Diarrhea Caused by Rotavirus in Children Less than 5 Years of Age in Hanoi, Vietnam

Trung Vu Nguyen,^{1,2} Phung Le Van,¹ Chinh Le Huy,¹ and Andrej Weintraub^{2*}

Department of Medical Microbiology, Hanoi Medical University, Hanoi, Vietnam,¹ and Department of Laboratory Medicine, Division of Clinical Bacteriology, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden²

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Group A rotaviruses are the major cause of diarrhea in young children worldwide. From March 2001 to April 2002, 836 children less than 5 years of age were investigated in Hanoi, Vietnam. This included 587 children with diarrhea and 249 age-matched controls. Group A rotavirus was identified in 46.7% of the children with diarrhea and 3.6% of the controls, which was a significant difference. Within the diarrhea group, the highest prevalence was seen in children from 13 to 24 months of age, and the prevalence was higher in males than in females. The symptoms of acute diarrhea caused by rotavirus were watery diarrhea, vomiting, fever, and dehydration. A higher prevalence of rotavirus detection was obtained for children who had all of these symptoms, followed by those who had diarrhea with vomiting-dehydration, fever-dehydration, and dehydration. The high rates occurred from September to December, although the infection was encountered all year round. In 58 patients (21.2% of the rotavirus-infected children), rotavirus infection was detected in association with either diarrheagenic *Escherichia coli* or *Shigella* spp. The most frequent combinations were rotavirus-enteroaggregative *E. coli* and rotavirus-enteropathogenic *E. coli*. At least one enteropathogen was identified from about 64% percent of the samples. The bacterial infection may not have given rise to clinical symptoms of such severity. The present study demonstrates the burden of rotavirus diarrhea in Hanoi, Vietnam. Continuous surveillance of diarrhea caused by rotavirus in young children would play an important role in diagnosis, treatment, and prophylaxis in order to improve the health of children in Vietnam.

Diarrhea, especially acute diarrhea, remains a major public health problem in the world. In developing countries, an estimated 12 or more diarrheal episodes per child per year occur within the first 5 years of life. Annually, approximately 4.6 million pediatric deaths, about 25 to 30% of all deaths among children less than age 5 years, can be attributed to acute diarrhea (11, 17). Acute diarrhea also contributes considerably to morbidity and medical expenses in developed countries. In the United States, approximately 16.5 million children under 5 years of age develop 1.3 to 2.3 diarrheal episodes per year. This accounts for up to \$ 1 billion of direct and indirect expenses (11, 19).

Many different agents, including viruses, bacteria, and parasites, of which viruses have been intensively studied in recent years, can cause acute diarrhea. The most notable viral agents causing diarrhea are rotavirus, adenovirus, astrovirus, and Norwalk-like viruses (4). Rotavirus is a leading cause of infantile gastroenteritis worldwide and is responsible for approximately 20% of diarrhea-associated deaths in children under 5 years of age (17). Bishop et al. (5) first identified rotaviruses in humans in 1973 when they observed characteristic particles in the cytoplasm of duodenal epithelial cells from young children admitted to the hospital for treatment for acute diarrhea. Rotaviruses are members of the family *Reoviridae* and are characterized by their segmented (11 segments), double-stranded RNA genome. Rotaviruses have three important antigenic

specificities: group, subgroup, and serotype. Rotaviruses are classified into serogroups A through G. However, only groups A to C have been shown to infect humans and most animals, with rotavirus disease mainly being caused by group A. Rotaviruses are also classified further into types G and P on the basis of the antigens on the outer capsid proteins (VP7 and VP4). At least 14 G types and 20 P types have been identified among human and animal rotavirus strains (6, 14, 17, 25).

Previous studies have shown the burden of rotavirus diarrhea in many parts of the world (6, 8, 15, 18, 33). Investigations carried out from 1994 to 1999 in Vietnam demonstrated that the frequency of diarrhea due to rotavirus in Vietnamese children is substantial (23, 24). Therefore, updated information about rotavirus infections in correlation with clinical symptoms, epidemiological factors, and especially coinfections with other pathogens is important for pediatricians and health care workers. Such information will help not only to improve the diagnosis and treatment of diarrhea in children but also to provide useful information for vaccination in the near future. The objectives of this study were to investigate group A rotavirus infections in children less than 5 years of age in Hanoi, Vietnam, to determine the clinical symptoms of diarrhea caused by rotavirus and to assess the role of coinfections with other diarrheagenic pathogens.

