Revista Eletrônica
Acervo Saúde
Electronic Journal Collection Health ISSN 2178-2091

Norovirus in hospitalized children with acute diarrhea after rotavirus vaccine introduction in Recife

Norovírus em crianças hospitalizadas com diarreia aguda após a vacina contra rotavírus em Recife

Norovirus en niños hospitalizados con diarrea aguda tras la vacuna contra el rotavirus en Recife

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ABSTRACT

Objective: To determine the frequency of norovirus and its genogroups in fecal samples of children under two years of age, hospitalized for acute diarrhea in northeastern Brazil. **Methods:** A cross-sectional, prospective study between February 2007 and April 2010. Acute diarrhea was defined as the presence of three or more stools of decreased consistency or the presence of at least one stool with visibly present blood within a 24-hour period, lasting up to 14 days. Stool samples were analyzed using RT-PCR. **Results:** A total of 103 children were studied. Norovirus was identified in 30.1% of the children, with genogroup GII identified in 100% of the positive samples. Vomiting was present in 74.2% of children with norovirus-positive fecal samples, representing a statistically significant association. **Conclusion:** Norovirus was identified as a major enteric pathogen, detected in one-third of hospitalized children with acute diarrhea. The significant association with vomiting reinforces its distinct clinical role in pediatric gastroenteritis. These findings underscore the need for continued surveillance and further research to understand the evolving epidemiology of viral gastroenteritis in the post-rotavirus vaccine period in Brazil.

Keywords: Norovirus, Rotavirus, Diarrhea, Child.

RESUMO

Objetivo: Determinar a frequência de norovírus e seus genogrupos em amostras fecais de crianças menores de 2 anos, hospitalizadas por diarreia aguda no Nordeste do Brasil. **Métodos:** Estudo transversal e prospectivo entre fevereiro de 2007 e abril de 2010. Definiu-se diarreia aguda como a presença de três ou mais evacuações de consistência diminuída ou a presença de pelo menos uma evacuação com sangue visível em um período de 24 horas, com duração de até 14 dias. As amostras de fezes foram analisadas por RT-PCR. **Resultados:** Foram estudadas 103 crianças. O norovírus foi identificado em 30,1% delas, sendo o genogrupo GII detectado em 100% das amostras positivas. Vômitos estavam presentes em 74,2% das crianças com amostras fecais positivas para norovírus, representando uma associação estatisticamente significativa. **Conclusão:** O norovírus foi identificado como um importante patógeno entérico, detectado em

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SUBMETIDO EM: 6/2026 | **ACEITO EM: 7/2026** | **PUBLICADO EM: 9/2026**

um terço das crianças hospitalizadas com diarreia aguda. A associação significativa com vômitos reforça seu papel clínico distinto na gastroenterite pediátrica. Esses achados ressaltam a necessidade de vigilância contínua e de mais pesquisas para compreender a epidemiologia em evolução da gastroenterite viral no período pós-vacina contra rotavírus no Brasil.

Palavras-chave: Norovirus, Rotavirus, Diarreia, Criança.

RESUMEN

Objetivo: Determinar la frecuencia de norovirus y sus genogrupos en muestras fecales de niños hospitalizados por diarrea aguda en el noreste de Brasil. **Métodos:** Estudio transversal y prospectivo entre febrero de 2007 y abril de 2010. La diarrea aguda se definió como la presencia de tres o más deposiciones de consistencia disminuida o de al menos una deposición con sangre visible en un período de 24 horas, con una duración de hasta 14 días. Las muestras de heces fueron analizadas mediante RT-PCR. **Resultados:** Se estudiaron 103 niños. Se identificó norovirus en el 30,1% de ellos, detectándose el genogrupo GII en el 100% de las muestras positivas. Se observaron vómitos en el 74,2% de los niños con muestras fecales positivas para norovirus, lo que representa una asociación estadísticamente significativa. **Conclusión:** El norovirus fue identificado como un importante patógeno entérico, detectado en un tercio de los niños hospitalizados con diarrea aguda. La asociación significativa con vómitos refuerza su papel clínico distintivo en la gastroenteritis pediátrica. Estos hallazgos subrayan la necesidad de una vigilancia continua y más investigaciones para comprender la epidemiología cambiante de la gastroenteritis viral en la era posterior a la vacuna contra el rotavirus en Brasil.

