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Prospective study of diarrhoeal disease in a cohort of rural Mexican children: incidence and isolated pathogens during the first two years of life

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SUMMARY

Colonization of the intestine by putative pathogens was followed longitudinally in a cohort of 56 infants born during one calendar year in a rural Mexican village with faecal cultures taken every fortnight and every time a child had diarrhoea. The frequency of isolation of pathogens during episodes of diarrhoea was compared with that of matched controls from the same cohort. Incidence of diarrhoea during the first year of life was 98%, diminishing to 93% during the second year. The incidence curves for each year were not significantly different ($P > 0{\cdot}1$). Isolation of enteropathogenic *Escherichia coli*, enterotoxigenic *Escherichia coli* producing heat-stable (ST) and/or heat-labile (LT) enterotoxins and rotaviruses was significantly higher in infants with diarrhoea during the first 2 years of life. In the case of shigella, although no significant differences were found by semester of life, 13 of 16 children in which these strains were found had diarrhoea. Isolation of *Salmonella* spp., *Campylobacter* spp. and protozoa were not significantly different in the two groups during the period studied. Strains showing localized adherence to HEp-2 cells or the presence of colonization factor antigens I or E8775 were found with significantly higher frequency in children with diarrhoea. Eighty-two percent of ST^+ or LT^+ ETEC strains isolated produced one of the three known colonization factors.

INTRODUCTION

There is no doubt that improvement in the environmental conditions of a community, especially with reference to adequate water supply and waste disposal, will bring about a rapid decrease in the incidence of diarrhoeal disease. These works of sanitary engineering are expensive, and most developing countries do not possess the resources or the technological knowledge to carry them out (Feachem *et al.* 1978). For these reasons, research groups in different countries have looked for alternative strategies to reduce morbidity and mortality caused by diarrhoea in infants.

One of these strategies has been the widespread use of oral rehydration for

prompt treatment at home of infants with diarrhoea. This approach requires a structure of health and educational services that will have to be created or improved in most developing countries, so that every child at risk of dehydration has access to this type of treatment.

A different strategy has been to make the host more resistant to infection through the use of vaccines against microorganisms frequently isolated from sick children. The knowledge of how bacteria produce a secretory response in the intestine and of the structures used by these organisms to adhere to intestinal epithelial cells has opened the possibility of developing specific vaccines against diarrhoeal disease (Levine *et al.* 1983; Gaastra & de Graaf, 1982). This approach requires initial epidemiological studies on the incidence and aetiology of diarrhoea in different parts of the world, to focus the development of these vaccines on the organisms most frequently isolated from children with diarrhoea at different ages.

Prospective studies of intestinal colonization have been carried out previously in several developing countries. Some of these investigations were done before pathogenic mechanisms were known (Mata, Urrutia & Gordon, 1967; Mata & Urrutia, 1971). More recent studies have used groups of children of different ages who have been followed for a number of years after treatment of a diarrhoeal episode in a specialized centre (Black *et al.* 1980).

In the present study we decided to follow from birth the colonization of the intestine by bacteria, rotaviruses and parasites in a cohort of infants born during one calendar year in a rural village in Central Mexico. The main objective of this investigation was to study the changes in the faecal flora during periods of health and disease in a group of rural children. This paper describes the design of the study, the incidence of diarrhoea during the first 2 years of life and the pathogens isolated during these episodes.

MATERIALS AND METHODS

The village. The study was carried out in a rural village of the state of Morelos, approximately 180 km southwest of Mexico City. 'The Village of the Stone Houses' has an area of 6·57 km^2, with a warm subtropical climate modified by an altitude of 947 m above sea level, and a median annual temperature between 23 and 25 °C. The rainy season is generally from June to October. According to a 1982 census it had 1283 inhabitants, divided into 205 families. The families had an average of five persons, with 49% of the population below 15 years of age.

