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Rotavirus type A and other enteric pathogens in stool samples from children with acute diarrhea on the Colombian northern coast

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Abstract The present study, conducted from March 1998 to July 2000, determined the etiology of acute diarrhea in 253 young children and infants from Cartagena and Sincelejo, Colombia. In 253 stool samples, the following enteric pathogens were recovered: rotavirus type A (36.6%) as the major agent, *Salmonella* spp (9.0%), *Shigella* spp (8.0%), enteric pathogenic *Escherichia coli* (6.0%), enteric hemorrhagic *Esc. coli* (2.8%), *Providencia alcalifaciens* (2.8%), *Aeromonas hydrophila* (2.0%), *Yersinia enterocolitica* (0.8%), *Entamoeba histolytica* (10%), *Giardia lamblia* (4%), *Endolimax nana* (3.2%), *Ascaris lumbricoides* (2.8%), *Ent. coli* (1.2%), *Balantidium coli* (0.8%), *Blastocystis hominis* (0.8%), *Dypilidium caninum* (0.4%) and hook worm sp. (0.4%). Infection with more than one pathogen occurred in 96 (37.9%) patients. Rotavirus and enteric pathogenic *Esc. coli* were frequent. Concurrent infection by more than one parasite occurred in 18.6% of the infants. Most rotavirus infections (76.7%) occurred in infants under 12 months. Vomiting, severe dehydration and fever were frequent in children with rotavirus infection. At least one fecal marker of inflammatory diarrhea was registered in patients with bacterial infection. To our knowledge, this is first report of *P. alcalifaciens* associated with infantile diarrhea in Colombia and the first description of *Esc. coli* O157:H7 and *Y. enterocolitica* in our region.

Keywords Rotavirus · Enteric pathogens · Diarrhea

Introduction

Acute diarrhea (AD) remains a common disease affecting young children and infants in developing countries. The persistency of various etiological agents remains a serious public health concern. In Latin America and the Caribbean region, as in other developing geographic regions, rotavirus is one of the most common causal agents of viral diarrhea in young children [7, 9, 14, 17, 23, 28, 29]. However, other agents such as diarrheagenic *Escherichia coli* strains, *Salmonella*, *Shigella* and *Aeromonas* spp, *Campylobacter yeyuni*, *Yersinia enterocolitica* and parasites have been also described [1, 11, 18, 19, 25].

Previous studies that we carried out over the past two decades demonstrated that rotavirus type A (RTVA) was responsible for around 20–35% of the cases of AD in children under 3 years in Cartagena, Colombia (10° 25' N, 75° 33' W) [29, 31]. Nevertheless, infection by enteropathogenic *Esc. coli* (EPEC), *Salmonella*, *Shigella*, *Aeromonas* ssp and *Vibrio cholerae* have also been reported [8, 16, 30]. This work describes the prospective study we carried out to determine the prevalence of rotavirus and other enteric pathogens in young children and infants with AD in Cartagena and in Sincelejo, Colombia (9° 18' N, 75° 24' W), where the etiology of diarrhea in this sector of the population had not been studied.

Material and methods

Study sites, study population and general design

The study was carried out from March 1998 to July 2000 and included 153 young children and infants with AD, recovering at the Hospital Infantil Napoleón Franco Pareja or treated at the Primary Health Care Centers in Cartagena, and 100 young children and infants recovering at the Regional Hospital or treated at the Primary Health Care Centers in Sincelejo. (Cartagena has a population of 900,000; Sincelejo has a population of 350,000.) Of the

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253 patients included in this study—most of whom were less than 3 years old—159 (62.8%) were male and 94 (37.1%) were female. Most of them (78.8%) were from low- to below-average-income families. Of the remainder, 21.1% were from the lower to middle socio-economic level. All of them lived in urban areas. The 253 stool specimens collected were all cultured for bacteria and also examined for parasites; and 246 of them were analyzed for RTVA.

A questionnaire filled out for each patient covered demographic information, area of residence, information about any symptom associated with the disease (fever, vomiting, dehydration status), type and duration of diarrhea and history of antibiotic therapy—if any—before the clinic visit. AD was defined as abnormal fecal discharge characterized by frequent—at least three times per day—liquid or semi-liquid loose stools, accompanied by symptoms such as nausea, vomiting and fever and involving dehydration and electrolyte loss. The socio-economic level of the children was defined according to local norms of the district authorities both in Cartagena and Sincelejo. Five or more fecal leukocytes per field, and in blood, were considered markers for inflammatory bacterial diarrhea; and a positive reducing-carbohydrate test was considered as suspicious of rotaviral diarrhea.

