ORIGINAL ARTICLE

Microbial Etiology of Acute Gastroenteritis in Hospitalized Children in Taiwan

Shan-Ming Chen,[1,2] Yen-Hsuan Ni,[1] Huey-Ling Chen,[1] Mei-Hwei Chang[1]*

Background/Purpose: Viral infections are the most common causes of acute infectious diarrhea in the pediatric population. To explore any possible microbial etiologies of acute gastroenteritis in children, we detected stool viral antigen including rotavirus, adenovirus, norovirus and astrovirus. We also studied the possible precipitating factors.

Methods: During a period of 1 year (from October 2003 to September 2004), children from birth to 15 years old admitted to the pediatric ward were prospectively surveyed. Stool specimens were collected within 48 hours after admission and then frozen at −40°C until analysis. Enzyme immunoassay was used to detect rotavirus, astrovirus, norovirus and adenovirus. Bacterial culture was performed at the same time.

Results: During the study period, 82 stool samples were collected due to acute gastroenteritis and fit the definition of a diarrhea episode. Forty-two (51.2%) patients with viral infection, 11 (13.4%) with bacterial infection, and six (7.3%) with mixed viral and bacterial infection were detected. The most prevalent virus was rotavirus (35.4%), followed by norovirus (29.3%). The most prevalent cause of bacterial infection was *Salmonella* (19.5%). With regard to clinical severity, rotavirus resulted in longer hospital stay, higher rate of vomiting, stool occult blood, leukocytosis, lower rate in stool pus cell, and C-reactive protein elevation more than 5 mg/dL as compared with norovirus. Only the difference in hospital stay reached significant statistical difference.

Conclusion: Norovirus is an important cause of acute gastroenteritis in children, although rotavirus is still the leading cause of pediatric acute gastroenteritis. [*J Formos Med Assoc* 2006;105(12):964–970]

Key Words: diarrhea, gastroenteritis, norovirus, rotavirus

Diarrhea is one of the major causes of morbidity and mortality in children.[1,2] According to a World Health Organization 2003 report, the median incidence of diarrhea in children aged under 5 years was 3.2 episodes per child-year.[3] Deaths are more commonly a result of dehydration, but malnutrition also plays an important role. It has been estimated that 25% of the growth differential between children in developing countries and children in North America is related to diarrhea.[4] Viruses are the most important causes of diarrhea and cause approximately 70–80% of cases.[5] Rotavirus, enteric adenovirus, astrovirus, and norovirus are now known to be the most common causes of viral gastroenteritis.[4,6,7] Rotaviruses are the most prevalent cause of severe diarrhea in infants and young children in Taiwan.[8–13] Enteric adenoviruses may be the second most common cause of viral diarrhea, and their clinical characteristics in children have been delineated in Taiwan.[4,14] However, the incidence rate of enteric adenovirus as well as norovirus and astrovirus responsible

[1]Department of Pediatrics, College of Medicine and Hospital, National Taiwan University, and [2]Department of Pediatrics, Chung Shan Medical University Hospital, Taipei, Taiwan.

Received: March 16, 2006
Revised: April 24, 2006
Accepted: June 6, 2006

***Correspondence to:** Dr Yen-Hsuan Ni, Department of Pediatrics, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail: yhni@ha.mc.ntu.edu.tw

for acute diarrhea in children are unknown. Even if bacteria are not the main pathogens of acute gastroenteritis in children, bacterial infection results in high morbidity and mortality if not treated early. The aims of this study were to explore the possible etiologies of acute gastroenteritis in hospitalized children. In addition, the clinical features of viral and bacterial gastroenteritis are outlined in order to provide clues for physicians to make a tentative diagnosis early and to begin treatments promptly.

Materials and Methods

Definitions

An episode of diarrhea was defined as passage of loose stools occurring three or more times within 24 hours, and excluded noninfectious causes. Children with diarrhea of more than 10 days' duration were also excluded. Dehydration was estimated by using a clinical assessment described by Narchi et al.[15] Severity of illness was assessed by a modification Dennehy et al's method, which was based on the severity of diarrhea, vomiting, associated fever and dehydration (Table 1).[16]

Table 1. Clinical score system for assessing the severity of illness

Symptom	Score*
Maximum number of stools per day	
3	1
4–5	2
≥6	3
Duration of diarrhea (d)	
1	1
2–4	2
≥5	3
Vomiting	
No	0
Yes	1
Maximum temperature	
No fever	0
>38°C	1
>39°C	2
Dehydration	
None	0
≤5%	1
>5%	2

**Clinical severity score defined as: mild (<6); moderate (6–8); severe (>8).*

Sample collections

From October 2003 to September 2004, children aged from birth to 15 years admitted to the pediatric ward at National Taiwan University Hospital were prospectively surveyed. A stool sample was collected within 48 hours after admission from children whose diarrhea conformed to the study definition. All stool samples were frozen and stored at −40°C until viral testing. The clinical data for each patient were recorded, including sex, age, gastrointestinal symptoms, and laboratory test results.

