Original Article

Profiles of enteropathogens in asymptomatic children from indigenous communities of Mérida, Venezuela

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Abstract

Introduction: In Latin America, gastrointestinal infections represent one of the main causes of death among indigenous groups, with a mortality rate three times greater than in the general population. In this study, the carrier state of enteropathogens and the epidemiological risk factor in asymptomatic children from indigenous communities of Mérida, Venezuela, were determined.

Methodology: Fifty-eight healthy children, 5 years of age and under, were clinically and epidemiologically evaluated. Fecal samples were tested for a range of classic enteropathogens. Antimicrobial susceptibility tests (AST) were performed by dilution methods.

Results: Of the specimens studied, there were 34 (58.6%) positive samples, and a single enteropathogen was detected in 22 (64.6%) of these. Associations of two and three enteropathogens were observed in 10 (29.3%) and two (5.8%) cases, respectively. *Blastocystis hominis* (16; 47.0%) and *Salmonella* spp. (15; 43.9%) were the most frequently detected enteropathogens. Carriage of enteropathogens was most frequent in children older than two years. The variety of food in the daily diet was the risk factor strongly associated with the presence of parasites and/or enteric bacteria ($p = 0.024 < 0.05$ and $p = 0.000 < 0.05$, respectively). The majority of these bacteria were susceptible to the antibiotics tested *in vitro*.

Conclusion: This study shows a high prevalence of enteropathogen carriage in asymptomatic children aged five and under from indigenous communities; this result is statistically related to the consumption of food. These findings stress the need of continuous epidemiological surveillance in vulnerable populations, as an important step to prevent the morbidity and mortality due to gastrointestinal infections.

Key words: Enteropathogens; Asymptomatic children; Indigenous communities.

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Introduction

Diarrhoeal diseases constitute a major worldwide public health problem, especially in poor or developing countries [1]. The World Health Organization (WHO) has estimated that annually 4.6 to 5 million children under five years of age die of diarrhoea in Asia, Africa, and Latin America [2]. In Venezuela, during 2006, intestinal infectious diseases occupied the fifteenth place among the causes of death for the general population, and the third place in children under 5 in the state of Mérida [3]. Several reports have confirmed that viruses, parasites and bacteria can be involved in the etiology of infectious diarrhoeal disease in children [4]. Nevertheless, the epidemiological characteristics, etiologic agents, and clinical presentation of diarrhoea vary depending on the country, region or community, as well as on the racial or ethnic group [5-7].

The indigenous communities are among the population groups most vulnerable to suffering from infectious diseases [8,9]. Several studies conducted in Latin America revealed that diarrhoea is one of the main causes of death among the indigenous groups, with a mortality rate three times greater than in the general population, due to poor supply or bad quality of water, and insalubrious and malnutrition-specific risk factors [8-11].

Venezuela has an indigenous population of approximately 314,000 inhabitants (1.55% of the total population). The majority live in conditions of marginality and poverty in regions bordering Colombia, Brazil, and Guyana. In the state of Mérida, specifically in the district of Sucre, are localized the indigenous communities of Guazábaras, Quinaroes and Quinanoques. According to the epidemiological registry from this district, incidence of diarrhoea in

children younger than five years of age have increased in recent years, and currently are among the five main causes of morbidity and mortality [3].

The microbiological analysis of feces has an epidemiological importance, especially in asymptomatic individuals. The carriage of enteropathogens, characterized by intermittent or continuous shedding of microorganisms in the stool without clinical signs of infection, constitutes the reservoir that keeps these microorganisms circulating in the environment, favoring the risk of the appearance of cases, outbreaks or epidemics, especially in impoverished populations [11-14]. To determine the carrier state of enteropathogens and epidemiological risk factors in a susceptible population, fecal samples of asymptomatic children five years of age and under, belonging to three indigenous communities, Guazábaras, Quinaroes and Quinanoques, from the district of Sucre of the state of Mérida, Venezuela, were collected and microbiologically evaluated.

