

ETIOLOGY OF ACUTE DIARRHEA AMONG CHILDREN IN RIBEIRÃO PRETO-SP, BRAZIL

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SUMMARY

To study the main enteropathogens causing diarrhea in the region of Ribeirão Preto regarding serogroups and serotypes, the feces of 1836 children under 10 years old, from both sexes, attack of acute gastroenteritis, were analysed during a period of 4 years in Adolfo Lutz Institute – Ribeirão Preto, SP. The pathogens identified by standard methods were the following: *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., and *Cryptosporidium* spp. Positive samples were 22.8% (419) with 1.7% association of pathogens. Larger isolates were mainly from children 0 to 11 months old. Enteropathogenic *E. coli* (EPEC) was most frequent (8.7%) with predominance of serogroup O119 (40.2%), followed by *Shigella* (6.2%), 63.6% of which *S. sonnei*.

KEYWORDS: Enteropathogens; Diarrhea; Children.

INTRODUCTION

Several factors can contribute to intestinal infections: duration of maternal breast feeding, food and environmental contaminations^{18,30}, maternal education and employment, home location, family income, infant's birth weight²⁷, access to treated water and basic sanitary conditions^{18,32}, seasonal variations and in special, the age of the host¹¹.

The morbidity of the infectious diseases associated with malnutrition is one of the main causes of delayed physical growth in children²⁷. In Asia (excluding China), Africa and Latin America 4.6 to 6 million children under five years old die of diarrhea each year¹⁵. In Ethiopia in 1993, nearly 25% of deaths in this age bracket were due to diarrhea²⁷.

To prevent dehydration and death, children are admitted to hospitals in underdeveloped countries^{5,10}, not only increasing costs to society but also aggravating a public health problem by being communicators and transmitters in hospital infections²⁵. Thus, knowledge of the etiology of acute diarrhea is of great importance for the control and prevention of disease.

Bacterial agents that most commonly cause diarrhea in Brazil are: enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *E. coli* (ETEC), *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp.^{2,11,30}.

This study was conducted to investigate the frequency and etiology of diarrhea in children admitted in hospitals or outpatient clinics in the region of Ribeirão Preto- SP, characterizing the enteropathogens isolated in terms of serogroups and serotypes.

MATERIALS AND METHODS

Subjects: From January 1994 to December 1997, were investigated enteropathogens agents in 946 children of outpatient clinics and 890 of patients admitted to pediatric hospitals, all under ten years old, with diarrhea. Routine diagnostic tests on stool samples included culture for enteropathogenic *Escherichia coli* (EPEC), enteroinvasive (EIEC), enterohemorrhagic *Escherichia coli* O157:H7 (EHEC), *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Yersinia enterocolitica*. In selected cases special tests for *Cryptosporidium* spp. were also performed.

Collection of specimens: Coprocultures were made from feces transported at room temperature in a Cary Blair medium⁹. *Cryptosporidium* spp., were made from feces kept in refrigeration until taken to the laboratory of the Adolfo Lutz Institute (IAL) in Ribeirão Preto-SP.

Bacteriologic studies: The isolation and presumptive identification of EPEC, EIEC, *Salmonella* spp., and *Shigella* spp. were performed according to the method described previously²²; EHEC O157:H7 was studied in a MacConkey medium containing sorbitol⁹. Biochemical characterization was made by the method of EDWARDS & EWING⁷. The serotypes of samples of *Shigella*⁷ and *Salmonella*²³ were determined in the laboratories of IAL (main branch) in São Paulo. *Yersinia enterocolitica* was isolated and identified biochemically by the methods of respectively, GRAY¹³ and FARMER & KELLY⁹; serotyping and phage typing were performed in the laboratory of "Universidade Estadual Paulista" (UNESP) in the city of Araraquara (reference laboratory)^{20,33}. The isolation and identification of *Campylobacter* spp. was made

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according to MORRIS & PATTON¹⁹, MAGALHÃES & ANDRADE¹⁷ and SKIRROW & BENJAMIN²⁸.

Cryptosporidium spp.: Was detected from concentrated formalin-fixed stool samples by the modified Kinyoun method, based on the acid-resistance of the protozoon, described by SMITH & BARLETT²⁹.

RESULTS

One or more enteropathogens were identified in 419 samples (22.8%) of the 1836 studied. This total, 388 (21.1%) were positive for only one microorganism, in 31 (1.7%) cases mixed infections occurred, 30 with associated of the two enteropathogens and one with three. EPEC and *Campylobacter* spp. were the enteropathogens most commonly associated with other microorganisms. EHEC O157:H7 was not detected.

