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DIARRHOEAL DISEASE IN A COHORT OF GUATEMALAN VILLAGE CHILDREN OBSERVED FROM BIRTH TO AGE TWO YEARS

L. J. MATA, J. J. URRUTIA AND J. E. GORDON
Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.
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INTRODUCTION

For a decade the Institute of Nutrition of Central America and Panama (INCAP) has conducted field studies on the interrelationships between infectious disease and malnutrition in Guatemalan infants and young children. Acute diarrhoeal disease is the leading cause of death during early childhood in Guatemala[1]. Protein-calorie malnutrition, kwashiorkor, also ranks high, its importance largely unrecognized because reporting of deaths is by lay officials unfamiliar with that disease as a cause of death[2]. That protein-calorie malnutrition was commonly precipitated by an attack of diarrheal disease in an already malnourished child was a significant finding[3].

Studies have established the principle of an interlocking relationship between diarrhoeal disease and a depleted nutritional state, a synergism especially manifest in early childhood and intimately associated with the weaning process[4]. A recently completed five-year field study in Guatemala dealt with diarrhoeal disease in preschool children as it occurred in the context of their family and community associations[5]. The information gained proved useful in public health action toward control of the immediate situation. An arresting conclusion was that, to understand the fundamental issue of what brought these situations into being, something more was needed on the developing resistance to repeated attacks of diarrhoea; on the progressive deterioration of the nutritional state, and on the combined effect of nutrition and infection on growth and development. The indicated means was a long-term, prospective study of an identified and fixed population at ages where impact was greatest.

As a consequence, investigation turned from the mass community effect of diarrhoeal disease within a prescribed time to the sequential evolution of malnutrition and infectious disease within a smaller group of persons continuously and carefully observed from birth to a projected five years of age, and especially during the critical early period of growth and development[6]. This is a report on the results of that investigation as the children reached two years of age.

STUDY AREA AND STUDY PLAN

Santa María Cauqué, a primitive village of 1125 persons in the Guatemalan highlands, is 36 km from Guatemala City along the Pan American Highway. Although the villagers are in frequent communication with the city, the community itself is

semi-isolated. The population is predominantly Mayan Indian. Most adults are illiterate and observe poor personal hygiene. Ancient customs and habits are slow to change. The village had no electricity. Spring water is piped to a central reservoir and to public faucets scattered through the village. Household supplies consequently are limited, for water has to be carried to homes in earthen jars. Few households have an individual water supply. Disposal of faeces and other wastes is inadequate. Childbirth is under primitive conditions, as is the rearing and feeding of children.

Field headquarters were established in a central village clinic, built to provide facilities for medical care, for a laboratory, and as a base for field workers. The study plan was to follow, in a cohort of children, all cases of acute diarrhoeal disease occurring from birth onwards.

From February 1964 to February 1966, 95 live births and 11 stillbirths occurred in the village corresponding with a live birth rate of 43‰. With two exceptions, all were attended by either one of the two folk midwives, both of whom followed the traditional procedures of child delivery. Including the first child, 84 of the 95 babies have been followed for varying times, 82 of them from birth. Nine were lost by death and five within the neonatal period. One infant migrated from the village and 29 were discharged from the cohort, either because of unsatisfactory cooperation by the parents or to adjust to numbers that the field and laboratory staff could manage efficiently, the aim being to have representative numbers by month of birth. The result was a cohort of 45 babies, or approximately one half of the number born during the two-year period.

METHODS

Case finding of impending births was by daily home visits of the study staff throughout the village and through cooperation of the village midwives who notified the field station of expected deliveries in advance *(Fig. 1)*. The result was that most pregnant women were interviewed and examined at the clinic well before the expected date of delivery At least one of the staff nurses living at the clinic was present at each birth. She recorded the result and traditional practices in childbirth, but took no part in deliveries except in emergencies and on request of the midwife.

Infants were examined by a physician within 18 hours of birth, then weekly during the first month, and fortnightly thereafter. Weight, height, and head circumference were measured at birth, at each subsequent visit during the first year, and then monthly *(Fig. 2)*. Weekly home visits by a dietician yielded information on food intake and feeding practices, and similarly by a nurse, on illnesses and injuries. Events of clinical significance, if not already reported, were referred to the clinic physician, who visited the patient daily or every other day until recovery or death.

