Epidem. Inf. (1989), **102**, 129–145
Printed in Great Britain

Longitudinal study of rotavirus infections among children from Belém, Brazil

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(*Accepted 14 February 1988*)

SUMMARY

From December 1982 to March 1986 a group of 80 children between 0 and 3 years old who lived in the peripheral area of Belém, Brazil, were followed up for episodes of diarrhoea. A total of 441 diarrhoeal episodes were recorded and 36 (8·2%) were associated with rotavirus. This agent was the only pathogen in 50% of rotavirus-related episodes of acute diarrhoea, and strains were characterized by analysis of RNA in polyacrylamide gels. Forty-one belonged to subgroup II (long pattern) and five to subgroup I. Reinfections by rotavirus were noted in 12 children involving either the same or different subgroups. Ten distinct electrophoretypes were detected in the study period and the predominant one had the '1N2L' profile. The cumulative age-specific attack rate for diarrhoea reached 2·8 by the end of the first year of life; a frequency of 2·3 episodes of diarrhoea per child per year was observed throughout the complete investigation. In comparing the age-specific attack rates for diarrhoea between breast-fed and bottle-fed children, a peak at 6 months of age was noted in the former, and at 1 month in the latter. A comparison by Fischer's exact test ($P = 0{\cdot}21$) provided no evidence for protection against clinical rotavirus disease by maternal milk. By the same test, however ($P = 0{\cdot}021$), we found significant evidence that early rotavirus infections were more likely to be asymptomatic and that infections after 4 months were more likely to be symptomatic. The clinical picture in children with rotavirus-related diarrhoea was more severe than in those suffering from acute diarrhoea due to another agent.

INTRODUCTION

Rotavirus has been shown to be a major enteropathogen throughout the world, affecting mainly children less than 5 years of age in both temperate (Davidson *et al.* 1975; Kapikian *et al.* 1976; Konno *et al.* 1983) and tropical countries (Linhares *et al.* 1983; Mata *et al.* 1983*a*,*b*; Paul & Erinle, 1982). Most of these studies were made on patients in hospital.

Few prospective community-based investigations, however, have been reported. A longitudinal study of 24 infants and young children carried out in rural Guatemala (Wyatt *et al.* 1979) showed that rotaviruses were associated with

14·2% of diarrhoeal episodes. These agents were the predominant enteropathogens identified in three Canadian communities (Gurwith *et al.* 1983). Evidence of rotavirus infections was noted in 44 of 81 children followed up from birth to 3 years old in Melbourne, Australia (Bishop *et al.* 1983).

In Brazil rotavirus particles were first demonstrated in 1976 by electron microscopy in stools from children with acute gastroenteritis admitted to a public hospital (Linhares *et al.* 1982). Several other hospital-based studies (Baldacci *et al.* 1979; Coelho *et al.* 1983; Coiro *et al.* 1985) have established the importance of rotaviruses as causative agents of acute infantile diarrhoea in Brazil. A prospective study of diarrhoeal illnesses carried out in northeastern Brazil demonstrated that enterotoxigenic *Escherichia coli* and rotaviruses were the most common enteropathogens (Guerrant *et al.* 1983). Similarly, rotavirus has been found to be a major enteropathogen in the Amazon region in children less than 6 years old living in Belém, Brazil, and accounted for at least 30% of all episodes of acute gastroenteritis (Linhares *et al.* 1983). Infections in adults have also been reported, mainly among isolated Indian communities (Linhares *et al.* 1981; Linhares *et al.* 1986).

The present report describes the results of a longitudinal community-based study carried out in Belém over 3 years. This investigation studied the epidemiology and clinical aspects of rotavirus infections from birth to 3 years of age. In addition an epidemiological study of rotavirus infections by subgroup/serotype was carried out.

MATERIALS AND METHODS

Study area

Belém is located in the eastern Amazon at the confluence of the Guamá and Pará rivers, 1° 30′ 20″ S, 48° 39′ 00″ W. The climate is that of tropical rain forest (Köppen), with very heavy rainfall during the months of February, March and April. The annual mean temperature ranges from 24 to 28 °C and the relative air humidity is regular and always high at about 8% throughout the year.

Patients and specimens

When the project began in October 1982, 217 pregnant women (6th to 9th months of pregnancy) belonging to a low socio-economic level were selected. All lived in the poorest housing found in the suburbs of Belém. Although special care was taken to select women who were less likely to drop out of the programme, we were only able to get the co-operation of 88.

Deliveries occurred between the end of November 1982 and the middle of March 1983 in a single public hospital. During this time technicians from our laboratory spent the day in the delivery ward to collect the necessary specimens; during the night these were collected by previously trained nursing staff. At birth the following specimens were taken (Fig. 1): sera from cord blood and from the mother, maternal milk and faeces from the baby. While in hospital faeces were collected daily from the newborn children. When the child went home faecal samples were obtained on alternate days up to day 14, and faeces, maternal milk,

