Prog. Fd. Nutr. Sci., Vol. 7, pp. 167–177, 1983
Printed in Great Britain.

0306-0632/83 $0.00 + .50

NATURAL HISTORY OF ROTAVIRUS INFECTION IN THE CHILDREN OF SANTA MARIA CAUQUE

L. Mata, A. Simhon, J. J. Urrutia and R. A. Kronmal

Instituto de Investigaciones en Salud (INISA), University of Costa Rica; Instituto de Nutrición de Centro América y Panamá (INCAP); Organización Panamericana de la Salud (OPAS); and School of Public Health and Community Medicine, University of Washington

ABSTRACT

A prospective observation of 45 cohort children from birth to three years of age permitted the collection of 5689 weekly stool specimens, along with frequent morbidity, dietary and growth data. Specimens tested by the ELISA showed that all children became infected with rotaviruses during the first three years of life, many repeatedly. The overall rotavirus incidence was 10.6 per 100 child-months (or 1.3 infections per child per year). Rotaviruses exhibited a high pathogenic potential estimated in 65%, but only about 10% of all diarrheas appeared associated with them. Rotaviruses occurred throughout the year but clustering was evident in August through December, with epidemics of greater severity in particular months of certain years. During outbreaks, from a third to one half of all children became infected with rotaviruses in a given month. This and the frequent association of rotaviruses with other enteric agents (34 %) suggest that fecal-oral transmission is the main source of infection, a net result of the prevailing low socioeconomic level in the village setting.

KEY WORDS: Rotavirus, diarrhea, breast-feeding, growth, longitudinal study, less developed society

INTRODUCTION

Many questions remain to be answered regarding the natural history of enteric infection, particularly in traditional and transitional populations where diarrheal disease and malnutrition exhibit their highest incidence. Such information can best be obtained by prospective observation of children in their natural ecosystem, through application of adequate field and laboratory

Reprints from: Dr. L. Mata, Instituto de Investigaciones en Salud (INISA), University of Costa Rica, Ciudad Universitaria Rodrigo Facio, Costa Rica

procedures. A data file and a bank of weekly fecal specimens belonging to a cohort of children from the village of Santa María Cauqué (1), offered the unique opportunity to learn about the incidence of rotaviruses and other pathogenic agents, and their interrelation with diarrheal disease and the nutritional status. This knowledge is fundamental to assess the relative importance of rotavirus infection in the overall diarrheal disease and malnutrition problem, and for logical recommendation of measures for prevention and control of infection in the community.

FIELD AND LABORATORY PROCEDURES

The study was based on 45 cohort children from the Cakchiquel village of Santa María Cauqué in the Guatemalan Central Highlands (1). The village is traditional inasmuch as poverty, illiteracy, low socioeconomic level, and low hygiene and environmental sanitation prevail. Almost all deliveries occurred in the home in the squatting or kneeling position and maternal-infant bonding and breast-feeding was effected in all cases (1). Exclusive breast-feeding extended for 6 to 9 months, and weaning was protracted and ended between 15 and 49 months of age, with a mode around 27 months (1, 2).

At the beginning of the study, the population was about 1100 inhabitants in 200 families crowded in the village. Infant mortality was 93 per 1000 live births and 1-4 yr mortality was 39 per 1000 (1). Population growth was 3 % per year during the study period, 1963-1969, and growth or attrition by migration was negligible. The cohort represented one half of all children born between February 1964 and January 1966, and was chosen at random from survivors present at the beginning of 1965. Two children died (Nos. 67 and 77) and one withdrew (No. 43) in their third year of life. No differences were noted in relative frequencies of preterm and small-for-gestational age newborns, or in physical growth and infant and preschool mortalities, between the cohort and the other children born during the recruitment period (1).

