

# FREQUENCY OF *CAMPYLOBACTER JEJUNI* AND OTHER AGENTS OF ACUTE DIARRHEA IN VENEZUELAN CHILDREN<sup>1</sup>

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## INTRODUCTION

Diarrheal diseases have great public health significance, being a very common cause of illness and malnutrition in young children (1-3). In Venezuela, approximately seven children die every day of these diseases (4).

Studies in various parts of the world have shown rotavirus and enterotoxigenic *Escherichia coli* (henceforth referred to as "ETEC") to be the most frequent agents of acute diarrhea (5-7). However, another microorganism known since 1972 to be associated with enterocolitis in man and animals is *Campylobacter jejuni* (8-10). This microbe, identified in both sick and apparently healthy persons, has been found in many countries (9-14).

At present, *Campylobacter* infection is considered a zoonosis. In some areas, including the industrialized

countries, animals are the reservoirs of this infection; whereas in some areas of the developing world humans also play an important role in dissemination. These circumstances, and the fact that *C. jejuni* is excreted for a long time after the acute phase of the disease, make the epidemiology of the infection complex enough to require additional research. In this connection, it should be noted that 36 *C. jejuni* serotypes have been identified on the basis of heat-labile antigens (15), and more than 50 serotypes have been associated with different heat-stable surface antigens (16); so far as is known, any of these could be involved in intestinal colonization.

The present study is the first to deal with the prevalence of *C. jejuni*

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among infants and young children with acute diarrheal disease in Venezuela. Its specific purpose was to investigate the prevalence of this pathogen, clinical disease characteristics, and associations between this agent and other diarrheal disease agents in Venezuelan children.

## MATERIALS AND METHODS

From June 1982 through May 1983 a study was made of 196 children under two years of age who attended the J. M. de los Ríos and Miguel Pérez Carreño hospitals in Caracas with diarrheal disease syndromes of varying symptomatology and severity. Of this total, 149 (76%) were treated as outpatients and 47 (24%) were hospitalized. Fifty percent of these children were 0–6 months old, 22.8% were 7–12 months, 13.0% were 13–18 months, and 14.2% were 19–24 months. In addition, a control group of 27 asymptomatic children with a similar background, age distribution, and sex ratio was studied.

Stool samples were collected fresh and were preserved at  $-20^{\circ}\text{C}$  for detection of rotavirus by enzyme-linked immunoassay (ELISA) (17). A portion of each sample was used for a parasitologic study performed within 60 minutes of collection by examining part of the specimen suspended in saline and Lugol's solution under a light microscope.

Samples for bacteriologic diagnosis were obtained directly with rectal swabs, preserved in a semisolid Cary-Blair transport medium (0.16% agar), and processed in conventional culture media. The enterobacteria present were identified by conventional methods (18). In each case, five to 10 colonies of *Escherichia coli* were isolated, pooled, and sown in trypticase soy broth. These

pools were tested for enteropathogenicity and invasive capacity, respectively, by agglutination with commercial antisera (BBL, Difco) and by the Sereny test (19). The Dean test (20) was used to assess production of heat-stable (ST) toxin, and the ELISA technique (21) was employed to assess production of heat-labile (LT) toxin.

For detection of *Aeromonas* spp. a portion of the sample was plated onto blood agar containing ampicillin (22), and for detection of *Yersinia enterocolytica* it was plated onto media containing Salmonella-Shigella and MacConkey agar and kept at room temperature for 48 hours (23).

For isolation of *Campylobacter jejuni*, a portion of each sample was sown onto Campy-Bap medium (24) containing 5.0% human blood. The plates were then incubated at  $42^{\circ}\text{C}$  in a roughly 5%  $\text{CO}_2$  atmosphere (jar with candle-flame) for 48 hours. Identification was effected by observation of curved Gram-negative bacilli, by the results of oxidase and catalase tests, by detecting  $\text{H}_2\text{S}$  production, and by finding absence of growth at  $25^{\circ}\text{C}$ , susceptibility to nalidixic acid (30  $\mu\text{g}/\text{ml}$ ), and resistance to cephalothin (30  $\mu\text{g}/\text{ml}$ ) (24).