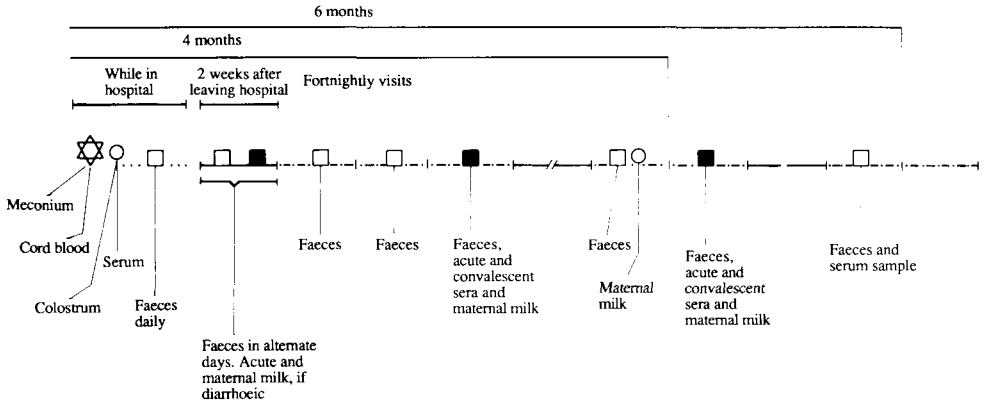

Fig. 1. Diagram outlining the follow-up study into children from Belém, Brazil. ✡, Birth; □, non-diarrhoeic child; ■, diarrhoeic child; ○, mother.

acute and convalescent sera were collected during any diarrhoeal episode. After day 14, faeces were collected fortnightly or whenever signs of diarrhoea were present. Faeces, maternal milk, acute and convalescent serum samples were collected throughout every diarrhoeal episode. The mothers were told to diagnose as diarrhoea those episodes in which faeces were either watery or semi-solid, and where (usually) more than three evacuations were made per day. Diarrhoeal children were always visited by a physician from our team who, apart from taking both clinical and dietary records, routinely administered oral therapy if required. Sera were routinely collected every 6 months and maternal milk every 4 months. As 6 children died and 11 moved away from Belém, the final number of children regularly followed up was 71. There were no marked ethnic differences among them.

Faeces, serum and milk samples were frozen and kept at −20 °C until processing, with the latter being delipidized beforehand as described by Totterdell, Chrystie & Banatvala (1980). All faecal samples were tested for rotavirus antigen with the World Health Organization kit prepared at the Regional Virus Laboratory, East Birmingham Hospital, Birmingham, England (Beards *et al.* 1984). Blocking tests were performed with all samples reaching an optical density greater than 0·1. Sera were tested for antibody by a newly developed enzyme-linked immunosorbent (ELISA) blocking test (Linhares *et al.* 1986), and milk samples by an ELISA technique previously described by Yolken *et al.* (1978). In addition, rotavirus positive faecal specimens were sent to Birmingham, UK, for serotyping by ELISA (Beards, 1987). Analysis of rotavirus RNA by polyacrylamide gel electrophoresis was done essentially as described by Lourenço *et al.* (1982). Isolation and identification of enteroviruses were done by conventional methods (Melnick & Wenner, 1969). Stools from diarrhoeal children and controls were examined for both bacteriological and parasitological agents. The methods used for isolation and identification of bacteria were those described by Edwards & Ewing (1972). Identification of *E. coli* heat-labile enterotoxin was made with a ganglioside immunosorbent assay (Svennerholm & Holmgren, 1978). No tech-

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Table 1. *Occurrence of viruses, bacteria and parasites in 144 episodes of diarrhoea and from routinely collected specimens (in apparently normal children). Belém, Brazil, Dec. 1982 to mid Dec. 1983*

Enteropathogens	Diarrhoeic		Non-diarrhoeic	
	No.	%	No.	%
Viruses				
Rotavirus*	9	6·2	8	0·4
Enterovirus†	66	45·8	62	43·7
Adenovirus†	2	1·4	7	4·9
Bacteria‡				
Salmonella	7	5·2	NE	
Shigella	5	3·7	NE	
EPEC	5	3·7	NE	
ST⁺ ETEC	6	5·4	NE	
LT⁺ ETEC	10	9·1	NE	
ST⁺ ETEC + LT⁺ ETEC	2	1·8	NE	
Parasites§				
G. intestinalis	13	10·6	NE	
E. hystolitica	—	—	NE	
Fungi	20	16·3	NE	

This table includes children aged from 0 to 11 months.

* 144 specimens from diarrhoeic children and 1869 from non-diarrhoeic ones were examined.

† 144 specimens from diarrhoeic children and 142 specimens from non-diarrhoeic ones have been inoculated in tissue-culture (Vero, HEp 2 and G 293 cells) and into suckling mice. These specimens were also tested for adenovirus by an ELISA test.

‡ 134 specimens from diarrhoeic children were examined. 110 of these were tested for the presence of *E. coli* toxins.

§ 123 specimens from diarrhoeic children were examined.

NE. Not examined.

niques for isolating campylobacters were available in our laboratory during the study period.

RESULTS

Table 1 lists the results obtained from virological, bacteriological and parasitological tests on faecal samples collected during 144 episodes of diarrhoea which occurred between December 1982 and mid December 1983. During this period faecal specimens from both diarrhoeal and non-diarrhoeal children were routinely processed for rotavirus antigen. However, stools collected from normal children were examined neither bacteriologically nor parasitologically. Rotaviruses were demonstrated in 9 (6·2%) of the 144 specimens from diarrhoeal patients, and in 8 (0·4%) of the 1869 samples from non-diarrhoeal children. Salmonellae, Shigellae and pathogenic *E. coli* were recovered from 35 (26%) specimens. *Giardia intestinalis* was found in 13 (10·6%) of specimens examined for parasites; fungi (mainly *Candida albicans*) were detected in 20 (16·3%). Enteroviruses were isolated from the faeces of 66 (45·8%) and 62 (43·7%) of the diarrhoeal and non-diarrhoeal children, respectively. Adenoviruses were detected in faeces from two of the diarrhoeal children and in 7 of the normal ones.

