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First detection of group C rotavirus in children with acute diarrhea in Spain

Brief Report

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Summary. Group C rotavirus causes sporadic cases and outbreaks of acute diarrhea in children and adults in many countries, but has never been detected among children in Spain. In a recently conducted surveillance study to screen fecal specimens for bacteria and viruses from a cohort of 822 young children who were treated for acute diarrhea in Madrid, no pathogens were detected in fecal specimens from 238 (29%) children. In this study, we examined 147 of those specimens for group C rotavirus by EIA and PCR and found 22 (15%) were positive. Our findings demonstrate that group C rotavirus is an important cause of childhood diarrhea in Spain.

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Rotaviruses are a major cause of acute diarrhea in animals and humans. They are divided into seven groups on the basis of their antigenic and genetic properties [7]. Group A rotaviruses are the most common cause of severe diarrhea in children worldwide and in various animal species [7]. Group B rotavirus has been responsible for large outbreaks of diarrhea and sporadic cases in adults of China and India, respectively, and among several mammalian species [4, 7]. Groups D to G cause diarrhea in poultry [7]. Group C rotaviruses remain the second most common variety of rotavirus found in humans and have an epidemiology quite distinct from that of group A rotaviruses. While both group A and C rotaviruses

have a worldwide distribution, group A rotaviruses infect all children in their first few years of life. Group C rotaviruses infect both children and adults, causing outbreaks in hospital, school, or military settings [2, 3, 5–7].

The key impediment in understanding the epidemiology of the group C rotaviruses has been the absence of a suitable and sensitive diagnostic test capable of screening a large number of specimens in epidemiologic or clinical studies. Group C rotaviruses cannot be detected with the routine immunoassays (e.g., EIA, Latex) commonly used for the diagnosis of group A rotavirus in clinical settings. Electron microscopy (EM) can detect group C rotaviruses when they are present in high concentration but cannot distinguish a group C rotavirus from a group A rotavirus by morphology. Polyacrylamide gel electrophoresis (PAGE) can identify group C rotaviruses based on their distinct and characteristic RNA migration pattern, but this method is relatively insensitive since group C viruses are excreted in much lower titer than group A viruses [3]. Molecular methods, including RT-PCR, have been useful to detect and confirm the presence of group C rotaviruses but are difficult and expensive to use in large field studies. Consequently, while many studies have documented the presence of group C viruses in focal settings, their true epidemiologic significance remains to be determined. The greatest advances in our understanding of this virus have come from studies of small outbreaks or surveys of etiology that have yielded specimens positive for rotavirus by EM but negative for group A rotavirus by EIA.

Between October 1996 and September 1997 children <4 years old with diarrhea who visited the Emergency Room of Severo Ochoa Hospital in Madrid were enrolled in a study to examine the etiology of diarrhea in Spanish children [8]. Uniform epidemiologic and clinical information was collected on all patients, including age, sex, admitting diagnosis, presenting symptoms, and duration of illness prior to admission. Fecal specimens were screened for etiologic agents of diarrhea by using routine culture methods for bacteria and commercial EIAs or RT-PCR for viruses (group A rotavirus, adenovirus, astrovirus, and calicivirus). Informed consent was obtained from all parents or guardians. The Institutional Review Board of Centers for Disease Control and Prevention exempted this study from IRB review because the study was conducted as part of routine surveillance for gastroenteritis agents. The specimens were tested anonymously and therefore no patients were identified.

Among the 822 children enrolled in the study, no etiologic agent could be identified in 29% (238) of the specimens submitted for diagnosis. Of these 238 specimens, 147 (62%) had adequate volume to assay further for group C rotaviruses. Specimens from these 147 children were representative of the total group in terms of age and symptom profiles, and seasonal distribution. In addition, 50 control fecal specimens were collected from children without diarrhea, ranging in age from 1 to 24 months, who were hospitalized at the same facility for treatment of other illnesses, including respiratory and urinary tract infections. All specimens were first screened for group C rotaviruses by EIA using reagents specific to the porcine Cowden strain [3]. Specimens positive by EIA were further examined for confirmation by RT-PCR and nested PCR, using primers

