis are shown in Table 2. Most of these patients (426, 97%) had diarrhea; a minor proportion (3%) had vomiting and fever but no diarrhea. Fever was noted in 354 patients (81%), while vomiting was noted in 265 (60%). Bloody stool was noted in 81 patients, 11 (14%) of whom had mixed infection. Bloody stool was noted in 74% (42/57) of patients with bacterial infection and 8% (19/244) of patients with rotavirus infection. Bacterial infection was more likely to cause bloody stool than rotavirus infection ($p \leq 0.001$). Seizure attack occurred in 17 patients, of whom 12 (71%) were found to have rotavirus infection.

Table 2. Clinical manifestations of community-acquired infectious gastroenteritis of different etiologies in children

Symptoms	Number of cases (%)							p^a
	Total (n = 441)	Rotavirus (n = 244)	Adenovirus (n = 36)	Bacteria (n = 57)	Rotavirus and adenovirus (n = 17)	Rotavirus and bacteria (n = 13)	Unknown (n = 136)	
Diarrhea	426 (97)	229 (94)	33 (92)	56 (98)	16 (94)	13 (100)	136 (100)	0.183
Fever	354 (80)	192 (79)	25 (69)	50 (88)	14 (82)	10 (77)	110 (81)	0.122
Vomiting	265 (60)	178 (73)	18 (50)	18 (32)	8 (47)	5 (39)	64 (47)	≤0.001
Dehydration	362 (82)	214 (88)	28 (78)	41 (72)	15 (88)	10 (77)	103 (76)	0.003
Bloody stool	81 (17)	19 (8)	2 (6)	42 (74)	2 (12)	9 (69)	28 (21)	≤0.001
Seizure	17 (4)	12 (5)	1 (3)	2 (4)	0	0	2 (2)	0.649

[a]Comparison between symptoms/signs in rotavirus and bacterial infection.

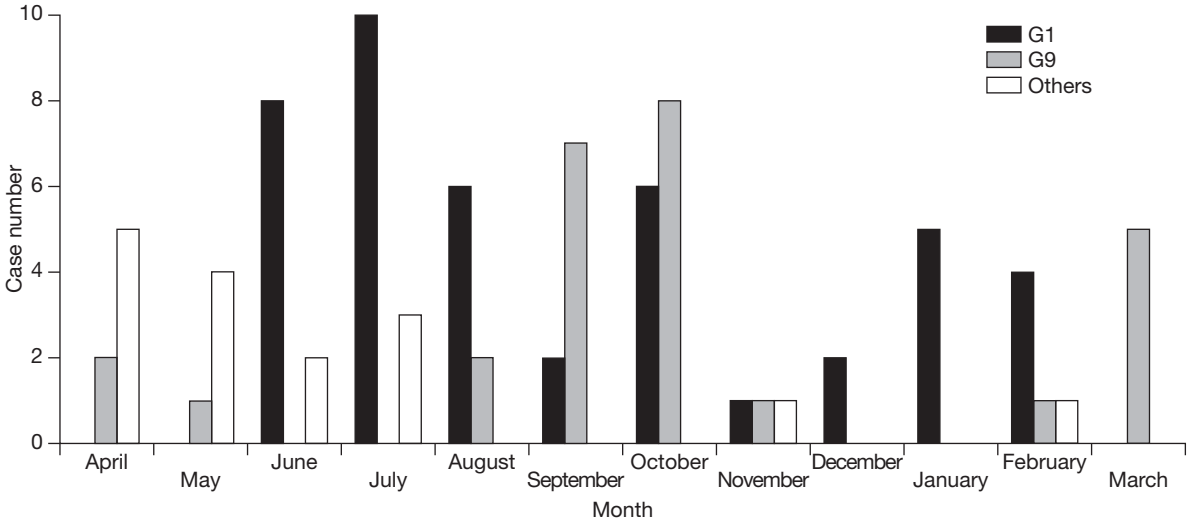

Fig.1. Monthly distribution of different serotypes of rotavirus infection in children, April 2001 to March 2002.

In the winter period between December 2001 and February 2002, 159 children (n = 467, 34%) were hospitalized due to infectious gastroenteritis, 106 of whom (67%) had rotavirus infections. Patients with infectious gastroenteritis caused by rotavirus in the winter period accounted for 40% of the 267 patients hospitalized for rotavirus infection during the 1-year study period. A peak number of 67 patients were hospitalized for infectious gastroenteritis (n = 467, 14%), and positive results of rotavirus detected by EIA also peaked in January 2002. Of the 67 patients, 46 (31%) had rotavirus infection. On the other hand, the proportion of patients with bacterial gastroenteritis ranged from 3 to 23% of patients hospitalized for infectious gastroenteritis in different months of the year. The lowest proportions (3-6%) were noted in the period from November 2001 to January 2002.