Table 2. *Occurrence of viruses, bacteria and parasites in faeces collected in 297 episodes of (diarrhoea, and from routinely collected specimens in well children). Belém, Brazil, mid Dec. 1983 to March 1986*

	Diarrhoeic*		Non-diarrhoeic†	
Enteropathogens	No.	%	No.	%
Viruses				
Rotavirus*	27	9·1	16	0·5
Enteroviruses†	86	66·1	69	48·6
Adenoviruses†	5	3·8	10	7·0
Bacteria‡				
Salmonella	11	4·0	6	2·1
Shigella	23	8·3	4	1·4
EPEC	6	2·2	10	3·4
LT^+ ETEC	23	8·8	17	6·1
ST^+ ETEC	12	4·6	3	1·1
ST^+ LT^+ ETEC	4	1·5	4	1·4
Parasites§				
Giardia intestinalis	61	21·7	71	24·2
Entamoeba hystolitica	22	7·8	9	3·1
S. stercoralis	8	2·8	2	0·7
Fungi	53	18·9	45	15·4

* 297 specimens from diarrhoeic children and 3·370 from non-diarrhoeic children were examined.

† 130 specimens from diarrhoeic children and 142 from non-diarrhoeic children were examined.

‡ 276 specimens from diarrhoeic children and 291 from non-diarrhoeic ones; 262 specimens from diarrhoeic children and 278 from control group were processed for the presence of *E. coli* toxins.

§ 281 specimens from diarrhoeic children and 293 from non-diarrhoeic ones.

Between mid-December 1983 and March 1986, stools were obtained in comparable numbers from both diarrhoeal children and controls, and were routinely processed for pathogenic bacteria and parasites. As shown in Table 2 rotavirus, Shigellae, heat-stable toxin producing (ST), *E. histolytica* and *S. stercoralis* were found more frequently in the stools of diarrhoeal children than in the control group.

The association of rotavirus with other enteropathogens is shown in Table 3. In 18 (50%) of the 36 rotavirus-positive faecal samples no enteropathogens other than rotavirus were detected. In the remaining specimens one or more other enteropathogens were found associated with rotavirus.

The temporal distribution of rotavirus electrophoretypes, according to the classification proposed by Moosai *et al.* (1984) is presented in Fig. 2. Clear profiles were obtained for 46 (76·7%) out the 60 rotavirus-positive specimens tested. The most frequent pattern was '1N2L' and accounted for 65·2% of the electrophoretypes. Rotaviruses with this electrophoretype were detected mostly during the first 2 years of the investigation. Nine other different electrophoretypes were detected during the whole 3 years of the investigation, in both symptomatic and asymptomatic infections.

Fig. 3 shows the temporal distribution of reinfections with rotavirus in 12

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Table 3. *Enteropathogens in faeces from 36 rotavirus-positive children with acute diarrhoea*

Enteropathogens	No.	%
Only rotavirus	18*	50·0
Rotavirus and one enteropathogen		
Rotavirus + enterovirus	3†	8·3
Rotavirus + fungi	1	2·7
Rotavirus + *E. hystolitica*	2	5·5
Rotavirus + LT$^+$ *E. coli*	3	8·3
Rotavirus and more than one enteropathogen		
Rotavirus + enterovirus + fungi	2	5·5
Rotavirus + Shigella + LT$^+$ *E. coli*	1‡	2·7
Rotavirus + LT$^+$ *E. coli* + fungi	1	2·7
Rotavirus + enterovirus + LT$^+$ *E. coli* + ST$^+$ *E. coli*	2	5·5
Rotavirus + enterovirus + Salmonella + fungi	1	2·7
Rotavirus + enterovirus + Salmonella + LT$^+$ *E. coli*	1	2·7
Rotavirus + Salmonella + LT$^+$ *E. coli* + *E. hystolitica*	1	2·7

* Two specimens were not processed for bacteriological agents: one was not processed for bacteriological and parasitological agents; one was not processed for parasitological agents.
† One specimen was not processed for bacteriological and parasitological agents.
‡ Not processed for parasitological agents.

children living in Belém, Brazil, indicating whether the infections were apparent or inapparent, together with the patient's age and subgroup of rotavirus. All infections during the first year of life were caused by subgroup II (long pattern), four of them being asymptomatic. The shortest time between two infections was 3 months, and the longest was 22 months. In three children (A, C and D) three successive infections were detected; in one case (C) the third infection was symptomatic, and associated with a possibly atypical rotavirus.