Most (80%) of the adult male population were dedicated to agricultural activities as labourers, small landowners or tenants. The main crops of the area were sugar cane, corn and different types of fruits. A family-type pottery industry had recently been developed in the village, where the main working force was young women. Six per cent of the adult population were dedicated to small businesses or were professionals (mainly schoolteachers).

Design of the study. All the children born between 15 March, 1982 and 14 March, 1983 were enrolled in the study after oral informed consent was obtained from both parents. The medical personnel of the group living in the village examined the 61 children born during the year of induction of the cohort in the first 24 h after birth. Three infants emigrated from the village during the first week of life.

The parents of these infants had relatives living in the village but were not permanent residents. Two more infants died at birth, probably due to congenital malformations although no *post-mortem* studies were authorized by the families for confirmation, thus leaving 56 infants for follow-up studies. None of these 56 infants emigrated or died during the duration of the study.

Twenty-three (41%) of the 56 infants in the cohort were male and 33 (59%) were female. These proportions were not significantly different by Fisher's exact probability test (Siegel, 1956). The distribution of births during the four seasons of the year was not significantly different.

A person from the field team visited each of the 56 families every 48 h to detect the presence of diarrhoea. For the purpose of this study diarrhoea was defined as four or more bowel movements in 24 h with a liquid or semi-liquid consistency of the stools or the presence of blood or mucus, as detected by the mother or caretaker of the child and confirmed by the examining physician.

Microbiological study. Every fortnight from birth a sample of faeces was collected from the children into sterile containers. Within 60 min the sample was inoculated on MacConkey, Tergitol 7 and Xylose-lysine-desoxycholate (XLD) agars (Merck, Darmstadt, West Germany) in a field laboratory in the village. The culture was enriched with selenite and tetrathionate broths (Merck). Plates and tubes were incubated overnight at 37 °C in the village and then transported to Mexico City for identification.

The enrichment broths were inoculated on MacConkey, XLD, brilliant green and Shigella–Salmonella (Merck) agars and incubated for 18 h at 37 °C. All types of different colonies growing on the direct and enriched media were identified by biochemical methods (Cowan & Steel, 1974). When a single colony type was found, at least five colonies from each plate were picked for identification. Independent of the number found, all *Escherichia coli*, salmonella and shigella strains found were kept for further analysis. Cultures were maintained on Dorset egg medium at 4 °C.

For the isolation of campylobacter strains, the faeces were inoculated in the laboratory in the village on CAMPY-BAP media (BBL, Rockville, Maryland) and incubated at 42 °C for 48 h in a microaerophilic environment produced with a CAMPY-PAK (BBL) envelope. Putative campylobacter colonies were identified by biochemical methods (Kaplan, 1980).

The rest of the faecal sample was refrigerated and sent for identification of rotaviruses by electron microscopy (Rodríguez *et al.* 1977) and eggs and cysts of parasites and protozoa by a concentration method (Faust, Russell & Jung, 1970). In a pilot study it was found that refrigerating the samples gave similar results to transporting them in polyvinyl alcohol, as long as the concentration study was carried out within 48 h of obtaining the sample.

When a child had diarrhoea a second faecal sample was obtained and processed as before. For comparison, that same day a second sample was also obtained from all infants born during the same calendar month as the sick child, thus serving as controls. For the present analysis each case of diarrhoea was matched with a control for age and sex who had not had diarrhoea during the previous fortnight to the moment of obtaining the sample and who lived closest to the child with diarrhoea.

Pathogenicity tests. A minimum of 5 and a maximum of 10 strains identified as

E. coli from each faecal culture were agglutinated with commercial antisera against the so-called enteropathogenic serotypes (Behringwerke AG, Marburg, West Germany). Those positive by slide agglutination were confirmed by tube agglutination using boiled cultures (Ørskov & Ørskov, 1975). The serotype of some of these strains was kindly confirmed by Dr Bernard Rowe, Division of Enteric Pathogens, Central Public Health Laboratory, London, UK.

