

Enteropathogens Associated with Diarrheal Disease in Infants of Poor Urban Areas of Porto Velho, Rondônia: a Preliminary Study

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One hundred and thirty cases of diarrhea and 43 age-matched controls, 0 to 5 years old, were studied in a pediatric outpatient unit from a poor peri urban area of Porto Velho, Rondônia. Eighty percent of diarrheal cases were observed in the groups under 2 years of age. Rotavirus (19.2%) was the most frequent enteropathogen associated with diarrhea, followed by Shigella flexneri (6.15%) and S. sonnei (1.5%) and Salmonella sp. (6.9%). Four cases of E. coli enterotoxigenic infections (3.1%), E. coli enteropathogenic (EPEC)(2.3%) one case of E. coli enteroinvasive infection (0.8%) and one case of Yersinia enterocolitica (0.8%) were also identified. Mixed infections were frequent, associating rotavirus, EPEC and Salmonella sp. with Entamoeba histolytica and Giardia lamblia.

Key words: diarrheal disease - enteropathogens - rotavirus infections - epidemiology - Rondônia - Brazil

Diarrheal disease accounts for an estimated annual five million deaths among infants under 5 years all around the world (Georges et al. 1984). The main risk factors for the morbi-mortality of diarrhea are well known and relate to the poor quality of life, lack of sanitation and clean water supply for most of the population living in poor areas of developing countries.

Reports show that diarrhea is present as an important childhood illness in many areas of Latin America countries and accounts for more than 20% of childhood mortality (Bern et al. 1992). In Brazil, diarrheal disease is present all over the country and still represents a major cause of children mortality in the poorest peri urban and rural areas of both Northeast and Northern regions (Guerrant & Kirchhoff 1983).

A longitudinal study carried out from 1990 to 1992 in the city of Belém, in the eastern Amazon region, yielded rates of 5.9 episodes of diarrhea per child/year (Linhares 1997).

Less recent data from Fortaleza, in the country's Northeast region, showed that the number of diar-

rheal episodes in the 6 to 12 months age-group exceeds 7 and 9 per person year among families in the poor urban and poor rural areas, respectively, decreasing progressively to two episodes year person among the adults (Guerrant & Kirchhoff 1983). To date, no survey has been performed in Rondônia as well as in the western Amazon Region with the purpose of establishing diarrheal disease incidence, however, records from the official health services indicate that diarrhea is the main cause of mortality among 0 to 5 year old children (Plano Plurianual de Saúde de Rondônia 1996).

Several studies have been developed in the South and Southeast regions of Brazil in order to identify etiological agents associated with diarrhea, as well as to assess both epidemiological and clinical features of the disease (Riley et al. 1984, Trabulsi et al. 1985a, Gomes et al. 1991). A few studies have been carried out in the Northeast region, particularly in Fortaleza (Guerrant & Kirchhoff 1983) and Recife (Leal et al. 1988). No study has been performed in the northern Amazon region, of Brazil, with exception of specific studies on rotavirus in Belém.

We present here preliminary data on the etiology and clinical features of diarrhea among infants from 0 to 5 years old, living in a poor urban area, who were referred to a pediatric, outpatient unit in Porto Velho, Rondônia (western Amazon region). The relative frequencies of enteropathogens are similar to that described in the Northeast region of Brazil.

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MATERIALS AND METHODS

Study area - The study was conducted between July 1998 and March 1999 by the staff of the "Centro de Pesquisa em Medicina Tropical (CEPEM)", Porto Velho, in the outpatient health unit "Policlínica Hamilton Gondin". In Porto Velho, capital, there has been a significant increase in population during the last three decades, resulting in migration from different areas of Brazil following the construction of the "BR364" road that connects the city to the country Central and South regions. The present population of the city is around 200,000 inhabitants. The city has a modern geometrical design but lacks an adequate sanitation system. The "Policlínica" is located in the poor northern city outskirts. Potable water supply is limited to a low proportion of age residences and sew network is absent, every house having pit toilets.

Study overview - One hundred and thirty infants aged 0 to 5 years old were brought by mothers to the "Policlínica" because of diarrheal disease; in addition, 43 age-matched non-diarrheal control infants from to the "Policlínica" were selected for the study. CEPEM staff pediatricians interviewed the accompanying adults, obtained written informed consent, completed a questionnaire and performed physical examination. Special attention was given to past history of symptoms, number of diarrhea episodes, fever (axillary temperature > 37^o.8), vomiting, dehydration and presence of mucus and blood in the stools.