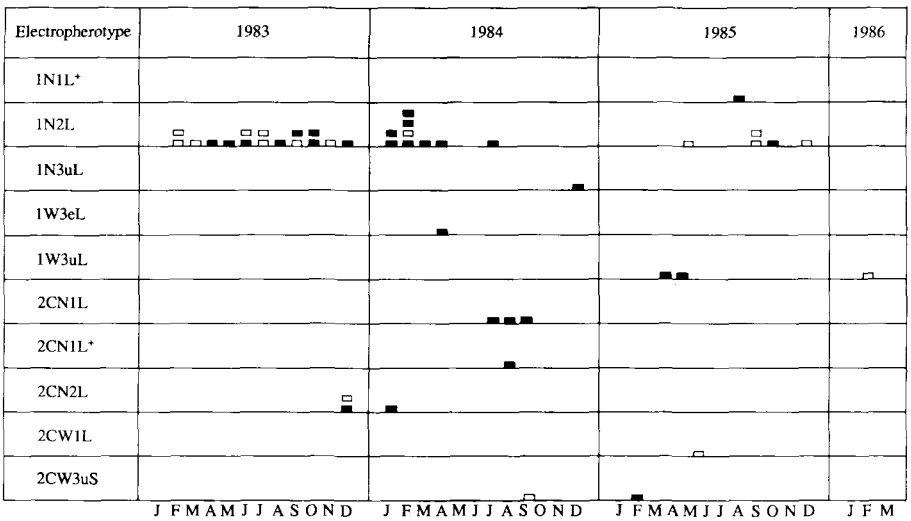

Fig. 2. Temporal distribution of rotavirus electrophoretypes. ■, Symptomatic; □, asymptomatic.

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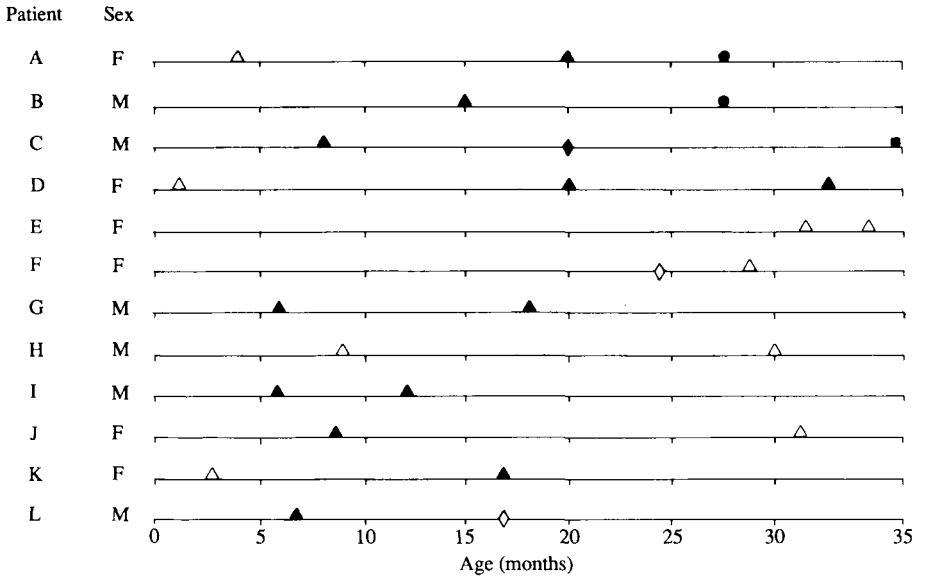

Fig. 3. Temporal distribution of reinfections by rotavirus in children from Belém, Brazil. ▲, Symptomatic, subgroup II; △, Asymptomatic, subgroup II; ●, Symptomatic, subgroup I; ◇, Asymptomatic, subgroup not determined; ◆, Symptomatic, subgroup not determined; ■, Symptomatic, atypical rotavirus (?).

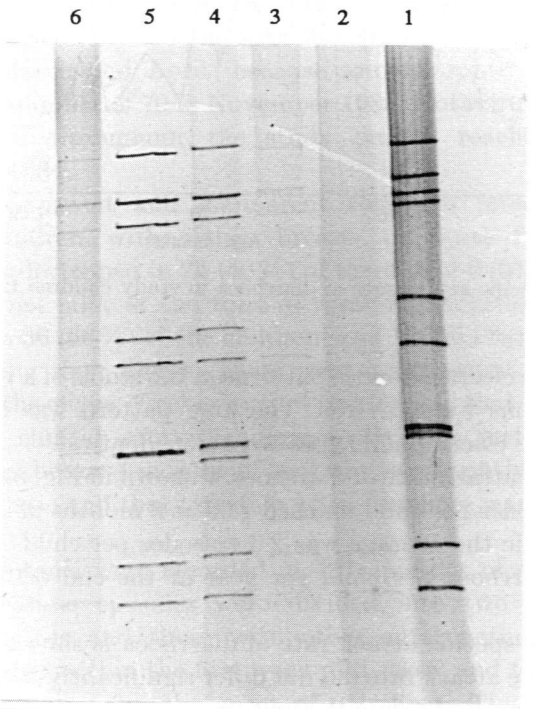

Fig. 4. Three successive rotavirus infections in a child from Belém, Brazil: electrophoretic profiles. 1, SA 11; 2, Long pattern, control; 3, Short pattern, control; 4, Patient D, first infection; 5, Patient D, second infection; 6, Patient D, third infection.