## RESULTS

*Campylobacter jejuni* was found in 18 (9.2%) of the sick children but in none of the control children. The enteric disease agent most often isolated was ETEC (from 41.8% of the patients), followed in order by rotavirus (14.1%), enteropathogenic *E. coli* (12.2%), and *Klebsiella pneumoniae* (11.2%). Among the parasites, *Giardia lamblia*

(in 3.5% of the patients) and *Entamoeba histolytica* (also in 3.5%) were both identified. Fifty samples were tested for *Yersinia enterocolitica* and *Aeromonas* spp., but neither of these microorganisms was detected. In 21.4% of the cases a mixed infection was diagnosed, but in 29.6% no agents were recognized. A high proportion of the asymptomatic (control) children were found to be infected with ETEC producing heat-stable toxin and with *G. lamblia* (18.5% and 17.6%, respectively).

A comparison of the hospitalized patients and outpatients (Table 1) revealed statistically significant dif-

ferences only with respect to heat-stable (ST) ETEC infections.

As Table 2 shows, *C. jejuni* was not found in association with other etiologic agents in most cases; however, in five of the six cases where it was found in association with another agent, that agent was ETEC. In no instance was *C. jejuni* found in association with rotavirus.

Those 12 subjects from whom *C. jejuni* was isolated as the sole etiologic agent exhibited the symptoms indicated in column 1 of Table 3. Ten (83%) had blood in their feces, 10 (83%) had mucus, three (25%) had fever, five (42%) vomited, and four (33%) had abdominal pain. Only three (25%) exhibited dehydration.

Overall, the symptoms of subjects with *C. jejuni* alone were simi-

TABLE 1. Diarrheal disease agents isolated from the feces of 196 study children with diarrhea and from 27 asymptomatic (control) children.

Agent	Sick children						Asymptomatic children (n = 27)		p <sup>d</sup>
	Hospitalized (n = 47)		Outpatients (n = 149)		Total (n = 196)		No.	(%)	
	No.	(%)	No.	(%)	No.	(%)			
<i>C. jejuni</i>	5	(10.6)	13	( 8.7)	18	( 9.2)	0	(0)	NS
Enterotoxigenic <i>E. coli</i> (ETEC)	22	(46.8)	60	(40.2)	82	(41.8)	9	(33.3)	NS
Heat-stable toxin (ETEC-ST)	16	(34.0)	22	(14.8)	38	(19.4)	1	( 3.7)	S
Heat-labile toxin (ETEC-LT)	6	(12.8)	31	(20.8)	37	(18.9)	5	(18.5)	NS
Heat-labile and heat-stable toxin (ETEC-ST/LT)	0	(0)	7	( 4.7)	7	( 3.6)	3	(11.1)	NP
Enteropathogenic <i>E. coli</i>	10	(21.3)	14	( 9.4)	24	(12.2)	0	(0)	NS
Invasive <i>E. coli</i>	0	(0)	10	( 6.7)	10	( 5.1)	0	(0)	NS
<i>K. pneumoniae</i>	5	(10.6)	17	(11.4)	22	(11.2)	0	(0)	NS
<i>Salmonella</i> spp.	1	( 2.1)	3	( 2.0)	4	( 2.0)	0	(0)	NS
<i>Shigella</i> spp.	1	( 2.1)	3	( 2.0)	4	( 2.0)	0	(0)	NS
Rotavirus <sup>a</sup>	9	(22.5)	14	(11.4)	23	(14.1)	0	(0)	NS
<i>G. lamblia</i> <sup>b</sup>	2	( 6.0)	2	( 2.5)	4	( 3.6)	3	(17.6) <sup>c</sup>	NP
<i>E. histolytica</i> <sup>b</sup>	1	( 3.0)	3	( 5.8)	4	( 3.6)	0	(0)	NP
More than one agent	9	(19.1)	33	(22.1)	42	(21.4)	0	(0)	NS
No agent	7	(14.9)	51	(34.2)	58	(29.6)	18	(66.7)	S

<sup>a</sup> n = 163.