The same *E. coli* strains from each faecal culture were tested for production of heat-labile (LT) enterotoxin with a GM_1-ELISA test (Svennerholm & Holmgren, 1978), using purified ganglioside kindly provided by Professor Lars Svennerholm, Institute of Neurochemistry, Gothenburg, Sweden, and for heat-stable (ST) enterotoxin with the infant mouse assay (Dean *et al.* 1972).

The presence of adhesive factors in all strains of *E. coli* isolated was studied with the HEp-2 assay designed by Cravioto *et al.* (1979); adhesion to HEp-2 cells was classified as localized and diffuse according to Scaletzky, Silva & Trabulsi (1984). All strains were also tested for mannose-resistant haemagglutination (MRHA) with human group A and bovine erythrocytes (Cravioto, Scotland & Rowe, 1982). Those giving a positive test were assayed for the presence of colonization factor antigens I, II and E8775 (Cravioto, Scotland & Rowe, 1982; Thomas *et al.* 1982) with an Ouchterlony immunodiffusion test using specific antisera against these antigens raised in rabbits (Cravioto, Scotland & Rowe, 1982).

Differences in the frequency of isolation of the various pathogens found in children with diarrhoea and controls were analysed with Fisher's exact probability test (Siegel, 1956). Differences in the incidence curves for each year of life were examined with a one-tail Kolmogorov–Smirnov test (Siegel, 1956).

RESULTS

Incidence of diarrhoea, taken as new cases per year, was calculated separately for each year of life (Fig. 1). During the first year, all but one child (98%) had at least one episode of diarrhoea. By the fifth month of life 62% of the children had suffered a bout of diarrhoea. There was an average of three episodes of diarrhoea per child per year for this period.

During the second year of life the incidence was 93%, very similar to the year before (Fig. 1). This time the curve rose more sharply during the first 6 months, with 87% of the children having a new episode of diarrhoea during this period. The curve then levelled off during the second half of the year. The average number of episodes of diarrhoea per child during the second year of life was also three. Differences in incidence curves for each year were not statistically significant ($P > 0{\cdot}1$).

The analysis of associated pathogens isolated from children with diarrhoea and controls was done by semesters of life. These cuts were done arbitrarily, considering that each age chosen was associated with different epidemiological conditions that put a child at risk of developing diarrhoea (Mata, 1978).

In infants below the age of 6 months (Table 1), only enteropathogenic *E. coli* (EPEC) ($P = 0{\cdot}001$) and enterotoxigenic *E. coli* (ETEC) strains producing both ST and LT ($P = 0{\cdot}012$) or ST only ($P = 0{\cdot}031$) were isolated more frequently from infants with diarrhoea than from controls. A pathogen or pathogens could be

