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PROSPECTIVE STUDY OF ENTEROPATHOGENS IN TWO COMMUNITIES OF MISIONES, ARGENTINA

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SUMMARY

Children under five years of age, from two communities of different socio-economic strata (97 from Zaiman and 55 from Las Dolores) were examined epidemiologically during 2 years, by means of quarterly visits of the working team, who carried out the collection of faecal samples.

During the study, one or more enteropathogens were identified in 73.9% of samples in children from Zaiman and in 58.3% of the samples from Las Dolores, being associated to diarrhoea in 70.5% and to asymptomatic infections in 65.7%. The number of diarrheic episodes was higher in Zaiman (15.45%) than in Las Dolores (12.35%), being more frequent in the spring-summer seasons.

In Zaiman, the bacterial enteropathogen proportion was relevantly higher ($p< 0.005$) in children with diarrhoea, whereas the presence of parasites was more frequent in asymptomatic children ($p< 0.01$). Rotavirus had an even distribution within diarrheic and asymptomatic children. In Las Dolores, no relevant differences were found in the detection of enteroparasites between diarrheic and asymptomatic children. Mixed infections were detected; enterotoxigenic *Escherichia coli* (ETEC)-rotavirus and ETEC-parasites being the most frequent ones. ETEC was involved in 85% of these infections.

These data, together with the high enteropathogen carriage, suggest an elevated level of environmental contamination. The latter plays an important role in diarrheic diseases, and added to the most extreme poverty, it affects children's lives.

KEYWORDS: Acute diarrhoea; Epidemiology; Infection; Risk factor; Morbidity.

INTRODUCTION

Diarrhoea is one of the three main causes of disease and death in children under 5 years of age in Latin America. The WHO has estimated that in 1989, 223 million diarrheic episodes broke out in the 57 million children under five years of age in this continent. In Argentina, 9.7 million episodes were reported during that period for a population of 3.2 million children under five years of age[45]. Misiones is not an exception regarding this subject, presenting alarming figures: in 1990 the morbidity rate was 33.6% in children under two years of age[5].

Prospective studies carried out in different communities showed that enterotoxigenic *Escherichia coli* (ETEC) and rotavirus were the pathogens most commonly associated to diarrhoea in Latin America[7,21,29].

Carriage of enteropathogens, characterized by the intermitent or continuous faecal excretion of microor-

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ganisms without clinical manifestation of infection, constitutes the reservoir that keeps these microorganisms in the environment, favouring contamination in marginal areas and causing disease or epidemic outbreaks.

In order to evaluate the state of carriage and diarrheic disease and their association with poverty and seasonal changes, we have carried out a prospective study of epidemiological surveillance in children under five years of age, without previous antibiotic treatment in two socio-economically different communities of Posadas, Misiones.

MATERIAL AND METHODS

Study area

Geographical Features: Misiones is a province with a sub-tropical warm weather, an annual average temperature that ranges from 21°C to 25°C and an annual average rainfall of 157.9mm. Misiones is surrounded by numerous streams, tributaries to the Parana River.

Geographical Location: The area under study is located in the outskirts of the Capital District and National Road 12, the most important access way to Posadas.

The delimitation of the geographical boundaries comes out when selecting the target population for this study due to their exposure to a possible risk factor: Zaiman creek, tributary to the Parana River, the main current that passes through Posadas. Two districts were selected in the area: Zaiman and Las Dolores. The latter was taken as the sentry district because its inhabitants do not use water from the Zaiman creek, which is, on the contrary, used by people from Zaiman for consumption, hygiene and recreation.

Study Population: Ninety-seven children from Zaiman and 55 from Las Dolores, all under five years of age, and without a former antimicrobial treatment were selected after verbal consent of one of their parents.

During both years of study, all the children were visited weekly by sanitary agents who recorded the health state and registered the presence of diarrhoea or other pathologies. Faecal samples were collected quarterly. During the study there were no casualties and hospitalization was not required.

Socio-economic Featuring: It was carried out taking into account 38 families from Zaiman and 24 from Las Dolores. Except for a family of 3 members, the remaining ones had an average of five members. The most numerous family had fourteen members. Featuring was determined from the families of the children who completed all the quarterly samplings along the two years of study.

According to the information obtained regarding the houses, a simple index was elaborated in order to characterize them, taking into account the building materials used in walls, roof and floor.