Palabras clave: Norovirus, Rotavirus, Diarrea, Niño.

INTRODUCTION

Diarrheal diseases (DD) are one of the most prevalent causes of morbidity and mortality in childhood and one of the main public health problems worldwide. The etiological agents of childhood DD have been identified as viruses, such as the rotavirus and the adenovirus and bacteria, fungi and protozoa (WHO, 2024).

Despite the overall decline, diarrhea alone was responsible for more than 443,832 deaths among children aged under five years, most of them in poor countries. Excluding neonatal causes, DDs alone are responsible for the second largest number of deaths, second only to pneumonia (WHO, 2024).

In the Brazilian Northeast alone, between 2019 and 2023, more than 44,790 hospitalizations due to diarrhea were recorded in children up to 5 years old (ARANHA MC, et al., 2024). In 2009, diarrhea in children aged under five years was the cause of 2.5% of deaths in Brazil and 5.0% in the State of Pernambuco (BRASIL MS, 2009).

Brazil has been a pioneer in introducing a National Immunization Program involving two doses of monovalent rotavirus vaccine (Rotarix®) for children under three months of age in March 2006 (PARASHAR UD, et al., 2003, CORREIA JB, et al., 2010). Since 2008, studies have demonstrated that the advent of the rotavirus vaccine brought about a change in the position of etiological agents for viral gastroenteritis, with a tendency for the rotavirus to decline and more cases of infection by the Norovirus (NoV) to be found (SARTORI AMC, et al., 2008).

NoV was originally called “Norwalk virus”; it was identified during an outbreak of diarrhea in the 1970s and was later classifieded in two genera: the Noroviruses and the Sapoviruses (KAPIKIAN AZ, et al., 1974). Its identification is difficult due to the impossibility of cultivating a cell culture and the most reliable method for identification is "reverse transcription polymerase chain reaction" (RT-PCR). It is characterized as an RNA virus without envelope, with a capsid and nucleic acid measuring around 27 - 30nm in diameter and is

subclassified into genogroups GI, GII, GIII, GIV and GV of which only GI, GII and GIV have been registered in humans, with the GII genogroup being considered the most important from an epidemiological point of view (CHHABRA P, et al., 2019).

It is believed that nearly 100% of adults have already been exposed to at least one NoV infection in their life and it is now considered the main viral agent responsible for gastroenteritis, taking all age groups all around the world into consideration (PATEL MM, et al., 2012).

It is estimated that each year the NoV causes 900,000 episodes of gastroenteritis that require medical attention and around 64,000 hospitalizations of children aged five years or under in poor countries (PATEL MM, et al., 2008).

Even though there are some studies describing the identification of the NoV in Brazilian children using the RT-PCR technique, data for the Northeast region during the period after the introduction of the vaccine are scarce and there is also a need for long-term monitoring of the modifications that have occurred during and after this period, for future evaluations and the development of appropriate prevention protocols. The aim of this study was to determine the frequency of norovirus and its genogroups in stool samples from children aged under two years admitted to hospital with acute diarrhea in Recife, Pernambuco, Brazil, in the post-rotavirus vaccine introduction period.

METHODS

A cross-sectional, prospective study was conducted from 2007 to 2010 with children under two years of age hospitalized for acute diarrhea at a public reference hospital in Recife, in northeastern Brazil. Acute diarrhea was defined as the presence of three or more bowel movements of decreased consistency or the presence of at least one bowel movement with visible blood within a 24-hour period, for 14 days or less.

For data collection, a specific instrument containing clinical and epidemiological information about the children was used. Stool collection occurred from February 2007 to April 2010 by a nursing technician, trained from Monday to Friday between 7 a.m. and 5 p.m. The fresh feces were collected with a spatula from the diaper and the material placed in cryotubes, identified with numerical codes, and stored at -20ºC for up to a week.

For detection of the NoV, the samples were placed in hemolysis tubes and diluted to 1:10, in the following proportions: 500 uL of stool in suspension + 4500 uL of 0.9% NaCl. Then they were centrifuged at 4000 x g for 20 minutes and the supernatant of the mixture put through a 0.22 µm filter (Millipore, USA).