Laboratory methods - Fecal samples were collected after either natural evacuation or through stimulation with a glycerin suppository. Samples were divided in two fractions: one fraction was used for parasitological examination and testing for rotavirus using Slidex Kit (Biomérieux). The second aliquot was cultured according to standard bacteriological procedures. Briefly, about 3 g of stools were suspended in 3 ml of PBS and, placed onto following selective media: MacConkey, SS and brilliant green agar (after enrichment in tetrathionate broth containing 40 µg/ml novobiocin) for isolation and identification of the following enteropathogenic bacteria: enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic (Shiga-like toxin-producing) *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), *Shigella*, *Salmonella* sp., *Yersinia* species, *Campylobacter jejuni* and *C. coli*. Plates were incubated at 37°C for 24-48 h excepting those used to identify *Campylobacter* species; these were detected using plates with modified Skirrow, followed by incubation at 42°C for 48 h in Gaspak jar.

Five positive colonies typical of *E. coli* and one lactose-negative colony of different morphologic

types were grown to MacConkey agar plates as well as lactose positive and negative colonies isolated on SS and brilliant green agar plates were identified by biochemical tests using a EPM millicitrate Kit (ProBac, Brasil). Colonies resembling *Campylobacter* species, growing on Skirrow modified plates, were submitted to Gram's stain and catalase and oxidase tests for confirmation of genus. *E. coli* strains isolated on MacConkey agar were submitted to slide agglutination tests using polyvalent sera against EPEC serogroups A, B and C. Non agglutinating isolates were tested with antiserum against EHEC serogroup O157 and with polyvalent antisera against EIEC serogroups A and B. Isolates identified by biochemical tests as *Shigella* and *Yersinia* species were serotyped by standard techniques. Commercially available antisera from ProBac and Promicro (São Paulo) were used for serotypes determinations.

E. coli isolates not belonging to the EPEC, EHEC or EIEC serotypes were tested for production of heat-labile enterotoxin (LT), and of heat stable (ST) enterotoxin by PCR. The primers employed (Tornieporth et al. 1995) are complementary to the following positions:

59 to 76 (ET-LT1,5'GCGACAAATTATACCGTGCT3')
765 to 746 (ET-LT2,5'GCGACAAATTATACCGTGCT3')

79 to 98 (ET-ST1,5'CTGTATTGCTTTTTTCACCT3') and
260 to 241 (ET-ST2,5'GCACCCGGTACAAGCAGGAT3').

Amplification was performed by 30 cycles in a Perkin-Elmer thermal cycler, as follows: denaturation for 2 min at 92°C, annealing for 1 min at 50°C, and extension for 2 min at 72°C. The amplification process was terminated by a 5 min extension at 72°C, and the tubes were rapidly cooled at 4°C. Fifteen µl of each sample was subjected to electrophoresis on a 2% ethidium bromide-prestained agarose gel at 100V for 2 h to visualize the PCR product.

RESULTS

Age-group distribution of diarrheal disease - A clear higher incidence in the age groups from 0 to 2 years old in the "Policlínica" was observed among the 130 diarrheal cases disease. Infants below 1 year of age were particularly affected, accounting for 62 cases (47.7%), of which 28 (21.4%) were less than 6 months old (Table I).

Prevalence of enteropathogens - Among the 130 cases of diarrheal disease, laboratory examinations were negative for enteropathogenic bacteria and rotavirus in 16 infants. Table II shows the distribution of pathogens found in stools of 53 infants with diarrhea (40.7%) and in 2 controls (4.6%). Rotavirus was the most frequent pathogen found (19.2% of diarrheal cases and 2.4% of controls). *Shigella* species were found in 10 cases

(7.7%) and not among controls, with high frequency of *S. flexneri* (8/10) in relation to *S. sonnei* (2/10). *Salmonella* sp. was found in 9 cases (6.9% of diarrheal cases and 1 control - 2.4%). ETEC was found in 4 diarrheal cases, all of which having both LT and ST enterotoxins. EPEC was found in 3 diarrheal cases (2 *E. coli* O111 and 1 *E. coli* O125). One case with EIEC and one with *Y. enterocolitica* were identified among diarrheic children. *Campylobacter* was found either in diarrheal cases or in controls. Parasitological examinations showed 8 cases of diarrheal disease cysts of *E. histolytica*. Cysts of *G. lamblia* were found in 19 cases of diarrhea and in 4 controls. Mixed infections were relatively frequent with the following distribution: 2 cases of infections by *S. flexneri* and rotavirus; 3 cases of infections involving *Salmonella* sp. and *G. lamblia*, and 2 cases of concomitant infection by EPEC and *E. histolytica*.

