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Epidemiologic Trends of Diarrhea-causing Virus Infection Analyzed by Multiplex Reverse Transcription PCR in Cheonan, Korea, 2010-2018

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Gastroenteritis with diarrhea is one of the most infectious diseases in the world following respiratory infections. Notably, diarrhea-causing viruses (DVs) cause more than 70% of such cases. In this study, 3,065 stool specimens from patients with diarrhea (median age, 1.1 years; range, 0.0–91.1 years), who were admitted to the DanKook University Hospital, were examined using multiplex reverse transcription PCR (mRT-PCR). The target viruses were astrovirus (AstV), enteric adenovirus (EAdV), group A rotavirus (RotV), norovirus GI (NoV-GI), and norovirus GII (NoV-GII). The mRT-PCR results were analyzed based on various factors such as seasonality, age, presence of co-infection, and analyzed trends. The detection rate of the DVs during the study period was found to be 30.8% (n = 943/3,065). When the detection rate was analyzed monthly, the DV detection rate was found to be highest between December to January. Of the detected DVs, NoV-GII was the most common, accounting for 45.5% of the detected viruses (n = 446/980). Notably, 86.5% (n = 848/980) of the pathogens were detected in individuals who were less than 5 years of age. During the study period, NoV-GII and RotV showed alternating trends. In addition, both the number and rate of co-infections increased.

Keywords: **Gastroenteritis with diarrhea, diarrhea-causing virus, surveillance, multiplex reverse transcription PCR**

Introduction

Gastroenteritis with diarrhea has been reported worldwide with approximately 3 to 5 billion patients per year and approximately 2 million deaths worldwide [1, 2]. Gastroenteritis with diarrhea is one of the most infectious diseases after respiratory infection worldwide [3, 4] and is known to be caused by diarrhea-causing viruses (DVs) in more than 70% of cases [5]. DVs include astrovirus (AstV), enteric adenovirus (EAdV), group A rotavirus (RotV), and norovirus (NoV) [6].

The incidence of gastroenteritis with diarrhea tends to increase with the active trade of agricultural products and foodstuffs among countries [7–9], and it has been reported that gastroenteritis with diarrhea is caused by DVs present in a community in many cases [10]. Except for RotV, there are no vaccines for DVs, and it is difficult to prevent infections [11, 12]. In addition, RotV vaccines are not widely available in developing countries with relatively high incidences of gastroenteritis with diarrhea, and the incidence and mortality of RotV-induced gastroenteritis with diarrhea remain high in developing countries [13].

Because of the public health implications of gastroenteritis with diarrhea, laboratory surveillance of gastroenteritis with diarrhea has been performed in preference to other diseases, and diarrheal disease monitoring is currently being conducted in many countries [14]. Korea is in the process of conducting acute diarrheal disease laboratory monitoring (EnterNet-Korea)

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[14], and the European Union is running EnterNet, which is a member of the National Reference Lab in 36 countries [15]; similar programs include FoodNet in the United States [16], OzFoodNet in Australia, and National Enteric Surveillance Program (NESP) and C-EnterNet in Canada [17].

To date, there have been many studies on short-term reports of DVs infection trends at the national level and on single virus species. However, studies on long-term reports and trend analyses of multiple virus species in a single local or single institution are rare. This study was based on a study by Kim *et al.* [18], and reported the long-term (2010–2018) infection pattern of DVs in Cheonan province. The purpose of this study was to identify the DVs infection trends in the Cheonan area of Korea and to use these trends as baseline data for the prevention of socioeconomic damage caused by gastroenteritis with diarrhea.

Materials and Methods

This study was approved by Dankook University Research Ethics Committee (IRB No. 2018-06-006).

Materials

From July 2010 to May 2018, 3,065 stool specimens were tested in the Department of Laboratory Medicine, Dankook University Hospital, using multiplex reverse transcription PCR (mRT-PCR) targeting five species of DVs (AstV, EAdV, RotS, Nor-GI, Nor-GII). The samples were subjected to nucleic acid extraction within 24 h.

Methods

Nucleic acid extraction: Stool specimens were diluted with 1 ml of distilled water, and 200 µl of the diluted solution was extracted with a Minelute RNA Spin Kit (Qiagen, Germany). The extracted nucleic acid was used to synthesize cDNA using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada) according to the protocol provided by the manufacturer. This cDNA was used for mRT-PCR.