The table 1 summarizes: the frequency of enteropathogens isolated from single or multiple infections. The prevailing EPEC serogroups were O119 (40.2%), O128 (15.7%), O111(11.3%), O26 (10%), O55 (8.8%) and other serogroups. The most frequent *Shigella* serotype was *sonnei*

(63.16%), among samples of *Shigella flexneri*, the type 2 was the most common. *Campylobacter* spp. was the third most frequent (5.4%) organism isolated, with predominance of *C. jejuni*. There were 12 *Salmonella* serotypes with predominance of *S. Enteritidis* (36.6%), followed by *S.I 4,5,12:i:-*, *S. Braenderburg*, *S. Infantis*, *S. Mbandaka*, *S. Agona*, *S. Indiana*, *S. Braenderup*, *S. Javiana*, *S. Give*, *S. Worthington* and *S. Typhimurium*. The isolated EIEC belonged to serogroups O167, O143 and O124. *Yersinia* was characterized as *Y. enterocolitica* 4 – O:3–VIII .

Of the 451 enteropathogens isolated, including mixed infections (Table 1), 51.7% were from the group of children with ages between 0 and 11 months for pathogens in general, but EPEC was the most frequent one in this age group (Table 2).

The Figure 1 shows the distribution of cases according to the origin of the samples. Of 890 (48.4%) samples from children admitted to hospitals, 207 (23.3%) were identified enteropathogens, while 946 (51.5%) samples from outpatient clinics were positive in 244 (25.8%) of the cases.

Table 1
Prevalence of enteropathogens in the stools of 1836 infants with diarrhea, Ribeirão Preto, SP, Brazil, 1994-1997

Enteropathogens*	1994 n=824		1995 n=557		1996 n=307		1997 n=148		TOTAL n=1836	
	N	%	N	%	N	%	N	%	N	%
EPEC	81	9.8	45	8.1	21	6.8	12	8.1	159	8.7
<i>Shigella</i> spp.	45	5.5	47	8.4	18	5.9	04	2.7	114	6.2
<i>Campylobacter</i> spp.	45	5.5	39	7.0	12	3.9	03	2.0	99	5.4
<i>Salmonella</i> spp.	12	1.4	19	3.4	07	2.3	03	2.0	41	2.2
<i>Cryptosporidium</i> spp.	22	2.7	08	1.4	04	1.3	-	-	34	1.8
EIEC	02	0.2	-	-	-	-	01	0.7	03	0.2
<i>Yersinia</i> spp.	-	-	-	-	-	-	01	0.7	01	0.05
Total	207	25.1	158	28.4	62	20.2	24	16.2	451	24.6

NOTE: EPEC = enteropathogenic *Escherichia coli*. EIEC = enteroinvasive *Escherichia coli*.; * *Escherichia coli* O157:H7 were not detected

Table2
Distribution of 451 enteropathogens from diarrhea children's feces, according to age, in Ribeirão Preto, SP, Brazil, 1994-1997

Enteropathogens (N)	Ages													
	0-11m		1-2y		2-4y		4-6y		6-8y		8-10y		NI	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N°	(%)
EPEC (159)	121	(76.1)	19	(11.9)	07	(4.4)	03	(1.9)	03	(1.9)	02	(1.3)	04	(2.5)
<i>Shigella</i> spp.(114)	28	(24.6)	18	(15.8)	27	(23.7)	17	(14.9)	07	(6.1)	10	(8.8)	07	(6.1)
<i>Campylobacter</i> spp. (99)	47	(47.5)	27	(27.3)	15	(15.1)	01	(1.0)	-	-	02	(2.0)	07	(7.1)
<i>Salmonella</i> spp. (41)	20	(48.8)	12	(29.3)	03	(7.3)	03	(7.3)	01	(2.4)	02	(4.9)	-	-
<i>Cryptosporidium</i> spp.(34)	15	(44.1)	15	(44.1)	02	(5.9)	-	-	-	-	-	-	02	(5.9)
EIEC (03)	02	(66.7)	-	-	-	-	-	-	-	-	01	(33.3)	-	-
<i>Yersinia enterocolitica</i> (01)	-	-	-	-	01	(100.0)	-	-	-	-	-	-	-	-
TOTAL (451)	233	(51.7)	91	(20.2)	55	(12.2)	24	(5.3)	11	(2.4)	17	(3.8)	20	(4.4)

% relative to the number of samples of each enteropathogen; NI: no information of age

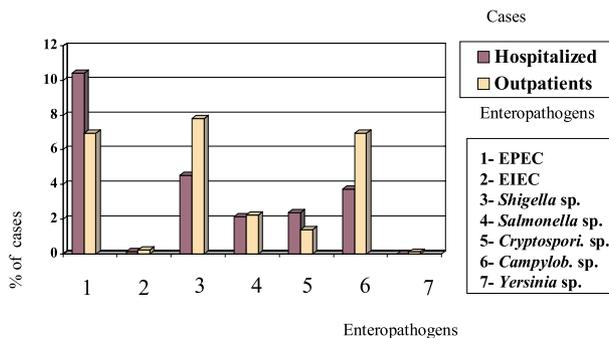


Fig. 1 - Percentages (%) of enteropathogens isolated from hospitalized and outpatients children attended in the region of Ribeirão Preto from 1994 to 1997.