Laboratory methods

Rotavirus, adenovirus, norovirus and astrovirus were detected in stool samples by specific commercial enzyme immunoassay (EIA) kits (Ridascreen®; R-Biopharm, Darmstadt, Germany). The assays for rotavirus, adenovirus and astrovirus were performed according to the manufacturer's instructions as follows: 100 μL of liquid stool or equivalent solid stool was suspended in 1 mL of the stool diluent. The sample diluent was mixed thoroughly and settled for 10 minutes before testing. Then, 100 μL of samples and controls were respectively added to microwell strips coated with monoclonal antibodies against these three viruses, and 100 μL of peroxidase-conjugated monoclonal antibody was added. The mixture was incubated at room temperature (20–25°C) for 60 minutes. After five wash cycles with 300 μL wash buffer, 100 μL of substrate were added to each well and incubated for 15 minutes in the dark. After this, the reaction was stopped by the addition of 50 μL stop reagent. The photometric measurements were made at wavelength of 450 nm. The measurements of each negative control had to be less than 0.2 at 450 nm. The cut-off values were calculated from the measured extinction for the negative control plus 0.15 extinction units. The kits for detection of rotavirus and adenovirus have a good

sensitivity and specificity (100% and 88.8–98.6%, respectively, for rotavirus; 93.3% and 94.3–100%, respectively, for adenovirus).[17] The sensitivity and specificity for detection of astrovirus were 89.2% and 87.3%, respectively, compared with reverse transcription–polymerase chain reaction (RT–PCR), according to the product's instruction leaflet.

The assay for norovirus was as follows: 200 μL of liquid stool or equivalent solid stool was suspended in 1 mL of the stool diluent. The sample diluent was centrifuged at 5000 rpm (approximately 2300–2500 g) for 5 minutes. Then, 100 μL of particle-free supernatant of suspension and controls were respectively added to microwell strips coated with monoclonal antibodies against noroviruses and incubated at room temperature (20–25°C) for 60 minutes. After five wash cycles with 300 μL wash buffer, 100 μL of peroxidase-conjugated monoclonal antibody were added and incubated at room temperature for 30 minutes. After another five wash cycles with 300 μL wash buffer, 100 μL of substrate were added to each well and incubated for 15 minutes in the dark. Finally, 50 μL stop reagent were added, and photometric measurements at wavelength 450 nm were made. The sensitivity and specificity for detection of norovirus were 95.0% and 95.2%, respectively, compared with RT–PCR (according to the product's instruction leaflet).

All stool samples were sent for bacterial culture simultaneously, including *Salmonella* species, *Shigella* species, and *Campylobacter jejuni*. Laboratory tests, including white blood count, C-reactive protein (CRP), stool occult blood, and stool pus cell, were performed at the standard central laboratory. Data on the monthly weather in Taipei, including mean temperature, precipitation and relative humidity, were provided by the Central Weather Bureau.

Statistical analysis

Statistical software SPSS version 10.1.3C (SPSS Inc., Chicago, IL, USA) for Windows was used for recording data and analyzing results. Nonparametric Kruskal-Wallis analysis of variance, Mann-Whitney *U* test, and Fisher's exact test for categorical data were used as appropriate; *p* value less than 0.05 was considered statistically significant.

Results

A total of 82 infants and children were enrolled during the study period. All stool samples were processed for four different viruses by EIA and bacterial culture. The male-to-female ratio was 1.83 (53/29). The median age was 31 months (ranged from 26 days to 165 months).