In order to classify the country's population according to socio-economical features, the National Institute of Statistics and Census (INDEC) has elabored the Unsatisfied Basic Needs Index (UBNi) which discriminates grossly the poor from the not poor. UBNi comprehends a series of variables (over crowded homes, sanitary service, education, etc.) which denote the minimum goods and services necessary for the development of an appropriate life within the Argentine society. The poors are defined as "those individuals that lack some or all of the above mentioned services". Poverty is relative, depending on the living standard in each society.

The maximum index was given to the most comfortable houses (concrete or tiled floor, zinc roof and brick walls) and the minimum, to the most rustic ones, built with disposable materials (hay or cardboard roof, wooden panels, untiled floor and partition walls), (rural houses). An intermediate group, classified as a "typical" house from the outskirts of Posadas, featured untiled floor, wooden walls and cardboard and tar roof.

Specimen Sampling: During the two years of study, 667 faecal samples of 152 selected children were studied, 415 from Zaiman and 252 from Las Dolores.

Faecal samples were taken at the beginning of the study and quarterly, to investigate bacterial, parasitic and viral enteropathogens. A portion of each faecal specimen was stored at -20°C prior rotavirus testing.

In every faecal sample the search for leukocytes was carried out by microscopic observation in fresh with 400X enlargement.

The faeces were analyzed according to the methodology suggested by the Enteric Infections Manual of the World Health Organization (WHO)[28]. Colonies suspicious of *Shigella* or *Salmonella* were identified biochemically according to EWING[14] and serologically using polivalent and monovalent sera provided by the National Institute of Microbiology "Dr. Carlos Malbran" of Buenos Aires (INM). Colonies suspicious of *Aeromonas* were detected according to LENNETTE[44] and the phenotypical markers of pathogenesis analysed according to JANDA[25]. *Campylobacter* was searched for by spreading the faeces in the semisolid enrichment medium proposed by CHAN & MACKENZIE[10], incubating at 42°C for 24

TABLE 1
Socio-economic analysis in Zaiman and Las Dolores Districts

	Zaiman	Las Dolores
Number of families	38	24
Members of the families (average and range)	5(3-14)	5(3-10)
Classification of the houses		
rural	47.3%	29.2%
intermediate	36.8%	29.2%
comfortable	15.8%	41.7%
Sanitary facilities		
Bathroom		
installed	2.6%	37.5%
privies	86.8%	62.5%
No data	10.5%	-
Electricity service		
with electricity service	52.6%	79.2%
without electricity service	42.1%	20.8%
No data	5.2%	-
Water supply		
wells/wells and public taps	84.2%	83.0%
only public taps	15.8%	-
Use of water from the creek		
not used	34.2%	96.0%
used for washing hygiene or recreation	55.3%	4.0%
only for recreation or washing	10.5%	-
Refuse treatment		
exposed	44.7%	8.3%
burying	13.2%	8.3%
burning	39.5%	62.5%
collecting system	-	12.5%
No data	2.6%	8.3%
Head parents educational level		
illiterate	52.6%	-
incomplete elementary level	28.9%	16.6%
complete elementary level	2.6%	12.5%
incomplete secondary level	-	12.5%
complete secondary level	-	41.6%
No data	15.8%	16.6%

hours and subsequently spreading in Skirrow medium, which was incubated at 42ºC during 24-48 hours, with gasses mixture. Biochemical identification was performed according to BUTZLER[8].

The search for enteropathogenic *Escherichia coli* (EPEC) and enterotoxigenic *Escherichia coli* (ETEC) was carried out with 5 lactose-fermenting colonies. EPEC were characterized biochemically and serologically with polyvalent and monovalent antisera (provided by INM) for detection of enteropathogenic serogroups (018, 020, 026, 044, 055, 086, 0111, 0114, 0119, 0125, 0126, 0127, 0128 and 0142). *E. coli* isolates were subcultured onto trypticase soya agar, emulsified in normal saline, and then mixed with a drop of polyvalent antiserum on a serologic slide. After 1 min, the suspension was observed for agglutination. If agglutination ocurred, the suspension was then tested against the appropriate monovalent antisera.

ETEC were characterized biochemically and sent to INM to search for heat-labile (LT) and heat-stable (ST) enterotoxins by enzyme-immunoassay (GM1-ELISA) with monoclonal antibodies[38,39]. For the detection of enteroinvasive *E. coli* (EIEC), the non-fermenting or slow lactose fermenting colonies, biochemically confirmed as *E. coli* were sent for the keratoconjunctivitis test in the conjunctival bag of the Guinea pig[37] at INM.