Isolation and identification of enteric pathogens

Neither rectal swabs nor transport media were used. Stool specimens were collected in sterile receptacles, kept at 8 °C and immediately transported to the laboratory of microbiology of the University of Cartagena. Fresh stool specimens obtained from patients were macroscopically examined for consistency, blood and mucus. The pH was measured using the standard strip (Merck, Darmstadt, Germany); and the presence of carbohydrates on feces (positive response to reducing substance or presence of glucose) was determined using the Clinitest (Ames Bayer, Mexico). Direct wet mount of samples of non-concentrated feces were also examined for blood and leukocytes, fat globules, fiber, mycelia and for the presence of *Entamoeba histolytica*, *Ent. coli* and commensal parasites. Stool samples were also cultured for EPEC, enterohemorrhagic *Esc. coli* (EHEC), *Salmonella*, *Shigella*, *Yersinia*, *Aeromonas*, *Vibrio* and *Providencia* spp by standard methods [10, 15].

Primary isolation and subculture

All fecal samples were inoculated on EMB agar (Oxoid, Basingstoke, UK), sorbitol MacConkey (Oxoid) and TCBS (BBL, Cockeysville, Md.) plates and incubated overnight at 37 °C. They were also cultured in peptone broth (BBL) and incubated up to 4 h. When there was growth in peptone broth, an inoculum was subcultured on TCBS plates. Non-fermentative colonies from EMB agar were subcultured in Hecktoen agar (BBL).

Biochemical tests

Fermentative and non-fermentative colonies from EMB and sorbitol MacConkey plates were confirmed by biochemical tests, including catalase (J.G.B. Cali, Colombia), oxidase (Merck), TSI (Difco, Detroit, Mich.), lysine decarboxylase (Merck), urea (Difco), SIM (Difco), Simons citrate (Difco) and MRVP broth (Merck).

Bacterial typing

Isolated colonies typical of *Salmonella*, *Shigella* and EPEC were serotyped by slide agglutination, using A, B polyvalent standard antisera (BBL; also Becton Dickinson, Cockeysville, Md.). Transparent colonies, such as those of EHEC, that grew in sorbitol MacConkey were typed using the *Esc. coli* O157:H7 latex test (Oxoid).

Enzyme immunoassay

Stool samples were examined for RTVA antigen by enzyme immunoassay, using two commercial kits, Rotazyme II (Abbott, North Chicago, Ill.) and Pathfinder Sanofi-Pasteur (Biorad, Redmond, Wash.), according to the instructions of the manufacturers. Readings were taken in a Quantum II (Abbott Irving, Tex.) at 492 nm and in a Spectronic 601 (Bausch and Lomb, Milton Roy, Colo.) at 450 nm.

Statistical analysis

Statistical analyses were performed with Epi-Info 6.04 A and Pharma/PCS 4 software. Relationships between proportions were analyzed by χ^2 tests at the 95% confidence level. Values of $P \leq 0.05$ were considered to indicate statistically significant differences.

Results

Enteric pathogens

A total of 228 pathogens were detected from stool samples. Table 1 shows the distribution of the enteric pathogens recovered (no *Vibrio* sp. isolate was recovered). The frequencies of most enteric bacteria and parasites were similar in both cities. In contrast, the frequency of RTVA infection was significantly higher in Cartagena than in Sincelejo.

Incidence of enteric pathogens and age

Table 2 shows the incidence of pathogens in relation to patient age. The incidences of pathogens were significantly higher in infants under 12 months. In those children, RTVA was identified in 69 cases, enteric bacteria in 63 cases and parasites in 44 cases.

Rotavirus, bacteria, parasites, and mixed infections

Of the 228 pathogens identified, infection with more than one pathogen occurred in 96 of 253 patients (37.9%). Infection with more than one pathogen occurred with rotavirus and bacterium, rotavirus and parasite, and with enteric bacteria and parasites. No patient was infected with two enteric bacterial strains.

RTVA and bacteria

Mixed infection occurred as follows: of 90 cases of rotavirus infection, 34 patients (37.7%) were infected with seven types of pathogenic bacteria (13 were infected with EPEC, seven with *Shigella* spp, five with *Salmonella* spp, five with EHEC O157:H7, two with *Aeromonas hydrophila*, one with *Providencia alcalifaciens*, one with *Y. enterocolitica*). RTVA and EPEC were the pathogens most commonly associated, with a frequency of 14.4%.