specific for the VP6 and VP7 genes of group C rotavirus. For RT-PCR, VP6 gene was amplified using primers BMJ 44 (5′-AGCCACATAGTTCACATTTC-3′) and BMJ 145 (5′-AGTCCGTTCTATGTGATTC-3′), whereas VP7 gene was amplified with primers BMJ13 (5′-AGCCACATGATCTTGTTT-3′) and BMJ107 (5′-TGTTTGGAGATGTGATGA-3′), following the previously described method [3]. The primers for nested PCR included BMJ 43 (5′-AGCGATGCCAGCTGGAACT G-3′) and BMJ144 (5′-CCTTCTGGGGATCATCCAT-3′) of VP6 gene, or BMJ 27 (5′-CAGATGAACACTGCTCAA-3′) and BMJ 143 (5′-CATGATCTTGTTTACG CAT-3′) of VP7 gene. The nested PCR was performed as RT-PCR but with omission of reverse transcription and with annealing temperatures of 53 °C and 48 °C for VP6 and VP7 genes, respectively. Southern hybridization and chemiluminescent detection were performed as previously described [1], with digoxigenin-labeled oligonucleotide probes: BMJ 157 (5′-TCTATGCGGCCTACATACCAT G-3′) for VP6 gene and BMJ 155 (5′-TTGTATAAAATATAGTCCACCAT-3′) for VP7 gene.

Group C rotaviruses were detected by EIA in 23 (16%) of the 147 pathogen-negative children tested. Of these, 22 were confirmed by nested PCR and Southern hybridization (Fig. 1). This figure ($n = 22$) was then extrapolated to the total group of patients for whom no agent was detected ($n = 238$) to provide the total number expected ($n = 36$), which when extrapolated to the total population of children indicated that a minimum of 4.4% of children surveyed (i.e., 36/822) would have been infected with group C rotavirus. None of the 50 controls were positive for group C rotavirus, suggesting that group C rotavirus was a causative agent of gastroenteritis.

Epidemiologic and clinical data of the 22 patients infected with group C rotavirus were reviewed and compared with those of patients infected with group A rotavirus (Table 1). Patients infected with group C rotavirus were older than those with group A rotavirus infection (median age 18 vs. 13 months), but this difference was not significant. Group C rotavirus did not appear to be seasonal and was detected year-round. Group C and A patients had similar clinical features, such as duration of diarrhea and vomiting. However, group C patients tended to have less severe disease with fewer episodes of vomiting per day, less dehydration, and no hospitalization despite with a higher median temperature.

These results demonstrate that group C rotavirus is an etiologic agent of diarrhea among children in Spain. Our study provides the lowest possible estimate of the magnitude of the problem (4.4%) because we screened by EIA only those specimens that had no other pathogens. Our previous study demonstrated that EIA was much less sensitive than RT-PCR for the detection of group C rotavirus in fecal specimens [3]. Even so, group C rotaviruses would represent a significant cause of diarrhea identified in this setting. Furthermore, the difference between the prevalence of group C rotaviruses among children with diarrhea and controls indicates that this virus is a likely cause of diarrhea. This study was initiated as a pilot project to determine whether it would be worthwhile to look further for group C in this population. Our findings confirm results of studies in Brazil, Japan, Sweden, and the United States which showed that group C rotavirus infections