Enteroparasites were searched for by testing the faeces in fresh and by culturing in sterile earth and activated coal for the differentiation of *Strongyloides stercoralis* and Uncinarias[2]. The search for *Cryptosporidium* was carried out by Ziehl-Nielsen modified in cold as described by GARCIA et al.[18].

For rotavirus a "sandwich" ELISA was used according to the methodology described by YOLKEN & LEISTER[47]. The results were confirmed by an ELISA Kit of commercial origin (Rotazyme II, Abbot).

Statistic Analysis: It was carried out according to the test method used to analyse the hypothesis of existence of statistical significant differences between proportions with an indicative level of 5%[12].

RESULTS

Socio-economic evaluation

Table 1 shows the results obtained from the socio-economic analysis carried out in both neighbourhoods.

Zaiman District

Based on the index that we elaborated, 47.3% of the houses of this district were classified as rural houses; 15.3% as comfortable and the rest as intermediate.

Regarding sanitary facilities, an installed bathroom was practically non-existent (only one house); in general all the houses had privies (open air) and were classified as rural. This variable was of little discrimination within this sample.

Another variable used to evaluate living conditions was the accessibility electricity service. It was observed that just 52.6% of the 37 houses from Zaiman (there are no data for one of them), had this service, only 5 of these houses were of the rural kind.

Regarding the origin, approximately 75% of the families obtained water from wells (small superficial excavations) and 50% of them combined the use of this type of water with water from public taps or watersheds. Only six houses used exclusively water from public taps. Considering the use of water from the stream, only 34.2% of the families declared not to make any use of it; more than 50% used it for washing, hygiene and recreation. The rest of the families used it only for recreation or washing.

The treatment of refuses was variable. More than 40% left refuses exposed, coinciding with those families of most rural houses, whereas 13.2% buried them and the rest declared to burn them.

Regarding the educational level, more than 50% were illiterate; only 28.9% had reached an incomplete elementary level. No information is available about the rest. Correlating the behaviour of this latter variable, it was evident that the potential income of these workers was very low, due to the low-qualified tasks, the instability and the scarce skill for working.

Las Dolores District

When applying the same index for house quality as in the former district, it was observed that 41.7% were comfortable houses and the rest showed a similar distribution between rural and intermediate.

Considering the elimination of excrets, 62.5% had prives and the remaining 37.5% had water closets with water discharge. These latter were associated to comfortable houses.

If the availability of electricity service was taken into account, it was observed that out of the 24 houses, 79.2% had this service; the percentage that lacked electricity was mostly from the rural kind: 4 out of 5 houses.

The water that they used came mainly from wells; only two families complemented the use of the well with public taps and/or water sheds. The remaining four families obtained water from excavations; their houses were comfortable.

When considering the use of stream water, it was observed that only one family (that of an "olero") used it for washing, hygiene and recreation. The rest of the families declared not to make use of water from the stream.

Refuses received diverse treatment as in Zaiman district. Burning was employed in 62.5% of the families; 12.5% had a collecting system and the rest either burned them or alternatively left them exposed.

Analyzing some features of the family heads, it was observed that as regards to the educational level, more than 41.6% had completed the secondary school. The elementary level had been completed by 12.5%, 16.6% had not completed it, and there is no information about the rest. The jobs of the family heads varied, ranging from maids and masons, to clerks and shop assistants; it was an outstanding feature that 40% of these workers had social security. Due to the educational level, the stability of some jobs and the requested qualifications for their perfomance, we could estimate that the income of these workers was higher than that of the family heads of Zaiman. The features of the houses and the kind of available service supported this hypothesis.

If the UBN index is taken as a reference, it is observed that the population of both communities, Zaiman

TABLE 2
Distribution of enteropathogens identified in two communities in Argentina

Microorganisms Identified	ZAIMAN (n=415)		DOLORES (n=252)		Test**
	N°	(%)	N°	(%)	
ETEC	65	(15.6)	31	(12.3)	Ns[a]
EPEC	25	(6.0)	10	(3.9)	NS
Shigella	10	(2.4)	2	(0.8)	NS
Aeromonas	4	(1.0)	1	(0.4)	NS
Campylobacter	2	(0.5)	2	(0.8)	NS
EIEC	1	(0.3)	2	(0.8)	NS
Salmonella	0	-	2	(0.8)	ND[b]
Total Bacteria	107	(25.7)	50	(19.8)	NS
Parasites*	190	(45.7)	79	(31.3)	0.001
Rotavirus	10	(2.4)	15	(6.0)	0.05
Total Enteropathogens	307	(73.9)	144	(57.1)	
Mixed Infections					
with two agents	62	(14.9)	22	(8.7)	ND
with three or more agents	13	(3.1)	10	(3.9)	ND