Methodology

Study area and population

The indigenous communities of Guazábaras, Quinaroes, and Quinanoques are located on the west part of the Sucre District, Mérida State, Venezuela, at the geographical coordinates 8º 18' N and 71º 29' W. This population is dispersed in various hamlets mainly located in rural areas called La Alegría Alta, Pueblo Viejo, Los Azules and San Martín. The district has a warm, rainy season between March and October and a dry season the rest of the year.

The studied indigenous communities have an estimated population of 720 inhabitants and approximately 24% are children. The main economic activities of this population are agriculture, handicrafts and tourism. The water supply comes from a rural aqueduct which supplies untreated water on an intermittent basis.

Subjects and sample collection

The study was conducted between April and June 2008. Fecal samples were collected from 58 children five years of age and under, not showing any gastrointestinal symptoms. Inclusion criteria mandated the following: no antimicrobial therapy in the 45 days prior to enrollment; no severe malnutrition status; and absence of congenital or immunological diseases. The average age of the studied children was three years; 55.17% were male. All children had normal nutritional conditions. Clinical and epidemiological data were recorded on a spreadsheet designed for this purpose. Informed consent was obtained from parents or close relatives. The distribution of children enrolled by indigenous community was as follows: Guazábaras 29 (50%); Quinanoques 21 (36.3%); and Quinaroes 8 (13.7%). The study was reviewed and approved by the ethical committees of the Council of Scientific, Humanistic and Technological Studies (CDCHT) of Los Andes University (Mérida, Venezuela).

Laboratory analysis

Fifty-eight fecal samples were collected after natural evacuation. Stools were divided into two aliquots: one was used for parasitological examination for helminth eggs and protozoan cysts by direct stool observation and concentration of fecal techniques (Kato-Katz and formalin-ether). The presence of *Cryptosporidium* and *Cyclospora* was established using modified Kinyoun carbolfuchsin staining [15].This same aliquot was tested for the presence of rotavirus using an agglutination kit (Slider Rota Kit 2, BioMerieux, Marcy-L´Etoile, France) according to the manufacturer's instructions. The other fraction of stool was cultured in various solid selective media (MacConkey, Xylose lysine deoxycholate (XLD) agar for *Salmonella* and *Shigella* isolation, *Salmonella Shigella* (SS) agar, cefsulodin-irgasan-novobiocin (CIN) agar for *Yersinia* and *Aeromonas* isolation, Campylobacter medium for Campylobacter, and Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar for *Vibrio* isolation, all Oxoid, London, UK), selenite broth for *Salmonella* enrichment and alkaline-peptone water for *Vibrio* enrichment. Plates were incubated for 18 to 24 hours at 36ºC, except for CIN and *Campylobacter* medium, which were incubated for 48 hours at 25ºC and 42ºC, respectively, the latter under microaerophilic conditions (gas pack, Oxoid, London, UK). After 6 to 8 hours' incubation, the selenite broth was sub-cultured onto MacConkey, XLD and SS agar, whereas the alkaline-peptone water (pH 8.6) was subcultured onto TCBS. Initially, the enteropathogenic bacteria identification was performed by colonial morphological characteristics and standard biochemical tests, followed by analysis using bacterial identification test strips API20E (BioMerieux, Marcy-L´Etoile, France). *Campylobacter* spp. were identified based on morphologic characteristics and oxidase test, then confirmed by Gram staining [15]. *Salmonella*, *Shigella* and *Vibrio* strains were agglutinated using

Table 1. Patterns of enteropathogenic distributions according to age group in asymptomatic children from three indigenous communities.