The extraction of RNA was carried out using 140 uL of the stool sample filtrate on a QIAamp® Viral RNA Mini Kit from Qiagen® (cód. 52906, Chattlesworth, CA), in accordance with the manufacturer's instructions. After extraction, the RNA samples were concentrated in a Concentrator 5301® (Eppendorf AG, Germany). The analysis of the concentration of the RNA from the samples was carried out on a Nanovue® nano spectrophotometer (GE health care, USA).

Identification of NoV Groups I (GI) and II (GII) were performed using a RIDA®GENE NoV I & II RT-PCR real-time (cod. Nr.: PG1415, R-Biopharm AG, Germany), according to the manufacturer's instructions. This kit is a Molecular Biology test used to detect NoV GI and GII in samples of RNA from human feces. The reaction was read using Real-Time PCR - ABI 7500 (Applied Biosystems, USA). The interpretation of the results obtained from the real-time PCR reactions was performed according to the manufacturer's recommendations.

The data were put in a database specifically created for the purpose using the open source Epi-Info 3.4.1. The frequency distribution for the studied, categorical variables was calculated using the measures of central tendency and dispersion for continuous variables (median and quartile). To test the association between the presence of NoV diarrhea and the variables under study, a bivariate analysis was performed using the chi-square test or Fisher's exact test when appropriate. The strength of the association between the variables was measured using the prevalence ratio. Medians were compared using the Mann-Whitney test. The significance level adopted was 5%.

The project was approved by the IMIP Human Research Ethics Committee under protocol number 0361 and 1170 — 08, CAAE: 0033.0.099.000-08.All the responsible parties who agreed signed the Informed Consent Form before the start of the research.

RESULTS

In total 103 children under two years of age admitted to hospital with acute gastroenteritis were studied. The demographic and clinical characteristics of the study population are presented in **Table 1**. The mean age was 9.6 months (SD±6.64), of whom 59 (57,3%) were male and most came from the Metropolitan Region of Recife (69,9%). As for clinical characteristics, in most of the children, the episode of diarrhea began up to 48 hours before admission to hospital (65.0%), vomiting and dehydration were present in 60 (58.3%) and 45 (43.7%) respectively and the diarrhea was watery in 90 (87.4%) of cases. The NoV was identified in 31 stool samples, corresponding to 30.1% of the total number of hospitalized children studied. In 100% of these stool samples the NoV was classified as belonging to the GII genogroup. The children with stool samples testing positive for the NoV had a mean age of 11 months (SD±6.8), with the episode of diarrhea commencing no more than 48 hours prior to admission to hospital (58.1%) and remained in hospital for an average of six days.

Table 1 - Demographic and clinical characteristics of children under two years of age hospitalized with acute diarrhea (N = 103).

Characteristic	Value(N=103)
Age (months)	9.6 (± 6.64)
Male Sex	59 (57.3%)
Origin	
Recife Metropolitan Region	72 (70.0%)
State Interior / Other	31 (30.0%)
Clinical Characteristics on Admission	
Diarrhea Onset ≤ 48h	67 (65.0%)
Presence of Vomiting	60 (58.3%)
Presence of Dehydration	45 (43.7%)
Type of Diarrhea: Watery	90 (87.4%)
Length of Hospital Stay (days), Median [IQR]	6 [4 - 8]
NoV Detection (RT-PCR)	31 (30.1%)
Genogroup GII (among positives)	31 (100%)

***Note:** SD = Standard Deviation; IQR = Interquartile Range.
Source: Germano EM, et al. (2026). Primary data collected from hospitalized children at IMIP, Recife, Brazil, from February 2007 to April 2010. Statistical analyses performed by the authors.*

When the children whose stool sample tested positive for the NoV were compared with those whose sample did not, a statistically significant difference was observed in relation to the occurrence of vomiting (51.4% of the children testing negative for the NoV and 74.2% of those testing positive) ($p<0.05$). When analyzed, age, place of origin, type of diarrhea, dehydration, duration of diarrhea on admission and duration of hospitalization did not show any statistically significant differences. (**Table 2**)

Table 2 - Comparative analysis of demographic and clinical characteristics among children hospitalized with acute diarrhea, according to NoV detection.