MATERIALS AND METHODS

Study design. A total of 836 children from 0 to 60 months of age, including 587 children with diarrhea attending three different hospitals and 249 age-matched healthy controls, were included in the study. The healthy children were enrolled from one day care center and one health care center in Hanoi, Vietnam. They had not had diarrhea for at least 1 month before collection of the fecal sample. The children were enrolled in the study for a 1-year period starting in March 2001 and ending in April 2002. Diarrhea was characterized by the occurrence of

* Corresponding author. Mailing address: Department of Laboratory Medicine, Division of Clinical Bacteriology, F-82, Karolinska Institute, Karolinska University Hospital, Huddinge, S-141 86 Stockholm, Sweden. Phone: 46 8 585 87831. Fax: 46 8 711 3918. E-mail: andrej.weintraub@labmed.ki.se.

TABLE 1. Attributes of 587 children in diarrhea group

Rotavirus detection	No. (%) of children by:								
	Age (mo) ^a					Gender ^b		Patient status ^c	
	≤12 (<i>n</i> = 240)	13–24 (<i>n</i> = 177)	25–36 (<i>n</i> = 95)	37–48 (<i>n</i> = 41)	49–60 (<i>n</i> = 34)	Male	Female	Inpatient	Outpatient
Positive (<i>n</i> = 274)	111 (46.3)	102 (57.6)	42 (44.2)	12 (29.3)	7 (20.6)	181 (49.6)	93 (41.9)	237 (52.1)	37 (28)
Negative (<i>n</i> = 313)	129 (53.7)	75 (42.4)	53 (55.8)	29 (70.7)	27 (79.4)	184 (50.4)	129 (58.1)	218 (47.9)	95 (72)

^a $P = 0.00285$ by chi-square for trend.^b $P = 0.06$ for males versus females.^c $P < 0.0001$ for inpatient versus outpatient.

three or more loose, liquid, or watery stools or at least one bloody loose stool in a 24-h period. An episode was considered resolved on the last day of diarrhea, followed by at least 3 diarrhea-free days. An episode was considered persistent if it continued for ≥ 14 days (2). Vomiting was defined as the forceful expulsion of gastric contents at least once in a 24-h period. Fever was defined as a temperature measured under the arm of $>37.2^{\circ}\text{C}$ (99°F). Thresholds of 37.2 to 39°C and $>39^{\circ}\text{C}$ were considered moderate and high fevers, respectively. Dehydration levels were assessed according to the recommendations of the World Health Organization Program for Control of Diarrheal Diseases (32) and were carried out by the pediatricians. After informed consent was obtained, a pediatrician specifically assigned to the study examined each patient and filled out the demographic data and information on clinical symptoms and illness onset on a standardized questionnaire.

Fecal samples (one from each subject) from children without diarrhea were collected in a clean container by their parents when the children defecated. All feces were collected in a special container with Cary-Blair transport medium, kept at 4°C , and transported to the Microbiology Laboratory, Hanoi Medical University, Hanoi, Vietnam, within 24 h. One stool specimen was collected from each of the children with diarrhea within 24 h of hospital admission, kept at 4°C , and transferred to the microbiology laboratory of Hanoi Medical University within 24 h. The remains of each sample after the first culture on the media was kept at -70°C for further work.

The collection of samples in Vietnam stopped for 2 weeks for the Tet (New Year) holidays in February 2002.

Rotavirus detection. Stool samples were analyzed for rotavirus A by using the IDEA Rotavirus enzyme-linked immunosorbent assay kit (DAKO Ltd., Ely, United Kingdom), according to the instructions of the manufacturer. This test is a qualitative enzyme immunoassay for the detection of rotavirus (group A) in human fecal samples.

Stool samples were also cultured on the surface of sorbitol MacConkey agar (Labora, Stockholm, Sweden) for the selection of *Escherichia coli* isolates, on other media such as thiosulfate citrate bile salt cholera medium (Labora) for the selection of *Vibrio*, and on deoxycholate citrate agar (Sigma-Aldrich, Stockholm, Sweden) for the selection of *Shigella* and *Salmonella*. The cultures were incubated overnight at 37°C . All samples were tested for *Vibrio*, *Shigella*, and *Salmonella* by using colony morphology, biochemical properties, and agglutination with specific sera. A multiplex PCR was used for the identification of diarrheagenic *E. coli*.