<sup>b</sup> n = 112.

<sup>c</sup> n = 17.

<sup>d</sup> Statistical significance (chi-square) of differences between the figures for hospitalized subjects and outpatients S = significant (p < 0.05), NS = not significant, NP = not performed.

**TABLE 2.** The number of patients from whom *Campylobacter jejuni* was the only diarrheal disease agent isolated, as compared to the number from whom it was isolated with another agent.

Agent(s) isolated	Study subjects infected with <i>C. jejuni</i>	
	No.	(%)
<i>Campylobacter jejuni</i>	12	( 66.7)
<i>C. jejuni</i> and ETEC	5	( 27.8)
<i>C. jejuni</i> and enteropathogenic <i>E. coli</i>	1	( 5.5)
Total	18	(100)

**TABLE 3.** Initial enteritis symptoms apparently caused by *Campylobacter*, rotavirus, enterotoxigenic *E. coli*, and other agents.

Symptoms	% of ill study subjects with listed symptoms out of those from whom the following were isolated:				% of all study subjects (n = 196) with indicated symptom
	<i>C. jejuni</i> alone (n = 12)	<i>C. jejuni</i> (n = 18)	Rotavirus (n = 23)	ETEC (n = 82)	
Watery diarrhea	17	33	27	17	30
Abdominal pain	33	33	22	26	28
Vomiting	42	44	89 <sup>c</sup>	42	45 <sup>c</sup>
Fever	25 <sup>a</sup>	39 <sup>a</sup>	89 <sup>c</sup>	42	48 <sup>c</sup>
Blood	83 <sup>b</sup>	67	11 <sup>d</sup>	32	35 <sup>b,d</sup>
Mucus	83 <sup>b</sup>	83	22	63	44 <sup>b</sup>
Dehydration	25	33	78 <sup>d</sup>	29	24 <sup>d</sup>

<sup>a</sup> Difference between the two groups from which *Campylobacter* was isolated is statistically significant ( $p < 0.05$ ).

<sup>b</sup> Difference between patients from whom *Campylobacter* was isolated and all patients is statistically significant ( $p < 0.01$ ).

<sup>c</sup> Difference between patients from whom rotavirus was isolated and all patients is statistically significant ( $p < 0.001$ ).

<sup>d</sup> Difference between patients from whom rotavirus was isolated and all patients is statistically significant ( $p < 0.05$ ).

lar to those of subjects with *C. jejuni* in association with another diarrheal disease agent. However, a statistically significant ( $p < 0.05$ ) difference was found between the proportion of subjects with fever in the *C. jejuni* groups, the proportion being higher among those with *C. jejuni* and other pathogens.

Blood and mucus were found more often in the feces of patients with *C. jejuni* alone than they were in the feces of all subjects ( $p < 0.01$ ). The average duration of the disease for those in-

fected with *C. jejuni* alone was 9.1 days, within a range of two to 30 days.

As the Table 4 data show, study subjects 0–6 months old were the most likely to yield *C. jejuni* isolates, 72% of the *C. jejuni* infections being found in this youngest group. Two of these infected infants were under a month old. One of them, a newborn

TABLE 4. The age groups of the 18 ill study children from whom *C. jejuni* was isolated.

Age group (in months)	No. from whom <i>C. jejuni</i> was isolated	No. tested	% positive	Cumulative % of subjects from whom <i>C. jejuni</i> was isolated
0-6	13/98		13.3	72.2
7-12	2/45		4.4	83.3
13-24	3/53		5.7	100.0

seven days old, had its infection diagnosed while it was still in the maternity ward. The latter had diarrhea with bloody stools but no fever. *Campylobacter* was not isolated from the mother's vagina or feces, and it was not possible to determine the origin of the infection. The newborn was treated with erythromycin (40 mg/kg/day) and stopped excreting *C. jejuni* at the end of the seventh day of treatment.