Multiplex RT-PCR analysis: mRT-PCR was performed using the Seeplex® Diarrhea-V ACE detection kit (Seegene, Korea) according to the manufacturer's instructions with a PTC-200 PCR system (MJ Research, USA). The following target genes for DVs were used: ORF1a for AstV, Hexon for EAdV, VP4 for RotV, and ORF2 for NoV-GI/GII. The internal mRT-PCR control was the Cesa3 gene of Arabidopsis. The negative control was distilled water (Table 1). The mRT-PCR for the five viruses was performed using a Seeplex® Diarrhea-V ACE detection kit with dual specificity oligonucleotides (Seegene) as primers, and the five pathogens were detected in a single test tube.

Table 1. Target genes for diarrhea-causing viruses.

Target viruses	Target region	Gene size (bp)
Arabidopsis (Internal control)	Cesa3	1,000
Astrovirus (AstV)	ORF1a	650
Enteric adenovirus (EAdV)	Hexon	411
Group A Rotavirus (RotV)	VP4	541
Norovirus G-I (NoV-GI)	ORF2	304
Norovirus G-II (NoV-GII)	ORF2	214

Electrophoresis: The mRT-PCR products were electrophoresed in a 2% agarose gel with ethidium bromide for 30 min at 100–150 V. The agarose gel was rinsed with distilled water, amplification was assessed by visualizing the gel on a UV trans illuminator, and the results were analyzed after a photograph was taken.

Statistics: The viruses detected by mRT-PCR were analyzed according to various criteria such as month, age, and virus.

Results

Overall statistics

Out of a total 3,065 stool specimens, (from 1,730 male and 1,335 female donors), 943 tested positive for the virus resulting in a detection rate of 30.8%. A total of 980 viruses were detected in these 943 virus-positive stool specimens. The detection rates were 31.5% (n = 545/1,730) and 29.8% (n = 398/1,335) for males and females, respectively. The mean age of the subjects was 6.5 years (median age, 1.1 year; range, 0.0–91.1 years). The mean age of the patients positive for a virus was 2.8 years (median age, 1.3 years; range, 0.0–87.5 years). NoV-GII was the most frequently detected virus, accounting for 45.5% of detected viruses (n = 446/980), and RotV was

Table 2. Percentage of diarrhea-causing viruses at Dankook University Hospital, Cheonan, Korea, 2010 to 2018.

Average age (years)	6.5	
Median age (years)	1.1	
	Number	(%)
Patients	3,065	100.00
Positive patients	943	30.8
Male	1,730	56.4
Male positive patients	545	31.5
Female	1,335	43.6
Female positive patients	398	29.8
Pathogen	Number	(%)
Astrovirus (AstV)	44	4.5
Enteric Adenovirus (EAdV)	86	8.8
Group A Rotavirus (RotV)	389	39.7
Norovirus-GI (NoV-GI)	15	1.5
Norovirus-GII (NoV-GII)	446	45.5
Total	980	100

the second most frequently detected virus, accounting for 39.7% of detected viruses (n = 389/980) (Table 2).

Statistics by month

The rate of virus detection was the highest in December at 48.1% (n = 136/283), and the detection rate was the lowest in July at 12.5% (n = 35/279). The highest detection rate of NoV-GII was from November to January, and the highest detection rate of RotV was from February to July. The detection rate of AstV was the highest in June at 17.1% (n = 7/41), and the highest rate of EAdV was in September at 27.5% (n = 11/40) (Fig. 1).

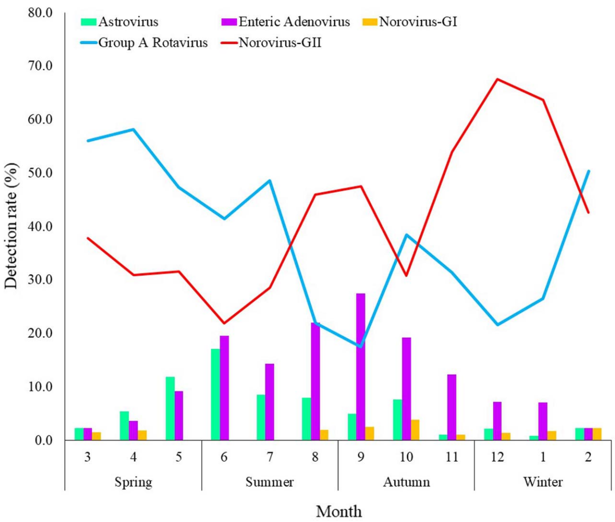

Fig. 1. Detection rate of diarrhea virus at Dankook University Hospital, Cheonan, Korea, 2010-2018, aggregated by month.