Analysis. Differences in proportions were assessed by a chi-square test. In cases in which the expected value for a cell was <5 , Fisher's exact test was used. Comparisons between two groups were assessed by a Mann-Whitney U test (for nonparametric data). P values <0.05 were considered statistically significant.

RESULTS

Rotavirus infection. From March 2001 to April 2002, 836 fecal samples, including 587 samples from a group of children with diarrhea and 249 samples from age-matched healthy controls, were obtained. Of the samples from the diarrhea group, 274 (46.7%) were positive for rotavirus; and 9 (3.6%) samples from the healthy controls were positive for rotavirus. The rotavirus detection prevalence was significantly different between the two groups ($P < 0.0001$). Within the diarrhea group, the prevalence of detection of rotavirus in children less than 2 years of age was 51.1%, which was significantly different ($P <$

0.001) from that (35.9%) for the older children. The age, gender, and inpatient and outpatient status of the 587 children in the diarrhea group enrolled in the study are shown in Table 1. The children enrolled in the study were divided into five age groups. Rotavirus infection was most prevalent in children in the group ages 13 to 24 month and was the second most prevalent in children ≤ 12 months of age and children from 25 to 36 months of age, although infections were also seen in the older children. There was a trend for a significant decrease in rotavirus prevalence with age (chi-square test for trend, 8.904; $P < 0.005$).

Slightly more males were admitted to the hospital due to diarrhea caused by rotavirus than females ($P = 0.06$). The ratio of infected males to infected females was 1.9 (181 males and 93 females). Eighty-six percent (237 of 274) of the children who had diarrhea caused by rotavirus came from the inpatient group. All children infected with rotavirus had acute diarrhea. Nine samples from children in the healthy group were positive for rotavirus; however, these children were asymptomatic, and rotavirus-positive samples were detected in healthy children in all five age groups.

In addition to the age distribution of rotavirus infection, the seasonality of rotavirus infection was also determined and is shown in Fig. 1. The infection occurred all year round; but the prevalence trend was higher in September, October, November, and December. During the other months of the year, the number of infected cases decreased. February was the Tet (New Year) holidays in Vietnam, resulting in a low number of diarrhea samples.

Rotavirus infection in relation to clinical symptoms. For all children with diarrhea, the main clinical symptoms, such as fever, vomiting, dehydration, type of stool, and number of episodes of diarrhea per day, are shown in Table 2. Fever,

FIG. 1. Seasonal distribution of rotavirus infection in the diarrhea group.

vomiting, and dehydration were common symptoms in rotavirus-infected children; dehydration occurred in 89% (243 of 274) of the rotavirus-positive children. The incidences of vomiting and dehydration in children positive for rotavirus were significantly different from those in children negative for rotavirus ($P < 0.0001$ and $P < 0.001$, respectively).

Eighty-one percent of rotavirus-positive children had watery stools; 8.4% had mucous stools. Of 222 watery stool samples from the rotavirus-infected children, 174 (78.4%) were due to rotavirus alone and 48 (21.6%) were due to rotavirus in association with diarrheagenic *E. coli* or *Shigella*. The mean number of episodes of diarrhea per day in the rotavirus-positive group differed significantly ($P < 0.001$) from that in the rotavirus-negative group. Among 274 children infected with rotavirus, the most frequent combination of symptoms was fever, vomiting, and dehydration (42%). The next most frequent combinations were vomiting-dehydration (20%) and fever-dehydration (14%). Of the 49 of 587 children without fever, vomiting, or dehydration, 13 were positive for rotavirus. Similar distributions of the combination of symptoms were observed in all children with diarrhea (Fig. 2).