It was also noted that seasonal variations in the frequency of *C. jejuni* infections differed markedly from seasonal variations in the frequency of rotavirus infections (Table 5). Specifi-

cally, the highest frequencies of *C. jejuni* infection were found in the hot and rainy months (June through October), whereas the highest frequencies of rotavirus infection were found in the relatively cool and dry months (November through March). ETEC was isolated during all parts of the study period except May and July.

TABLE 5. Seasonal variations in study children testing positive for *C. jejuni*, rotavirus, and ETEC.

Months of the June 1982-May 1983 study period	No. of cases studied (n)	Cases from which <i>C. jejuni</i> was isolated		Cases from which rotavirus was isolated		Cases from which ETEC was isolated	
		No.	% of cases studied	No.	% of cases studied	No.	% of cases studied
June	14	3	21.43	NP <sup>a</sup>	NP	3	21.43
July-August-September	38	4	10.53	3	7.89	14	36.84
October-November-December	67	7	10.45	4	5.97	30	44.78
January-February-March	41	3	7.32	14	34.15	28	68.29
April-May	36	1	2.77	2	5.56	7	19.44
Total	196	18	9.18	23	11.73	82	41.84

<sup>a</sup> NP = Test not performed.

## DISCUSSION

The present study was performed to investigate the presence of *C. jejuni* in children under two years of age with acute diarrhea. This agent was identified in 9.2% of the sick children studied, a percentage similar to that found in other Latin American countries (25, 26).

Earlier studies have described the presence of *C. jejuni* in the feces of healthy persons (10, 12-14). In this study, however, *C. jejuni* was not isolated in any healthy child. This noteworthy finding is consistent with results obtained in industrialized countries, where isolation of *Campylobacter* has always been associated with disease (10). (In developing countries, especially very poor countries like India and Bangladesh, the rate of endemicity is high, reaching up to 35%.)

As in other developing regions (11, 12), the pathogen was isolated most frequently from children under 12 months old. This pattern differed from that found in industrialized countries, where the risk of infection is greater in older children (10, 27). Moreover, 72% of the infected study children in Venezuela were under six months old, and two were neonates. One of these neonatal infections manifested itself in the maternity ward, as previously described elsewhere (28, 29); however, it could not be established that transmission had taken place during delivery. Both infected neonates were afebrile, a circumstance that parallels previously reported findings (29).

The symptoms observed among patients with *C. jejuni* diarrhea in this study agree essentially with those described by other authors (9, 10). It is important to note that most of these patients had stools with the dysenteric characteristics of blood or mucus (Table 3). This suggests an invasive pathogenic mechanism associated with production of a cytotoxin (30). However, studies in Bangladesh (12) have found that a large proportion of cases with *C. jejuni* had watery diarrhea without mucus or blood, a symptomatology that differs from that described in developed countries (10). This watery diarrhea could be associated with a heat-labile toxin, similar to the heat-labile toxin of *Vibrio cholerae* and that of ETEC described by Ruíz-Palacios (31).

It remains to be determined whether this variation in the reported symptoms of *Campylobacter* infections arises from differences in the host or in the agent strains involved. On the one hand, it is known that not all persons exposed to *Campylobacter* develop the disease (32); however, Klipstein (33) has demonstrated a correlation between disease and toxin production in symptomatic subjects that contrasts with an absence of toxin in strains isolated from asymptomatic carriers. These observations, and the epidemiologic differences noted between subjects in developed and developing countries, suggest that much remains to be learned about the pathophysiology of *C. jejuni* and its pathogenic role in acute diarrhea.