Table 1 Distribution of enteric pathogens identified in stool samples from 253 young children and infants in the cities of Cartagena and Sincelejo, Colombia (153 in Cartagena, 100 in Sincelejo). Mycelia were excluded from totals

Pathogen	Cartagena (%)	Sincelejo (%)	Total (%)
Virus			
RTVA	62 (44.2)	28 (28.3)	90 (36.6)
Bacteria			
<i>Salmonella enteritidis</i>	11 (7.2)	11 (11.0)	22 (8.7)
<i>Shigella flexneri</i>	10 (6.5)	7 (7.0)	17 (6.7)
Enteric pathogenic <i>Escherichia coli</i> B	5 (3.3)	2 (2.0)	7 (2.7)
Enteric pathogenic <i>Esc. coli</i> A	4 (2.6)	4 (4.0)	8 (3.1)
Enteric hemorrhagic <i>Esc. coli</i> O157:H7	4 (2.6)	3 (3.0)	7 (2.8)
<i>Providencia alcalifaciens</i>	4 (2.6)	3 (3.0)	7 (2.8)
<i>Aeromonas hydrophila</i>	3 (2.0)	2 (2.0)	5 (2.0)
<i>Shi. dysenteriae</i>	2 (1.3)	0	2 (0.8)
<i>Sal. paratyphi</i>	1 (0.7)	0	1 (0.4)
<i>Shi. sonnei</i>	1 (0.7)	0	1 (0.4)
<i>Yersinia enterocolitica</i>	1 (0.7)	1 (1.0)	2 (0.8)
Parasites			
<i>Entamoeba histolytica</i>	16 (10.5)	9 (9.0)	25 (9.9)
<i>Ent. coli</i>	3 (2.0)	0	3 (1.2)
<i>Giardia lamblia</i>	2 (1.3)	8 (8.0)	10 (3.9)
<i>Endolimax nana</i>	5 (3.3)	3 (3.0)	8 (3.2)
<i>Ascaris lumbricoides</i>	3 (2.0)	4 (4.0)	7 (2.8)
<i>Balantidium coli</i>	0	2 (2.0)	2 (0.8)
<i>Blastocystis hominis</i>	2 (1.3)	0	2 (0.8)
<i>Dipylidium caninum</i>	1 (0.7)	0	1 (0.4)
Hook worm sp.	0	1 (1.0)	1 (0.4)
Total (%)	140 (91.5)	88 (88.0)	228 (90.1)

Table 2 Age prevalence of enteric pathogens in 253 young children and infants with acute diarrhea in Cartagena and Sincelejo, Colombia. n Number of patients in each age-group

Microorganisms identified	Percentage of positive cases by age-group (months)								
	Cartagena (%)				Sincelejo (%)				Total (%)
	0-4 (n=44)	5-8 (n=43)	9-12 (n=45)	>12 (n=21)	0-4 (n=19)	5-8 (n=28)	9-12 (n=20)	>12 (n=33)	
RTVA	16 (36)	17 (39)	20 (44)	9 (45)	5 (26)	9 (32)	2 (10)	12 (36)	90 (36.6)
Enteric bacteria	9 (20)	15 (35)	15 (35)	7 (33)	8 (42)	10 (36)	6 (30)	9 (27)	79 (31.2)
Parasites	7 (16)	12 (28)	9 (21)	4 (19)	6 (32)	2 (7)	8 (40)	11 (33)	59 (23.3)
Total (%)	32 (21)	44 (29)	44 (29)	20 (13)	19 (19)	21 (21)	16 (16)	32 (32)	228 (90.1)

RTVA and parasites

Among the 90 patients with rotavirus infection, 13 (14.4%) were infected with three kinds of pathogen parasites (nine were infected with *Ent. histolytica*, two with *Giardia lamblia*, two with *Ascaris lumbricoides*).

Enteric bacteria and parasites

Of the 79 patients with bacterial isolates found in their feces, 20 (25%) were infected with a single enteric bacterial pathogen, while 50 (63.3%) were infected with two pathogens. Of these 50, 14 patients (28.0%) were infected with six types of enteric bacteria and five types of parasites, as follows:

1. Of five patients with *Salmonella enteritidis* isolates, two were infected with *Ent. histolytica*, one was infected with *Blastocystis hominis*, one was infected with

Dipylidium caninum and one was infected with *Ent. nana*.