ETEC: Enterotoxigenic *Escherichia coli*; EPEC: Enteropathogenic *E. coli*; EIEC: Enteroinvasive *E. coli*.
* Parasites: *Cryptosporidium, Giardia lamblia, Hymenolepis nana, Uncinarias, Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura.*
** Hypothesis of existence of relevant differences between proportions.
a Not significant
b Not determined

and Las Dolores, should be listed as poor; however, it is seen that in Zaiman life conditions are more critical than in Las Dolores.

Etiologic agents

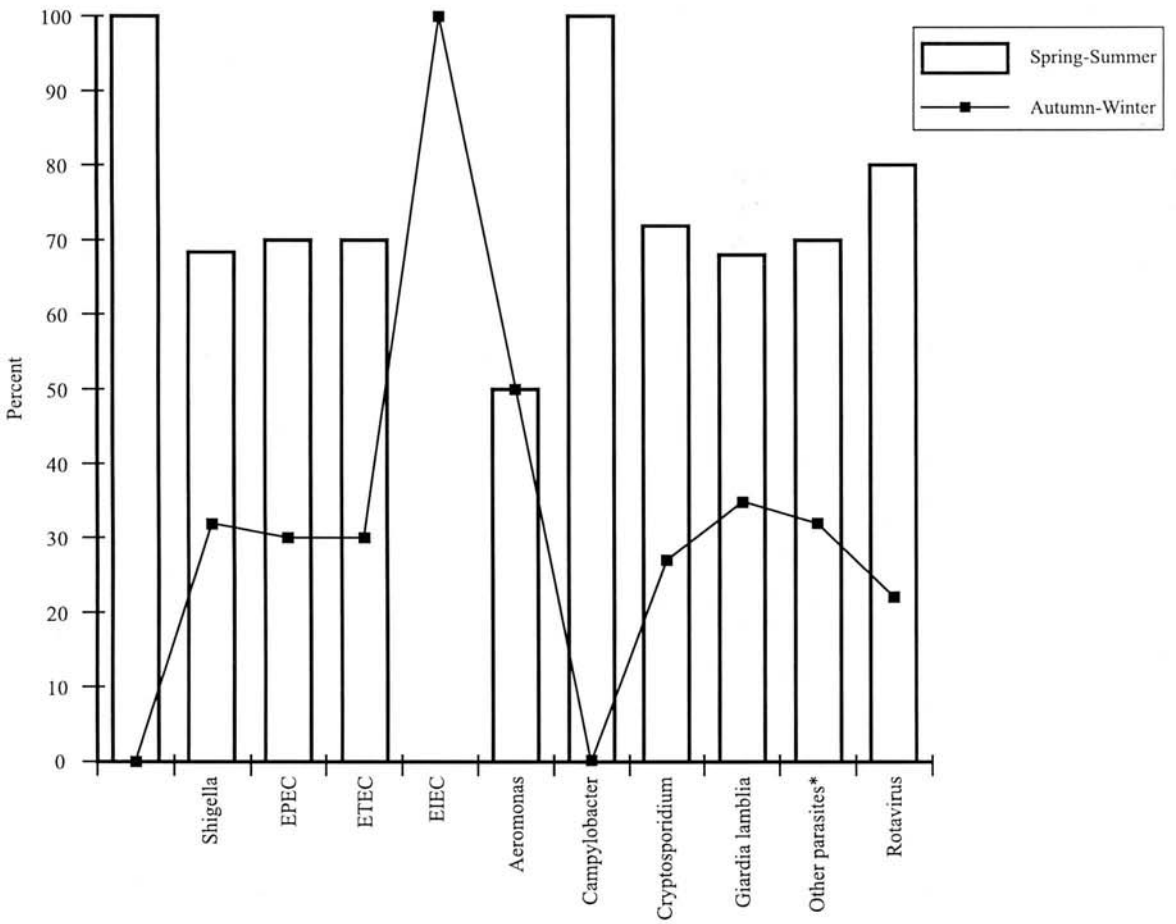
Table 2 shows the different enteropathogens (EP) identified in both communities. In Zaiman the detection of enteroparasites was more frequent than in Las Dolores ($p< 0.001$), this difference was highly significant. On the other hand, rotavirus had a more relevant occurrence in Las Dolores ($p< 0.05$). No relevant differences were found for bacterial EP ($p> 0.05$). The prevailing EPEC serogroups detected were 026, 055 and 0125, the percentage was slightly higher in children with diarrhoea. Serogroups not usually associated with diarrhoea were detected in asymptomatic children (018, 044, 086, 0126, 0127, 0142). The most frequent *Shigella* serotypes were *flexneri* 3 and *sonnei*. Regarding *Aeromonas* strains in this study, 4 were *A. hydrophila* and 1 *A. sobria*. All of them produced beta-hemolysis on human blood agar and presented at least two phenotypical markers of pathogenesis.