Statistics by age

The detection rate was the highest in the greater than 1–3 years old group at 50.7% (n = 332/655), and the second highest detection rate was in the less than 1 year old group at 25.7% (n = 381/1,481). Of the total positive specimens, 86.5% (n = 817/943) were from individuals less than 5 years old (Fig. 2).

Statistics by co-infection

The co-infection rate was 3.9% (n = 37/943). The mean age of patients with co-infection was 4.1 years (range, 0.0–63.0 years; median age, 1.7 years). The co-infection rate of NoV-GII among the examined viruses was 81.1% (n = 30/37). The co-infection rate of NoV-GII and RotV was the highest at 40.5% (n = 15/37), and the co-infection rate of NoV-GII with EAdV was the second highest at 24.3% (n = 9/37).

Trends

NoV-GII had the highest detection rate in 2010, 2011, 2012, 2014, 2016, and 2017, and RotV had the highest detection rate in 2013, 2015, and 2018 (Fig. 3). Note that AstV, EAdV, and NoV-GI were excluded from the graph in Fig. 3 for ease of viewing. The co-infection rate was

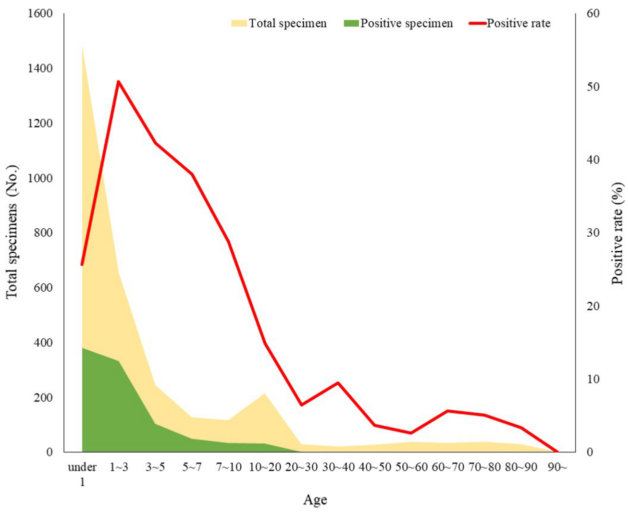

Fig. 2. Age distribution of diarrhea virus-infected specimens and positive rate, 2010-2018, Cheonan, Korea.

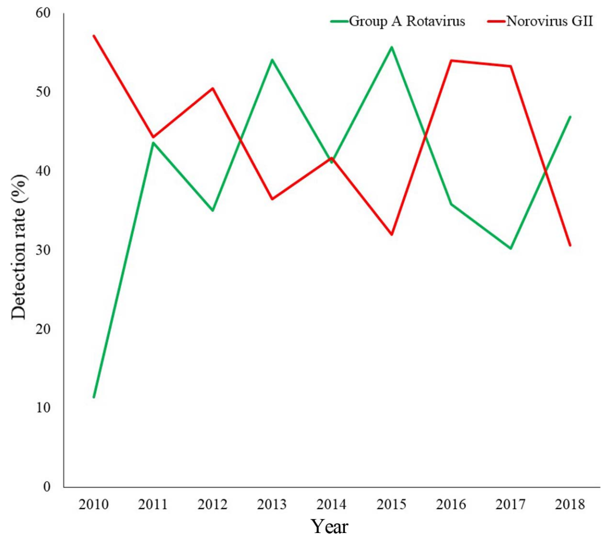

Fig. 3. Detection rate of diarrhea virus at Dankook University Hospital, Cheonan, Korea, 2010-2018.

the highest at 5.8% (n = 10/172) in 2017.

Discussion

Overall statistics

Despite improvements in living and sanitary conditions, the detection rate of DVs is not declining [3, 9]. During the study period, the DVs detection rate was 30.8% (n = 943/3,065) at Dankook University Hospital in Korea. This was similar to the 30.6% detection rate reported for the acute viral laboratory surveillance at the Korea Centers for Disease Control and Prevention. There was no significant difference between males and females, as 31.5% (n = 545/1,730) of males and 29.8% (n = 398/1,335) of females were positive for DVs. The mean age of the subjects with a DVs was 2.8 years, and the mean age of the subjects was 6.5 years. DVs tend to occur more frequently in children younger than 5 years of age [10], suggesting that the average age of the DVs detection group is low. The highest detection rate during the study period was in 2016 (35.5%) (n = 131/369). Among the detected DVs, the detection rate of NoV-GII was the highest at 45.5% (n = 446/980). This was in line with the results of studies in Korea, Taiwan, and Japan over a similar period [19–22].

