

## Coinfection of Viral Agents in Korean Children with Acute Watery Diarrhea

Currently, there are a few reports on viral coinfection that causes an acute watery diarrhea in Korean children. So, to evaluate the features of coinfectious viral agents in children with acute watery diarrhea, we enrolled 155 children with acute watery diarrhea from July 2005 to June 2006. Fecal samples were collected and evaluated for various viral infections such as rotavirus, norovirus, adenovirus and astrovirus. The mean ( $\pm$  standard deviation) age of the children was  $2.71 \pm 2.37$  yr. The detection rate of viral agents was most common in children between the ages of 1 and 3 yr. Rotavirus was detected in 63 children (41.3%), norovirus in 56 (36.2%), adenovirus in 11 (7.1%), and astrovirus in 1 (0.6%). Regarding rotavirus, there were 38 (60.3%) cases with mono-infection and 25 (39.7%) with coinfection. For norovirus, there were 33 (58.9%) cases with mono-infection and 23 (41.1%) with coinfection. Coinfection with rotavirus and norovirus was most common, and occurred in 20/155 cases (12.9%) including coinfection with adenovirus. So, rotavirus and norovirus were the most common coinfectious viral agents in our study population with acute watery diarrhea.

**Key Words :** Coinfection; Watery Diarrhea; Rotavirus; Norovirus; Children

Hong Koh, Seoung Yon Baek,  
Jae Il Shin, Ki Sup Chung,  
and Young Mee Jee\*

Department of Pediatrics, Yonsei University College of  
Medicine, Severance Children's Hospital, Seoul; the  
Korean National Institute of Health\*, Seoul, Korea

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### Address for correspondence

Ki Sup Chung, M.D.  
Department of Pediatrics, Yonsei University College of  
Medicine, Severance Children's Hospital, 250  
Seongsan-ro, Seodaemun-gu, Seoul 120-752, Korea  
Tel : +82.2-2228-2050, Fax : +82.2-393-9118  
E-mail : alacrima@naver.com

## INTRODUCTION

There are more than 20 viruses known to cause acute watery diarrhea. Among them, four viral groups including group A rotavirus, norovirus, enteric adenovirus type 40/41, and astrovirus are frequently associated with acute watery diarrhea (1-3). There is limited information on coinfections in Korean children with watery diarrhea. Guerrero et al. demonstrated coinfection with another pathogen in 11 (42%) of the 26 Mexican infants with acute watery diarrhea (4). Simpson et al. also reported an incidence of causative agents (rotavirus in 28%, norovirus in 13%, sapovirus in 1%, and coinfections in 9%) in American children (5). Recently, Sanchez-Fauquier et al. showed that coinfection of rotavirus with other viral infections was detected in 6.3% of Spanish children with watery diarrhea in 2005 (6). The aim of this study was to assess the coinfectious viral agents causing watery diarrhea including age and seasonal distribution in this population.

## MATERIALS AND METHODS

### Subjects

The present study was conducted according to good clinical practice and was approved by our hospital ethics com-

mittee. Stool specimens were collected from 155 children with acute watery diarrhea that were admitted to Severance Children's Hospital, Seoul, Korea from July 2005 to June 2006. Acute watery diarrhea was defined as more than three liquid stools per day lasting for more than 24 hr (7).

### Sample collection and analysis

For long-term preservation of the viral samples, they were stored at  $-70^{\circ}\text{C}$  until use within two weeks. Rotavirus was detected by the rapid stick test (Biocard Rotastick™, Ani Biotech Oy, Vantaa, Finland) using a sandwich type immunoassay. Adenovirus and astrovirus were detected with the Viro-Capture™ Adenovirus Detection Kit (Bioicell, Houston, TX, U.S.A.) and Amplified IDEIA™ Astrovirus Kit (Dako, Glostrup, Denmark), respectively, according to the manufacturer's instructions using polyclonal and monoclonal antibodies in a solid phase immunoassay. The stool samples were also sent to the Korean National Institute of Health for the detection of norovirus. The samples were suspended in phosphate-buffered saline to a concentration of 10-20% and were tested immediately or stored at  $-70^{\circ}\text{C}$ . The stool suspensions were clarified by centrifugation at  $4^{\circ}\text{C}$ , 5,000 g for 10 min. The supernatants were mixed with an equal volume of fluorocarbon and centrifuged at  $4^{\circ}\text{C}$ , 3,500 g for 10 min; the supernatants were then collected and centrifuged at  $4^{\circ}\text{C}$ ,