Mixed infections were frequent (multiple pathogens), with two agents in 14.9% and 8.7%, and with three or more agents in 3.1% and 3.9% in Zaiman and Las Dolores respectively, the most frequent ones were those caused by EPEC or ETEC or rotavirus associated to other pathogens. Out of the 96 infections where ETEC was isolated, 40 were associated to other pathogens: 15 to *Giardia lamblia*, 5 to *Cryptosporidium*, 4 to *Aeromonas*, 3 to rotavirus, 3 to EPEC and the remaining ones to other enteroparasites, ETEC-LT being involved in 85% of these mixed infections.

In Table 3 the detection of EP is analyzed in children with diarrhoea and asymptomatic ones from both communities. During the follow-up of the sentry chidren, 95 diarrheic episodes were registered. (In Table 3: Total amount of children with diarrhoea).

When comparing the number of diarrheic episodes in both communities, it was observed that Zaiman had a higher frequency (15.4%) than Las Dolores (12.3%); however no relevant difference was found. Considering the isolation of EP in both communities, all bacterial and parasitic agents were identified with a high frequency in Zaiman whereas rotavirus was of a higher frequency in Las Dolores. However, none of these differences were statistically significant with the applied methods.

In Zaiman, the isolation of bacterial enteropathogens in children with diarrhoea was statistically significant ($p< 0.05$) with regard to asymptomatic



EPEC: Enteropathogenic *Escherichia coli*; ETEC: Enterotoxigenic *Escherichia coli*; EIEC: Enteroinvasive *Escherichia coli*. Other parasites*. *Hymenolepis nana, Uncinarias, Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura.*

Fig. 1 - Seasonal changes in the prevalence of pathogens isolated in children in two communities of Argentina.

children; whereas the parasites were found with a higher frequency ($p< 0.01$) in the latter. Although the detection of rotavirus was higher in sentry children with diarrhoea, no statistically relevant differences were found.

In Las Dolores, no relevant differences were found in the detection of bacterial, parasitic and viral enteropathogens. Rotavirus had a higher occurrence in Las Dolores, both in symptomatic and sentry children.

ETEC and rotavirus were the most frequently detected agents in diarrheic episodes. The detection of rotavirus and ETEC was statistically more relevant ($p< 0.05$) than in the samples from asymptomatic children. Among the ETEC strains, the ST procedures were more frequently associated to diarrheic episodes ($p< 0.05$), but this was not observed for ETEC-LT. For the remaining bacterial EP no statistically relevant differences were found ($p> 0.05$).

Between the first and second year of the study, no changes were found in the frequency of EP, except for EPEC, which was most frequently isolated during the second year in children with diarrhoea than in the first year ($p< 0.05$).

Age distribution

Table 4 shows the analysis of the distribution of enteric pathogens according to age group. It revealed that only parasites presented in older children a highly relevant difference ($p< 0.001$).

Seasonal distribution

When analysing the seasonal distribution of diarrhoea in both communities, Zaiman and Las Dolores, the

detection was more frequent in Zaiman in spring-summer time, though the differences found had no statistic relevance (26.7% vs. 20.3%). See Figure 1.

DISCUSSION

An important factor in the genesis of diarrhoea is the great environmental microbiological contamination which, together with other factors, is responsible for recurrent infections[1]. More data from our population would be necessary in order to attribute the great number of diarrheic episodes to its sanitary level or to some other cultural or physical factors, though studies carried out by FIGUEROA et al.[17] showed that appropriate hygienic habits could relevantly lower the prevaling levels of contamination. The use and the source of water constitutes one of the pillars to evaluate sanitary conditions of habitation. Employment and educational levels of the head parents of the families are considered to be key indicators to estimate life conditions in these families. In our study, the declared jobs showed scarce qualification and except for nine cases that declared subordination, the rest were independent workers with a high instability due to the kind of tasks performed: ambulant sellers, brickleayers and "oleros" (rudimentary and artisan producers of bricks). When analysing the data about the poverty level (water supply, sanitary installations, socioeconomic level, etc.) of both communities and the number of diarrheic episodes detected in children under 5 years of age during the quarterly surveys, a higher occurrence was registered in Zaiman, where the majority of the families did not have any kind of sanitary installations.