Table 2 shows the number of patients with acute diarrhea and enteropathogens detected in their stool. Enteric viruses accounted for more than half (58.5%) of the total of 82 samples and were the most common pathogens in our study. Enteric bacteria were detected in 17 (20.7%) of 82 cases. Twelve (14.6%) of 82 cases had a mixed infection. A total of 59 (71.9%) infants and children, including those with mixed infection, were screened out enteropathogens in fecal samples. Most (74.5%) of them were under 5 years of age (Table 3). Of the 29 patients with rotavirus infection, 14 (48.2%) were less than 2 years old and 25 (86.2%) were less than 5 years old. The respective figures in the norovirus group were six (25%) and 17 (70.8%), and in the *Salmonella* group were four (25%) and 12 (75%).

The clinical features and laboratory data of patients affected with a single pathogen are summarized in Table 4. The clinical features of the *Salmonella* group were more severe than the other two agents in terms of duration of diarrhea, maximal body temperature, length of hospital stay, severity score and CRP value. In addition, the *Salmonella* group revealed a high ratio in positive stool pus cell and stool occult blood test. With regard to clinical severity, rotavirus resulted in longer hospital stay, higher rate of vomiting, stool occult blood, leukocytosis, and lower rate of stool pus cell and CRP elevation more than 5 mg/dL compared to norovirus. However, we did not find any statistically significant difference except in duration of hospital stay. In our study, mixed infections

Table 2. Number of patients who tested positive for the indicated pathogens (from 82 stool specimens)

Pathogen	Positive (*n*)	Positive rate (%)
Virus	42	51.2
Rotavirus	24	29.3
Norovirus	12	14.6
Astrovirus	0	0
Norovirus + Rotavirus	5	6.1
Norovirus + Adenovirus	1	1.2
Virus + Bacteria	6	7.3
Norovirus + *Salmonella* sp.	5	6.1
Norovirus + *Campylobacter jejuni*	1	1.2
Bacteria	11	13.4
Salmonella sp.	11	13.4
Total	59	71.9

Table 3. Age distribution of patients with pathogen detected (including patients with mixed infections)*

	Rotavirus (*n* = 29, 35.4%)	Norovirus (*n* = 24, 29.3%)	*Salmonella* sp. (*n* = 16, 19.5%)
Age (mo)			
< 6	2 (2.4)	2 (2.4)	0 (0)
6–24	12 (14.6)	4 (4.9)	4 (4.9)
25–60	11 (13.4)	11 (13.4)	8 (9.8)
> 60	4 (4.9)	7 (8.5)	4 (4.9)
Mean age ± SD (mo)†	29.3 ± 19.9	47.0 ± 38.2	51.9 ± 44.5

**Values are presented as number of cases (percentage, number of positive/number of tests); †p = 0.167, Kruskal-Wallis test.*

(including virus-virus and virus-bacteria) were detected in 14.6% (12/82) of patients. Norovirus was the most common pathogen found in mixed infection. Half (12/24) of norovirus infections were mixed infection (Table 5). No statistical difference in the severity of illness was noted between mixed infections and isolated pathogen infections.

The monthly distribution of cases with rotavirus and norovirus infection was plotted with reference to mean temperature, precipitation and relative humidity in the Taipei area (Figure). We found that the peak incidence of both viruses was during cold and relatively lower precipitation weather, but was not related to relative humidity. In addition, the norovirus group showed an earlier peak than the rotavirus group (November *vs.* December). During the winter (December to February), 72.7% (16/22) of 22 children admitted were affected with rotavirus and 31.8% (7/22) with norovirus. In contrast, in autumn (September to November), 13.8% (4/29) of 29 children were infected with rotavirus and 41.4% (12/29) with norovirus.

Discussion

We attempted to investigate the viral and bacterial etiology of pediatric acute diarrhea in northern Taiwan. In this study, rotavirus accounted for the most cases (35.4%) and most rotavirus infections occurred in children under 2 years of age. Norovirus (29.3%) was the second most common pathogen. In contrast to rotavirus, norovirus attacked children older than 2 years. A similar pattern of age distribution has been described in a report from Finland.[18] Norovirus has been reported to

Table 4. Comparison of clinical characteristics and laboratory data among rotavirus, norovirus and *Salmonella* gastroenteritis*