Characteristic	NoV Positive (n=31)	NoV Negative (n=72)	PR (95% CI)	*p*
Demographics				
Age (months), Mean (±SD)	11.0 (± 6.8)	9.0 (± 6.5)	-	0.15[1]
Male Sex	19 (61.3%)	40 (55.6%)	1.13 (0.74 - 1.73)	0.58[2]
Origin (Outside RMR)	9 (29.0%)	22 (30.6%)	0.95 (0.50 - 1.82)	0.88[2]
Clinical Presentation				
Diarrhea Onset ≤ 48h	18 (58.1%)	49 (68.1%)	0.85 (0.56 - 1.29)	0.32[2]
Presence of Vomiting	23 (74.2%)	37 (51.4%)	2.06 (1.02 - 4.16)	0.03[2]
Presence of Dehydration	13 (41.9%)	32 (44.4%)	0.94 (0.57 - 1.56)	0.81[2]
Watery Diarrhea	26 (83.9%)	64 (88.9%)	0.94 (0.76 - 1.17)	0.34[3]

***Note:** SD = Standard Deviation; PR = Prevalence Ratio; CI = Confidence Interval; RMR = Recife Metropolitan Region.** [1]t-test; [2]Chi-square test; [3]Fisher's Exact Test.*
Source: Germano EM, et al., 2026.

DISCUSSION

This is the first study conducted in the Northeast Region of Brazil identified in the databases studied involving children aged under two years admitted to hospital with acute gastroenteritis who were recruited in the period subsequent to the introduction of the rotavirus vaccine and which used the RT-PCR method to identify the NoV. This fact has practical implications for the construction of public policies for Brazil.

NoV was identified in a third of the 103 stool samples studied (30.1%). The frequency of a positive test for the NoV reported in the international literature has varied from 1.9% in India (NATARAJU SM, et al., 2010) to 47.5%, in Vietnam (TAMURA T, et al., 2010), and the findings for Korea and Tunisia (a little over 36%) were similar to those of the present study (KOH H, et al., 2008); (SDIRI-LOULIZI K, et al., 2008).

Hospital based studies conducted in Brazil using the RT-PCR technique have revealed a frequency of testing positive for the NoV of between 7.8% in Rio de Janeiro in 2003 and 54.0% in Rio de Janeiro in 2005 before and after vaccine Rotarix® respectively (FERREIRA MSR, et al., 2012, FERREIRA MSR, et al., 2008). A study in Porto Velho and Paraná (Brazil) with hospitalized children up to two years of age identified very similar positivity, around 8% (AMARAL MSC, et al., 2015, RABONI SM, et al., 2014).

Frequencies similar to those reported in the present study were found in another study conducted in Belém between January to June 2016 (24.6%) and one carried out in Manaus between January 2010 to December 2014 (38.1%). However, the child age group is not available and hospitalized children under 10 years of age were included, respectively (da SILVA LD, et al., 2017; HERNANDEZ JM, et al.,2018).

It is important to consider, however, the publication of other recent studies carried out with fecal samples in the laboratory, different chronologies and different age groups. Some studies with children who did not require hospitalization or did not have diarrhea are also available.

A study published with 190 samples collected from children up to 11 years of age in Espírito Santo, through monitoring of gastroenteritis in outpatient and hospitalized children, showed a detection of NoV in 26.8% of the samples, while another study carried out with 1546 samples collected from sporadic cases and outbreaks that occurred in 10 Brazilian states between January 2017 and December 2018, involving 1546 adults and children from the South, Southeast and Northeast of Brazil, observed a positivity of 32.1%, with the most affected group being in the age group between 6 and 24 months of age (BARREIRA DMPG, et al., 2016; BARREIRA DMPG, et al., 2021).

We also identified a study carried out in Manaus, Amazonas, with 426 fecal samples from hospitalized children aged 0-14 years between 2010 and 2011 that was positive for NoV in 35.2% of the samples, mainly among children from 1 to 2 years of age (50%) (COSTA STPD, et al., 2017). These studies are extremely important to identify the association of NoV with outbreaks of acute gastroenteritis and circulating genotypes, but they do not respond to the impact of the virus on the hospitalization of children due to GAE in Brazil.