Clinical studies

The field procedures used in the Cauqué Study have been described in detail elsewhere (1). Weekly home visits provided good information on breast-feeding and food supplementation, physical growth, morbidity, and survival. Illnesses appeared in rather complex episodes, often formed by several morbidity events linked with others. One day or more without disease separated two different morbidity events; however, diarrheal diseases and other illnesses generally were well separated from each other. Due to prevailing scientific knowledge and ethical considerations at the time of the study, no antimicrobial treatment was provided for diarrhea. Children were breast-fed through bouts of diarrhea, and oral fluids available at that time were recommended by the field staff. Occasionally, intravenous fluid therapy was indicated, but this and other practices of Western medicine were not readily accepted by the villagers (1).

Laboratory studies

Fecal specimens were collected weekly from each child during the first three years of life or longer. Specimens generally brought to the field laboratory within one hour of evacuation, were made into 10-20 % suspensions in Hanks' solution with skimmed milk and antibiotics. Suspensions were stored at -15°C for the rest of the day, and were then kept frozen at -70°C. Suspensions were clarified by two cycles of cold centrifugation at 1100 x g for 15-30 minutes (1). Supernatants were stored frozen at -70° or -85°C and were thawed at least four times since the time of preparation (1964-1969).

Screening ELISA. All fecal supernatants were tested for rotavirus antigen by an ELISA (3, 4). Flexible U-bottom Microtiter plates (Dynatech Cat. 1-220-24, Lot M12, Alexandria, Va.) were coated with NIH's goat anti-human rotavirus antiserum (Goat 930, 10th week p.i.) at a dilution of 1:10,000, and plates were incubated overnight at 4°C. After this and subsequent steps, plates were washed thrice with PBS containing 0.05 % Tween 20 (PBS-T). One percent BSA in PBS-T (Fraction V, devoid of rotavirus antibody) was added to each well and plates were incubated at 37°C for 30 min. Fecal supernatants were added to wells (50 ul/well) containing 50 ul of PBS-T plus 0.5 % BSA and 0.5 % normal goat serum (PBS-T-BSA-NGS). A pool of rotavirus-positive fecal supernatants adjusted to give a final absorbance of 1.0 was included as control in each plate. A pool of rotavirus-negative supernatants (absorbance < 0.1) served as control. A saline control was also included. After overnight incubation at 4°C, guinea pig anti-human-rotavirus-type 2 hyperimmune serum (NIH V-710-501-558) diluted 1:5,000 in PBS-T-BSA-NGS was added to each well and plates were incubated at 37°C for one hour. Anti-guinea pig-alkaline phosphatase conjugate diluted 1:500 in PBS-T-BSA-NGS was added to each well and plates were incubated at 37°C for one hour. Conjugates were prepared using rabbit anti-guinea pig globulins (Dako Cat.Z108, Lot 077, Copenhagen) and alkaline phosphatase (Sigma type VII Cat. P4502, Lot 30F-0161, St. Louis, Mo.). Sigma 104-105 substrate diluted in diethanolamine buffer was allowed to react for 30 min at 37°C, stopping the reaction with 3N NaOH. Visual reading was made by two independent workers. Specimens with a color equivalent to 0.2 absorbance or more were considered presumptive positive.

Confirmatory ELISA: Steps were identical to those described for the screening ELISA except that alternate rows of wells were coated with NIH's preimmunization Goat 930 serum and Goat 930 hyperimmune serum, both diluted 1:10,000. Fecal supernatants were added to both preimmune and hyperimmune antiserum-coated wells. Confirmed rotavirus positives were those giving at least twice the absorbance in hyperimmune serum-coated wells as in preimmune serum-coated wells. Specimens yielding the same color in both sets of wells were considered false positives. Rotavirus virions were visualized in selected ELISA-confirmed positives, by examining supernatants in formvar coated-grids stained with phosphotungstic acid, examined in a Hitachi H300 transmission electron microscope (TEM). Such specimens had been frozen 13-18 years.