Samples of faeces were collected weekly, with additional specimens during diarrhoeal attacks. Specimens were processed at the field laboratory within an hour after evacuation. The laboratory was equipped with a kerosene incubator, a propane gas refrigerator, and other facilities suited to field conditions.

Suspensions of faeces were prepared for investigation of intestinal parasites. Agar and broth media were inoculated for identification of enteric pathogenic bacteria; and suspensions were made in a preservative solution for investigation of enteroviruses

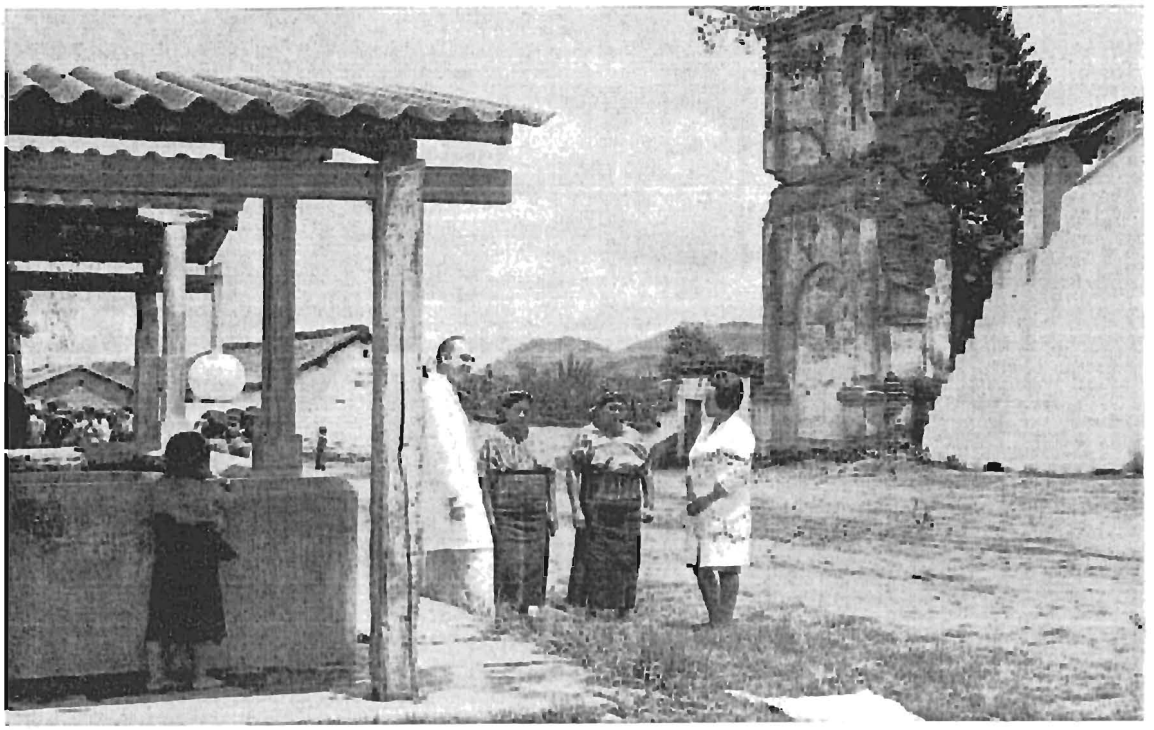

Fig. 1. A casual consultation in the village square with the two local midwives about impending births in the community.