The higher attack rates of diarrhoea illness registered in the poorest community, Zaiman, was not substantially higher than that found in Las Dolores, a less poor community, what differs from the report of GUERRANT et al.[21]. Other studies of children with diarrhoea in developing countries showed that less than 20% of those episodes were associated to classical bacterial pathogens: *Shigella*, *Vibrio* and *Salmonella*[16,20,29]. On the contrary, 35% of the episodes were associated to two recognized enteropathogens: ETEC and rotavirus. In previous studies that we carried out in children with diarrhoea under 5 years of age, that required medical assistance, the proportion of cases associated to one or more enteropathogens was of 57.4%, with 12.7% of enteroparasites[42].

In this paper on epidemiological surveillance in children that did not require medical assistance, one or more enteric pathogens were associated to diarrheic episodes in 70.5%. We estimate that the high percentage of parasites (28.5%) identified in this population elevated the percentage of isolation of enteropathogens. In our

TABLE 3
Enteropathogens in children with diarrhoea and asymptomatic children in two communities in Argentina

Microorganisms identified	With diarrhoea						Without diarrhoea					
	Zaiman (n=64)		Dolores (n=31)		Total (n=95)		Zaiman (n=351)		Dolores (n=221)		Total (n=572)	
	N°	(%)	N°	(%)	N°	(%)	N°	(%)	N°	(%)	N°	(%)
ETEC												
ETEC-LT	9	14.0	5	16.1	14	14.7	39	11.1	18	8.1	57	10.0
ETEC-ST	5	7.8	2	6.4	7	7.4	7	2.0	3	1.4	10	1.7
ETEC-LT/ST	0	-	0	-	0	-	5	1.4	3	1.4	8	1.4
EPEC	7	10.9	0	-	7	7.4	16	4.5	12	5.4	28	4.9
Shigella	2	3.1	0	-	2	2.1	8	2.3	2	0.9	10	1.7
Aeromonas	1	1.5	0	-	1	1.0	3	0.8	1	0.4	4	0.7
Campylobacter	0	-	0	-	0	-	2	0.6	2	0.9	4	0.7
EIEC	0	-	0	-	0	-	1	0.3	2	0.9	3	0.5
Salmonella	0	-	0	-	0	-	0	-	2	0.9	2	0.3
Total Bacteria	24	37.5	7	22.6	31	32.6	81	23.1	45	20.4	126	22.0
Parasites*	21	32.8	6	19.3	27	28.4	169	48.1	73	33.0	242	42.3
Rotavirus	5	7.8	4	12.9	9	9.5	5	1.4	11	5.00	16	2.8
Total Enteropathogens	50	78.1	17	54.8	67	70.5	255	72.6	129	58.4	384	67.1

ETEC: Enterotoxigenic *Escherichia coli*; EPEC: Enteropathogenic *E. coli*; EIEC: Enteroinvasive *E. coli*.
LT: heat-labile enterotoxin ST: heat-stable enterotoxin.
* Parasites: *Crytosporidium, Giardia lamblia, Hymenolepis nana, Uncinarias, Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura.*

TABLE 4
Age-distribution of enteropathogens identified in two comunities

Microorganisms identified	<1 y* (n=92)		1-2 y (n=160)		>2 y (n=412)	
	N°	(%)	N°	(%)	N°	(%)
ETEC						
LT	8	8.7	20	12.5	42	10.2
ST	3	3.2	4	2.5	10	2.4
LT/ST	0	-	0	-	9	2.2
EPEC	5	5.4	11	6.9	19	4.6
Shigella	1	1.1	2	1.2	9	2.2
Aeromonas	0	-	1	0.6	4	1.0
Campylobacter	0	-	1	0.6	3	0.7
EIEC	0	-	0	-	3	0.7
Salmonella	0	-	2	1.2	0	-
Total Bacteria	17	18.5	41	25.6	99	24.0
Parasites**	8	8.7	47	29.4	214	51.9
Rotavirus	6	6.5	3	1.9	16	3.9
Total Enteropathogens	31	33.7	91	56.9	329	79.8