Fecal specimens were also investigated for enteric bacteria (obviously excluding enterotoxigenic Escherichia coli, Vibrio and Campylobacter), intestinal parasites and cultivatable enteric viruses (1). Clinical, anthropometric and microbiologic data were displayed and summarized by the Conversational Computer Statistical System (CCSS) (5). A rotavirus event was defined as a weekly sample positive for rotavirus antigen. Two different rotavirus events were considered independent if they were separated by at least three weekly specimens negative for rotavirus antigen. Prevalence (all rotavirus events) and incidence (new independent rotavirus events) were expressed per 100 child-weeks or child-months. A rotavirus event was assumed associated with diarrhea if it appeared one week before, during, or one week after the diarrheal episode.

RESULTS

Rotavirus infection in the first week of life

The frequency of rotavirus infection at or shortly after birth was studied by testing meconium and feces collected in the first week of life. Specimens were available for about one half of the 45 cohort children in days first to seventh; only one of the samples (feces collected on the second day of life) was

positive for rotavirus antigen, and this corresponded to the only infant born in a hospital. The prevalence in the cohort was 2.2 % for the first week of life, and 0.54 % for the total number of specimens collected in the first week of life, namely 185.

Rotaviruses in the first three years of life

The shedding of rotaviruses by the 45 cohort children according to the weekly sampling was as in Figure 1. All children shed rotaviruses during the first three years: one child (No. 91) had only one rotavirus event; ten had two; eleven had three; nine had four; eight had five; four had six; one had seven; and one child (No. 59) had eight rotavirus events. Most rotavirus-positive weeks were well separated from each other, but sometimes two or three rotavirus-positive weeks were found in proximity. No significant differences in rotavirus excretion were noted between term and preterm and small-for-gestational age infants.

Prevalence and incidence of rotavirus events

It was assumed that two or more rotavirus events (positive weeks) separated by no more than two rotavirus-negative weeks were related, that is, they were the same infection. Eleven children (22 %) had infections lasting two weeks; six (13 %) lasting three weeks; four (9 %) lasting four weeks; and two (4 %) had infections lasting five weeks.

The number of children at risk and the corresponding number of expected and observed weeks (those tested for rotavirus antigen) are summarized in Table 1. According to the number of children in each age period, 6890 weeks were expected, but about 10 % of the weekly specimens could not be collected, and an additional 10 % were not available for ELISA testing because the material was insufficient, or because the specimens had been removed for other studies and were not readily available. The highest number of observed weeks, 86.5 %, corresponded to the first trimester of life, and the lowest, 77.3 %, to the

TABLE 1

Prevalence and Incidence of Rotavirus Events, Cauqué Children Observed from Birth to Three Years of Age, 1964-1969

Age in weeks	Number of children	Number of child-weeks		Number of rotavirus events and (rate per 100 child-weeks)		Ratio: prevalence/ incidence
		Expected	Observed(%)*	Prevalence**	Incidence***	
0-12	45	585	506(86.5)	7(1.38)	5(0.99)	1.4
13-25	45	585	480(82.0)	7(1.46)	7(1.46)	1.0
26-38	45	585	476(81.2)	10(2.10)	10(2.10)	1 0
39-51	45	585	485(82.9)	21(4.33)	16(3.30)	1.3
52-64	45	585	452(77.3)	24(5.31)	18(3.98)	1.3
65-77	45	585	462(78.8)	13(2.81)	11(2.38)	1.2
78-90	45	585	466(79.7)	19(4.08)	14(3.30)	1.2
91-103	45	585	477(81.5)	17(3.56)	15(3.14)	1.1
104-116	43	559	474(84.8)	20(4.22)	16(3.38)	1.2
117-129	43	559	483(86.4)	11(2.28)	10(2.07)	1.1
130-142	42	546	465(85.2)	13(2.80)	12(2.58)	1.1
143-155	42	546	463(84.8)	7(1.51)	6(1.30)	1.2
Total		6890	5689(82.6)	169(2.97)	140(2.46)	1.2

* In parentheses percent of the expected weeks

** Computed using all rotavirus-positive weeks in the period

*** Computed using only new rotavirus-positive weeks in the period

52-64 weeks-of-age period (Table 1). Overall, observed weeks represented 82.6 % of the expected weeks, a suitable number to describe epidemiological phenomena.