Research previously carried out in Recife, in the same hospital where the present study was conducted, between 2004 and 2005, involving 290 children aged under five years of age revealed a frequency of testing positive for the NoV of 15.0% using the immunoenzymatic assay technique (ELISA). The smaller percentage found may be interpreted as resulting from the greater accuracy of the RT-PCR method compared to ELISA for diagnosis of NoV infection, as cited by other authors (NAKAGOMI T, et al., 2008).

However, it is important to bear in mind that this study, which preceded the introduction of the rotavirus vaccine, studied children aged up to five years, which may also have influenced the results, since the occurrence of rotavirus infection tends to diminish with age. On the other hand, the possibility cannot be ruled out that, with the decline of the rotavirus after the introduction of the vaccine, the relative importance of the NoV has grown in the hospital under study. A previous published study by our research group noted a decline in the proportion of cases testing positive for the rotavirus from 35.0% in the period prior to introduction of the vaccine to 16.3% thereafter (MONTENEGRO FMU, et al., 2007).

A systematic review examined 235 articles and identified 31 original articles that used the RT-PCR technique to identify the NoV in a period of one year in various countries. In these studies, the overall proportion testing positive for NoV was 12%, considering only those studies that involved children (PATEL MM, et al., 2008).

The variation in the frequency of testing positive for the NoV observed in the literature may be put down to methodological differences, such as the age of the children included, the time elapsed since the onset of the disease, the way the stool samples were analyzed (since this may interfere with the virulence of the virus and reduce the chances of detecting it), the definition of the diarrheal episode, the seasonal nature of the virus, or the socio-economic conditions of the children. Hence the importance of conducting studies that standardize these aspects so as to produce valid results that can be compared with one another.

In the present study, the mean age of the children infected by NoV was eleven months. This is similar to the findings of other studies that showed that the disease is more likely to occur in the first two years of life (SANTOS VS, et al., 2017).

NoV is a highly infectious pathogen that is easily transmitted through vomiting or contaminated food. Furthermore, the virus remains active on dry surfaces as well as in the stool of asymptomatic individuals (PATEL MM, et al., 2008).

In the first year of life children use bottles and pacifiers and are also in the process of learning to crawl, walk with assistance and explore the surroundings and it is very common for them to put their hands or objects in their mouths. Thus, frequent exposure to these potentially contaminated elements makes children more vulnerable to the virus and this may be exacerbated by unfavorable socio-economic circumstances (WHO, 2009).

Studies have shown that maternal breastfeeding is the most cost-effective strategy for the prevention of morbidity and mortality from infectious diseases in children, since, apart from the already well-known benefits, it plays an important role in reducing contamination during the preparation of feeding bottles (JIANG X, et al., 2019).

Diarrhea is generally a self-limited disease and most cases respond well to oral rehydration therapy (ORT) and there is no need for hospitalization, still less high complexity care. Therefore, children who need to be admitted to hospital must present severe clinical characteristics, such as vomiting that is so frequent it rules out the use of ORT or severe dehydration with the need for venoclysis (WHO, 2021).

In the present study of 103 children admitted to hospital with gastroenteritis 43.7% were considered to be dehydrated and 58.3% were vomiting at the time of admission to hospital. These data corroborate the need for hospitalization for intravenous hydration due to dehydration or vomiting, which makes the use of oral rehydration therapy impossible (WHO, 2021).

Furthermore, it was also observed that 30% of the children admitted to hospital with acute diarrhea were from outside the Recife Metropolitan Region. It is therefore important to investigate why these children were referred to a tertiary hospital, to which they had to be transported, resulting in delay in the start of treatment, which may lead to a less favorable prognosis (WHO, 2021; JIANG X, et al., 2004).

This may be explained by the possibility of bacterial infection having given rise initially to diarrhea and the need for empirical treatment with antibiotics, especially in children with other associated risk factors. However, even so, the use of such medication is only recommended under special circumstances (MOURA RSA, et al., 2012, HALL AJ, et al., 2011).

In the present study, it was observed that vomiting was more frequently observed in children infected by the NoV (74.2%) compared with those not infected (51.4%) ($p<0.05$) (**Table 2**). Vomiting is an important symptom in NoV infection as well as a viral vector for disease transmission (KIRBY AE, et al., 2016).

This finding is similar to the findings of other studies such as those carried out in Brazil, China and South Korea that identified vomiting as one of the main characteristics of norovirus-induced gastroenteritis, occurring in 60.7%, 80.3% and 61.9% respectively of the hospitalized children studied (DAI Y-C, et al., 2006; PARK K, et al., 2012).