100,000 g for 90 min. The pellets were re-suspended in H<sub>2</sub>O and examined after negative staining with 2.0% uranyl acetate. This suspension was centrifuged at 4°C, 20,000 g for 10 min, and viral RNA was extracted from 140 µL of the supernatant using a commercial RNA extraction kit (QIAmp viral RNA extraction kit; QIAGEN, Hilden, Germany) according to the manufacturer's instructions. After treating with DNase I (Invitrogen, Carlsbad, CA, U.S.A.) at 37°C for 5 min, the RNA sample was used for reverse-transcriptase-polymerase chain reaction (RT-PCR) immediately. Two sets of primers, NV82+SM82/NV81 (8) and Yuri22F/Yuri22R (9) were used for PCR to amplify the RNA-dependent PNA polymerase region. For nested PCR, two outer primer sets, 36/35' (10) and MR3/MR4 (11) were used for the first PCR; and then NV82, SM82/NV81, and Yuri22F/Yuri22R were used for the second PCR, respectively (12, 13).

All fresh stool specimens were cultured to isolate *Salmonella*, *Shigella*, and *Campylobacter* respectively. But other bacterial cultures of enteropathogens such as *E.coli* O-157, *Clostridium difficile*, *Vibrio*, and *Yersinia* were not performed before storage.

**Statistical analysis**

SPSS version 12.0 statistical package (Chicago SPSS Inc., Chicago, IL, U.S.A.) was used for the statistical analysis of frequency, one sample t-test and cross table method.

**RESULTS**

**Detection rates of infectious agents**

There were 115/155 (74.2%) positive cases. Rotavirus was detected in 63/155 (41.3%), norovirus 56/155 (36.2%), adenovirus 11/155 (7.1%), and astrovirus 1/155 (0.6%). For the rotavirus, 38/63 (60.3%) cases had mono-infection, and 25/63 (39.7%) coinfection. For the norovirus, 33/56 (58.9%)

**Table 1.** Incidence of viral infections in children with acute watery diarrhea

	Mono-infection	Coinfection	Total
Rotavirus	38 (60.3%)	25 (39.7%)	63/155 (41.3%)
Norovirus	33 (58.9%)	23 (41.1%)	56/155 (36.2%)
Adenovirus	2 (18.2%)	9 (81.8%)	11/155 (7.1%)
Astrovirus	1	0	1/155 (0.6%)

**Table 2.** Coinfectious viral agents and its incidence

Coinfection	No. of patients
Rotavirus+Norovirus	18/28 (64.3%)
Rotavirus+Adenovirus	5/28 (17.9%)
Rotavirus+Norovirus+Adenovirus	2/28 (7.1%)
Norovirus+Adenovirus	2/28 (7.1%)
Norovirus+ <i>Salmonella</i>	1/28 (3.6%)

cases had mono-infection, and 23/56 (41.1%) coinfection. One child had coinfection with *Salmonella* and norovirus. Among 11 cases with adenovirus, 9 (66.7%) cases had coinfection and only 2 (18.2%) cases had mono-infection. Astrovirus was detected in 1 case (Table 1).

As shown in Table 2, coinfection with another pathogen was observed in 28/155 (18.1%) cases. Coinfection with rotavirus and norovirus was the most common, and occurred in 20/155 (12.9%) including coinfection of adenovirus. In bacterial cultures, *Shigella* and *Campylobacter* were not cultured, but *Salmonella* was detected in only one child coinfecting with norovirus.

**Clinical manifestations of infectious agents**

The mean age of the children with acute watery diarrhea was 2.71 ± 2.37 yr of age with a slight male preponderance (M:F=88:67). Fever was present in 98 (63.2%) children and vomiting in 96 (61.9%). The diarrhea lasted from 3 days in 18 cases to 8 days in 10 cases. There was no significant difference between the cases with mono-infection compared to those with coinfection (data not shown).

**Age and seasonal distribution of infectious agents**

Thirteen (20.6%) children infected with rotavirus were less than 1 yr of age, and 35 (55.6%) were between 1 and 3 yr. Eighteen (32.1%) children infected with norovirus were less than 1 yr of age and 23 (41.1%) were between 1 and 3 yr. The peak age for both viruses was between 1 and 3 yr. Adenovirus infection was slightly higher in the children between 1 and 3 yr of age compared to other age group. However these differences were not statistically significant (Table 3).

Rotavirus outbreak was observed between December and March with its peak incidence in February. That is, 47 (74.6%) cases occurred during winter season. Norovirus had three peaks in February, June, and October without an apparent seasonal variation. Adenovirus infection showed an even occurrence throughout the year.