Rotavirus and coinfections. In the present study, 190 bacterial pathogens were identified. The bacterial etiology consisted of 162 diarrheagenic *E. coli* isolates, including 86 enteroaggregative *E. coli* (EAEC), 12 enteroinvasive *E. coli* (EIEC), 50 enteropathogenic *E. coli* (EPEC), and 14 enterotoxigenic *E. coli* (ETEC) isolates. The diarrheagenic *E. coli* strains were isolated from both groups of children, while *Shigella* spp. were found only in the diarrhea group. Among the 28 *Shigella* spp. detected, 20 were *Shigella sonnei*, 7 were *S. flexneri*, and 1 was *S. boydii*. No *Salmonella* spp. or *Vibrio cholerae* strains were isolated. As shown in Table 3, coinfections were detected in 9.9% (58 of 587) of the children in the diarrhea group and 0.8% (2 of 249) of the healthy children. The most common association was rotavirus and EAEC, with a prevalence of 5.3% (31 of 587), followed by rotavirus and EPEC at 3.4% (20 of 587). In total, 211 (35.9%) fecal samples from children in the diarrhea group were negative for either rotavirus or diarrheagenic *E. coli* and *Shigella* spp.

The clinical symptoms were different for children with rotavirus infection only and children with bacteria-associated rotavirus infection. Table 4 shows the relationships between viral and bacterial infections in the diarrhea group in terms of clinical symptoms. Overall, among the children in the diarrhea group, the clinical symptoms seemed to be more severe in children who were infected with either bacteria or rotavirus, or both, than in those from whom no rotavirus, diarrheagenic *E. coli*, or *Shigella* sp. was identified. In general, however, coinfection did not cause an increase in the severity of the clinical symptoms compared to those in children infected only with rotavirus or compared to those in the group with diarrhea in whom we could not identify potential pathogens.

DISCUSSION

Rotavirus infection. Many studies have shown the important role of rotavirus as a cause of diarrhea in children in both developed and developing countries (2, 4, 6, 8, 11, 14, 30). Most of the cases occur in children less than 5 years of age. Overall, the prevalence of rotavirus-positive children with di-

TABLE 2. Rotavirus detection prevalence versus clinical symptoms for 587 children in the diarrhea group

Rotavirus detection	No. (%) of children by:										No. of episodes/ day ^d					
	Fever		Vomiting ^a		Dehydration ^b			Kind of stool ^c				Mean (median)	SD			
	Moderate	High	Yes	No	Yes	No	Watery	Bloody	Mucous	Little blood	Mucous- bloody			Semisolid	Rice water	Other
Positive (<i>n</i> = 274)	154 (50.2)	8 (3.3)	182 (57.6)	92 (33.9)	243 (50.1)	31 (30.4)	222 (81.1)	0 (0)	23 (8.4)	2 (0.7)	8 (2.9)	12 (4.4)	5 (1.8)	2 (0.7)	7.78 (7.00)	3.8
Negative (<i>n</i> = 313)	153 (49.8)	16 (6.7)	134 (42.4)	179 (66.1)	242 (49.9)	71 (69.6)	168 (53.7)	1 (0.3)	100 (31.9)	9 (2.8)	12 (3.9)	21 (6.8)	1 (0.3)	1 (0.3)	6.46 (6.00)	3.1

^a $P < 0.0001$.

^b $P < 0.001$.

^c The prevalence of children with watery diarrhea was statistically significantly different compared to those of the other types of diarrhea ($P < 0.00001$).

^d $P < 0.001$ by Mann-Whitney U test.

FIG. 2. Relationships between rotavirus infection and clinical symptoms. Overlapping areas show the numbers and proportions of children with two or more symptoms.

arrhea ranges from 30 to 50% (30). The purpose of our study was to estimate the prevalence of rotavirus infection in children with diarrhea admitted to three different hospitals in Hanoi, Vietnam, during a 1-year period.

Our study showed a rotavirus prevalence of 46.7% in children with diarrhea. Similar values were obtained in two previous studies in Vietnam (23, 24). A significant difference was seen when the diarrheal group was compared to the healthy group (3.6%). Not many studies on rotavirus detection in healthy children worldwide have been carried out. A study by Nath et al. (22) showed a prevalence of 4%. Other studies also showed a low prevalence of rotavirus detection in fecal samples in this group (21, 27, 31). It was reported that asymptomatic infection with rotavirus was not infrequent, especially in neonates, in whom only mild or subclinical symptoms were seen (17, 29). However, most of children infected with rotavirus showed one or several clinical symptoms.