The prevalence and incidence of rotavirus events, expressed as rates per 100 child-weeks in Table 1 were low in the first six months and at the end of the third year, and high between 6 and 30 months of age. Rates became higher as

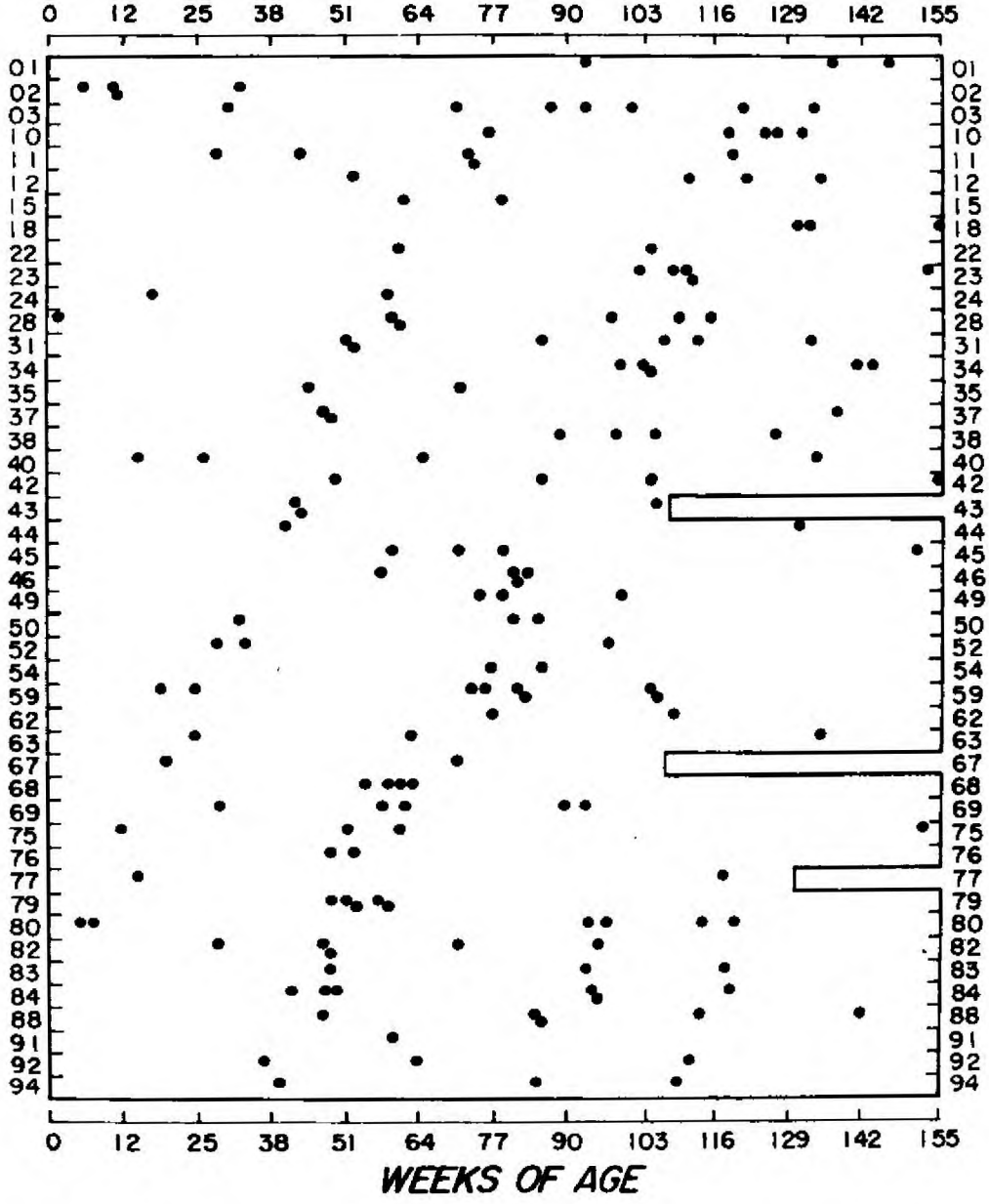

FIG. 1. Rotaviruses revealed by testing 5689 weekly stool specimens from 45 cohort children. Each dot is a week positive for rotavirus antigen. All children became positive at least once during the study period.

weaning began in the second semester, and attained the highest values in the second year of life when children eventually left the breast. Prevalence/incidence ratios fluctuated around 1.1 indicating the acuteness of the process and a lack of persistence of the virus at detectable levels (Table 1).

Seasonality of rotavirus infection

The cohort was recruited during two chronological years, February 1964-January 1966, and the total period of observation then extended to January 1969. The data shows a clustering of rotavirus events in certain months, evident in Figure 1. Monthly rotavirus events were expressed as rates per 100 child-months. To compute rates, different denominators were used as the number of children present in each month varied. Due to the small number of children at risk in 1964 and 1969, the following description applies only to the period 1965-1968. The highest prevalence of rotavirus events was observed in August-December of 1966, Table 2. Almost one half of all children were infected in September (20 out of 45 children) and in November (21 out of 45); again, in August of 1967, 15 of 38 children (40 %) had rotavirus events. However, the attack rate was nil or low in September-December of that year, and in the corresponding months of 1968, likely because all cohort children were at least two years old and had already been infected with rotaviruses. The epidemic in January-February of 1968 was unusual in that it affected one fifth