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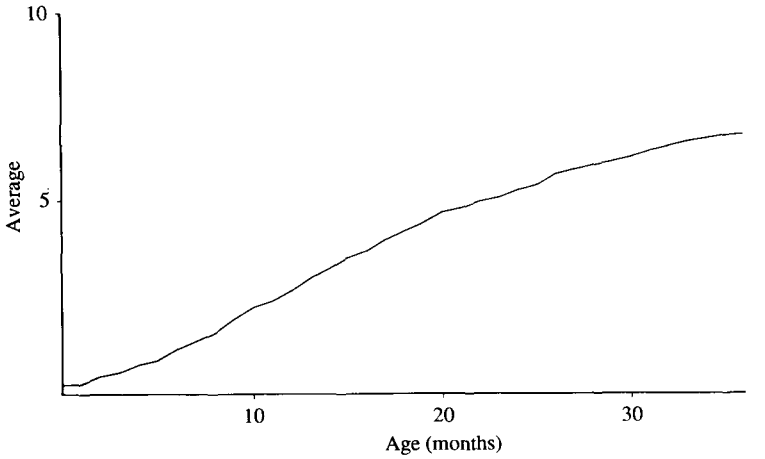

Fig. 5. Cumulative attack rate of diarrhoea by age in study children from Belém, Brazil.

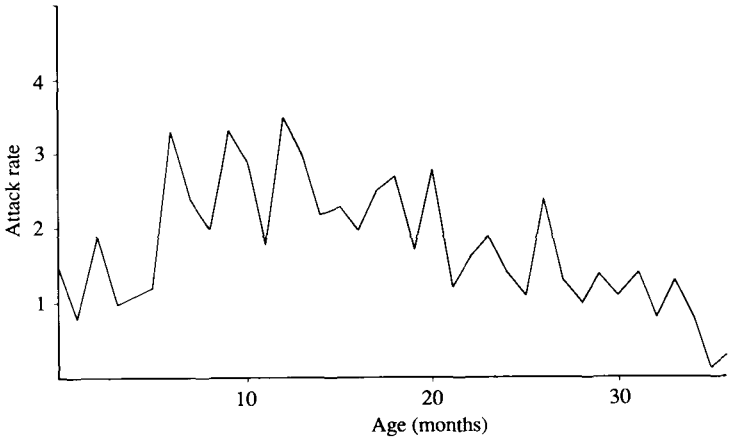

Fig. 6. Age-specific attack rate of diarrhoea in study children from Belém, Brazil.

Fig. 4 shows the electrophoretic patterns in the stools of a patient who had three successive infections by rotavirus. The long pattern was detected in all three infections, but the electrophoretypes were not identical.

The cumulative attack rate of diarrhoea is shown in Fig. 5. The average number of diarrhoeal episodes per child reached 1·09 at 6 months of age and by the end of the first year of life the average was 2·6 episodes per child. The average number of episodes of diarrhoea per child per year at the end of the 3-year study was 2·5.

The overall age-specific attack rate of diarrhoea is shown in Fig. 6. From 0 to 5 months of age the attack rate did not differ significantly between the age groups. There was a first peak at 6 months and, from this age to 20 months, the level is slightly higher than that of the 0–5 months age group. After this age the attack rate clearly decreases. Fig. 7 shows the age-specific attack rate of diarrhoea according to this type of feeding. In those children who were breast-fed there was

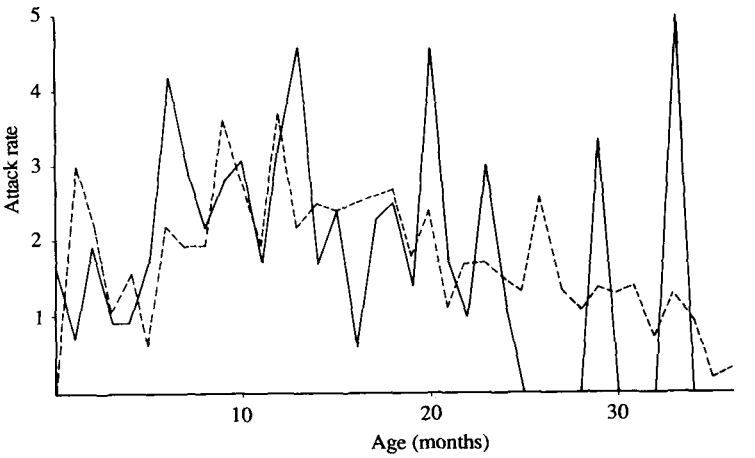

Fig. 7. Age-specific attack rates of diarrhoea in study children from Belém, Brazil, by method of feeding. ——, Breast-fed children; – –, bottle-fed children.

a peak at 6 months, whereas in the bottle-fed group the peak occurs earlier, at the age of 1 month.

From December 1982 to March 1986, 456 episodes of diarrhoea were recorded (Fig. 8), 36 (7·9%) of them associated with rotavirus. As births occurred from the end of November 1982 to the middle of March 1983, the number of children studied increased from the first to the fifth month of investigation, reaching a maximum of 82 in March and April; because some dropped out, there was a reduction to the final number of 70 in November 1985. Rotavirus-related episodes of diarrhoea occurred throughout the study period, reaching a maximum frequency in August 1984.

Fig. 9 correlates apparent and inapparent rotavirus infections among 60 rotavirus-positive children with either breast- or bottle-feeding and age. Inapparent infections developed in 24 (40%) of them, and 9 (37·5%) out of these were receiving maternal milk at the time of rotavirus infections. Symptomatic infection was noted in 36 (60%) of the children, and 13 (36·1%) out of these were being breast-fed during their illness. There were no apparent infections among children under 3 months of age. Fischer's exact test showed that maternal milk did not protect against clinical rotavirus disease. By this test, however, early rotavirus infection (i.e. before 4 months of age) was significantly more likely ($P = 0·02$) to be asymptomatic and that infection after 4 months was more likely to be symptomatic.