	Rotavirus ($n=24$)	Norovirus ($n=12$)	*Salmonella* sp. ($n=11$)	p
Diarrhea (maximum number/d)	6.4 (3–12)	6.1 (3–11)	6.6 (3–10)	0.763†
Duration of diarrhea (d)	4.8 (3–8)	4.4 (3–6)	7.3 (5–11)	0.002†
Vomiting (%)	92.0	66.7	45.0	0.14‡
Fever (maximum °C)	38.5 (36.0–39.8)	38.3 (36.4–40)	39.7 (38.7–4)	0.002†
≥39°C (%)	33.3	8.3	81.8	0.21‡
Severity score§	7.9 (5–10)	7.3 (5–10)	9.3 (8–10)	0.012†
Hospital stay (d)‖	3.1 (2–5)	2.3 (1–5)	4.7 (2–8)	0.002†
≥4 days (%)	29.1	8.3	72.7	0.22‡
Stool occult blood (%)	29.1	16.7	63.6	0.68‡
≥3+++ (%)	12.5	8.3	45.5	1.0‡
Stool pus cell (%)	4.2	16.7	63.6	0.25‡
Serum WBC ($\times10^3/mm^3$)	11.9 (6.11–27.8)¶	10.1 (5.8–18.5)	9.17 (5.9–20.6)	0.194†
$>15\times10^3/mm^3$ (%)	29.1	8.3	9.1	0.22‡
CRP (mg/dL)	1.5 (0.03–14.5)	3.0 (0.01–7.40)	9.7 (0.9–26.7)	<0.001†
>5 mg/dL (%)	4.2	25	72.7	0.98‡

**Data are presented as mean (range); †p value (Kruskal-Wallis test) for difference among rotavirus, norovirus and Salmonella; ‡p value (Fisher's exact test) for difference between rotavirus and norovirus; §clinical severity score defined as mild (<6), moderate (6–8), severe (>8); ‖p<0.05 (Mann-Whitney U test) for difference between rotavirus and norovirus; ¶in one case, data on serum WBC was not collected because of clotted blood sample. WBC = white blood cell count; CRP = C-reactive protein.*

Table 5. Clinical characteristics and laboratory data among patients with mixed infection*

	Norovirus + Rotavirus ($n=5$)	Norovirus + Adenovirus ($n=1$)	Norovirus + *Salmonella* ($n=5$)	Norovirus + *Campylobacter* ($n=1$)
Diarrhea (maximum number/d)	5.4 (3–10)	4	6.6 (3–10)	8
Duration of diarrhea (d)	3.4 (3–4)	7	8.4 (5–11)	4
Vomiting (%)	80.0	0	100.0	0
Fever (maximum °C)	38.9 (37.3–40)	38.0	39.6 (39.4–40.1)	38.8
Severity score	7.4 (5–10)	6	9.8 (8–11)	8
Hospital stay (d)	2.2 (2–3)	2	6.4 (3–8)	1
Stool occult blood (%)	0	0	60.0	100
Stool pus cell (%)	20.0	0	80.0	100
Serum WBC ($\times10^3/mm^3$)	9.5 (4.3–12.7)	12.6	8.0 (1.8–14.5)	6.2
CRP (mg/dL)	1.8 (0.14–4.1)	0.07	9.2 (3.8–14.6)	2.5

**Values are presented as mean (range) or percent. WBC = white blood cell count; CRP = C-reactive protein.*

result in a less severe illness than rotavirus.[19] Hence, the incidence of norovirus infection may be underestimated because milder cases may not be hospitalized in this study.

According to the prevalence data in Taiwan from the detection of serum antibody of Norwalk virus in 1980, 5% of sera from the age of 3 months to 3 years were positive, and up to 15% and 24% from 3 years to 6 years, and from 6 years to 12 years, respectively, were positive.[20] On the basis of the above-mentioned and preliminary results of our study, norovirus-associated