Patterns of enteropathogens*	Total n (%)	Indigenous communities			Age group (years)	
		Guazábaras n (%)**	Quinanoques n (%)**	Quinaroes n (%)**	0-2 n (%)**	3-5 n (%)**
Absence of enteropathogens	24 (41.4)	9 (37.5)	13 (54.2)	2 (8.3)	10 (41.7)	14 (58.3)
Presence of enteropathogens	34 (58.6)	20 (58.8)	8 (23.5)	6 (17.6)	8 (23.5)	26 (76.5)
Total	58 (100)	29 (50.0)	21 (36.2)	8 (13.8)	18 (31.0)	40 (69.0)
Single patterns						
Salmonella spp.	8 (23.5)	3 (37.5)	3 (37.5)	2 (25.0)	4 (50.0)	4 (50.0)
Shigella boydii	1 (2.9)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)
Aeromonas spp.	3 (8.8)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	3 (100)
Blastocystis hominis	7 (20.6)	5 (71.4)	1 (14.3)	1 (14.3)	2 (28.6)	5 (71.4)
Giardia lamblia	3 (8.8)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.7)
Two enteropathogens						
Salmonella spp.+*Aeromonas* spp.	1 (2.9)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
Salmonella spp.+*B. hominis*	3 (8.8)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	3 (100)
Salmonella spp.+*G. lamblia*	1 (2.9)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
Aeromonas spp.+*G. lamblia*	1 (2.9)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
B. hominis+*G. lamblia*	4 (11.8)	3 (75.0)	0 (0.0)	1 (25.0)	0 (0.0)	4 (100)
Three enteropathogens						
Salmonella spp.+*B. hominis*+ *I. butschlii*	1 (2.9)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
Salmonella spp.+*B. hominis*+ *G. lamblia*	1 (2.9)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)

* diarrhoeagenic *E. coli* and Rotavirus not detected

** percentages relate to n values reported in the first column

specific antisera (Fuvesin and Becton Dickinson, MD, USA, respectively). Identification of different diarrhoeagenic *Escherichia coli* (EPEC, ETEC, EAEC and EIEC) was performed by polymerase chain reaction (PCR) described elsewhere [16].

Antimicrobial susceptibility

Minimum inhibitory concentrations (MICs) were determined on Mueller Hinton agar (Difco, Detroit, MI, USA) by dilution methods according Clinical and Laboratory Standards Institute parameters [17]. The antimicrobial agents used were ampicillin, cephalothin, cefotaxime, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, tetracycline, chloramphenicol, and trimethoprim/ sulfamethoxazole. *E. coli* ATCC 25922 was used as the control strain for susceptibility testing.

Statistical analysis

Data analysis was performed using SPSS (version 15, IBM, IL, USA) statistical software. Proportions were compared by the X^2 Chi-square test and odds ratios (OR) and 95% confidence intervals (95% CIs) were calculated. All epidemiological data adjusted (OR), comparing enteropathogenic bacteria and parasites, were calculated by logistic regression models. A *P* value below 0.05 was considered statistically significant ($p < 0.05$).

Results

A total of 58 stool samples obtained from asymptomatic children from three indigenous communities of Mérida, Venezuela, were analyzed between April and June 2008. Patterns of enteropathogen distribution and age group in asymptomatic children from three indigenous communities are shown in Table 1. Of the 58 specimens studied, 34 samples were found to be positive for at least one enteropathogen (58.6%); of the positive samples, a single enteropathogen was detected in 22 (64.6%) samples, while associations of two and three enteropathogens were observed in 10 (29.3%) and 2 (5.8%) cases, respectively. *Blastocystis hominis* (16 of 58; 27.6%) and *Salmonella* spp. (15 of 58; 25.8%) were the most frequently found enteropathogens. Rotavirus, diarrhoeagenic *E. coli, Campylobacter* spp, *Cryptosporidium* and *Cyclospora* were not detected. The predominant patterns of association were bacteria + parasite (7 of 34 positive cases;

Table 2. Relation of socio-epidemiologic characteristics with enteropathogenic bacteria detection in asymptomatic children from three indigenous communities.