Age distributions - The age group distribution of the different enteropathogens found in association with the 114 cases of diarrheal disease is shown in the Figure. As already shown in Table I, 80.5% of diarrheal episodes occurred in age groups below 2 years. Rotavirus positivity rates are equally high in infants less than 1 year old, as well as in the 1-2 year old age group. Other pathogens are distributed by all age groups, but their related frequencies cannot be defined in view of the low number of diarrheal cases observed in children older than 2 years.

Epidemiological and clinical features - While all bacterial pathogens were equally found in diarrheal cases throughout the study period, the number of rotavirus-positive cases, 70%, were higher in the October-February period (rainy season) than that between May and September (dry season). Clinical signs and symptoms corded by pediatricians at admission are summarized in Table III; information from accompanying adults about vomiting is also included. Fever was found to occur

more frequently in rotavirus infections (60% χ^2 14.85, P= 0.011, 5df) than the presence of blood in stools was a common finding in cases of *S. sonnei* infections (80% χ^2 36.45, P= 0.00001 5df) as well

TABLE I

Age-group distribution of cases of diarrhea and age-matched controls

Age group (months)	Diarrhea cases		Controls	
	N	%	N	%
0 - 6	28	21.4	19	44.3
7 - 12	34	26.3	0	0
13 - 24	43	33	11	25.5
25 - 36	10	7.8	4	9.3
37 - 48	10	7.8	7	16.2
49 - 60	5	3.9	2	4.7
Total	130	100	43	100

TABLE II

Frequency of isolation of enteropathogens in 130 cases of diarrheal disease and 43 age-matched controls

Enteropathogen	Cases		Controls	
	N	% total	N	% total
Rotavirus	25	19.2	1	2.4
<i>Shigella flexneri</i>	8	6.1	0	0
<i>S. sonnei</i>	2	1.6	0	0
<i>Salmonella</i> sp.	9	6.9	1	2.4
ETEC LT/ST	4	3.1	0	0
EPEC	3	2.3	0	0
EIEC	1	0.8	0	0
<i>Yersinia</i>	1	0.8	0	0
<i>Campylobacter</i>	0	0	0	0
Negative	77	59.2	41	95.2
Total	130	100	43	100

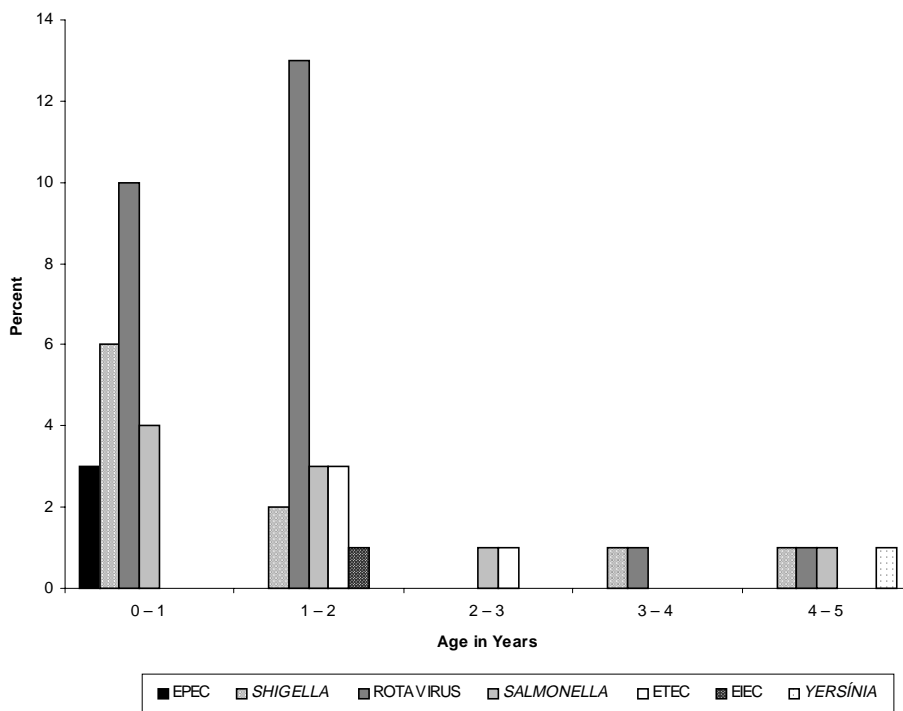
EPEC: enteropathogenic *Escherichia coli* (2 O111 and 1 O125); ETEC: enterotoxigenic *E. coli*; LT: heat-labile enterotoxin; ST: heat-stable enterotoxin; EIEC: enteroinvasive *E. coli*