2. Of three patients with EHEC O157:H7 isolates, two were infected with *Ent. histolytica* and one was infected with *A. lumbricoides*.
3. Of two patients with EPEC isolates, one was infected with *Ent. histolytica* and one with *Ent. nana*.
4. Two patients with *Shi. flexneri* isolates were infected with *Ent. histolytica*.
5. One patient with *Shi. sonnei* isolate and one patient with *Y. enterocolitica* isolate were infected with *Asc. lumbricoides* and *Ent. histolytica*, respectively.

Relation of bacterial isolates and inflammatory markers

Of the 79 bacterial isolates recovered from the 253 specimens studied, at least one marker of inflammatory

diarrhea was recovered in their fecal leukocytes (36.7%) and blood (59.5%; Table 3).

Symptoms associated with diarrhea; and history of antibiotics

In 240 of the 253 cases studied, the presence or absence of vomiting and fever was recorded. Vomiting was present in 175 cases, 185 patients had fever and 169 had severe dehydration. Of the 253 cases, 108 (42.6%) had received mono- or combined therapy with antibiotics commonly used, such as ampicillin, gentamicin, amoxicillin, cephalosporin and trimetropin-sulfa, prior the clinic visit. Ampicillin was used as the first election in 62 out of 108 (57.4%) patients.

Discussion

This study shows that RTVA (36.6%) was the major agent associated with diarrhea, followed by enteric pathogenic bacteria (31.2%) and parasites (23.3%; $P > 0.05$). RTVA occurrence was higher in Cartagena (44.2%) than in Sincelejo (28.3%; $P < 0.05$).

We observed an increase in rotavirus infection reports in Cartagena over the past few years. This might be the result of an improvement in laboratory diagnosis, or a direct result of displacement of people from rural areas, as a result of social conflicts, with subsequent expansion of human settlements in peripheral zones in the city. Although the Sincelejo region has a similar demographic background, including displacement phenomena, the first report of the diarrhea etiology showed that the RTVA frequency there (28.3%) was lower than the bacterial frequency (33%; $P > 0.05$).

As previously observed in Cartagena [29, 31] and documented in other studies [9, 11, 12, 14, 22, 24, 25, 27, 28, 29] the majority of RTVA infections (76.6%) occurred in infants less than 12 months of age. The peak age incidence of rotavirus infection was in 5–12 months infants: 40% of cases in Cartagena and 22.9% in Sincelejo ($P < 0.05$). In contrast, no significant difference

was observed for the incidence rate in infants under 4 months old: 36% of cases in Cartagena and 26% in Sincelejo ($P > 0.05$; see Table 2).

Vomiting (51.7%) and fever (48.6%) were noticeable in patients with rotavirus infection. Furthermore, the presence of fever in this study correlates with the results found by Riveron and Muraira [20, 24], who indicated 31% and 44%, respectively.

The occurrence of rotaviral infections correlates with the winter season in developed countries. In our region, diarrhea occurs throughout the year, including the dry season (December–February) and the rainy season (August–November). We were unable to establish any correlation between climatic variations and RTVA and other enteric pathogen prevalence. These results are similar to those reported by Gutierrez in Bogotá, Colombia [12], and other authors in Argentina and Australia [5, 6].

In this study, the prevalence of *Salmonella* and *Shigella* spp, with similar percentages (9.0%, 8.0%) in children less than 2 years of age, are in accordance with other reports [18, 19, 22, 25, 26, 32, 33]. Regarding the pathogens, we found a predominance of *Sal. enteritidis* and *Shi. flexneri*.

There was a strong positive correlation between the presence of EHEC O157:H7, *Shi. dysenteriae*, *Shi. flexneri*, *Sal. paratyphi* and *Sal. enteritidis* isolates and the presence of leukocytes or blood upon microscopic observation. For the remaining enteric bacterial isolates, such as *Shi. sonnei*, this correlation was either low or negative.

The identification of *P. alcalifaciens* in seven infants was unexpected; and five of them had only this bacterium isolated from their feces. *P. alcalifaciens* is a bacterium whose etiological role in diarrhea has not been well established. Nevertheless, its invasive nature by pathogenic mechanisms, such as actin condensation and inflammatory changes associated with invasiveness, has been confirmed in Hep2 cells and adult rabbit ileal loops from diarrheal stool samples in Bangladesh and Brazil [2, 3, 4, 13]. Recently, there was a confirmed large outbreak of foodborne diarrhea by this bacterium [21].

