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# Comparison of Group A, B and C Rotaviral Gastroenteritis among Children in Korea: Prevalence and Clinical Features

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**Purpose:** The aim of this study is that the prevalence of rotavirus infection was evaluated by each group and clinical features of group A, B and C rotaviruses infections were described respectively to compare one with another.

**Methods:** Between January 2010 and December 2010, we enrolled a group of children below 10 years of age admitted for management of acute diarrhea at the Catholic University of Korea Bucheon St. Mary's Hospital. A total of 310 stool samples documented to be free of common bacterial pathogens were collected from children with diarrhea. The presence of group A, B or C rotavirus is indicated by amplification of DNA segments of the expected lengths after the first and second PCRs

**Results:** In a total of 310 stool specimens, 40 (12.9%) specimens were positive for rotaviruses. These included 23 (7.4%) positive for group A, 5 (1.6%) for group B and 12 (3.9%) for group C rotaviruses. Group B rotavirus infected patients had significantly less diarrheas per day (group A: $P$=0.01, group C: $P$=0.01) and shorter duration of vomiting days (group A: $P$=0.03, group C: $P$=0.03) than those with group A and C rotaviruses infection respectively. All the group B rotaviruses had been isolated in March and October. Group C rotavirus infections were prevalent during late summer and early winter and peaked in October.

**Conclusion:** These findings indicate that group B and C rotaviruses are notable causes or the contributing causes of diarrhea among infants and children in Korea.

**Key Words :** Rotavirus, Gastroenteritis, Prevalence

## Introduction

Rotavirus is one of the most important causes of acute gastroenteritis in mankind and animals worldwidely[1]. Group A rotavirus has been considered as the major cause of severe diarrhea in mankind, whereas the clinical importance of group B and C rotaviruses gastroenteritis remains unclear[2].

Occasionally called adult diarrhea rotavirus, group B rotavirus is distinct genetically and antigenically from group A and group C rotaviruses[2]. The prevalence of group B rotavirus is not as high as that of group A rotavirus; however, serological findings indicate that group B rotaviruses are distributed so wide that most people could acquire antibodies to the virus early in life at least in prevalent areas[3].

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As few laboratories provide specific diagnoses of group B rotavirus, there is a scarcity of studies concerning group B rotavirus infections; therefore, the epidemiological patterns and clinical influence are poorly understood.

Averagely, the worldwide seroprevalence of group C rotavirus infection is nearly 33%, with a peak in the older age groups[4-7]. Despite a number of reported studies on sporadic cases of severe diarrhea or outbreaks of gastroenteritis caused by group C rotavirus in many countries, the prevalence of group C rotavirus and a clinical importance of the disease caused by this virus remain uncertain[8-15].

The clinical features of group A rotavirus-infected patients have been described broadly[16]. Most studies have reported fever, a high frequency of vomiting and dehydration, which lasted for 1-9 days[17]. The majority of patients infected with group B rotavirus experienced vomiting and/or abdominal pain along with diarrhea, but fever was reported in only a few patients (4.5%)[18]. Also, other studies have reported that group B rotavirus was responsible for severe cholera-like diarrhea, with vomiting and severe dehydration[19]. Group C rotavirus has been reported to be associated with relatively mild disease; fewer episodes of vomiting, less dehydration and fewer hospitalizations when compared to group A rotavirus infection in children[9, 12].

However, above studies based their conclusions on very small sample sizes for analysis of clinical data. Moreover, very few studies had been executed to compare the clinical presentations and prevalence of patients infected with group A, group B and group C rotaviruses one another, which was not far sufficient to provide knowledge helpful to physicians treating patients infected with rotavirus. It spurred us on trying this study even if this study still may mean preliminary. We analyzed 310 children with acute diarrhea for a year. Among them, the prevalence of rotavirus infection was evaluated by each group and clinical features of group A, B and C rotaviruses infections were described respectively to compare one with another.