In this study, we identified G serotype of rotavirus in 87 patients (Fig. 1), aged from 2 months to 7 years, with a mean age of 26 months. The male-to-female ratio of these patients was 1.8 (56/31). Among the isolates, 44 (51%) were G1 and 27 (31%) were G9, followed by G3 (n = 10, 12%), G2 (n = 3, 3%) and G4 (n = 3, 3%). Of these 87 patients, 2 had seizure attack. Serotype G9 rotavirus was isolated in the stool specimens of both of these patients. Clinical manifestations in the G9 rotavirus infection group included diarrhea in all patients, dehydration in 19 (70%), followed by fever (n = 18, 67%) and vomiting (n = 17, 63%). Only 1 patient complained of bloody stool. The clinical manifestations in patients infected with the 5 serotypes of rotavirus are summarized in Table 3. A peak of G9 infection occurred in September and October of 2001.

Table 3. Clinical manifestations in rotavirus infections of different G serotypes[a]

Symptoms	Non-G9				G9	p^b
	G1	G2	G3	G4		
Vomiting	29	2	8	1	17	0.737
Diarrhea	38	2	8	2	27	0.024
Bloody stool	2	0	0	1	1	0.789
Fever	36	2	9	2	18	0.124
Dehydration	34	1	10	2	19	0.422
Seizure	0	0	0	0	2	0.033

[a]One patient died of congenital heart disease.
[b]Comparison between non-G9 and G9 group.

Discussion

In this study, 43% (106/244) of cases of infectious gastroenteritis due to rotavirus occurred in winter. Rotavirus infection was most frequent in children less than 2 years old (163/267, 61%). This percentage is lower than that found by Lo et al [3] in a study from southern Taiwan in 1984 (83.6%) and Chiu et al [1] in northern Taiwan in 1993 (76%). In the present study from southern Taiwan, 36% (95/267) of rotavirus-infected children were within an age range from 2 years to 5 years. The lower rate of gastroenteritis due to rotavirus infection in children younger than 2 years in this study may have been due to a relatively higher incidence in older children.

Adenovirus is another important pathogen causing severe diarrhea in children younger than 2 years [10].

In this study, adenovirus infection was found in 18 patients (4%) with community-acquired infectious gastroenteritis. All of them were younger than 5 years old. *Salmonella* spp. and *Campylobacter* spp. are common causes of pathogenic gastroenteritis worldwide [11,12]. In this study, 7% (32/467) of fecal specimens were positive for *Salmonella* spp. and 3% (15/467) were positive for *Campylobacter* spp. These findings are within the ranges reported from previous studies in Taiwan [10,11,13]. Mixed infection was found in 7% of cases. It is difficult to tell from available data whether these patients had coinfection or were chronic carriers of rotavirus or *Salmonella*.

Rotavirus is also a frequent cause of nosocomial infection [14]. In our study, 92% (24/26) of cases of nosocomially-acquired gastroenteritis were caused by rotavirus. The distribution of etiologies in nosocomial infections was different from that of community-acquired infection (Table 1). Of all rotavirus infections, only 9% were acquired in the hospital. Previous studies found that 15 to 69% of rotavirus infections occurred in hospital settings [1,15]. Hand-washing and contact precautions should be emphasized in hospitals caring for patients with rotavirus infection.

Seizure attack occurred in 17 patients (4%) in this study, 12 (71%) of whom had rotavirus infection. Thus, 5% (12/244) of rotavirus infections were associated with seizure attack and 3% of patients (5/197) with non-rotavirus infection had seizure attack, p=0.1968.

Previous studies found that serotypes G1 to G4 rotaviruses were major human pathogens [16]. Serotype G9 rotavirus has been detected in approximately 0.5% of the circulating strains worldwide [17]. Since 1992, serotype G9 strains have been detected in at least 10 countries, including Thailand, India, Brazil, Bangladesh, Malawi, Italy, France, the United States, the United Kingdom and Australia. The ratio of serotype G9 infection has gradually increased, and has become the fifth common serotype worldwide, suggesting it may be an important serotype causing gastroenteritis in humans [16]. In our study, serotype G1 strains were the most prevalent overall; the second most common was serotype 9, followed by G3, G2 and G4. To our knowledge, G9 serotype rotavirus has not been previously reported as a major serotype in Taiwan.

The clinical features of serotype G9 infection, except diarrhea and seizure, were similar to those of other rotavirus serotypes. In our study, all of the G9-infected patients had diarrhea, while only 83% (50/60) of non-G9 cases had diarrhea. Prevention of dehydration in G9-infected patients thus appears to merit extra attention. Use of an oral hydration agent and education in hand-washing may be helpful in these aims. Of the 87 patients who had rotaviruses serotyped, 2 had seizure attack, and both of these patients were infected by serotype G9 rotavirus. Although our results showed a significant difference in clinical manifestations between the G9 and non-G9 group, the case numbers were too small to reach definitive conclusions.

Most of the serotype G9 rotavirus infections (26/27, 96%) were community-acquired. In September and October of 2001, a peak of G9 rotavirus infection (15/27, 56%) was found. None of these cases was nosocomial. However, whether these cases represent an epidemic outbreak of G9 infection in the community is difficult to determine because of the small number of cases.

This study indicates that serotype G9 rotavirus is an emerging pathogen in southern Taiwan. Incorporation of G9 rotavirus into vaccines should be considered.

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