Other clinical symptoms were recorded in 437 episodes of diarrhoea and are shown in Table 4. These episodes were divided into two categories: those associated with rotavirus and those with other enteropathogens. In general specimens were collected within the first week of disease, and two thirds of them were obtained within 72 h after the onset of diarrhoea. The rotavirus-related episodes of diarrhoea were shown to be more severe than those of other aetiology. The percentages of watery stools, vomiting, nausea, abdominal pain, fever and dehydration were higher in the rotavirus-related infections than in those of other

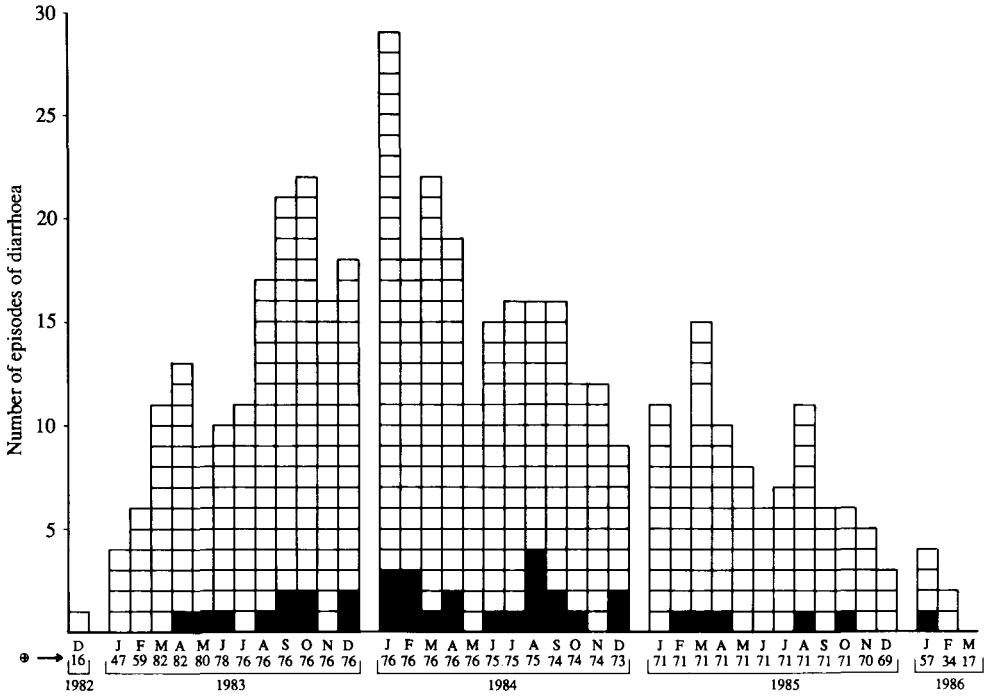

Fig. 8. Monthly frequency of diarrhoea episodes in study children from Belém, Brazil. ⊕, Number of children followed up until the end of the month; □, Non-rotavirus-related diarrhoea; ■, Rotavirus-related diarrhoea. This figure does not include 45 episodes reported by mothers from which we have no specimens, as follows: 10 in 1983, 23 in 1984, 12 in 1985.

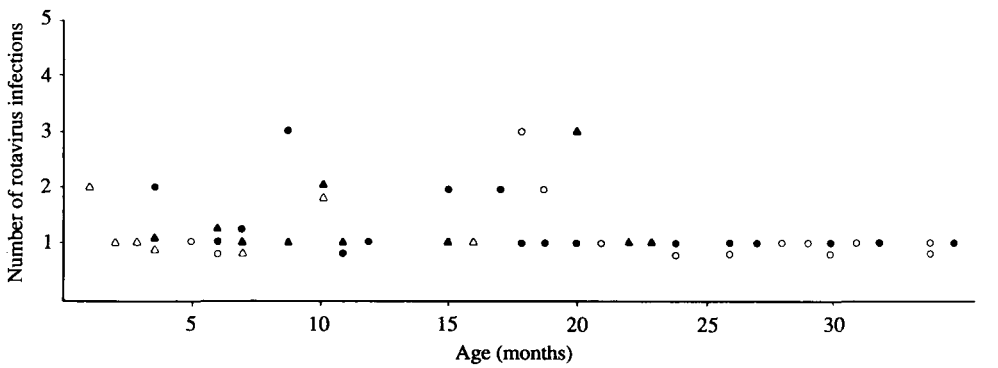

Fig. 9. Apparent and inapparent rotavirus infections among 60 children either breast- or bottle-fed, according to age groups. Apparent rotavirus infection: breast-fed, ▲; bottle-fed, ●. Inapparent rotavirus infection: breast-fed, △; bottle-fed, ○.