TABLE III

Signs and symptoms in 130 infants with diarrheal illness

Pathogen identified	Mucus in stools		Blood in stool		Fever		Vomiting	
	N	%	N	%	N	%	N	%
EPEC	3	100	1	33.3	2	66.6	2	66.6
Rotavirus	21	84	4	16	15	60	8	32
<i>Salmonella</i> sp.	5	55.5	1	11.1	3	33.3	5	55.5
<i>Shigella</i> sp.	8	80	8	80	2	20	9	90
ETEC	4	100	1	25	2	50	3	75
EIEC	1	100	1	100	1	100	0	0
<i>Yersinia</i>	1	100	0	0	1	100	1	100
Total	43	33	16	12.3	26	20	28	21.5

EPEC: enteropathogenic *Escherichia coli*; ETEC: enterotoxigenic EIEC: enteroinvasive *E. coli*



Enteropathogens in infant diarrhea. EPEC: enteropathogenic *Escherichia coli*; ETEC: enterotoxigenic *E. coli*; EIEC: enteroinvasive *E. coli*

as the occurrence of vomiting (χ^2 18.84, $P=0.002$). The presence of mucus in stools was observed in all diarrheal cases irrespective to the etiology.

DISCUSSION

In the present study, 130 cases of diarrhea and 43 age-matched controls in the age group of 0 to 5 years old were studied in a pediatric outpatient unit in the peri urban area of Porto Velho. Diarrheal cases were concentrated in the age groups below 2 years of age (80%), suggesting the progressive development of immunity against enteropathogens. However, as the diarrheal cases were detected passively, the parental and social habit of increased attention to younger children may partially explain the bias observed in the age distribution of cases. A longitudinal survey performed in Fortaleza, in which an active search for diarrheal cases was performed, a similar age distribution was observed (Guerrant & Kirchoff 1983) which confirms an important role of immunity to explain the age distribution profile.

Regarding the enteropathogens associated in the diarrheal episodes, it was found in the Porto Velho sample a high prevalence of rotavirus (19.2% of diarrhea cases and 2.4% of controls).

Rotavirus is the most frequent enteropathogen found in the three studied regions (around 20%).

In Porto Velho, as already observed in São Paulo (Trabulsi et al. 1985) and Belém (Linhares 1997), rotavirus is more frequently associated with fever (60% of cases in Porto Velho).

ETEC was the most frequent sub group of *E. coli* associated with diarrhea in the Guerrants' Fortaleza survey, while it was responsible for a lower proportion of patients in Trabulsi's survey in São Paulo (Trabulsi et al. 1985b) and in the present survey in Porto Velho. While LT toxin positive *E. coli* strains were always found in São Paulo and Fortaleza's samples, both LT and ST toxins were found in the four ETEC isolates of Porto Velho.

Salmonella and *Shigella* infections, rare in the São Paulo surveys (Gomes et al. 1991), are more frequent in the Northeast (Guerrant & Kirchoff 1983) and in Porto Velho, where they were associated with 6.9% and 7.7% of diarrheal disease episodes respectively. *Shigella* infections, which are equally frequent in other developing countries like South Africa (Scragg et al. 1978), Mexico (Munhoz et al. 1979) and Bangladesh (Stool et al. 1982) are responsible for the most severe forms of diarrhea and, in the Porto Velho sample, were frequently associated with vomiting (90%) and the presence of blood in stools (80%), indicating a serious damage of the intestinal mucosa.

No case of *Campylobacter* species infection was detected in diarrheal cases in Porto Velho, while this enteropathogen is frequently observed in São Paulo (Gomes et al. 1991). One case of diarrhea associated with *Y. enterocolitica* was observed in the Porto Velho sample. While already described in diarrheal cases in Rio de Janeiro, *Y. enterocolitica*, was rarely found in other areas. It is worthy of note that it is frequently associated with diarrhea in Canada, suggesting a better adaptation of this species to cold environment.

In conclusion, the present survey has shown that the distribution of enteropathogens associated with diarrhea in Porto Velho is similar to that observed in northeast and eastern Amazon areas in the State of Pará. Also, some particularities observed in this study, raise the interest for further research on enteropathogens in western Amazon aiming to better identify groups, sub groups and variants of viral and bacterial pathogens present in the region and information on antibiotic resistance.

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