Socio-epidemiologic characteristics	Enteropathogenic bacteria Detection		*P* value	Odds ratio (95% CI)
	Positive n (%)*	Negative n (%)*		
Social class				
Working-class	11 (19.0)	19 (32.8)	0.717	1.22 (0.41-3.62)
Middle class	9 (15.5)	19(32.8)		
Formation of the family group				
Maximum 6 persons	9 (15.5)	23 (39.7)	0.258	0.53 (0.18-1.60)
At least 7 persons	11 (19.0)	15 (25.9)		
Water supply				
With treatment	14 (24.1)	24 (41.4)	0.602	1.36 (0.43-4.35)
Without treatment	6 (10.3)	14 (24.1)		
Variety of food in day diet				
Still breast-feeding	3 (5.2)	17 (29.3)	**0.024**	4.59 (1.15-18.31)
Two or more different type	17 (29.3)	21 (36.2)		
Contact with domestic animals				
Yes	18 (31.0)	31 (53.4)	0.400	2.03 (0.38-10.85)
No	2 (3.4)	7 (12.1)		
Annual episodes of diarrhoea				
None	7 (12.1)	7 (12.1)	0.161	2.39 (0.70-8.17)
At least one	13 (22.4)	31 (53.4)		
Communities (group A)				
Guazábaras	11 (22.0)	18 (36.0)	0.490	0.66 (0.20-2.19)
Quinanoque	6 (12.0)	15 (30.0)		
Communities (group B)				
Guazábaras	11 (29.7)	18 (48.6)	0.657	1.02 (0.20-5.13)
Quinaroes	3 (8.1)	5 (13.5)		
Communities (group C)				
Quinanoque	6 (20.7)	15 (51.7)	0.642	0.68 (0.12-3.71)
Quinaroes	3 (10.3)	5 (17.2)		

***** percentages relate to the total number of samples (58)

20.4%) followed by parasite + parasite (4 of 34; 11.8%). Only *Shigella boydii* was isolated without combination with other pathogens. Carriage of multiple enteropathogens was more frequent in children older than two years. In contrast, single pathogens were isolated in the two-year and under group. A more diverse pattern of enteropathogens was observed in children from Guazábaras community (20 of 34; 58.8%).

The relationship between socio-epidemiologic characteristics with enteropathogenic bacteria or parasites detection in asymptomatic children is shown in Tables 2 and 3. Surprisingly, there were no significant relations between social class, formation of the family group, water supply, contact with domestic animals, and annual episodes of diarrhoea variables and positive detection of enterobacteria or parasites. However, the variety of food in the daily diet was statistically related to the presence of bacteria and parasites (p = 0.024 and p = 0.000, respectively), with the presence of enterobacteria and parasites being more frequent in children with diets of two or more different food types, for which the risk of isolating enteric bacteria shows a fourfold increase (OR, 4.59; 95% CI, 1.15 to 18.31). In the cases with parasites, the risk increased by more than 20 times (OR, 21.1; 95% CI 2.56 to 174.01) when compared with breast-feeding children. There was no significant relationship between indigenous communities and the presence of bacteria in the samples studied. Nevertheless, children from Guazábaras and Quinanoque communities (group A) showed a higher risk of parasitosis than children of

Table 3. Relation of socio-epidemiologic characteristics with detection of parasites in asymptomatic children from three indigenous communities.