Table 3 Number of enteric pathogenic bacteria isolates and presence of inflammatory markers (fecal leukocytes, blood) in stool samples from children and infants with acute diarrhea in Cartagena and Sincelejo, Colombia

Bacterial strains	Number (%)	Number (%) of positive stool samples	
		Leukocytes	Blood
<i>Sal. enteritidis</i>	22 (27.8)	6 (27.3)	17 (77.3)
<i>Shi. flexneri</i>	17 (21.5)	8 (47.0)	14 (82.4)
Enteric pathogenic <i>Esc. coli</i>	15 (18.9)	6 (40.0)	2 (13.3)
Enteric hemorrhagic <i>Esc. coli</i> O157:H7	7 (8.8)	3 (42.9)	7 (100.0)
<i>Providencia alcalifaciens</i>	7 (8.8)	3 (42.9)	3 (42.9)
<i>Aeromonas hydrophila</i>	5 (6.3)	0	1 (20.0)
<i>Shi. dysenteriae</i>	2 (2.5)	2 (100.0)	2 (100.0)
<i>Yersinia enterocolitica</i>	2 (2.5)	0	1 (50.0)
<i>Shi. sonnei</i>	1 (1.3)	0	0
<i>Sal. paratyphi</i>	1 (1.3)	1 (100.0)	0
Total	79 (100.0)	29 (36.7)	47 (59.5)

To our knowledge, this is the first report of *P. alcalifaciens* associated with infantile diarrhea in Colombia and the first description of the isolation of *Esc. coli* O157:H7 and *Y. enterocolitica* in our region. We propose to carry out a controlled-case study in order to establish the true role of *P. alcalifaciens* in diarrheal cases in our population. In relation to *P. alcalifaciens*, EHEC O157:H7, *Y. enterocolitica* and *C. yeyunus*, we suggest increasing the search along the Colombian north coast and other areas in Colombia.

With respect to the use of antibiotics, we found that 42.6% of all patients had received previous antibiotic treatment. In addition, of the 79 patients from whom bacterial isolates were recovered (31.2%), 39 of them had a history of antibiotic therapy previous to hospital or health center visit. Apparently, up to 11% of the infants had been treated unnecessarily or had not been treated properly with antibiotics.

The isolation of multiple enteric pathogens from stool samples is common in many developing countries, including Colombia [1, 8, 16, 18, 28, 29]. This problem may reflect the high degree of fecal contamination in the environment, or the high probability of person-to-person transmission as a result of poor hygiene measures or lack of water.

In this study population, the parasitic rate of infection was higher in Sincelejo (27%) than in Cartagena (20.9%; $P > 0.05$). So far, *Ent. histolytica*, followed by *G. lamblia* were the most common and concurrent infections, with more than one parasite observed in 18.6% of the stool samples. Identification rates of parasites were not significantly different by age in both cities, except for infants of 5–8 months of age. However, a high frequency of parasitic infection of 28% (12 cases) was recovered among children of this age-group in Cartagena, as compared with 7.1% (two cases) among infants in Sincelejo ($P < 0.05$; see Table 2).

Because approximately 78% of the young children and infants live in marginal areas, we expected high levels of parasitic infections and mixed infections. Two different enteric pathogens were observed in 42.1% of the stools; and half of those were parasites. These findings are consistent with other reports in communities from tropical areas of the world with poor hygiene [11, 18, 22, 26, 29, 30, 32]. In fact, some of the reports indicated frequencies up to 56% [11, 24]. Our findings suggest an early exposure of children to endemic enteric pathogens. This may be indicative of poor access to quality drinking water, sewerage systems and perhaps even a lack of basic education in these marginal communities. These and other social factors may play major roles in the high endemicity of enteric pathogens in these communities.

RTVA being one of the major diarrhea-causing agents as compared with bacterial and parasitic infections in infants, the training of medical staff in the proper use of antibiotic therapy is imperative. There is a strong need for the education of the medical community with regard to the high incidence of viral and parasitic diseases which do not necessitate antibiotic

therapy. The prescription and use of antibiotics before the identification of the etiological agent may be counterproductive. Our results suggest that all protocols used in primary health care programs in this region should be reviewed.

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