## Materials and Methods

### 1. Subjects

Between January 2010 and December 2010, we enrolled a cohort of children below 10 years of age admitted for treatment of acute diarrhea at the Catholic University of Korea Bucheon St. Mary's Hospital. A total of 310 stool samples documented to be free of common bacterial pathogens were collected from children with diarrhea.

### 2. DNA preparation by RT-PCR

The fresh or frozen stool used with in the first steps of the protocol. Stool samples are lysed in buffer ASL (QIAGEN GmbH, Hilden, Germany). DNA-damaging substances and PCR inhibitors present in stool sample are adsorbed to inhibit EX matrix. The inhibit EX matrix is pelleted by centrifugation and the DNA and RNA in the supernatant is purified on QIAamp mini spin columns (QIAGEN GmbH, Hilden, Germany).

Viral nucleic acid was extracted from 200 mL of the filtered supernatant, using a QIAamp DNA stool kit and QIAamp Viral RNA mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Five microliters of the extracted nucleic acid was first incubated with 500 mM dNTPs and

50 ng random primers at 65℃ for 5 min, followed by reverse transcription using 100 U SuperScript II reverse transcriptase (Invitrogen), 20 U RNase inhibitor, The reaction was incubated at 25℃ for 5 min, 50℃ for 60 min and heat inactivated at 70℃ for 15 min. Synthesized cDNA was used in PCR detection of the viruses.

### 3. DNA Amplication

The presence of group A, B or C rotavirus is indicated by amplification of DNA segments of the expected lengths after the first and second PCRs: 1062 and 257 bp for group A, 489 and 434 bp for group B, and 356 and 327 bp for group C rotavirus, respectively. For first PCR, primer pairs A1-A4, B1-B4 and C1-C4 (Table 1) were used separately in individual reverse transcription polymerase chain reaction (RT-PCR) assays of the three human rotavirus groups[20]. For second PCR, primer pairs A2-A4, B1-B3 and C1-C3 (Table 1, Fig. 1) were used in RT-PCR assays[20].

### 4. Statistical analysis

Statistical analysis to compare clinical features among group A, B and C rotavirus-infected patients was performed using SPSS (SPSS Inc., version 18.0, Chicago, IL, USA) for Windows by chi-square test or Fisher's exact test. A *P* value under 0.05 was considered statistically significant.

### 5. Results

40 (12.9%) cases were positive for rotaviruses in 310 stool specimens. These included 23 (57.5% of the rotavirus infections) positive for group A, 5