Socio-epidemiologic characteristics	Parasite detection		*p*-value	Odds ratio (95% CI)
	Positive n (%)*	Negative n (%)*		
Social class				
Working-class	10 (17.2)	20 (30.0)	0.637	0.77 (0.26-2.26)
Middle class	11 (19.0)	17 (28.0)		
Formation of the family group				
Maximum 6 persons	13 (22.4)	19 (32.8)	0.437	1.54 (0.52-4.59)
At least 7 persons	8 (13.8)	18 (31.0)		
Water supply				
With treatment	13 (22.4)	25 (43.1)	0.663	0.78 (0.26-2.39)
Without treatment	8 (13.8)	12 (20.7)		
Variety of food in day diet				
Still breast-feeding	1 (1.7)	19 (32.8)	**0.000**	**21.1 (2.56-174.01)**
Two or more different type	20 (34.5)	18 (31.0)		
Contact with domestic animals				
Yes	17 (29.3)	32 (55.2)	0.576	0.66 (0.16-2.80)
No	4 (6.9)	5 (8.6)		
Annual episodes of diarrhoea				
None	7 (12.1)	7 (12.1)	0.218	2.14 (0.63-7.30)
At least one	14 (24.1)	30 (51.7)		
Communities (group A)				
Guazábaras	14 (28.0)	15 (30.0)	**0.012**	**5.60 (1.35-23.23)**
Quinanoque	3 (6.0)	18 (36.0)		
Communities (group B)				
Guazábaras	14 (37.8)	15 (40.5)	0.621	0.93 (0.20-4.47)
Quinaroes	4 (10.8)	4 (10.8)		
Communities (group C)				
Quinanoque	3 (10.3)	18 (62.1)	0.068	0.17 (0.03-1.06)
Quinaroes	4 (13.8)	4 (13.8)		

***** percentages relate to the total of samples (58)

Table 4. Susceptibilities of bacterial enteropathogens isolated in asymptomatic children from three indigenous communities.

Antibiotic	MIC (µg/mL)	Bacterial enteropathogens					
		Salmonella spp. (*n*= 15)		*Aeromonas* spp. (*n*=5)		*S. boydii* (*n*= 1)	
		%		%		%	
	Range	S	R	S	R	S	R
Ampicillin	2- >128	13.3	86.7	NT	NT	100	0
Cephalothin	2-128	100	0	NT	NT	100	0
Cefotaxime	2-256	100	0	100	0	100	0
Amikacin	4-256	100	0	100	0	100	0
Gentamicin	1-64	93.3	6.7	100	0	100	0
Netilmicin	2-128	93.3	6.7	100	0	100	0
Tobramycin	1-64	93.3	6.7	100	0	100	0
Tetracycline	1-64	80.0	20	80	20	100	0
Chloramphenicol	2-128	100	0	100	0	100	0
Trimethoprim	2-64	93.3	6.7	100	0	100	0
Sulfonamides	64-2048	93.3	6.7	100	0	100	0
Ciprofloxacin	0.25-16	100	0	100	0	100	0

NT: not tested; S: sensitive; R: resistant

other indigenous communities ($p = 0.012 < 0.05$; OR, 5.60; 95% CI, 1.35 to 23.23).

The susceptibility of bacterial enteropathogens to different antibiotics is shown in Table 4. The only *S. boydii* strain isolated, and all the isolated *Aeromonas* strains were sensitive to all the antibiotics tested, with the exception of one *Aeromonas* strain that showed resistance to tetracycline (MIC = 16 μg/ml). Fourteen of the 15 (86.7%) *Salmonella* isolates were resistant to ampicillin (MIC >128 μg/ml), three (20%) isolates were resistant to tetracycline and one (6.7%) strain was resistant to gentamicin, netilmicin, tobramycin and trimetoprim-sulfamethoxazole. Eleven out of 14 (78.6%) resistant *Salmonella* isolates presented a single pattern of resistance (ampicillin) and the three remaining (21.4%) isolates were resistant to two, three or more antibiotics. All isolates were sensitive to cephalothin, cefotaxime, amikacin, chloramphenicol and ciprofloxacin.

Discussion

It has been recognized that in developing countries, enteric pathogens can frequently be encountered in healthy children, making it more difficult to determine their true etiological role in the diarrhoeal disease [7,13,18,19]. This is the first study conducted among asymptomatic indigenous children from Mérida, Venezuela, to assess the profile of enteropathogens and their relationship with socio-economic factors. The results of this study showed the presence of potential enteropathogens in more than half of the children studied. The presence of multiple enteropathogens was more frequent in children older than two years. In contrast, single pathogens were isolated in the group aged two years and younger. Previous studies have shown that children living in impoverished areas are colonized soon after weaning and are probably re-infected during the rest of their childhood [1,5].