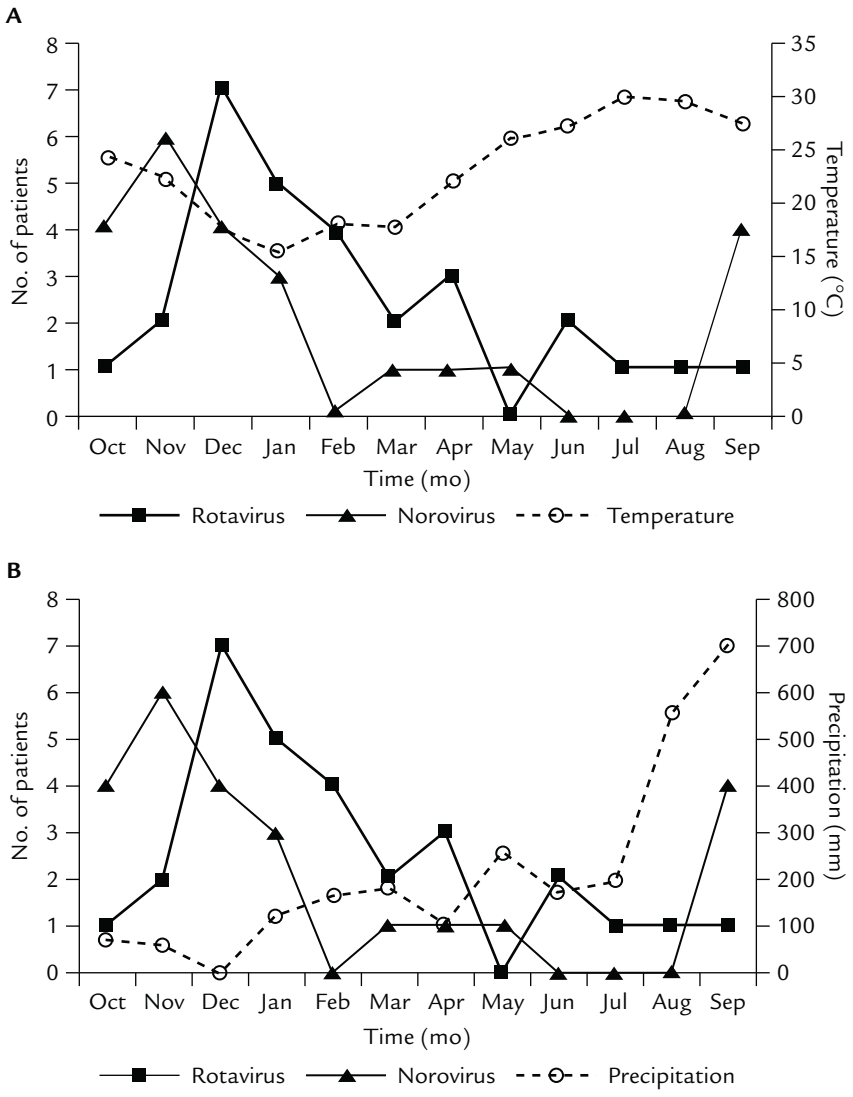

Figure. Relationship between monthly distribution of viral infection and climate in Taipei: (A) mean monthly temperature; (B) mean monthly precipitation.

gastroenteritis is not at all rare in the pediatric population in Taiwan. Meanwhile, the age-related pattern of antibody acquisition suggests that it ought to play a major role in acute gastroenteritis in older children.

In clinical severity, *Salmonella* infection was more severe than the other main viral agents. Norovirus infection resulted in significantly shorter hospital stay compared with rotavirus. In addition, it also tended to result in a lower rate of vomiting, stool occult blood and leukocytosis, but a higher rate of stool pus cell and CRP elevation. It was easy to differentiate bacterial from viral infection by clinical symptoms, but it was difficult to discriminate between rotavirus and norovirus without any laboratory tests. Rotavirus was still the most frequent viral pathogen in winter. A similar finding was delineated in most previous reports in Taiwan.[5,8,9,11,12] Furthermore, a prominent seasonality was demonstrated in norovirus infection in this study. However, norovirus had an earlier peak time in incidence than that of rotavirus, and was more common than rotavirus in autumn (41.4% *vs.* 13.8%). Mounts et al reviewed 12 norovirus surveys conducted during a 21-year period in eight countries, and found a peak in winter in 10 of 12 surveys for both sporadic cases and outbreak-associated gastroenteritis.[21] It also tended to be related to cold and relatively low precipitation weather in this study. Nevertheless, a recent study in Jakarta, Indonesia reported that the seasonal distribution

did not correlate with rainfall.[22] They demonstrated that rotavirus peaks in June and July and norovirus peaks in August and September. Therefore, the trend of seasonality of acute gastroenteritis associated with norovirus appeared to vary according to different geographic areas. Further prospective longitudinal surveillance is necessary to provide more reliable epidemiologic data, because the knowledge of seasonality of norovirus infection is an important clue for diagnosis.

In Lin et al's study of enteric adenovirus infection in children in northern Taiwan, 76.6% of 64 cases were younger than 2 years.[14] However, very few cases of adenovirus and astrovirus infection were found in this study. A negative result does not completely exclude astrovirus infection. It may be caused by an intermittent excretion of the virus or a small quantity of antigen in the fecal sample. A more extensive study may be mandatory to investigate the true incidence.