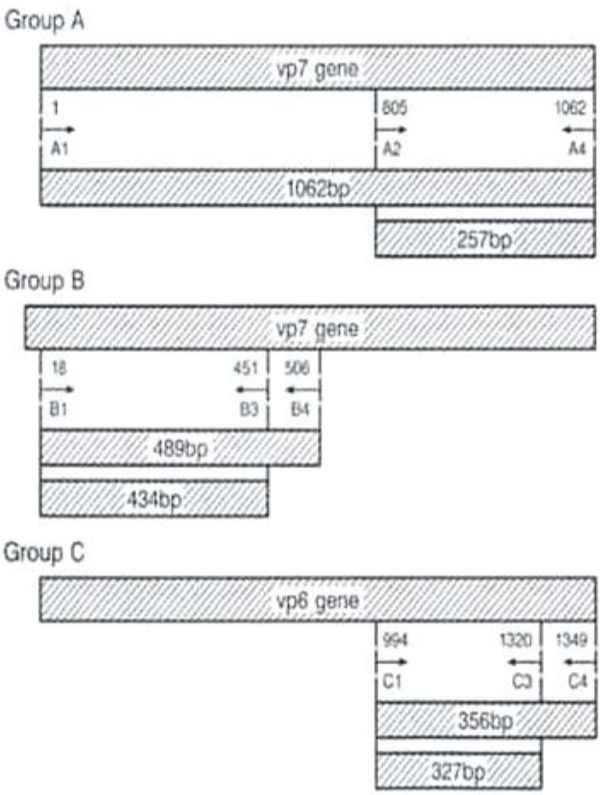


**Fig. 1.** PCR primers for detection of rotavirus groups A, B and C.

**Table 1.** Primers used for RT-PCR

| Primer (nested) | Name | Oligonucleotide Sequence (5'-3') |
|---|---|---|
| Rotavirus A (VP7) | A1 | GGC TTT AAA AGA GAG AAT TTC CGT CTG G(forward) |
| | A2 | GGA CCA AGA GAA AAC GTA GC (reverse) |
| | A4 | GGT CAC ATC ATA CAA TTC TAA TCT AAG (reverse) |
| Rotavirus B (VP7) | B1 | CTA TTC AGT GTG TCG TGA GAG G (forward) |
| | B3 | CGA AGC GGG CTA GCT TGT CTG C (reverse) |
| | B4 | CGT GGC TTT GGA AAA TTC TTG (reverse) |
| Rotavirus C (VP6) | C1 | CTC GAT GCT ACT ACA GAA TCA G (forward) |
| | C3 | GGG ATC ATC CAC GTC ATG CG (reverse) |
| | C4 | AGC CAC ATA GTT CAC ATT TCA TCC (reverse) |

(12.5%) for group B rotavirus and 12 (30%) for group C rotavirus.

Epidemiologic and clinical data of patients infected with group A, B, and C rotaviruses were examined and compared with one another (Table 2). The age range of children excreting group A (2-72 months) and excreting group C rotaviruses (4-78 months) were higher than that of children infected with group B rotavirus (6-36 months), but the difference was not significant ($P$=0.12).

Although these three groups of patients had similar overall clinical features such as hospitalization, diarrhea and fever days, vomiting per day, group B rotavirus patients had significantly less diarrheas per day than those with group A and C rotaviruses infection (group A: $P$=0.01, group C: $P$=0.01). Patients infected with group B rotavirus tend to have shorter duration of vomiting days than those infected with group A and C rotaviruses significantly (group A: $P$=0.03, group C: $P$=0.03). Amongst patients infected with group A rotavirus, only 2 patients showed severe dehydration and one patient suffered seizure during hospitalization.

Monthly distribution of group A, B and C rotavirus-positive cases was showed in Fig. 2. Group A rotavirus infections were prevalent during late winter, autumn, and summer, All the group B rotaviruses were isolated in spring and autumn. Any case of group B rotavirus infection was not detected in winter (January-February and November-December).