of children older than two and a half years of age. The monthly global prevalence, also in Table 2, shows that 70.4 % of all rotavirus events occurred in the period August-December. January and February seem to reflect the aftermath of an exhuberant virus transmission in the preceeding months.

TABLE 2

Prevalence of Rotavirus Events by Year and Month,
Cauqué Children Observed from Birth to Three Years of Age

Month	1964	1965	1966	1967	1968**	Total
January		1(5.9)*	3(6.7)	1(2.2)	5(20.0)	10(7.5)
February	0	1(4.8)	2(4.4)	6(13.9)	5(22.7)	14(10.6)
March	1(33)	0	1(2.2)	4(9.5)	0	6(4.5)
April	1(33)	1(4)	4(8.9)	0	0	6(4.5)
May	1(33)	1(3.7)	1(2.2)	2(4.8)	0	5(3.7)
June	0	0	3(6.7)	0	0	3(2.2)
July	0	3(10)	0	3(7.7)	0	6(4.5)
August	0	0	6(13.3)	15(40.5)	1(7.7)	22(16.7)
September	1(12)	0	20(44.4)	2(5.4)	1(9.1)	24(17.9)
October	2(15.4)	4(10.8)	13(28.9)	0	0	19(14.6)
November	0	12(29.3)	21(46.7)	0	0	33(24.4)
December	0	11(25.0)	8(17.8)	2(6.7)	0	21(15.5)
Total	6(8.2)	34(9.6)	82(15.2)	35(7.6)	12(7.3)	169(10.6)

* Number of rotavirus events (rate per 100 child-months)

** Only one child was studied in January 1969 and rotaviruses were not detected. These data are excluded from table.

The mean minimum temperature and the mean percent humidity were greater during August-November than in the remainder of the year. There were no marked differences or a trend in other variables (rainfall, and maximum and mean temperatures) and rotavirus seasonality.

Association of rotaviruses with diarrheal disease

The incidence of diarrhea in Santa María Cauqué during the study period ranged from 33.4 per 100 child-months during the first six months of life, to 87.4 per 100 child-months in the fourth semester (Table 3). Thereafter, the incidence slowly declined to 55 per 100 child-months in the second half of the third year of life. Overall, the rate of diarrhea and dysentery was 792.4 per 100 child-years, which is equivalent to 7.9 attacks per child per year (1).