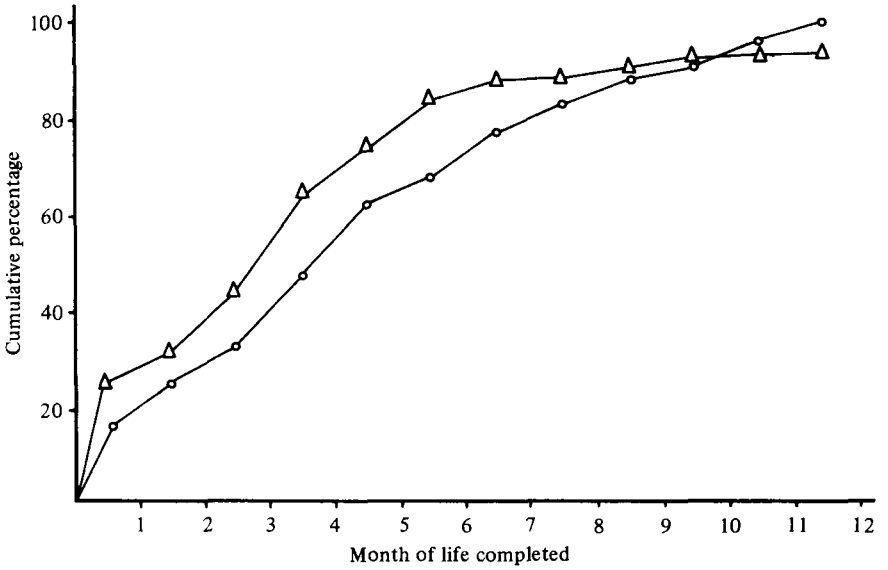

Fig. 1. Incidence of diarrhoea during the first (○) and second (△) year of life of the children in the cohort. Village of the Stone Houses, 1982–1984. ($P > 0{\cdot}1$ by Kolmogorov–Smirnov one-tail test.)

Table 1. *Frequency of isolation of putative pathogens from cases of diarrhoea and controls during the first 6 months of life (Village of the Stone Houses, 1982–1983)*

Isolated pathogens	Frequency		Fisher's exact probability test
	Cases (%)	Controls (%)	
EPEC	18 (19)	4 (4)	0·001
ETEC			
ST	10 (11)	3 (3)	0·031
LT	6 (6)	11 (12)	0·092
ST/LT	15 (16)	5 (5)	0·012
Rotavirus	6 (6)	2 (2)	0·106
Shigella	2 (2)	0	0·248
Salmonella	2 (2)	3 (3)	0·309
Campylobacter	9 (10)	8 (9)	0·194
Protozoa*	1 (1)	0	0·500
No pathogens	24 (26)	57 (61)	7×10^{-7}
Total	93	93	

*Trophozoites of *Entamoeba histolytica*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; ST, heat-stable enterotoxin; LT, heat-labile enterotoxin.

identified in 74% of children with diarrhoea compared with only 39% of the control group ($P = 7 \times 10^{-7}$).

EPEC ($P = 0{\cdot}040$) and ST/LT producing ETEC strains ($P = 0{\cdot}023$) were once again isolated more frequently from children with diarrhoea than from matched controls during the second semester of life, as shown in Table 2. During this period rotavirus were also found more frequently in children with diarrhoea ($P = 0{\cdot}028$),

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Table 2. *Frequency of isolation of putative pathogens from cases of diarrhoea and controls during the second semester of life (7–12 months) (Village of the Stone Houses, 1982–1983)*

Isolated pathogens	Frequency		Fisher's exact probability test
	Cases (%)	Controls (%)	
EPEC	8 (10)	2 (3)	0·040
ETEC			
ST	8 (10)	5 (6)	0·162
LT	3 (4)	12 (16)	0·011
ST/LT	16 (21)	7 (9)	0·023
Rotavirus	13 (17)	5 (6)	0·028
Shigella	7 (9)	2 (3)	0·066
Salmonella	6 (8)	9 (12)	0·156
Campylobacter	3 (4)	7 (9)	0·115
Protozoa*	1 (1)	0	0·500
No pathogens	12 (16)	28 (36)	0·002
Total	77	77	

*Includes *Entamoeba histolytica* and *Giardia lamblia*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; ST, heat-stable enterotoxin; LT, heat-labile enterotoxin.

Table 3. *Frequency of isolation of putative pathogens from cases of diarrhoea and controls during the first semester of the second year of life (13–18 months) (Village of the Stone Houses, 1983–1984)*

Isolated pathogens	Frequency		Fisher's exact probability test
	Cases (%)	Controls (%)	
EPEC	4 (3)	0	0·061
ETEC			
ST	3 (2)	2 (2)	0·308
LT	34 (26)	22 (17)	0·026
ST/LT	8 (6)	1 (1)	0·016
Rotavirus	12 (9)	2 (2)	5×10^{-4}
Shigella	3 (2)	0	0·124
Salmonella	11 (8)	12 (9)	0·168
Campylobacter	4 (3)	5 (4)	0·255
Protozoa*	9 (7)	9 (7)	0·192
No pathogens	41 (32)	76 (59)	7×10^{-6}
Total	129	129	

*Includes *Entamoeba histolytica* and *Giardia lamblia*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; ST, heat-stable enterotoxin; LT, heat-labile enterotoxin.

while LT-only ETEC strains were isolated more frequently from controls than from sick children ($P = 0·011$).