Asymptomatic infection with *B. hominis* appears to be common [20]. In this study, the high rate of asymptomatic carriage of *B. hominis* suggests that it may not play a consistent pathogenic role in these indigenous children. In some cases, *B. hominis* was associated with *G. lamblia* and/or *Salmonella* spp., which could indicate that a major route of transmission is fecal-oral. Among the enteropathogenic bacteria isolated, *Salmonella* spp. were observed in 25.8% of the total of cases. This indicates a very high rate of asymptomatic carriage of *Salmonella,* especially in children three to five years of age, from the studied indigenous communities. This finding is consistent with those of other authors who have found a significant increase in the isolation rate of *Salmonella* strains from symptomatic and asymptomatic aboriginal children in Western Australia [18], among asymptomatic children in day-care centers in Yucatán, México [21], and in children in low- and middle-income countries [22]. It is possible that repeated infections resulted in gradual acquisition of immunity, which over time may reduce the incidence of clinical disease.

In this study, *Aeromonas* spp. and *S. boydii* were isolated in low proportion. Although the prevalence of asymptomatic *Aeromonas* and *Shigella* infections that occur in children has not been established, some studies point out the clinical and epidemiological importance of these bacteria isolated from children without diarrhoea from marginal areas in developing countries [23,24]. On the other hand, many asymptomatic gastrointestinal infections are found when children are living under conditions with a high fecal-oral transmission rate, heavy environmental contamination, high prevalence of diarrhoea, and hyperendemicity of known pathogens [19].

It is noteworthy that multivariate analysis showed no interaction between the socio-epidemiological characteristics studied. However, the variety and preparation of food in the daily diet was the risk factor strongly associated with the presence of parasites or enteric bacteria, especially in the indigenous children three to five years of age. Hence, this association is probably due to both ingestion of a contaminated weaning diet and probably also a decrease in protection from the breast-feeding. It is possible that the unhygienic habits of family members who prepare food, enable the fecal-oral cycle to operate efficiently within the family group. This can also be interpreted as an indication that the housewife is not stringent enough about cleanliness and care in the preparation of food. Further studies to examine food preparation practices, custom hygienics, and their relation to enteropathogen infection in the home may be useful to reduce the incidence of colonization.

A dramatic increase in prevalence of fecal carriage of resistant *Enterobacteriaceae* in healthy populations from developing countries has been reported [25, 26]. In spite of the relatively small size of the sample, the results of this study provide useful information about antimicrobial resistance. All *Salmonella* strains were resistant to at least one of the antibiotics tested, but these strains were totally susceptible to cephalothin, cefotaxime, amikacin,

chloramphenicol and ciprofloxacin. *S. boydii* and most *Aeromonas* spp. strains showed high susceptibility to a wide range of antimicrobial agents. The much needed data on antibiotic susceptibility in indigenous communities is still not available and we hope this study will contribute toward such information.

In conclusion, results of this study show a high prevalence of asymptomatic carriage of enteropathogens in children under five years of age from indigenous communities. This finding was statistically associated with the consumption of food. Moreover, most enteric bacteria were susceptible to first-line antimicrobial agents used to treat severe gastrointestinal infection in children. Nevertheless, active monitoring for the emergence of antimicrobial resistance is necessary because of the public health implications of a potential spread of resistance clones.

Although the pattern of prevalence of enteropathogens in asymptomatic children from indigenous communities remains unknown for many tropical countries, these findings open the door to further studies. Understanding the environmental epidemiology, as well as the risk factors in the microbial ecosystem and the immunity that predisposes healthy children to colonization with enteropathogens is an important step in planning effective actions to prevent the morbidity and mortality due to gastrointestinal infection.

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