The numbers and corresponding rates of new rotavirus events by semester of life are shown in Table 3. As indicated above, 17 % of the specimens were not available; thus, rates were adjusted by dividing by the percent of expected samples. The adjusted rates of rotavirus-associated diarrhea and of rotavirus-not associated with diarrhea are also in Table 3. Evidently, the number of diarrheal episodes associated with rotaviruses was relatively small, and it was no more than 11.9 per 100 child-months during the third semester. For the first

TABLE 3

Incidence of Diarrhea and of Rotavirus Events Associated with Diarrhea, 45 Cauqué Children Observed from Birth to Three Years of Age

Incidence per 100 child-months	Age in months					
	0-5 N=270*	6-11 N=270	12-17 N=270	18-23 N=270	24-29 N=255	30-35 N=250
Diarrheal disease	33.4	63.0	77.8	87.4	78.0	55.0
Observed rotavirus events** associated with diarrhea	6(2.2)***	18(6.7)	25(9.3)	20(7.4)	14(5.5)	8(3.2)
Observed rotavirus events not associated with diarrhea	6(2.2)	8(3.0)	4(1.5)	9(3.3)	12(4.7)	10(4.0)
Adjusted**** rotavirus events associated with diarrhea (a)	(2.6)	(8.2)	(11.9)	(8.6)	(6.4)	(3.8)
Adjusted rotavirus events not associated with diarrhea (b)	(2.6)	(3.7)	(1.9)	(4.1)	(5.5)	(4.7)
Ratio a/b	1.0	2.2	6.3	2.1	1.2	0.8

* Number of child-months

** It refers only to new rotavirus events (incidence) in age group

*** Number of events (incidence per 100 child-months)

**** Adjusted to account for specimens not collected or not available (see text)

three years of life, the total incidence of rotavirus-associated diarrhea was 83.0 episodes per 100 person-years. This rate represents 0.8 rotavirus-associated diarrhea episodes per child per year, which is equivalent to about 10 % of all diarrheas. Nevertheless, the number of rotavirus events associated with diarrhea was larger (65 % of the total) than that of rotavirus events not associated with diarrhea (35 %), indicating the distinct pathogenic capacity of rotaviruses. The pathogenic potential varied with age. It was less in the first, fifth and sixth semesters; and greatest in the third semester of life, as revealed by the ratio 6.3 (Table 3).

Enteric infection and growth

Rotavirus infection is just antoher pathogen among several viruses, bacteria and parasites which often induce acute or chronic diarrhea and malnutrition (6). Even though rotavirus infections were short-lived, more than one half of them coincided with bouts of diarrheal disease and with periods of weight loss or growth stagnation.

Multiple infections

Two thirds of all rotavirus events appeared singly. The rest were double infections (21 %) mainly with Giardia; or triple infections (13 %), mainly with Giardia, Shigella and E.histolytica (Table 4).

TABLE 4

Etiologic Association in All Rotavirus Diarrhea,
45 Cauqué Children Observed from Birth to Three Years of Age

Agent	Age in months						
	0-5	6-11	12-17	18-23	24-29	30-35	Total
Rotavirus only	6	16	18	12	6	2	60
Rotavirus with:*							
Giardia		1	4	3	3	1	12
Shigella			2	1			3
E.histolytica					1	1	2
Salmonella				1			1
Enteropath. *E.coli*(E.E.C.)					1		1
Giardia & Shigella			1	2	1	2	6
E.histolytica & Shigella						2	2
Giardia & E.histolytica					1		1
Shigella & Salmonella					1		1
Giardia & E.E.C.				1			1
Shigella & E.E.C.		1					1
Total	6	18	25	20	14	8	91