In children 6–12 months of age, 84% of those with diarrhoea had an associated pathogen. This frequency was significantly higher ($P = 0·041$) than that found in children with diarrhoea during the previous 6 months of life. The number of controls in which a pathogen was isolated during this period was also significantly

Table 4. *Frequency of isolation of putative pathogens from cases of diarrhoea and controls during the second semester of the second year of life (19–24 months) (Village of the Stone Houses, 1983–1984)*

Isolated pathogens	Frequency		Fisher's exact probability test
	Cases (%)	Controls (%)	
EPEC	0	0	1·000
ETEC			
ST	5 (7)	2 (3)	0·166
LT	13 (18)	11 (15)	0·160
ST/LT	4 (6)	0	0·059
Rotavirus	6 (8)	4 (6)	0·209
Shigella	2 (3)	0	0·248
Salmonella	4 (6)	7 (10)	0·162
Campylobacter	5 (7)	5 (7)	0·266
Protozoa*	9 (12)	13 (18)	0·121
No pathogens	25 (34)	31 (42)	0·081
Total	73	73	

*Includes *Entamoeba histolytica* and *Giardia lamblia*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; ST, heat-stable enterotoxin; LT, heat-labile enterotoxin.

higher than the number found in the previous 6-month period ($P = 6 \times 10^{-4}$). Six of ten children between 6 and 12 months of age had a pathogen isolated from their faeces without the presence of diarrhoea.

During the first 6 months of the second year of life the frequency of isolation of pathogens diminished significantly (Table 3). EPEC strains continued to be found more frequently in children with diarrhoea, but the frequency was not statistically different from the one found in the controls ($P = 0{\cdot}061$). As in the previous age groups, the isolation of ST/LT producing ETEC strains ($P = 0{\cdot}016$) and rotaviruses ($P = 5 \times 10^{-4}$) was significantly higher in cases than in controls (Table 3).

Finally, as shown in Table 4, in children 18–24 months of age only ST/LT producing ETEC strains were found with significantly higher frequency in children with diarrhoea than in controls ($P = 0{\cdot}059$). In this age group the isolation of putative pathogens between children with diarrhoea and controls was not significantly different ($P = 0{\cdot}081$).

It seems worth mentioning that in the case of *Shigella* spp. 9 of 11 strains isolated during the first year of life and the 5 strains isolated during the second year of life were found in children with diarrhoea. In this case, analysis by semester of life probably masked significant differences due to the low rate of isolation of this pathogen. In the case of *Salmonella* spp., *Campylobacter* spp. and protozoa, a statistical analysis by year instead of semester of life did not show significant differences between the two groups.

More than one pathogen per culture was isolated in 9% of cases of diarrhoea during the first 12 months of life, and 16% of cases during the second 12 months of life (data not shown). Most of these cases showed isolation of ETEC or rotavirus together with salmonella or campylobacter strains. The percentages and type of

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Table 5. *Serotypes of enteropathogenic* Escherichia coli *(EPEC) associated with the presence of diarrhoea in children of the Village of the Stone Houses, 1982–1984*

Children	EPEC serotypes (number of children)				Total
With diarrhoea	026(2),	055(5),	0111(16),	0119(7)	30
Without diarrhoea	026(1),	086(3),	0114(1),	0128(1)	6

Table 6. *Patterns of adherence to HEp-2 cells of* Escherichia coli *strains isolated from children with or without diarrhoea in the Village of the Stone Houses, 1982–1984*

Type of adherence	Children: With diarrhoea (%)	Children: Without diarrhoea (%)	Total
Localized	25 (92)	2 (7)*	27
Diffuse	10 (24)	32 (76)†	42
Total	35 (51)	34 (49)	69

*$P = 6{\cdot}328 \times 10^{11}$;
†$P = 1{\cdot}290 \times 10^{-6}$. Both by Fisher's exact probability test.