This pathogen infects not only children but also adults (12, 16), and rotavirus infection may occur repeatedly in humans from birth to old age (17). Young children are the most vulnerable subjects, and the prevalence of infection differs by age. Generally, the prevalence of rotavirus infection was significantly higher in the group less than 2 years of age than in the older group ($P < 0.01$). The highest prevalence was seen in children from 13 to 24 months of age (57.6%), followed by those less than 1 year of age (46.3%), and the prevalence decreased in the older children (Table 1). This result was similar to those of other studies (4, 10, 30). Many studies have shown a rotavirus infection prevalence of 15 to 20% in children less than 6 months of age (4, 6, 10, 30). In our study, it was 35% (36 of 103). Even 34.2% (13 of 38) of children less than 3 months of age had rotavirus infection, which shows that rotavirus infection may occur early in a child's life.

There is a difference in the age distributions of rotavirus

TABLE 3. Detection of rotavirus and other diarrheagenic pathogens in both groups of children

Group of children and rotavirus infection status	No. (%) of children from whom the following bacteria were isolated:								Total (n = 836)
	EAEC	EIEC	EPEC	ETEC	<i>S. boydii</i>	<i>S. flexneri</i>	<i>S. sonnei</i>	Negative	
Diarrhea									
Positive	31 (11.3)	0 (0)	20 (7.3)	5 (1.8)	0 (0)	1 (0.4)	1 (0.4)	216 (78.8)	274
Negative	37 (11.8)	12 (3.8)	19 (6.1)	8 (2.6)	1 (0.3)	6 (1.9)	19 (6.1)	211 (67.4)	313
Subtotal	68 (11.6)	12 (2)	39 (6.6)	13 (2.2)	1 (0.2)	7 (1.2)	20 (3.4)	467 (74.5)	587
Healthy									
Positive	1 (11.1)		1 (11.1)	0 (0)				7 (77.8)	9
Negative	17 (7.1)		10 (4.2)	1 (0.4)				212 (88.3)	240
Subtotal	18 (7.2)		11 (4.4)	1 (0.4)				219 (88)	249

TABLE 4. Comparison of clinical symptoms by coinfection among children in the diarrhea group^a

Clinical symptom	RV (+), B (-) (n = 216)	RV (+), B (+) (n = 58)	RV (-), B (+) (n = 102)	RV (-), B (-) (n = 211)
No. (%) of children with:				
Fever				
Yes	129 (59.7)	33 (56.9)	64 (62.7)	105 (49.8)
No	87 (40.3)	25 (43.1)	38 (37.3)	106 (50.2)
Dehydration				
Yes	196 (90.7)	47 (81)	85 (83.3)	157 (74.4)
No	20 (9.3)	11 (19)	17 (16.7)	54 (25.6)
Vomiting				
Yes	148 (68.5)	34 (58.6)	48 (47.1)	86 (40.8)
No	68 (31.5)	24 (41.4)	54 (52.9)	125 (59.2)
Stool				
Watery	174 (80.6)	48 (82.8)	50 (49)	118 (55.9)
Others	42 (19.4)	10 (17.2)	52 (51)	93 (44.1)
No. of episodes/day				
Mean	7.9	7.3	6.9	6.3
Median	7	7	6	6
SD	3.9	3.4	3.4	2.9

^a RV (+), rotavirus detected; RV (-), rotavirus not detected; B (+), bacteria isolated; B (-), bacteria not isolated.

infections in developing and developed countries. In the former, the highest rates occur during the first year of life. However, in developed countries the peak rates occur in the second year of life. This could lead to the earlier application of rotavirus vaccine to children in developing countries. Moreover, our study indicated that there was a trend of decreasing rates of rotavirus infection in the older children. This might partly be explained by the fact that older children acquired protective immunity during previous exposures to rotavirus and are therefore more resistant to infection with this agent (13, 20).

In addition to the age distribution of rotavirus infection, many studies have indicated a higher ratio of infected males to infected females (8, 24, 26, 28, 30). The ratio in our study was 1.9. No reasonable explanations have yet been given for this distribution. As mentioned above, 86% of children with diarrhea caused by rotavirus were inpatients.