* With two agents = 19 cases; with three = 12

DISCUSSION

The Cauqué study, a long-term prospective field observation of a typical traditional village of remarkably constant health and demographic indicators, permitted description of the natural history of enteric infection through weekly fecal sampling of children in health and disease. Early neonatal rotavirus

infection was extremely low as only one child was positive (on day 2 of his life) and this child was the only one born in a hospital. Contrastingly, neonatal rotavirus infection has been described as endemic in Great Britain and Australia (7, 8). This finding in the Indian neonates is even more remarkable in that abundant soiling of babies with mothers' feces occurs in the home deliveries which usually are in the kneeling or squatting position. Infants infected with rotaviruses during the first trimester of life were not protected from infection or symptoms later on. All cohort children were infected at least once in the first three years of life, and rates were generally greater than those described in Canadian and Bangladeshi cohort children examined with comparable techniques (9, 10).

Rotaviruses were associated with only about 10 % of all diarrheal disease episodes. This may be an underestimate, as some antigen could be present in concentrations not detectable by the ELISA, or it may be bound to maternal milk immunoglobulins or other factors in these intensively breast-fed children. However, the pathogenic potential of rotaviruses was relatively high inasmuch as 65 % of all new rotavirus events (incidence) was associated with diarrhea. This accounted for similar incidence and prevalence rates which then could be used interchangeably in rotavirus epidemiology, a finding of potential interest in other field studies. Rotavirus diarrhea was recognized in a previous analysis of the Cauqué data as a very severe illness in children under two years of age (11) It should be kept in mind, however, that enterotoxigenic bacteria and Campylobacter were not investigated, and some of the rotavirus diarrheas might have been associated with these agents. In fact, 34 % of all rotavirus infections were associated with other enteropathogens. Rotavirus events were fewer in the first and sixth semesters of life, probably reflecting some protection derived from breast-feeding (12) and development of active natural immunity (13). However, such immunity did not prevent the occurrence of repeated infections, a common phenomenon in Cauqué children, suggesting existence of several rotavirus serotypes and subgroups.

Infections also increased during particular epidemic situations. In 1966 and 1967, outbreaks occurred between September and December in which 50-60 % of all cohort children became infected with rotaviruses. Peaks did not coincide with lowest minimum temperatures as has been described in industrialized countries with temperate climate (14, 15). In fact, there was no correlation with climactic variables, except high relative humidity, or with concurrent respiratory illness. On the other hand, the frequent association of rotaviruses with other enteric agents suggests fecal-oral transmission, favored by the prevailing poor personal hygiene and environmental sanitation.

Rotavirus diarrhea appeared positively correlated with periods of growth faltering or stagnation; many episodes, however, were related to other enteropathogenic agents.

The results here presented suggest that control and prevention of diarrheal disease ultimately depend on implementation of holistic interventions affecting personal and home hygiene, environmental sanitation, education and income.

ACKNOWLEDGMENTS

The authors will be always in debt with the families of Santa María Cauqué for their willing participation in the study. The goat and guinea pig anti-human rotavirus antisera were kindly provided by Drs. A. Z. Kapikian and R. G. Wyatt. The authors acknowledge the collaboration of the following staff from

INCAP (Bertha García, Olga Román, Raúl Fernández, and Roberto Rosales) and INISA (Guillermo Vargas, Roberto Padilla, Jorge Quesada and J. César Gamboa). The study was aided, in part, by the International Development Research Centre (IDRC), Ottawa, Canada.

REFERENCES

1. MATA, L.J. The Children of Santa María Cauqué. A Prospective Field Study of Health and Growth. The MIT Press, Cambridge, Mass., 1978.

2. MATA, L., MURILLO, S., ALLEN, M.A., JIMENEZ, P. and GARCIA, B. Child feedings in less developed countries: induced breast-feeding in a transitional society. In: Clinical Disorders in Pediatric Nutrition. F. Lifshitz (ed). Marcel Dekker Inc., N.Y. 1982, pp. 35-53.