Statistics by month

The results of DVs detection by month revealed that the highest detection rate was from November to April, and the detection rate decreased from May and remained low from June to September. These trends were similar to the results reported by Cho *et al.* in Korea in 2016 [10]. By virus, AstV showed a tendency to be common from April to October. The detection rate of EAdV increased from May to 27.5% (n = 11/40) in September. RotV had a high detection rate between January and May, which was similar to that reported by Shim [23] and Lee *et al.* [19]. The detection rate of NoV-GII increased from October and peaked in December (67.7%, n = 94/139). These results were similar to those of Chi *et al.* for a study in Taiwan in 2017 [21]. NoV-GI showed no detectable pattern.

Statistics by age

DVs are commonly known to have a high detection rate in groups less than 5 years of age [10, 20, 24]. In the present study, 86.6% (n = 817/943) of the DV-positive specimens were from the under 5 years of age group. Moreover, 93.0% (n = 80/86) of EAdV-positive specimens were from the under 5 years of age group. This is similar to the results reported by Cho *et al.* in 2016. In this study, we analyzed the group children who were under 10 years old, who accounted for 91.8% (n = 866/943) of DV-positive specimens, by dividing them into age groups with 2 year increments. The detection rate of DVs was the highest in the group below 1 year of age at 40.4% (n = 381/943), and it decreased with age according to the following trend: the younger the age, the higher the virus susceptibility and the higher the DVs detection rate.

Table 3. Number of co-infected specimens and percentage of co-infection

Co-infected pathogen		Number	(%)
AstV	EAdV	0	0.0
	RotV	2	5.4
	NoV-GI	0	0.0
	NoV-GII	4	10.8
EAdV	RotV	2	5.4
	NoV-GI	1	2.7
	NoV-GII	9	24.3
RotV	NoV-GI	2	5.4
	NoV-GII	15	40.5
NoV-GI	NoV-GII	2	5.4
Total		37	100

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Statistics by co-infection

The double infection rate of DVs was 3.92% (n = 37/943), and there was no triple infection of DVs during the study period. The double infection rate was higher than that reported by Lee *et al*. [25]. The most common co-infected DVs was NoV-GII (n = 30/37). The co-infection rate of NoV-GII and RotV was the highest (40.5%, n = 15/37), which was the same as the results reported by Thongprachum *et al*. [22] in Japan in 2015 and Koh *et al*. [24], and the number of co-infected specimens and co-infection rate were the highest in 2017 (5.8%, n = 10/172). During the study period, both the number of co-infections and the rate of co-infection tended to increase. Thus, this year by year increase in the co-infection rate makes it necessary to study and monitor co-infections. Moreover, according to a study by Lee *et al*. [20] in 2017, the co-infection rate of DVs and diarrhea-causing bacteria was as high as 23.2%; thus, further studies related to diarrhea-causing bacteria are needed.

Trends

Analysis of the trends of DVs during the study period revealed that NoV-GII and RotV exhibited alternating trends. The study by Cho *et al*. [10] in Korea in 2016 and the study by Thongprachum *et al*. [22] in Japan in 2015 reported similar trends. The change in the detection rate of NoV-GII is known to be highly related to variants emerging every 2 to 3 years [10, 26]. In Korea, the GII.4 Sydney variant, a variant of NoV-GII, appeared in 2012 [27], the GII.17 variant appeared in 2014, and GII.2 and GII.3 appeared in 2016. However, in this study, the detection rate of NoV-GII in 2012, 2014, and 2016 was higher than in each respective previous year. NoV-GII was reported to be prevalent in the cold season [28], but in this study, more than 30.0% of cases were detected each month, regardless of season. There is currently no commercialized vaccine or no special treatment for NoV-GII [12]. Therefore, constant attention and surveillance are needed, as it can be prevalent in the future. The detection rate of AstV was 6.7% (n = 11/163) in 2014, which was higher than its average detection rate of 4.5% (n = 44/980), possibly reflecting an outbreak in Korea in 2014 [29, 30].

Limitations

In this study, the DVs genotypes were not analyzed in detail, so it was difficult to accurately determine the genotypes of the DVs. Another limitation was that the statistics were for one local region in Korea. However, there have been a number of studies that have examined trends over a short period of 2–3 years or studies on long-term trends for single DVs. The results of this study will help to understand the trend of DVs and will be aid in the prevention and treatment of gastroenteritis with diarrhea caused by DVs.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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