In the present study, a clear seasonal pattern in rotavirus diarrhea was seen. Although not many samples were collected during February due to the traditional Tet (New Year) holidays, a common characteristic has been found in the north of Vietnam, where there are four seasons in a year. Rotavirus infection occurred all year round but peaked during the fall and winter months, from September to December. This pattern was not observed in the south, where there are only two seasons per year, the rainy and the dry seasons. Rotavirus infections occurred almost all year in the south, with less distinct seasonal differences (13, 20, 23, 24). Our results are similar to those of studies conducted in Korea, China, and Thailand but differed from those of a Japanese study, in which rotavirus was rarely detected from September to December (9, 20, 29, 33).

Rotavirus infection in relation to clinical symptoms. It is generally considered that rotavirus diarrhea is more likely to be associated with fever, vomiting, and dehydration than diarrhea caused by other pathogens (29). These symptoms may

occur alone or in combination, resulting in the hospitalization of children for treatment. Lundgren and Svensson (17) reviewed studies on the pathogenesis of rotavirus infection and proposed four hypotheses on the mechanism by which rotavirus evokes intestinal secretion of fluid and electrolytes. In the present study, watery stools were seen in 81.1% of the children infected with rotavirus. Infection only with rotavirus contributed to 78.4% (174 of 222) of the cases of this type of stool, and this could be the symptom suggestive of rotavirus diarrhea. Vomiting is the consequence of disturbed motor activity of the stomach, i.e., delayed emptying of fluid contents, resulting in dehydration (3). The outcome of vomiting and diarrhea is dehydration or even severe dehydration, which is life-threatening for children. In our study, fever, vomiting, and dehydration were seen at prevalences of 59.1, 66.4, and 89%, respectively, in the children infected with rotavirus. These prevalences differed significantly from those for non-rotavirus-infected children, indicating the role of rotavirus infection in diarrheal disease in Vietnamese children.

Among the children in all age groups, we detected rotavirus at the highest rate among those with all three symptoms. The combination of all three symptoms was most prevalent in the rotavirus-positive group (Fig. 2). Our study supports the conclusions from other studies that rotaviruses induce a clinical illness characterized by vomiting, diarrhea, fever, and dehydration (or some combination of these symptoms) (4, 6, 7, 28–30).

Having analyzed the clinical symptoms of acute diarrhea caused by rotavirus, many investigators emphasize the sudden onset of the disease, the higher body temperature, and the prevalence of vomiting at the initial stage of the disease, which usually precedes loose stools (3). This could be useful information for pediatricians and health care workers trying to diagnose the possible cause of diarrhea. As mentioned above, 13 children had diarrhea and rotavirus infection but did not develop fever, vomiting, or dehydration. Ten of these children

were less than 2 years of age. The clinical aspect of this finding could be relevant.

Rotavirus and coinfections. Taking into account two published studies on rotavirus diarrhea in Vietnamese children (23, 24), we examined the stool samples for other bacterial pathogens, focusing on diarrheagenic *E. coli*, *Shigella* spp., *Salmonella* spp., and *V. cholerae*. Neither *Salmonella* spp. nor *V. cholerae* was isolated from any of the groups of children. Thus, *Salmonella* spp. and *V. cholerae* do not play important roles as agents causing diarrhea in children in Vietnam. In contrast, both *Shigella* spp. and diarrheagenic *E. coli* were identified. In total, diarrheagenic *E. coli* and *Shigella* contributed to 27.3% (160 of 587) of diarrheal cases in the diarrhea group and 12% (30 of 249) of diarrheal cases in the control group (Table 3). Interestingly, we found that 60 children were infected with both rotavirus and either diarrheagenic *E. coli* or *Shigella*. The most common multiple infection was rotavirus and EAEC, followed by rotavirus and EPEC. Albert et al. (1) reported that rotavirus infection was associated with ETEC, EPEC, and *Shigella* spp. at prevalences of 17, 9.7, and 1.2%, respectively, in rotavirus-infected children. In a study carried out by Ming et al. (21) in China, only one child was reported to be infected with both rotavirus and ETEC. These prevalences are different from those detected in our study.

However, simultaneous rotavirus and bacterial infections had no significant collaborative influences on clinical symptoms compared to the influences of rotavirus infection or bacterial infection. Furthermore, the coinfections could cause difficulties for pediatricians and health care workers in terms of the diagnosis, treatment, and prophylaxis of diarrhea in children. More studies are necessary in order to evaluate this area further.

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