3. YOLKEN, R.H., KIM, H.W., CLEM, T., WYATT, R.G., KALICA, A.R., CHANOCK, R.M. and KAPIKIAN, A.Z. Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. Lancet 2: 263-266, 1977.

4. KAPIKIAN, A.Z., YOLKEN, R.H., GREENBERG, H.B., WYATT, R.G., KALICA, A.R., CHANOCK, R.M. and KIM, H.W. Gastroenteritis viruses. In: Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. E.H. Lennette and N.J. Schmidt (ed). American Public Health Association, Washington, D.C. 1979, pp. 927-995.

5. KRONMAL, R.A., BENDER, L. and MORTENSEN, J. A conversational statistical system for medical records. J. Roy. Stat. Soc. 19: 82-92, 1970.

6. MATA, L., JIMENEZ, P., ALLEN, M.A., VARGAS, W., GARCIA, M.E., URRUTIA, J.J. and WYATT, R.G. Diarrhea and malnutrition: breast-feeding intervention in a transitional ponulation. In: Acute Enteric Infections in Children. New Prospects for Treatment and Prevention. T. Holme, J. Holmgren, M.H. Merson and R. Mollby (ed). Elsevier/North Holland, 1982, pp. 233-251.

7. CHRYSTIE, I.L., TOTTERDELL, B.M. and BANATVALA, J.E. Asymptomatic endemic rotavirus infections in the newborn. Lancet 1: 1176-1178, 1978.

8. BISHOP, R.F., CAMERON, D.J.S., VEENSTRA, A.A. and BARNES, G.L. Diarrhea and rotavirus infection associated with differing regimens for postnatal care of newborn babies. J. Clin. Microbiol. 9: 525-529, 1979.

9. GURWITH, M., WENMAN, W., HINDE, D., FELTHAM, S. and GREENBERG, H. A prospective study of rotavirus infection in infants and young children. J. Infect. Dis. 144: 218-224, 1981.

10. BLACK, R.E., BROWN, K.H., BECKER, S., ALIM, A.R.M.A. and HUQ, I. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II. Incidence of diarrhea and association with known pathogens. Am. J. Epidemiol. 115: 315-324, 1982.

11. WYATT, R.G., YOLKEN, R.H., URRUTIA, J.J., MATA, L., GREENBERG, H.B., CHANOCK, R.M. and KAPIKIAN, A.Z. Diarrhea associated with rotavirus in rural Guatemala: a longitudinal study of 24 infants and young children. Am. J. Trop. Med. Hyg. 28: 325-328, 1979.

12. YOLKEN, R.H., WYATT, R.G., MATA, L., URRUTIA, J.J., GARCIA, B., CHANOCK, R.M. and KAPIKIAN, A.Z. Secretory antibody directed against rotavirus in human milk - measurement by means of enzyme-linked immunosorbent assay. J. Pediat. 93: 916-921, 1978.

13. GHOOSE, L.H., SCHNAGL, R.D. and HOLMES, I.H. Comparison of an enzyme-linked immunosorbent assay for quantitation of rotavirus antibodies with complement fixation in an epidemiological survey. J. Clin. Microbiol. 8: 268-276, 1978.

14. KAPIKIAN, A.Z., KIM, H.W., WYATT, R.G., CLINE, W.L., ARROBIO, J.O., BRANDT, C.D., RODRIGUEZ, W.J., SACK, D.A., CHANOCK, R.M. and PARROTT, R.H. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. N. Engl. J. Med. 294: 965-972, 1976.

15. KONNO, T., SUZUKI, H., IMAI, A., KUTSUZAWA, T., ISHIDA, N. and ADACHI, M. A long term survey of rotavirus infection in Japanese children with acute gastroenteritis. J. Infect. Dis. 138: 569-576, 1978.

Impreso en la Oficina de Publicaciones de la Universidad de Costa Rica