DISCUSSION

The results found in the present study are similar to those reported from other places in Brazil^{12,30} and other countries^{1,21}, where EPEC constitutes one of the most important etiological agents of acute diarrhea. Among enteropathogens isolated, EPEC stood out by its presence in 8.7% of the cases, including mixed infections. GOMES *et al.* (1991)¹¹, isolated EPEC in 26% of the cases of infantile diarrhea. In Argentina²¹ was found approximately 3 times greater percentage of EPEC (25.2%), compared to that reported in the present study (8.7%).

Our studies were restricted to EPEC serogroups identification, however the EPEC serogroups are very heterogeneous. There is extensive variation in virulence among bacterial strains demonstrating that identification of EPEC by serotype is very important^{11,12}.

The figure 1 shows that the majority of the enteropathogens were from the outpatients less EPEC and *Cryptosporidium* spp. 10.4% of the strains of EPEC were isolated from hospitalized children; this is a reason for concern from the epidemiological viewpoint, since these children represent an important reservoir of these microorganisms, capable of constituting a source of hospital infection^{11,32}.

The second most frequent etiological agent isolated, was *Shigella* spp. the most frequent pathogen in children over two years old. Similar data were found by HUILAN *et al.*¹⁶; they are explainable by the small infecting inoculum required to start this infection, as well as by the increased contact between children of this age range, mainly in day nurseries^{14,18}. *Shigella sonnei* appeared more frequently than *S. flexneri* in contrast to results of other authors in Brazil^{11,31} and Argentina²¹, who report that *S. flexneri* is the most prevalent species isolated. Among samples of *S. flexneri*, the type 2 was the most common, in agreement with findings by other authors^{2,21}.

Campylobacter spp. was the third enteropathogen found in our study, being more frequent than *Salmonella* spp. as the cause of enteritis. Its importance as a cause of diarrhea has been emphasized in Brazil^{11,30}, and other countries^{16,26}.

Was noted a predominance of the serotype Enteritidis (36.6%), in infections by the genus *Salmonella*, including mixed ones. Other authors

^{2,8}, observed an increase of this serotype in the last decade. According to EIGUER *et al.*⁸, this serotype has been one of the major causes of alimentary toxoinfection in man.

Considering the increasing importance of *Cryptosporidium* spp. in infantile diarrhea, the prevalence of this agent in our region was investigated; a frequency of 1.8% was found, mixed infections included. Other studies in Ribeirão Preto, showed higher values (13.2%³, and 27%²⁴). The necessity of the routine investigation in this aspect is therefore noticed.

EHEC was not found in the present study, in agreement with reports by GOMES *et al.*¹¹. Unlike results of authors which could not isolate this etiologic agent^{21,26}. In the present study was isolated one sample of *Yersinia* spp., similar to that found in São Paulo by TORRES *et al.*³⁰.

The age group most affected by EPEC was that of children less than 2 years old, with marked prevalence of ages 0-11 months, and decreasing with age, as previously reported¹. The greatest incidence of *Campylobacter* spp. occurred in children between 0 and 4 years, in disagreement with SKIRROW & BENJAMIN²⁸, who reported a predominance of this infection in older children and young adults. *Cryptosporidium* spp. is an opportunist parasite, infection is more common in children than adults because the host becomes more resistant with aging²⁴. This was confirmed in the present work, because the parasite was more frequent in children less than 2 years old, practically disappearing after 4 years, as also related by DIAS *et al.*⁶. In disagreement with earlier cited authors, BICHARA *et al.*⁴, found *Cryptosporidium* spp. in students 11 to 14 years old, demonstrating that besides immunity, sanitary hygiene and contact with animals are also important.

CONCLUSIONS

Enteropathogens EPEC, *Shigella* spp. and *Cryptosporidium* spp. were the most frequently found causes of diarrhea in children under ten years old in the region of Ribeirão Preto, with the serotype O119 of EPEC, *S. sonnei* and *C. jejuni* being the most isolated ones. Among positive cases, approximately 84% occurred in children under four years old and 52% between ages 0 and 11 months.

RESUMO

Principais enteropatógenos causadores de diarreia em crianças, na região de Ribeirão Preto-SP, Brasil

Com o objetivo de caracterizar os principais enteropatógenos causadores de diarreia na região de Ribeirão Preto, quanto aos sorogrupos e sorotipos, por um período de 4 anos foram estudadas fezes de 1836 crianças, menores de 10 anos de idade, de ambos os sexos, portadoras de gastroenterite aguda no IAL de Ribeirão Preto, SP. Foram pesquisados os seguintes enteropatógenos: *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., *Yersinia* sp., e *Cryptosporidium* sp., identificados através de metodologia tradicional. Foram positivas 419 (22,8%) amostras, com 1,7% de associação entre enteropatógenos. Houve predomínio na faixa etária de 0 a 11 meses. Destacou-se a *E.coli* enteropatógena (EPEC) (8,7%), sendo mais frequente o sorogrupo O119 (40,2%), seguida do gênero *Shigella* (6,2%), dos quais 63,2% corresponderam à *S. sonnei*.

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