In a systematic review, vomiting was found to be presented as an important symptom associated with infection by the NoV, especially in children aged over one year (PATEL MM, et al., 2009). A prospective

cohort study nested in a case-control study with 4,800 adults and children conducted in the Netherlands, which found a frequency of 19.7% testing positive for the NoV. When the clinical characteristics were evaluated, the study identified that 74% of the children infected by the NoV vomited.

However, the authors state that, when the children were analyzed in more detail, a greater proportion of the older ones, aged 5-11 years (95%) and over 12 years (82%) presented with vomiting compared to 59% of children of less than one year of age, and 75% for the 1-4 year age band (ROCKX B, et al., 2002). As the mean age of the children infected by the NoV in the present study was 11 months, it is possible that frequency of vomiting found could be higher if we had older children.

Owing to the difficulty of growing the NoV in a cell culture, its classification into the GI, GII, GIII, GIV to GX genogroups was only achieved recently. Only the GI, GII, GIV, GVIII and GIX genogroups have been documented in humans, with the GII genogroup being the most prevalent worldwide and responsible for more than 80% of all NoV infections (OLIVEIRA JGDS, et al., 2024).

Since the late 1990s the GII.4 genotype has caused at least four global epidemics and it has led to it being identified as the main genotype worldwide with a varying frequency always well above 50% of cases, sometimes reaching higher than 95%. It is believed that this epidemiological strength can be attributed to greater stability in the environment, greater virulence and a variety of forms of transmission, thereby facilitating contamination (OLIVEIRA JGDS, et al., 2024).

These data corroborate the results of the present study which identified the presence of GII in 100% of positive samples, although it was not possible to identify the genotype in the samples analyzed. There is a need for research to include the genotype classification to establish the molecular characteristics of the strains of NoV in various regions of the country (BÁNYAI K, et al., 2018).

It is also believed that, with the advent of the rotavirus vaccine, there has been a change in the frequency of occurrence of the etiological agents involved in viral gastroenteritis, with a tendency for greater protection from rotavirus infection and a higher rate of detection of NoV infection, although confirming this would require long-term monitoring of these modifications around the world.

Two Brazilian studies and others in United States and Nicaragua, carried out after the introduction of the vaccine, have already identified a drop in the occurrence of rotavirus infection and a proportional increase in NoV infection in children in these areas (FERREIRA MSR, et al., 2010; FERREIRA MSR, et al., 2012; EBELTS, 2012; BUCARDO F, et al., 2014).

The present study is one of the first Brazilian studies published after the introduction of the rotavirus vaccine to establish the frequency of NoV in hospitalized children using the RT-PCR technique, although it has certain limitations.

Additionally, stool samples were collected only on weekdays between 7 a.m. and 5 p.m., which may have excluded children admitted during evenings or weekends, potentially introducing selection bias. However, given the high volume of pediatric admissions at the study site, this limitation is unlikely to have substantially affected the overall findings.

Although data collection occurred between 2007 and 2010, this period coincides with the early post-rotavirus vaccine introduction phase in Brazil, for which epidemiological data on norovirus in hospitalized children remain scarce, particularly in the Northeast region. Thus, despite the time elapsed, these findings provide valuable baseline information on the early epidemiological dynamics of viral gastroenteritis following a major public health intervention, and may serve as a reference for future longitudinal and multicenter studies.

The source of information was the memory of the person responsible for the children, and this may have led to information bias. Even including all the children with diarrhea considered acute, the time elapsing from the onset of symptoms to the collection of a stool sample may have interfered in the viral load of the stool making it difficult in some cases to identify the virus.

CONCLUSION

NoV was detected in 30.1% of children under two years of age hospitalized with acute diarrhea in Recife, Brazil, during the period following rotavirus vaccine introduction, with a predominance of genogroup GII. The significant association with vomiting reinforces the clinical relevance of this pathogen in pediatric gastroenteritis. While the cross-sectional, single-center design of this study suggests caution in causal interpretations, it provides important baseline data that can inform future multicenter investigations into the evolving epidemiology of viral gastroenteritis in Brazil, as well as molecular studies to monitor strain diversity and evolution.

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