EPEC: Enteropathogenic *Escherichia coli*
ETEC: Enterotoxigenic *E. coli*
EIEC: Enteroinvasive *E. coli*.
LT: heat-labile enterotoxin ST: heat-stable enterotoxin.
* age in years
** Parasites: *Cryptosporidium, Giardia lamblia, Hymenolepis nana, Uncinarias, Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura.*

study, the distribution of bacterial enteropathogens was comparable in both populations, *Shigella*, ETEC and EPEC were isolated with a higher frequency in Zaiman, although this difference was not statistically significant; the most commonly identified parasite was *Giardia lamblia*. Rotavirus, on the contrary, was more frequently detected in Las Dolores. In diarrheic children from both communities, we observed that the proportion of the episodes associated to bacterial and parasitic enteropathogens was high in children from the poorest community, where not only the number of detected enteropathogens was high, but also the frequency of cases where more than one enteropathogen was identified. The high frequency of isolation of enteropathogens in the poorest areas stresses the concept of the prevalence of these agents in marginal areas. In this study, as well as in others[30,34], diarrhoea is clearly associated to poverty due to deteriorated nutritional states and the permanent contamination of the environment.

EPEC, one of the traditionally enteropathogenic groups, possesses a plasmid that encodes an enteroadherence factor (EAF) which is responsible for a characteristic pattern of a localized adherence to Hep-2 and HeLa cells and an eaeA gene which is associated to the "attachment and effacement" of epithelial cells of intestine. Recognized evidence supports the fact that EPEC excretion from breast fed infants could be considered as another aspect of environmental microbiological contamination, and also that symptomatic and asymptomatic infants and children older than five, asymptomatic adult carriers and pets could act as reservoirs for EPEC[32]. Most of this evidence, including ours, would help towards a better understanding of our findings, that support the hypothesis that children with or without diarrhoea would represent the most important reservoir for these microorganisms. Considered to be the enteropathogen that causes diarrhoea in small children and especially in developing countries, EPEC is also frequently found in asymptomatic children, though with a lower rate of isolation[33]. In our study, the percentage of isolation of EPEC was strongly higher during the second year of study on children with diarrhoea, though EPEC was very frequent in asymptomatic children, as referred by PRADO et al.[35] and MOTEJO et al.[32].

The frequency of ETEC associated to diarrhoea and in asymptomatic children was 22.1% and 12% respectively; these figures are comparable to studies carried out by BLACK et al.[5] in children in Bangladesh (28% and

4.5%, respectively), GUERRANT et al.[21] in the Northeastern of Brazil (20.8% and 12.5%, respectively), but contrast with those of REIS et al.[36] in São Paulo, Brazil (13.4% and 11.4%). Our results show that ST producing ETEC strains were statistically associated to diarrhoea. Other studies carried out in children with diarrhoea and in sentries reported as well a higher association of ETEC-ST strains in symptomatic children[5,15,26], unlike ETEC-LT. According to ECHEVERRIA et al.[13], GURWITH & WILLIAMS[24] and WÄDSTROM[45], ETEC is not a relevant cause of infantile diarrhoea in developing countries with subtropical climates. According to LORTIE et al.[27], ETEC is the major cause of infantile diarrhoea in developing countries and the most frequent agent of travellers diarrhoea as well. These data indicate the need of further studies for a higher comprehension of ETEC's role in infantile diarrhoea. The knowledge of its incidence and distribution within the infantile population is necessary for the development of vaccines to make the host more resistante to disease.

The frequency of rotavirus was highly superior in the less poor community, Las Dolores; however, in both communities, it was more associated to diarrhoea than to asymptomatic cases. Our findings coincide with those of other authors[9,22], who studied children under 2 years of age.

The participation of ETEC in mixed infections, together with rotavirus, has already been reported in pediatric enteric infections[13,23,31].

Previous studies, carried out between 1986 and 1987, about the frequency and distribution of enteropathogens in children under 5 years of age with diarrhoea, detected a different serovar of *Salmonella*: *Salmonella* Zaiman[41] which was recovered from Zaiman stream waters[3]. It was thought that this frequent recovery of *Salmonella* was due to the contamination of the stream with refuses and meat products disposed by a packing house, since the infections with *Salmonella* species are relatively less common in marginal areas where feeding of livestock "en masse" is uncommon[21]. As from 1988, by means of strategies to improve life standard of these riparian children and by the support of our laboratory, municipal authorities provide tools for the treatment of refuses from the packing house, previous to the discharge in the stream. This explains the recent low recovery of *Salmonella* in this community.