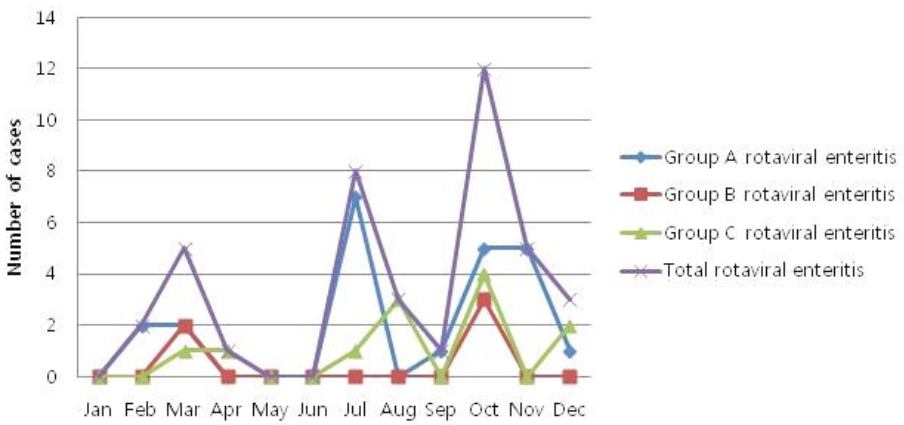


**Fig. 2.** Monthly distribution of group A, B and C rotavirus-positive cases in Korea.

**Table 2.** Comparison of Clinical Features of Patients Infected by Group A, B and C Rotaviruses

| | Group A (N=23) | | Group B (N=5) | | Group C (N=12) | | $P$-value |
|---|---|---|---|---|---|---|---|
| | Median | Range | Median | Range | Median | Range | |
| Age (months) | 12 | 2-72 | 14 | 6-36 | 17 | 4-78 | >0.05 |
| Hospitalization (days) | 6 | 2-11 | 6 | 3-7 | 5 | 1-11 | >0.05 |
| Diarrhea duration (days) | 5 | 3-9 | 3 | 2-5 | 4 | 2-9 | >0.05 |
| Diarrhea frequency (/day) | 9 | 3-20 | 4 | 2-6 | 6 | 3-8 | 0.01 |
| Vomiting duration (days) | 2 | 1-3 | 0 | 0-1 | 2 | 0-3 | 0.03 |
| Vomiting frequency (/day) | 4 | 2-10 | 0 | 0-3 | 3 | 0-6 | >0.05 |
| Fever (days) | 2 | 0-6 | 0 | 0-4 | 2 | 0-5 | >0.05 |

Group C rotavirus infections were prevalent during late summer and early winter, with most cases occurring between August and December.

## Discussion

There are several reasons why very few studies have reported in detail on the clinical manifestations of group A, B and C rotavirus infections in terms of comparison. Most of all, distribution of group B rotaviruses is restricted to one geographical area and as few as studies have investigated the epidemiology of these viruses.

China, India and Bangladesh, only three neighbouring countries, have reported group B rotavirus, with the current addition of Myanmar to the list. In India, during 2003-2004, group B rotaviruses constituted 2-4% of diarrhea patients attending Dhaka hospital in whom no common diarrhea pathogen was isolated. The present our study also detected a similar proportion (1.6%) of patients infected by this virus. However, a remarkably higher percentage (18.5%) of group B rotaviruses was isolated from Indian children during 2002-2004[18]. In spite of lack of published references, it is apparent that group B rotavirus infection accounts for exraordinary percentage of rotaviral gastroenteritis, which will require further researches.

After finding the prevalence and clinical features of group B and C rotavirus-involved children, we compared them with those of group A rotavirus-involved children. Our study confirms that group B and C rotaviruses have been circulating at Bucheon St. Mary's Hospital and that they are responsible for a substantial proportion of pediatric diarrheal illness.

When compared with the clinical features of group A rotavirus infection, it was found that group B infection included most of the symptoms of group A infection. However, dehydration was milder in group B rotavirus-infected patients than in group A rotavirus-infected patients. While many investigators described previously that most of the group B-positive patients were older than 18 years, all the group B-positive patients in our study were younger than 4 years. According to 5 cases, it is highly implicative that group B rotavirus infections can cause less significant symptoms in infants and children. In restricted communities of Korea during the surveillance period, small-scale of group B rotavirus outbreaks might occur and go undetected, particularly if the severity of the disease is mild and hospitalization may not be required.

The frequency of group C rotavirus infections detected in this work (3.9%) was higher than in other studies carried out with pediatric population in Korea (0.7%), Turkey (0.8%), Japan (1.2%), Nigeria (1.8%), Argentina (2.8%) and Malawi (3.3 %)[10-11, 21-24]. However, the prevalence of the infection was lower compared to Japan (10.2%), Spain (16%)[12, 13]. Group C rotavirus has also been found in co-infection with other enteric pathogens such as *Vibrio cholerae, Shigella flexneri* and group A rotavirus[10, 12, 14]. But, any mixed infections (Vibrio Cholerae, Shigella Flexneri) with group C rotavirus were not shown in this study.

The data presented above, together with other studies, demonstrate clearly that the selection of diagnostic methods can directly influence detection rates. With this very same collection, a 1% detection rate was reported previously using only one ELISA method for screening with nested PCR for confir-

mation. The incorporation of an additional ELISA method (CDC-ELISA), modifications in the set of primers for RT-PCR and nested PCR and the use of Southern blotting increased the estimate of group C rotavirus incidence to 3%[25].