	Rotavirus infections	
	Early (< 4 months)	Later (⩾ 4 months)
Asymptomatic	4	20
Symptomatic	0	36

Fisher's exact $P = 0{\cdot}02$

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Table 4. *Clinical symptoms in 437 episodes of diarrhoea, according to the presence of rotavirus in faeces**

Episodes of diarrhoea	No.	Symptoms (%)								
		Diarrhoea	Evacuations per day†	Watery stools	Vomiting	Nausea	Abdominal pain	Fever	Respiratory symptoms	Dehydration (> 5%)
Rotavirus-‡ related	33	100	3·2	91·0	59·0	59·0	69·0	66·0	53·0	19·0
Other	404	100	2·7	76·0	32·0	32·0	53·0	35·0	48·0	3·0

* This table does not include three rotavirus-positive children who were not followed for clinical observation.
† Mean number.
‡ In 48·6% of episodes rotavirus was associated with other enteropathogens.

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Table 5. *Clinical symptoms in 12 cases of reinfections by rotavirus in children from Belém, Brazil*

Patient	Infection	Age (months)	Sub-group	Maternal milk	Symptoms (duration in days) Watery stools	Nausea	Vomiting	Abdominal pain	Fever	Dehydration (Degree)	Other enteropathogens***
A	1st	4	II	No	4	3	3	—	3	I	*Shigella sonnei* and enterotoxigenic (LT+) *E. coli*.
	2nd	20	II	No	6	5	5	5	—	II	
	3rd**	27	I	No							
B	1st	15	II	No	4	—	—	—	1	—	Fungi
	2nd	27	I	No	5	2	2	5	2	I	—
C	1st	8	II	Yes	7	6	6	—	6	I	—
	2nd	20	?	Yes	2	—	—	2	2	—	—
	3rd	35	Non-group A	No	3	1	1	—	—	—	*E. hystolitica*
D	1st*	1	II	Yes	—	—	—	—	—	—	—
	2nd	20	II	No	2	1	1	—	—	—	*E. hystolitica*
	3rd****	32	II	No	3	1	2	1	—	—	—
E	1st*	31	II	No							
	2nd*	34	II	No							
F	1st*	24	?	No							
	2nd*	28	II	No							
G	1st	6	II	No	2	2	2	2	2	—	—
	2nd	18	II	No	4	—	—	4	3	—	—
H	1st*	9	II	Yes							
	2nd	30	II	No							
I	1st	6	II	Yes	2	2	2	—	—	—	Fungi
	2nd	12	II	Yes	3	3	3	3	3	II	Enterotoxigenic (ST+) *E. coli*.
J	1st	9	II	No	3	—	—	3	1	II	*Salmonella typhimurium* *E. coli* LT+
	2nd*	31	II	No							
K	1st*	3	II	No							
	2nd**	17	II	No							
L	1st	7	II	Yes	5	—	—	5	5	I	—
	2nd	17	?	No	1	—	—	—	1	—	Fungi

*, asymptomatic; **, not followed for clinical observation ***, Bacteriological and parasitological examination were done only in diarrhoeal cases. ****, Bacteriological examination was not done. ?, Unclear electrophoretic profile.

aetiology. The mean number of evacuations per day and the frequency of respiratory symptoms did not differ significantly between the groups.

A comparison of the clinical severity associated with reinfections is made in Table 5. Among those with two successive rotavirus infections the first episode was found to be more severe in one child (L); the second infection was more severe in one child (B); and in two patients (G and I) the episodes were of comparable severity. In those who had three successive infections by rotavirus, it was observed that in one (A) the third infection was not recorded in detail and in other (D) the first infection was asymptomatic. In one case (C) three successive apparent infections involved a strain which was possibly a non-group A strain.

DISCUSSION

Previous studies carried out in our region (Linhares *et al.* 1983; Linhares *et al.* 1982) had dealt only with rotavirus gastroenteritis affecting hospitalized children. So, data concerning the natural history of rotavirus infections were not available until the present community-based longitudinal investigations were carried out.

Data on the incidence of enteropathogens clearly show that rotaviruses were more frequent among diarrhoeic children than in apparently normal ones.

The overall incidence of rotavirus was 7·6% in the diarrhoeal children and 0·4% in the control group. These results agree with those obtained from other workers in developing countries (World Health Organization Diarrhoeal Diseases Control Programme, 1984). However, they differ from the results of Guerrant *et al.* (1983), who recorded a prevalence of 19% in a similar community-based study carried out in North-eastern Brazil. In considering the role of other enteropathogens, our data should be examined in two periods. In the first (December 1982–December 1983) specimens only from diarrhoeal patients were examined, for bacteria and parasites with the aim of specific treatment. However, from the second half of December 1983 onwards, stools from a comparable number of non-diarrhoeal children (Table 2) were examined for both bacterial pathogens and parasites. During this period major enteropathogens such as rotavirus, Shigellae, enterotoxigenic *E. Coli* (ST+), *E. histolytica* and *S. stercoralis* were found more frequently among diarrhoeal children than in the control group. It is likely that similar results would have been observed in the earlier (December 1982–December 1983) study period.

Rotavirus was associated with other enteropathogens in 18 (50·0%) out of the 36 children suffering from acute diarrhoea in our study. This finding does not agree with that of Mata *et al.* (1983*b*), who found rotavirus to be the only pathogen present in two thirds of their patients with diarrhoea. The difference between these two sets of results may have been due to the fact that we did not have techniques for detecting campylobacters. In a previous hospital based study (Linhares *et al.* 1983) carried out in Belém, Brazil, rotaviruses alone were found in 69·7% of the diarrhoeal cases. In our present study an attempt was made to classify the electrophoretic patterns according to Rotacode (Moosai *et al.* 1984). Forty-six (76·7%) of the 60 rotavirus-positive specimens gave clear profiles, allowing them to be classified. The long pattern (subgroup II) was more frequent than the short pattern (subgroup I), and agrees with the results obtained in Rio de Janeiro by Pereira *et al.* (1983).