Table 7. *Production of colonization factor antigens by enterotoxigenic* Escherichia coli *strains isolated from children with or without diarrhoea in the Village of the Stone Houses, 1982–1984*

Colonization factor antigen	Children: With diarrhoea (%)	Children: Without diarrhoea (%)	Total
I	23 (74)	8 (26)*	31
II	11 (65)	6 (35)†	17
E8775	18 (62)	11 (38)‡	29
Total	52 (68)	25 (32)§	77

*$P = 1{\cdot}3 \times 10^{-4}$; †$P = 0{\cdot}07$; ‡$P = 0{\cdot}04$; §$P = 9{\cdot}2 \times 10^{-6}$. All by Fisher's exact probability test.

microorganisms found in these children were not significantly different in patients with or without diarrhoea in each age group studied. For this reason, each pathogen found was taken as a separate isolation for the purpose of statistical analysis.

EPEC strains isolated from children with diarrhoea belonged to serogroups O26, O55, O111 and O119 (Table 5). EPEC strains isolated from children without diarrhoea belonged to serogroups O26, O86, O114 and O128. As shown in Table 6, there was a highly significant difference in the number of children with diarrhoea in which strains of *E. coli* able to adhere to HEp-2 cells with a localized pattern were found. Twenty-five (92%) of 27 children with isolation of this type of adhering strains were ill. The contrary was found with children in which *E. coli* strains showing a diffuse adherence pattern were found; of 42 children in which this type of strains was isolated, 32 (76%) did not have diarrhoea. These two sets of previous data were highly related, since the 25 strains showing localized adherence to HEp-2 cells isolated from children with diarrhoea belonged to

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serogroups O55, O111 and O119. Interestingly, the *E. coli* strains with localized adherence ability, isolated from the two children without diarrhoea (Table 6), did not belong to any of the EPEC serotypes. Only three of the strains showing diffuse adherence belonged to an EPEC serogroup, and all three belonged to serogroup O86 and were isolated from children without diarrhoea.

Table 7 shows the distribution of children with or without diarrhoea in which *E. coli* strains producing one of the three known CFAs were found. Such strains were found in 77 (82%) of 94 children in which ST-only or ST/LT ETEC strains were isolated. Fifty-two (67%) of these 77 children had diarrhoea, while 25 (32%) did not. This difference was highly significant ($P = 9{\cdot}2 \times 10^{-6}$). Considering each CFA separately, only CFA/I and E8775 were found with a significantly higher frequency in children with diarrhoea (Table 7).

DISCUSSION

Specific prevention of diarrhoeal diseases in young children of any country should be based on data obtained locally in different environments. These data should reflect with the highest possible fidelity what is causing disease in most of the population at risk. Since Black *et al.* (1981) have shown that severity of diarrhoea and risk of dehydration are related with different micro-organisms, aetiologic studies carried out in specialized paediatric hospitals of big cities can be misleading due to the type of patients who seek attention at this level. Prospective studies in a community tend to avoid this type of bias.

The results obtained in the present investigation demonstrate that EPEC and ETEC strains, along with rotaviruses and possibly shigella, were associated with approximately 75% of cases of diarrhoea in a rural community in Mexico. The type of agents found was similar to those isolated in previous transversal studies carried out during the 1970s in hospitals in Mexico City (Donta *et al.* 1977; Pickering *et al.* 1978). In these hospital studies the frequency of isolation of rotavirus and shigella was much higher than in the present community-based investigation. These differences were probably due to the severity of the diarrhoeal episode associated with these latter pathogens, so that specialized treatment in a paediatric hospital was required. A lower frequency of isolation of rotaviruses than expected from data obtained in urban hospitals has also been recently found by Simhon *et al.* (1985) in a longitudinal study in a rural community in Costa Rica.

