INTRODUCTION

Viral diarrhea is one of the most common illnesses in infants and children worldwide. It is estimated that approximately half a million infants and children died as a result of infectious diarrhea in 2015, with a mortality rate of almost 3.8 per 1,000 births (1). Even though mortality rates have decreased strikingly since the introduction of the rotavirus vaccine, infectious diarrhea caused by viruses is still common (1,2).

Rotavirus and norovirus are the most common causes of viral infectious diarrhea, while astrovirus and adenovirus are also important pathogens of this illness (3,4). Recently, other viruses such as sapovirus, Aichi virus, human parechovirus, enterovirus, human cosavirus, human bocavirus, and Saffold virus have been reported as causes of viral diarrhea (5-7). However, there is a lack of data on the prevalence of the various causative viruses in Korea. We must consider the possibility of viruses other than rotavirus and norovirus being present in cases of diarrhea.

MATERIALS AND METHODS

A total of 801 fecal specimens submitted to the clinical microbiology laboratory for rotavirus testing between July 2016 and July 2017 were included in this study. The study was conducted with the approval of the Institutional Review Board (IRB) of Inje University Busan Paik Hospital (No. 18-0053).

The viral nucleic acids were extracted using the STARMag 96X4 Universal Cartridge kit (Seegene, South Korea) according to the manufacturer’s instructions. The reverse transcription (RT) assay was carried out with the Super Script™ IV First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) after nucleic acid extraction. The reaction mixture with random hexamer was incubated at 23°C for 10 min, 53°C for 10 min, and inactivation initiated at 80°C for 10 min. The multiplex RT-polymerase chain reaction (PCR) was done with PCR primer sets and conditions described in a previous report (12). Initially,
five PCR sets (1 monoplex and 4 multiplex) were used with 5 primer sets (A, B, C, D, and E), and then two nested PCR analyses were done using two primer sets (F and G) for norovirus GII and primer set E using AccuPower® PCR PreMix (Bioneer, Daejeon, Korea). The PCR conditions of primer sets A, B, C, and D were as follows: 94°C for 3 min; 35 cycles of 94°C for 1 min, 50°C 1 min, and 72°C 1 min; and a final extension at 72°C for 7 min. The PCR conditions of primer set E were 94°C for 1 min, 30 cycles of 55°C for 1 min and 72°C 1 min, and a final extension at 72°C for 7 min. Nested PCR for norovirus GII after multiplex PCR set C was done at 94°C for 3 min and 35 cycles of 94°C for 1 min. Nested PCR using primer set G after multiplex PCR set E was performed for 35 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min to detect human cosavirus, human bocavirus, and Saffold virus. The PCR products were detected by electrophoresis on 2.0% agarose gel.

RESULTS

Of the 801 fecal specimens, 681 (85.0%), 59 (7.4%), and 61 (7.6%) were from patients < 1 year of age, 1 to < 5 years of age, and ≥ 5 years of age, respectively, and 434 (54.2%) were from male patients. At least one diarrheal virus was detected in 223 (27.8%) specimens (Table 1). Rotavirus A was the most common (17.7%; N = 137) followed by norovirus GII (5.0%; N = 40), enterovirus (4.2%; N = 34), adenovirus (1.0%; N = 8), astrovirus (1.0%; N = 8), human parechovirus (0.6%; N = 5), and human bocavirus (0.2%; N = 2). On the other hand, rotavirus B, rotavirus C, Aichi virus, human cosavirus, and Saffold virus were not detected in any samples. Among the samples, two viruses were found in 11 (1.4%) specimens. Mixed infection by rotavirus A and enterovirus was most common (0.5%), followed by rotavirus A and astrovirus (0.2%), rotavirus A and norovirus GII (0.2%), adenovirus and astrovirus, adenovirus and enterovirus, and rotavirus A and adenovirus (0.1% each).