Reinfection by either similar or different subgroups in the same child suggests that different serotypes may be involved and, to confirm this, serotyping should be done; in addition, re-infections involving the same serotype cannot yet be ruled out. Sequential rotavirus infections have been described by other authors (Bishop *et al.* 1983; Fonteyne, Zissis & Lambert, 1978; Rodriguez *et al.* 1978). However more studies are needed to demonstrate whether the same serotype of rotavirus can produce repeated infections in the same child, and the results will be particularly relevant to the production of vaccines. One of our study children (C) suffered three successive symptomatic rotavirus infections, with the third episode being due to a possibly atypical strain. The specimen was clearly negative for rotavirus when tested by ELISA, although positive by electron microscopy, while electrophoresis of the RNA showed an electrophoretype different from those of conventional (group A) rotaviruses. In another part of Brazil, Pereira *et al.* (1983) found an atypical rotavirus in the faeces of a child suffering from acute gastroenteritis.

There were approximately 2·5 episodes of diarrhoea per child per year during the 3 years of our longitudinal study (Fig. 5). As rotaviruses occurred in about 8% of cases of gastroenteritis, the average number of rotavirus diarrhoea episodes per child per year was 0·2. Mata *et al.* (1983*b*) recorded an average of 0·8 episodes of rotavirus diarrhoea per child per year in a cohort studied in Guatemala.

The overall age-specific attack rate of diarrhoea in our study was higher in children over 6 months old than in those between birth and 6 months of age. It was also noted that if the age-specific attack rate of diarrhoea was related to breast-feeding, there is a initial peak at 6 months in the breast-fed children, while in bottle-fed infants it occurs at 1 month of age. This suggests a relationship between breast-feeding and susceptibility to diarrhoea during the first year of life.

There were 456 episodes of diarrhoea during the 3 year study in which faecal samples were obtained for laboratory examinations. In another 45 cases these specimens were not obtained, as illness occurred in the interval between home visits and mothers were unable to inform us by telephone or to bring the child to the laboratory. Rotavirus was detected in 36 (7·9%) of the 456 cases of acute diarrhoea investigated, and the results do not seem to show a seasonal pattern. These findings do not agree with those of Guerrant *et al.* (1983) who showed that rotavirus–related diarrhoea in North-eastern Brazil occurs more frequently between June and October (the drier, slightly cooler months of the year).

Sixty rotavirus infections occurred during our 3-year longitudinal study, of which 24 (40%) were asymptomatic and no illness occurred in children infected under 4 months of age. Although Totterdell *et al.* (1982) found that factors present in maternal milk from both antibody positive and negative mothers conferred protection against clinical rotavirus disease, Fischer's exact test indicated no protection ($P = 0{\cdot}21$) in the present investigation.

A comparison of clinical severities was made between rotavirus-related episodes of acute diarrhoea and those with other aetiologies as shown in Table 4. Watery stools, vomiting, nausea, abdominal pain, fever and dehydration were prominent symptoms in children suffering from rotavirus-associated gastroenteritis. The significant difference between the frequency of dehydration in the two categories

(19·0% in the rotavirus-related diarrhoeas and 3·0% in the non-rotavirus related ones) strongly support the use of the WHO rehydration formula (World Health Organization, 1985) in the treatment of children with rotavirus diarrhoea. As dehydration may cause death, particularly in malnourished infants, the development of a rotavirus vaccine will certainly make a significant contribution in reducing the mortality. No significant differences between the groups were recorded with respect to the incidence of respiratory symptoms. Evidence for rotavirus-associated invasive gastroenteritis, as previously described by Clemens *et al.* (1983), were not recorded by us.

In comparing clinical severities of different episodes in the same child, it was observed that the first infection (either apparent or inapparent) did not always confer protection against subsequent severe disease, although Bishop *et al.* (1983) have reported that infection by one strain is sufficient to give protection against severe gastroenteritis caused by reinfection with a heterologous strain.

In about 25% episodes of diarrhoea we were not able to detect any enteropathogens which could be regarded as the causative agent. This led us to postulate that other possible pathogens (mainly viruses), not detectable by the conventional techniques used, could be involved in the aetiology of acute gastroenteritis among children in our region. Campylobacters may have also caused diarrhoea in the study children, but no techniques for their detection are yet available in our laboratory.

This work was supported by both the World Health Organization, Diarrhoeal Diseases Control Programme, and Fundação Serviços de Saúde Pública, Ministério da Saúde. We are grateful to Dr T. H. Flewett for supplying us with the ELISA kits. We are indebted to Mr Antonio Francisco P. Martins and Mr Antonio de Moura for technical assistance. We wish to thank Dr Melinda Moore and Dr J. J. Shaw for their comments and criticism on the paper and to Mrs Maria de Conceição Mendes Chagas for typing the manuscript. Thanks are also due to both Bacteriology and Parasitology staffs of Instituto Evandro Chagas for providing technical assistance, as well as to the staff of Secretary of Health of Pará State for logistical support.

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