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-
100,000 g for 90 min. The pellets were re-suspended in H2O 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 (QIAamp 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 RNA 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 monoinfection, and 25/63 (39.7%) coinfection. For the norovirus, 33/56 (58.9%) cases had monoinfection, 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 monoinfection. 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 infected 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 monoinfection 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
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 coinfected 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 coinfected 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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