Diarrheal viruses were most common in patients < 1 year old (78.6%) but less common in patients 1 to < 5 years old (15.0 %) and ≥ 5 years old (6.4%) (Table 2). Rotavirus, norovirus, and enterovirus were significantly common in patients < 1 years old at 89.1%, 55.0%, and 85.3%, respectively. Rotavirus A and norovirus GII were highly prevalent in the winter, although rotavirus A was detected all year round (Figure 1). In contrast, almost all enterovirus was detected in the summer. We
Prevalence of Diarrheal Viruses

Fig. 1. (Color online) Seasonal variation in prevalence of diarrheal viruses between July 2016 and July 2017.

could not confirm any seasonality of the other viruses due to their low prevalence.

**DISCUSSION**

Several viruses have been reported as important pathogens of diarrhea and cause high morbidity and mortality rates in children. Rotavirus and norovirus are well known as the most common causes of viral diarrhea; however, many other viruses also can cause diarrhea (7,12-14). In our study, 223 (27.8%) fecal specimens were positive for diarrheal viruses by multiplex RT-PCR. Nguyen et al. (14) reported the frequency of five common diarrheal viruses, namely rotavirus A, norovirus GII, adenovirus, sapovirus, and astrovirus, in Vietnam. In this report, rotavirus infection was extremely common, at 67.4%, whereas norovirus was present in only 5.5% of specimens. In the Ivory Coast, 34% of fecal specimens were found to contain rotavirus A (15). In Europe, the frequency of rotavirus A is between 25.0% and 64%, and the frequency is especially high in Norway and Sweden (16). The prevalence of rotavirus A was reported as 20.8% in China (17). In our study, rotavirus A was the most common, being detected in 17.1% of fecal specimens. Thongprachum et al. (13) investigated the prevalence of diarrheal viruses and reported that norovirus was most prevalent at 39.3%, despite rotavirus being at 20.1% in Japan. However, in this study, norovirus GII was the second most common after rotavirus at 5.0%, and its prevalence was similar to that of a previous report from Korea (18).

Adenovirus and astrovirus also are considered important pathogens of viral diarrhea. For this reason, several kinds of diagnostic kits have been created, including immunoassays and molecular methods, to detect four important diarrheal viruses, specifically rotavirus, norovirus, adenovirus, and astrovirus, at the same time (8,10). For the study period, the detection rates of adenovirus and astrovirus were only 1.0% each. Recently, enterovirus has been reported as a cause of viral diarrhea, although its relation to the disease is not well established. Alcala at al. (19) investigated the prevalence of diarrheal viruses using rotavirus-negative samples, and they found that enterovirus was the most common viral agent at 37.9%. In the report of Jain et al. (20), enterovirus was the second most common causative virus, at 5.6%, among rotavirus, norovirus, and enterovirus. In our study, enterovirus was the third most common, and the detection rate was 4.2%. Most enterovirus infections were found in July and August, and this seasonal variation is similar to that of previous reports (13,21). One needs to consider the possibility of an enterovirus infection if the common viral diarrheal tests are negative, in particular in the summer season.

For the other uncommon diarrheal viruses, rotavirus B, rotavirus C, norovirus GI, Aichi virus, human parechovirus, human bocavirus, sapovirus, human cosavirus, and Saffold virus were reported as causes of diarrhea in previous reports (5-7). In this study, we could detect human parechovirus and human bocavirus, although these were less prevalent. However, rotavirus B, rotavirus C, norovirus GI, sapovirus, Aichi virus, human cosavirus, and Saffold virus were not detected in this study. The fecal specimens included were intended for rotavirus testing, which may have caused prejudice to the specimen collection to detect various other kinds of diarrheal viruses. This is a limitation of this study.

In conclusion, we found that five diarrheal viruses, namely rotavirus A, norovirus GII, adenovirus, astrovirus, and enterovirus, are the most common causes of viral diarrhea. The possibility of other viruses besides the major five must be considered, even though the detection rate is very low.

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**Conflict of interest** None to declare.

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