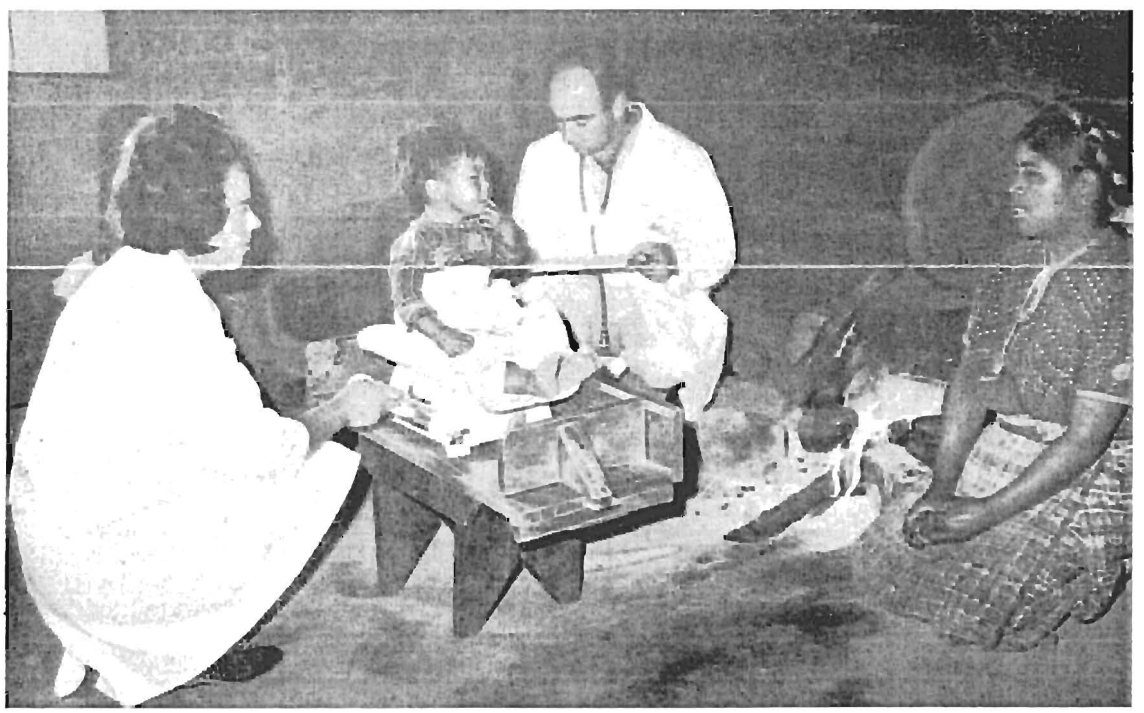

Fig. 2. Home examination of a village child by physician and nurse; the mother attends.

and adenoviruses. These and other more detailed examinations were made at the base laboratory in Guatemala City.

Based on clinical familiarity with the diarrhoeas of this region, diarrhoeal disease in the present study has been defined *as an illness associated with four or more abnormal bowel movements within 24 hours*. A liquid or semi-liquid consistency or the

presence of blood and mucus were criteria which independently or together characterized stools as abnormal. Less than four stools per day also was considered indicative of diarrhoea if blood and mucus were present, or if evacuations were liquid or semi-liquid, voluminous and violently expelled. Mushy or soft faeces in the required numbers in a child usually passing formed stools also was considered as diarrhoea. The essential feature was a significant departure from the normal pattern of bowel movement, in number and in character of stools.

A new case of diarrhoea dated from the first recognized abnormality of stools after at least 15 preceding days of normal bowel habit. One or more days without diarrhoea, but not more than 15 days, were included in the episode (case) when they came between bouts of clinically definite diarrhoea. A diarrhoeal attack was considered terminated when stools were three or less per day and no recurrence was noted within the succeeding two weeks.

Duration of diarrhoea was calculated as actual days during which diarrhoea was present, except that periods of one or two days with less than four bowel movements, but more than the usual number, and followed again by diarrhoea, were included. When a period of normality extended for two to 14 days with again a recurrent diarrhoea, only the initial day or days of diarrhoea in that period were considered in the computation. Diarrhoea lasting *from one to 14 days* was classed as *acute* diarrhoeal disease; *chronic* diarrhoeal disease was of *15 or more days* duration.

RESULTS

Age specific incidence of diarrhoeal disease. Although appreciable, the incidence of diarrhoeal disease during the first three months of life was less than at any other time within the first two years, *Table I:* 38 episodes occurred among 82 children during that period to give an attack rate of 4.1 cases per 100 person-weeks at risk, with weeks at risk taking into account losses through death or other circumstance.