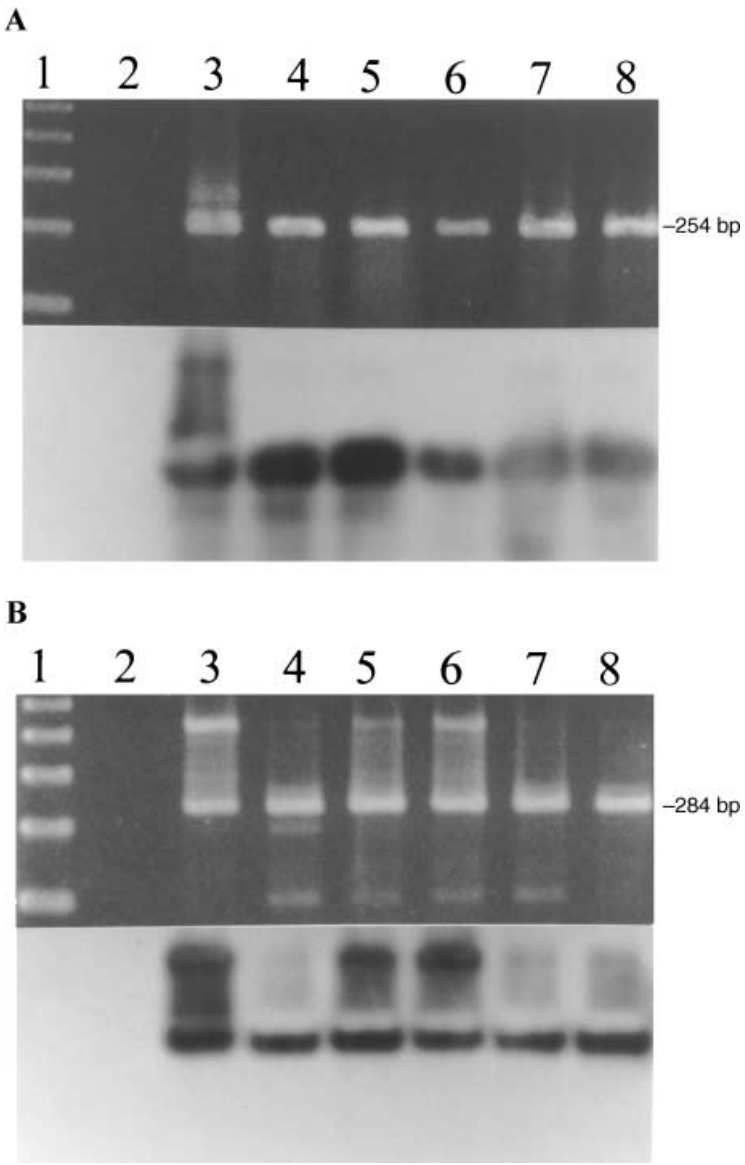

Fig. 1. Amplification of group C rotavirus genes 5 (**A**) and 8 (**B**) by PCR and Southern hybridization of amplified DNA products. Amplified products were analyzed on 3% agarose gels, stained with ethidium bromide, transferred to nylon membranes, and hybridized with digoxigenin-labeled oligonucleotide probes. **A**, lanes *1–8*: DNA molecular weight markers, H_2O, positive control RNA, and samples 561, 757, 783, 833, and 107, sequentially. **B**, lanes *1–8*: DNA molecular weight markers, H_2O, positive control RNA, and samples 501, 721, 771, 904, and 940, sequentially

were not uncommon, ranging in prevalence from 1% to 36% [2, 3, 5, 6]. Our findings further indicate that group C rotavirus is as common as other enteric pathogens, such as adenovirus (3%), calicivirus (6%), and astrovirus (9%), but less common than group A rotavirus (25%) in this collection (unpublished data).

Table 1. Comparison of symptoms from patients infected with group C or A rotavirus

	Rota C patient N = 22		Rota A patient N = 205		Statistical significance*
	median	range	median	range	p-value
Diarrhea (days)	1	1–7	1	1–8	0.2
Diarrhea (stools/day)	5	2–11	5	1–20	0.2
Vomiting (days)	1	0–6	1	0–5	0.5
Vomiting (episodes/day)	1	0–5	3	0–20	0.05
Maximum fever (°C)	38	36–40	37	36–40	<0.05
Dehydration (%)	10		41		<0.05
Hospitalization (%)	0		19		<0.05

*The Mann-Whitney U-test was used to compare the medians of symptom scores between patients with group C and A rotavirus infections. For comparison of proportions, chi square or Fisher's exact test was used. All tests were two-tailed and considered significant when $P \leq 0.05$

The current study suggests that appropriate application of modern diagnostic tests is likely to identify group C rotaviruses as a cause of diarrhea in children attending hospitals in many settings. Future studies should apply simpler but more-sensitive diagnostics, such as EIA using reagents specific to human group C rotavirus, to a larger proportion of clinical specimens and attempt to trace back the cases to identify epidemiologic clues that might link cases by common exposures or a common sequence. These studies could help determine the true burden of group C rotavirus diarrhea, the prevalence of mixed infection with group C rotavirus and other enteric viruses among children, and the possible source or vehicle of infection that might lead to future prevention and control measures.

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