Shigella was present in our area, with a frequency of 8.8%[43] to 9.4%[42] in young and poor children with acute diarrhoea, compelling a great number of cases to hospitalization. In other areas of our country, *Shigella* was isolated in 8.5% of children under 5 years of age, suffering from acute diarrhoea[33]. The percentage of *Shigella* isolation in this work was markedly low (children who did not require hospitalization).

The high carriage rate of parasites in asymptomatic and in older children from both communities makes it difficult to attribute a clear etiological role when these agents are detected in diarrheic episodes. This fact has already been observed in our previous studies [4,42,43], testifying the presence of a highly contaminated environment and the difficulty for these children, who lack proper sanitation, to avoid it.

A seasonal variation was registered in diarrheic episodes, having a highly statistic relevance ($p < 0.0001$) during spring-summer time, in both years of this surveillance (24.4% spring-summer vs. 10.5% fall-winter). These registers coincide with TIN-AYE et al.[40], who found a definite seasonal variation in diarrheic episodes in children under 5 years of age in a community of Burma, with GUERRANT et al.[21], in studies carried out in the Northeastern of Brazil and with CRAVIOTO et al.[11] in a prospective study of diarrhoeal disease in a cohort of rural Mexican children; the same was not observed by BLACK et al.[5], who found a slight seasonal variation in the incidence of diarrhoea in children in a community from Bangladesh. Among the enteropathogens analyzed, ETEC prevailed during summer time. The seasonal variation in the incidence of diarrhoea could be due to changes in the environment, which make enteropathogens transmission more feasible, or different enteropathogens could play their etiological role in different seasons, such as rotavirus with a higher incidence in cold months. Even if the infection by rotavirus is more frequent in winter[19,22], in countries with marked seasonal differences, in subtropical areas, as ours, the diarrhoea associated to rotavirus is present all year long. The relevance of the seasonableness of diarrhoea cannot be ignored for it is one of the determining factors of rural poverty[30]. Understanding the etiology of diarrhoea in developing countries with subtropical climate, such as our working area, is complex due to the great variety of risk factors that affect marginal areas and the high number of mixed infections.

The frequency of diarrhoea in the poorest area, with fallacies in water supply and sanitary installations, emphasizes the need to implement preventive rules and to improve the therapeutic techniques for this disease.

The knowledge of seasonal pattterns of diarrhoea, and the frequency in the isolation of enteropathogens according to socio-economic conditions, make it possible to implement preventive strategies and treatment in these areas of the country. This should be taken into account for

an effective fight against this disease that threatens the normal development and even the life of these children.

RESUMO

Estudio prospectivo de enteropatógenos en dos comunidades de Misiones, Argentina

Niños menores de cinco años de edad de dos comunidades de diferente condición socio-económica (97 del Barrio Zaimán y 55 del Barrio Las Dolores), fueron vigilados epidemiológicamente durante 2 años, mediante visitas trimestrales del equipo de trabajo, el que realizó la toma de muestras fecales.

Durante el estudio, se identificaron uno o más enteropatógenos en el 73,9% de las muestras de niños del Barrio Zaimán y en el 58,3% de Las Dolores, estando asociados a diarrea en un 70,5% y en infecciones asintomáticas en un 65,7%. El número de episodios de diarrea fue superior en Zaimán (15,5%) que en Las Dolores (12,4%), presentándose con mayor frecuencia en los meses de primavera-verano.

En el Barrio Zaimán la proporción de enteropatógenos bacterianos fue significativamente mayor ($p < 0.005$) en los niños con diarrea, mientras que los parásitos se presentaron con mayor frecuencia en niños asintomáticos ($p < 0.01$). Rotavirus tuvo una distribución comparable en niños con diarrea y asintomáticos.

En el Barrio Las Dolores, no se encontraron diferencias significativas en la detección de enteroparásitos entre asintomáticos y niños con diarrea.

Se detectaron infecciones mixtas, siendo ECET-rotavirus y ECET-parásitos, las más frecuentes. ECET estuvo involucrado en el 85% de las mismas.

Estos datos y la elevada portación de enteropatógenos, sugieren un alto nivel de contaminación ambiental, la cual juega un rol importante en la enfermedad diarreica, que sumada a la pobreza más extrema, afecta la vida de los niños.

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