These results did not differ significantly from those found in prospective studies carried out in other developing countries (Black *et al.* 1980; Guerrant *et al.* 1983; Freiman *et al.* 1977). The few differences in the percentage of isolation of different pathogens could be further cancelled if one compares the proportion of isolation of one micro-organism to another, regardless of the percentages found in each study. Thus in Bangladesh, Brazil, South Africa or Mexico one finds approximately three ETEC strains for each rotavirus isolated and one shigella for every two strains of salmonella or campylobacter.

What this study shows more than the others is the distribution of frequencies of isolation of different pathogens at specific ages. Most of the previous investigations have taken children with a wide range of ages. In some, this goes

from 1 month to 12 years of life (Donta *et al.* 1977). Others, like Black *et al.* (1980) in Bangladesh, do not give the distribution of children less than 2 years of age that they studied. As can be seen in the present report, with the exception of ETEC strains producing both ST and LT, the frequency of isolation of other pathogens differed significantly from one age group to another. This type of analysis allows a more realistic approach to the possible use of vaccines for the prevention of diarrhoea in children. While vaccines against EPEC and ETEC should be administered during the first months of life or perhaps through the breast milk of immunized mothers, a rotavirus vaccine could be given at a later age.

Localized adherence to HEp-2 cells and production of colonization factor antigens seem to have epidemiological importance in relation to the presence of diarrhoea in children. The results of the HEp-2 adhesive assays reported in this study confirm previous findings with a small number of selected strains isolated in other Latin American countries (Nataro *et al.* 1985) or with a large number of isolates obtained from strain collections (Scaletzky *et al.* 1985). In the case of the CFAs, the results presented are similar to those found in studies by Evans *et al.* (1978), Thomas & Rowe (1982) and Göthefors *et al.* (1985), who reported that 75–85% of freshly isolated ETEC strains were capable of producing one of these three known ETEC adherence factors.

The frequency with which children of this community were colonized with undesirable micro-organisms shows the high risk they have of suffering disease at an early age. The lack of clinical symptoms in the presence of a pathogenic agent could be due to active immunity produced by previous contact with similar organisms or, as has been shown in the case of cholera (Glass *et al.* 1983), to passive immunity produced by specific antibodies present in the breast milk 99% of these children received from birth.

What does not seem conceivable with these findings is that a micro-organism can produce diarrhoea only in children of certain age groups without the active participation of the host. While LT-producing ETEC strains were found throughout the first two years of life in both cases and controls, EPEC strains were only found in children less than 18 months of age and always more frequently in those with diarrhoea. Lack of isolation of EPEC strains after 18 months of life is difficult to explain after the high frequency with which these strains were found in younger children. If indeed these strains can cause diarrhoea by adhering to the microvillous surface of intestinal epithelial cells, as has been shown in clinical studies (Rothbaum *et al.* 1982), then the interaction between these bacteria and specific receptors in the cells of the host could prevent colonization by the same organism after initial exposure. Since EPEC strains have been shown to cause diarrhoea in adult volunteers (Levine *et al.* 1985) it seems doubtful that lack of colonization of older children could be due to the disappearance of specific receptors in the intestinal cells after a certain age, as has been shown in calves for the K99 adhesive factor (Runnels, Moon & Schneider, 1980). Early interaction between agent and host probably constitutes an important protection factor against pathogenic micro-organisms at later ages. The close correlation between adhesion of EPEC to HEp-2 cells and ability to cause diarrhoea in humans (Paulozzi *et al.* 1986) offers a good model for the future study of this interaction. The results of these investigations will offer a more appropriate biological approach to the prevention of diarrhoeal diseases in children.

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