In this study, we showed a portion of the possible etiologies of former undiagnosed acute gastroenteritis in hospitalized children. The high incidence of norovirus infection indicates that norovirus is an important viral etiology of pediatric acute gastroenteritis in Taiwan. At the same time, a nationwide surveillance system, including sporadic cases and outbreaks, should be considered as well.

References

1. Snyder JD, Merson MH. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. *Bull World Health Organ* 1982;60:605–13.
2. Bern C, Martines J, de Zoysa I, et al. The magnitude of the global problem of diarrhoeal disease: a ten-year update. *Bull World Health Organ* 1992;70:705–14.
3. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003;81: 197–204.
4. Gastanaduy AS, Begue RE. Acute gastroenteritis. *Clin Pediatr (Phila)* 1999;38:1–12.
5. Sung YL, Huang YF, Huang CF, et al. Emergence of G9 serotype rotavirus as a major cause of infectious gastroenteritis in southern Taiwan. *J Microbiol Immunol Infect* 2004; 37:322–6.
6. Blacklow NR, Greenberg HB. Viral gastroenteritis. *N Engl J Med* 1991;325:252–64.
7. Pickering LK, Cleary TG. Approach to patients with gastrointestinal tract infections. In: Feigin RD, Cherry JD, Demmler GJ, et al, eds. *Textbook of Pediatric Infectious Diseases*, 5th edition. Philadelphia: Saunders, 2004:610–45.
8. Chen HJ, Chen BS, Wang SF, et al. Rotavirus gastroenteritis in children: a clinical study of 125 patients in Hsin-Tien area. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1991; 32:73–8.
9. Lin CL, Huang FY, Chou SC, et al. Clinical observation of rotavirus gastroenteritis in children. *Acta Paediatr Sin* 1984; 25:407–11. [In Chinese]
10. Chyou SC, Leu YJ, Huang FY, et al. An etiological study of infectious diarrhea in infants and children in Taipei area. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1988; 29:213–20.
11. Lo YS, Huang BL, Tsai LY, et al. Clinical observations of infants and children with rotavirus gastroenteritis in southern Taiwan. *Gaoxiong Yi Xue Ke Xue Za Zhi* 1988;4:358–63.
12. Chiu TF, Lee CN, Lee PI, et al. Rotavirus gastroenteritis in children: 5-year experience in a medical center. *J Microbiol Immunol Infect* 2000;33:181–6.
13. Tsai CH, Chiu HH, Abe T. Epidemiologic features of rotavirus infection in Taiwan: a review. *Pediatr Int* 2000;42:411–4.
14. Lin HC, Kao CL, Lu CY, et al. Enteric adenovirus infection in children in Taipei. *J Microbiol Immunol Infect* 2000;33: 176–80.
15. Narchi H. Serum bicarbonate and the severity of dehydration in gastroenteritis. *Arch Dis Child* 1999;80:493.
16. Dennehy PH, Nelson SM, Spangenberger S, et al. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. *J Infect Dis* 2001;184:10–5.
17. Rabenau H, Knoll B, Allwinn R, et al. Improvement of the specificity of enzyme immunoassays for the detection of rotavirus and adenovirus in fecal specimens. *Intervirology* 1998;41:55–62.
18. Pang XL, Joensuu J, Vesikari T. Human calicivirus-associated sporadic astroenteritis in Finnish children less than two years of age followed prospectively during a rotavirus vaccine trial. *Pediatr Infect Dis J* 1999;18:420–6.
19. Matson DO. Caliciviruses and hepatitis E virus. In: Feigin RD, Cherry JD, Demmler GJ, et al, eds. *Textbook of Pediatric Infectious Diseases*, 5th edition. Philadelphia: Saunders, 2004:2087–101.
20. Cukor G, Blacklow NR, Echeverria P, et al. Comparative study of the acquisition of antibody to Norwalk virus in pediatric populations. *Infect Immun* 1980;29:822–3.
21. Mounts AW, Ando T, Koopmans M, et al. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J Infect Dis* 2000;181(Suppl 2):S284–7.
22. Subekti D, Lesmana M, Tjaniadi P, et al. Incidence of Norwalk-like viruses, rotavirus and adenovirus infection in patients with acute gastroenteritis in Jakarta, Indonesia. *FEMS Immunol Med Microbiol* 2002;33:27–33.