

Detection of Rotavirus in Busan, 1999

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Abstract

Rotavirus is highly contagious diarrhea virus, and is one of the most common cause of severe diarrhea-associated gastroenteritis in infants and children worldwide. Rotavirus was investigated while searching prevalence of enteric viruses in human. Twenty two cases of rotavirus was identified by the Latex agglutination test from human feces. Rotavirus was detected from 10 infants between 1 and 2 years old, 5 children aged from 3 to 5, 2 children of 7 years old, 2 children of 13 and 14 years old. And also 3 adults were found out to be virus positive. Virus outbreak appeared 3 cases in February, 4 cases in March, 1 case in July and 14 cases in December. Electron microscopic observation of negative-stained rotavirus showed typical appearance of wheel-shaped and about 70 nm in diameter.

Key Words : Rotavirus, diarrhea virus, Latex agglutination test

INTRODUCTION

Rotavirus is an icosahedral virus in reoviridae, has a distinct morphologic appearance. It is wheel-shaped RNA virus and named after its appearance. Rotavirus is a human pathogen and also animal-to-human transmission are known, but the latter does not appear to be common. Human strains of rotavirus

differ from those infect animal, but human rotavirus strains that possess a high degree of genetic homology with animal strains have been identified. Human rotavirus has a worldwide distribution and is one of the most common cause of severe acute gastroenteritis in children in the industrial world^{1, 4, 8, 9)}. Rotaviruses include seven major groups(A~G), most human strains belong to group

A, although groups B and C have occasionally been associated with human illness^{6, 7)}. Some children experience seizures with fever or chronic malabsorption and require a longer period of recovery or more complicated evaluation. Rotavirus infects intestinal cells and kill them. The diarrhea caused by this pathogen may result from the loss of absorptive area and the flux of water and fluid across the damaged surface. Some evidence showed that rotavirus produce an enterotoxin that causes or contributes to the development of diarrhea^{1, 3, 5)}.

Rotavirus cause approximately one third of diarrhea-associated hospitalizations and 800,000 deaths per year. By age 4, almost every child will have had a rotavirus infection. Throughout the world each year approximately 140 million cases of rotaviral gastroenteritis occur and more than 870,000 result in death due to severe dehydration and electrolyte loss³⁾.

In this study, the authors have detected rotavirus from patients while working on the epidemiological study of diarrhea infection in Busan as an annual program.

MATERIALS AND METHODS

Collection of specimens. Stools from suspected diarrhea patients were collected

from the designated hospitals in Busan in 1999.

Preparation of specimens. Approximately 10% suspension of the stool specimen in Extraction Buffer(one bottle containing 25ml of 0.1 M phosphate buffered 0.85% saline pH 7.3 with 0.1% sodium azide) was prepared. This operation should be carried out by transferring 1ml of Extraction buffer (two 0.5ml volumes from the calibrated dropper in the bottle) into a centrifuge tube. When a swab specimen is applied, it has to be put into a small tube containing 1ml of Extraction Buffer and mix thoroughly, remove swab and centrifugation is followed. Using a spatula or sampling stick add approximately 0.1ml of fecal sample, approximately one tenth of the volume of the Extraction Buffer, cap the tube and vortex to homogenize the pellet. Let samples to stand for at least 5 minutes at room temperature (18 to 25°C) and then centrifugation (1000×g, 10 min) was followed. The supernatant may be stored at 2-8°C for up to 3 days or at -20°C for long storage but thaw or re-freeze is not allowed as this may reduce the sensitivity of the test. All reagents used for this process was brought to room temperature, preferably 18 to 25°C.

Identification. The Murex Rotavirus Latex(ZL40, Murex Biotech limited, UK) agglutination test was used to detect rotavirus.

Test was performed by the supplier's direction. Positive result was read by the development of an agglutinated pattern showing clearly visible clumping of the latex particles within two minutes of mixing the latex with the fecal extract.

Electron microscopy. The supernatant prepared from stool was used for microscopic observation. Rotavirus was examined ($\times 120K$) under an electron microscope (JEM 1200 EX2, JEOL) after negative staining with 4% uranyl acetate for 1 min.

RESULTS AND DISCUSSION

Rotaviruses are ubiquitous, 95% of children worldwide are infected by 3 to 5 years of age and it is a major cause of diarrhea and gastroenteritis in babies and pre-school children²⁾. Our rotavirus isolation showed 15 out of 22 cases were 1 to 5 years of age, 2 of 7 years old and 2 of teen-ager. But in Pusan, 1999, 3 adults of 30, 40 and 57 years old showed positive in virus isolation (Table 1). This result indicates that rotavirus is no longer children's diarrhea agent.