The seasonal variation of the *Campylobacter*-associated disease observed in this study is similar to that described in other countries with tropical climates, where the disease typically occurs in rainy months (10, 34). The opposite is the case in temperate countries, where the disease is most prevalent in dry summer months (10, 35). Tempera-

ture could be a common factor here, for in general the months when the disease seems most prevalent are the warmest of the year.

Regarding other etiologic agents, ETEC was the one most frequently isolated (from 41.8% of the study subjects). The frequency of ETEC (ST) was found to be similar to that described by other authors (26, 36). The frequency of ETEC isolates producing heat-labile toxins (LT) was higher than that generally reported elsewhere (26, 35), but the pathogenic significance of this ETEC was reduced by the fact that such strains were also found in 18.5% of the asymptomatic children. In contrast, ETEC (ST) was found in only 3.7% of the asymptomatic children. The high frequency of ETEC (ST) isolated from the hospitalized children and the low frequency detected in healthy children suggests that this agent could be more of a problem in acute diarrheal disease in our area than is ETEC (LT).

Rotavirus was the pathogen isolated with the second-greatest frequency from the children with diarrhea. This isolation frequency (14.1%) is similar to that found in other communities (22, 34), and the seasonal distribution of these isolations resembles that found by other authors (26, 34, 37). However, the observed 14.1% frequency is low compared to figures indicated by data obtained from other studies in Venezuela, in which rotavirus was found among 30-40% of the patients (5, 17, 37). The difference could be explained by the origin of the samples studied here (most were obtained from outpatients), since rotavirus tends to be more prevalent among hospitalized patients (see Table 1).

The frequency with which *K. pneumoniae* was isolated (from 11.2% of the sick children but none of the

asymptomatic children) is noteworthy, since this microorganism has been associated with production of both heat-labile and heat-stable toxins (26).

*G. lamblia* was found in both sick and asymptomatic children, whereas *E. histolytica* was found only in sick children. The presence of *G. lamblia* in 17.6% of the healthy children studied indicates that it is necessary to study more about this agent's health implications and epidemiology, since a high prevalence of the parasite in healthy children could present a risk of transmission. In 29.6% of the cases no agent was identified. Part of this percentage might be accounted for by "new" etiologic agents such as *Cryptosporidium*, a parasite under study at our laboratory. (In a recent study, *Cryptosporidium* oocysts were identified in 10.8% of 120 children under two years old who were suffering from acute diarrhea—38).

Overall, the findings reported in this study indicate that the epidemiology of *Campylobacter* infection in Venezuela deserves special attention and more research, especially since it appears to share some features found in developed countries and others found in developing countries. Among other things, prospective studies that include *Campylobacter* serotyping are needed to determine the microorganism's reservoirs and transmission mechanisms, as well as the immunity to *Campylobacter* in marginal populations, among which *Campylobacter*-associated disease is very common.

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# SUMMARY

The frequency of *Campylobacter jejuni* and other enteric disease agents causing diarrheal illness was studied in 196 Venezuelan children under two years old who attended two Caracas hospitals from June 1982 through May 1983. A group of 27 asymptomatic (control) children was also included in the investigation.

This is the first reported study to examine the prevalence of *C. jejuni* infection among this age group in Venezuela. In all, *C. jejuni* was isolated from 18 (9.2%) of the ill study children but from none of the controls. Enterotoxigenic *E. coli* (ETEC) was isolated from 82 (41.8%) and rotavirus was isolated from 23 (14.1%).

*C. jejuni* was most prevalent among the study subjects 0-6 months old. (Two of these infected infants were neonates, and one was only seven days old.) Blood and mucus in the stools were very common symptoms of *Campylobacter* disease. Also, most of the *C. jejuni*-associated cases occurred from

June through October, a period characterized by heavy rainfall and warm temperatures in Caracas.

These findings suggest a noteworthy prevalence of disease associated with *C. jejuni*, indicating a need for additional prospective studies to identify transmission mechanisms, animal reservoirs, and asymptomatic infections in poor communities where *Campylobacter*-associated disease is very common.

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