**DISCUSSION**

Acute watery diarrhea is a common disease resulting in

**Table 3.** Infectious agents and age distribution of patients

Age (yr)	Rotavirus	Norovirus	Adenovirus	Astrovirus	None
<1.0	13 (20.6%)	18 (32.1%)	2 (18.2%)	0	22 (55.0%)
1.0-2.9	35 (55.6%)	23 (41.1%)	4 (36.4%)	0	13 (32.5%)
3.0-5.9	12 (19.0%)	8 (14.3%)	3 (27.3%)	1	1 (2.5%)
≥6.0	3 (4.8%)	7 (12.5%)	2 (18.2%)	0	4 (10.0%)
Total	63 (100%)	56 (100%)	11 (100%)	1	40 (100%)

high morbidity worldwide, especially during early childhood (14-16). Cubitt et al. reported that 28% of children with gastroenteritis had rotavirus, 6% adenovirus, 3% astrovirus, and 3% calicivirus in a 1985 study conducted in London (17). In France Bon et al. showed that the rotavirus group A was detected in 61% of cases, calicivirus in 14%, astrovirus in 6%, and enteric adenovirus in 3% in 1999 (2). Chung et al. demonstrated that isolation rates of rotavirus, norovirus, adenovirus, and astrovirus of acute gastroenteritis in Korean children were 16.9%, 11.6%, 4.0%, and 4.0%, respectively (18). Recently, Lee et al. studied the etiologic agents in 962 Korean children hospitalized with gastroenteritis that rotavirus, norovirus, adenovirus and astrovirus were detected in 25.7%, 13.7%, 3.0%, and 1.1% of the study population, respectively (19). In this study, rotavirus was detected in 41.3%, norovirus 36.2%, adenovirus 7.1% and astrovirus 0.6%. Therefore, results of the present study were somewhat consistent with previous reports.

In 1998, Guerrero et al. reported that astrovirus was detected in 26/510 (5%) of Mexican infants with diarrhea and the coinfection with another pathogen occurred in 11/26 (42%), including 1 rotavirus, 2 *Campylobacter* spp., and 4 enterotoxigenic *E. coli* (4). Torres et al. showed that in Uruguay coinfection with two or more agents was detected in more than one-third of children with diarrhea, and *E. coli* enteric virotypes, especially enteropathogenic *E. coli*, were shown to be prevalent (20). Simpson et al. in America demonstrated that rotavirus was detected in 28%, norovirus in 13%, Sapporo virus in 1%, and coinfections in 9% in 2003 (5). Rosenfeldt et al. documented one patient that had coinfection with rotavirus and astrovirus conducted in Denmark. They also identified *Campylobacter jejuni* in 2 patients and *Clostridium difficile* in 5 patients among the coinfecting young children (14). Yan et al. reported that norovirus, sapovirus and astrovirus were detected in 42, 16, and 4 of the 62 positive samples, respectively, but no coinfection was found among these samples from Japanese pediatric patients (21). However, Roman et al. showed that the most frequent mixed infections were rotavirus-astrovirus, and rotavirus-adenovirus in Spanish children (22). In addition, Li et al. demonstrated that a total of 3,577 fecal specimens were collected from children with acute gastroenteritis in Japan, Korea and Vietnam during the period of 1998 to 2001. Among the three countries, the detection rate of adenovirus was highest in Korea (8.7%) (23). Recently, Chung et al. studied in 812 Korean children with acute gastroenteritis that coinfection of viral agents was confirmed in 2.7% of the study population, most commonly with rotavirus and norovirus and with rotavirus and human astrovirus (18). In our study, coinfections were observed in 28/155 (18.1%). This finding showed that rotavirus and norovirus were the most common combinations occurring in 20/155 (12.9%) cases. Therefore, coinfection with rotavirus and norovirus was more frequent in this population. Furthermore, the present study reported that

astrovirus infection occurred less frequently than reported in other studies and especially the cases of coinfecting adenovirus were larger than those of monoinfected. The differences among studies reporting viral infections in different countries might be explained by the different age group, seasonal variations at the time of sampling, and the viral detection methods used. However, all studies, including ours, agreed that rotavirus was the most common etiological agent.

Rotavirus most commonly affects children from 6 months to 2 yr of age. Surveillance in Japan between 1994 and 1999 showed that the most frequent etiology of acute watery diarrhea was rotavirus in children younger than 3, and norovirus in children older than 3 yr of age (24). Lee et al. in Malaysia also reported that rotavirus infection was most frequent in the less than 3 yr age group in 2006 (25) similar to the results of our study.

The limitations of this study included the following: 1) not all specimens were confirmed by RT-PCR or gene amplification although the detection rate by immunoassay was relatively high, 2) specimens were not cultured for all bacterial pathogens such as *E. coli*, *Vibrio*, *Yersinia* and *Clostridium* species using specific agar.

In conclusion, the results of this study showed that rotavirus and norovirus were the most common coinfectious agents responsible for acute watery diarrhea in Korean children. The incidence of coinfection among various viral agents was relatively high in this population presenting with acute watery diarrhea.

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