Table 1. Isolation of rotavirus in Busan, 1999

Month	Age	Sex	Specimens
Feb	40	F	Stool
	2	M	◇
	1	M	◇
Mar	4	M	Stool
	1	F	◇
	3	M	◇
July	30	F	◇
	2	F	Stool
Dec	2	M	Stool
	4	F	◇
	57	M	◇
	1	F	◇
	3	M	◇
	7	F	◇
	14	M	◇
	7	M	◇
	2	M	◇
	5	F	◇
	13	M	◇
	1	F	◇
2	M	◇	
2	M	◇	

M : Male, F : Female

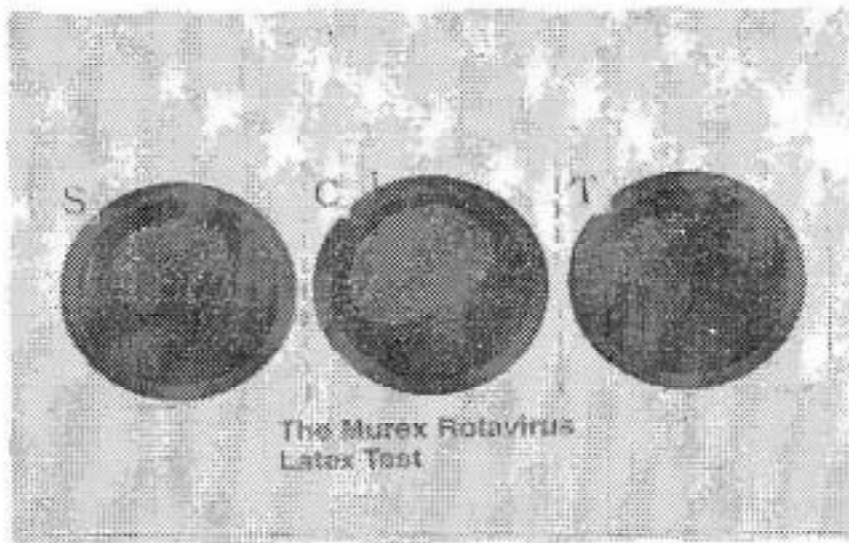


Fig. 1. Identification of rotavirus by Latex agglutination test. Symbols: S, Standard positive control, C, Negative control, T, Sample from patient's fecal supernatant.



Fig. 2. Electron micrograph of rotavirus particles observed in a stool supernatant prepared from 2 years old female. Magnification, $\times 120K$. Bar represents 50nm.

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023478 80.0KV X120K 50nm

Rotavirus has no susceptible cell, so Latex agglutination test is the only method to identify the virus. Direct agglutination test gave a clear reaction on virus confirmation as shown in Fig. 1. Rotavirus has a distinctive morphologic appearance by negative-stain electron microscopy. The morphological feature showed its typical appearance of wheel-shaped as shown in Fig. 2. Complete particles had a double-layered capsid and measured in 70nm in diameter.

The predominant mode of rotavirus transmission is fecal-oral, and it also spreads through respiratory secretions, person-to-person contact or contaminated environmental surfaces. It has been known that rotaviruses are shed in large numbers during episodes of diarrhea. All viruses we examined were from feces of the suspected patients. High infection in the first 3 years of life is related to the sanitation since babies or pre-school children are poor in hygiene. Rotaviruses are highly contagious and believed to survive for hours on human hands and for days on the surfaces of such objects as toys and countertops. Among infants and younger children hygiene is an important factor as an aspects of virus epidemics and it is required to be controlled under care. From the epidemics of Busan, 5 adults of virus isolation remained unclear.

In temperate climates, rotavirus diarrhea occurs predominantly during the fall and winter, in tropical settings and in developing countries seasonality is less marked and illness occur year round. Isolation of rotavirus in Busan, 1999, the outbreaks began in February. 3 in February, 4 in March, 1 in July and 14 in December. Peak in December suggests that this virus can be an active agent in winter months diarrhea. The reason for this pattern is unknown, but annual detection will give an answer whether this pattern is due to climate in this area or other factors involved.

Rotavirus infects virtually all infants and children between 1 and 5 years of age in both industrialized and developing countries, improving water, food and sanitation appeared unlikely to reduce disease incidence.

Rotaviral disease might be best be controlled through vaccination and decreased incidence of disease with increasing age¹³. Since human rotaviruses have a worldwide distribution continuous detection work will give a clue to surveillance. Community prevention measure include good hygiene practices, breast-feeding and available vaccination will prevent spread.

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