The median age of group C rotavirus detections in the current study were 17 months old. Previously, seroepidemiological investigations and detection data suggested a difference between age distribution in children with diarrhea caused by group A rotavirus, which typically infects children before 3 years of age, and group C rotavirus, which affects older children[7, 25, 26]. Although it is generally believed that group C rotavirus is more prevalent among older children (>4 years), detection of group C rotaviruses in both older and younger children is not surprising at all[9-12, 15]. However, the small number of samples in the present study did not allow us to draw solid conclusions about the prevalence in older children.

The sporadic nature of group C rotavirus infections along with the possibility of rapid dissemination among susceptible individuals in certain settings could be the cause of large outbreaks. It has been suggested that sporadic introduction from a hypothetical reservoir could explain at least in part the variability in detection rates observed in short studies[27]. This study does not suggest that the group C rotavirus have currently a major epidemiological impact, even after the introduction of a group A rotavirus vaccine.

The fact that the group C rotavirus strains do not remain circulating in humans like group A rotavirus, may suggest that this group does not achieve the fitness required to become a successful human pathogen[28]. It is an alternative hypothesis that the group C rotavirus may cause a subclinical infection in humans. Based on the study conducted in Hungary, the group C rotavirus were detected from raw sewage samples, suggesting that the virus is in circulation; however, a significant increase in the number of sporadic cases or outbreaks was not observed[29].

It is noted that the group B and C rotavirus incidence and associated disease remain unclear once sensitive tests for its detection are not available to clinical laboratories. The diagnosis is difficult since most of the ELISA assays do not recognize the group B and C rotavirus specific VP7, VP6 antigen. The RT-PCR using group B and C rotavirus specific primer is a convenient option[30]. However, it has not been used widely due to the large costs for routine surveillance.

In conclusion, these findings suggest that group B and C rotaviruses are notable causes of diarrhea among infants and children in Korea. Until now, as mentioned above, group B rotaviruses have been confined regionally and for this reason little attention has been paid to them worldwide. The studies of group C rotavirus also were insufficient. Continuous monitoring of group B and C rotaviruses both in hospitals and in the community may be helpful to evaluate the true clinical importance of group B and C rotaviruses.

## 한 글 요 약

### 한국 소아에서의 로타 장염군의 비교: 유병율과 임상증상

**배길성 · 배우리 · 김지훈 · 빈중현 · 김현희 · 이희진 이원배**

*가톨릭대학교 의과대학 소아과학교실*

**목적:** 본 연구의 목표는 로타 바이러스 감염의 유병률을

로타 바이러스 A, B, C 군으로 나누어 평가하고 각 군의 임상 양상을 서로 상대적으로 비교함에 있다.

**방법**: 2010년 1월에서 2010년 12월 사이에, 부천 성모병원에 급성 장염으로 입원한 10세 미만 환아를 대상으로, 총 310개의 대변표본이 추출되었고, PCR을 통해 DNA 증폭과정을 거쳐, A, B, C 군의 로타 바이러스 군을 구분하였다.

**결과**: 총 310개의 대변표본에서 40개(12.9%)에서 로타 바이러스 양성이 확인되었으며, 이중 23개(7.4%)가 로타 바이러스 A군에 양성, 5개(1.6%)가 B군, 12개(3.9%)가 C군에 양성 소견을 보였다. B군은 A군과 C군에 비교하여 유의하게 경한 설사증상과 짧은 구토기간을 보였다(group A: $P$=0.01, group C: $P$=0.01) 모든 B군 로타 바이러스는 3월과 10월에 발견되었으며, C군은 늦은 여름과 초겨울에, 그 중에서 10월에 최고의 유병률을 보였다.

**결론**: 국내에서 로타 바이러스 장염은 주로 로타 바이러스 A군에 의하여 발생하였다. 그러나 일부에서는 로타 바이러스 C군과 B군이 원인으로 확인된바 향후 이에 대한 지속적인 연구가 필요할 것으로 사료된다.

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