Incidence increased progressively with advancing age to give values in the second year twice those of the first three months. The observed attack rates of 5.7 per 100

TABLE I

DIARRHOEAL DISEASE AMONG INFANTS OF A COHORT OF 82, BIRTH TO AGE TWO YEARS
Santa María Cauqué, 1964–1966

Age (weeks)	*Number of infants*	*Person-weeks at risk*	*Diarrhoeal Disease*	
			Number of cases	*Cases per 100 person-weeks*
0–12	82	934	38	4.1
13–25	70	844	48	5.7
26–38	61	759	49	6.5
39–51	52	578	42	7.3
First year of life		3115	177	5.7
52–64	38	367	33	9.0
65–77	29	307	26	8.5
78–90	19	191	17	8.9
91–103	12	101	9	8.9
Second year of life		966	85	8.8
Total		4081	262	6.4

TABLE II

DURATION OF ACUTE AND CHRONIC RECURRENT DIARRHOEAL DISEASE, BY AGE
Santa María Cauqué, 1964–1966

Age of child (weeks)	*Number of cases*	*Duration in Days*					
		1–7		*8–14*		*15+*	
		Cases	*Percent all cases this age*	*Cases*	*Percent all cases this age*	*Cases*	*Percent all cases this age*
0–12	38	20	53	10	26	8	21
13–25	48	28	58	12	25	8	17
26–38	49	23	47	17	35	9	18
39–51	42	22	52	12	29	8	19
First year	177	93	53	51	29	33	19
52–64	33	14	42	12	36	7	21
65–77	26	14	54	6	23	6	23
78–90	17	11	65	3	18	3	18
91–103	9	7	78	0	0	2	22
Second year	85	46	54	21	25	18	21
Total	262	139	53	72	27	51	19

person-weeks during the first year and 8.8 in the second are in accord with the established increased frequency of diarrhoeal disease incident to weaning, the progressive loss of maternal immunity, and increase in number of personal contacts, and the more extensive exposure to a contaminated environment.

Duration of diarrhoeal disease. Acute diarrhoeal disease already said was by far the commonest clinical form accounting for 80 per cent of all attacks, *Table II*. Cases of short duration, 1 to 7 days, were 54 per cent of the 262 episodes experienced by the cohort within the two-year period. Chronic diarrhoeal disease, lasting 15 or more days and extending to as long as 97 days, accounted for 20 per cent of total attacks. The distribution of cases by duration was similar in the various age groups.

Observation on Causation. Epidemiologic evidence from numerous studies points to an infectious origin of diarrhoeal disease. In the first place, the disease is primarily of small children. Incidence increases with age up to a period marked in developing countries by the end of the weaning period. Incidence thereafter decreases to low levels, suggesting an immunizing process[7]. Secondly, in developing countries there is a succession of epidemic waves of the disease, of one to two years duration, spreading through a community. About three such events occur during each decade, giving a periodicity much like that of measles[8]. Furthermore, the index case within a family is normally the youngest child, while secondary cases arise mainly among other preschool children, rather than among school children. They are still fewer in number among adults[9].

The rate and nature of colonization of the intestine by viruses, bacteria, parasites and other infectious agents is a current feature of the present study[6]. An early entrance and excretion of infectious agents occurs shortly after birth, presumably the results of ingestion of maternal faeces, or through fluids or other materials contaminated by attendants. This has been manifested by the following observations: (a) 25 out of 82 children excreted *Candida* yeasts in the meconium and faeces during the first days of life; (b) rapid colonization of the intestine of newborns by coliforms,

streptococci, enterococci and other bacteria, to reach concentrations of 10^9 to 10^{10} per gram of wet faeces within 24 hours; (c) 2 out of 82 children had inapparent infection by *Shigella* a few weeks after birth; and (d) 15 out of 62 children excreted enteroviruses in the first week of life. A significant finding is that early infection with agents commonly recognized as pathogenic is far from uniformly associated with clinical diarrhoeal disease.

With increasing age, and especially toward the end of the first year, intestinal colonization by a variety of infectious agents usually present in the area—for example,

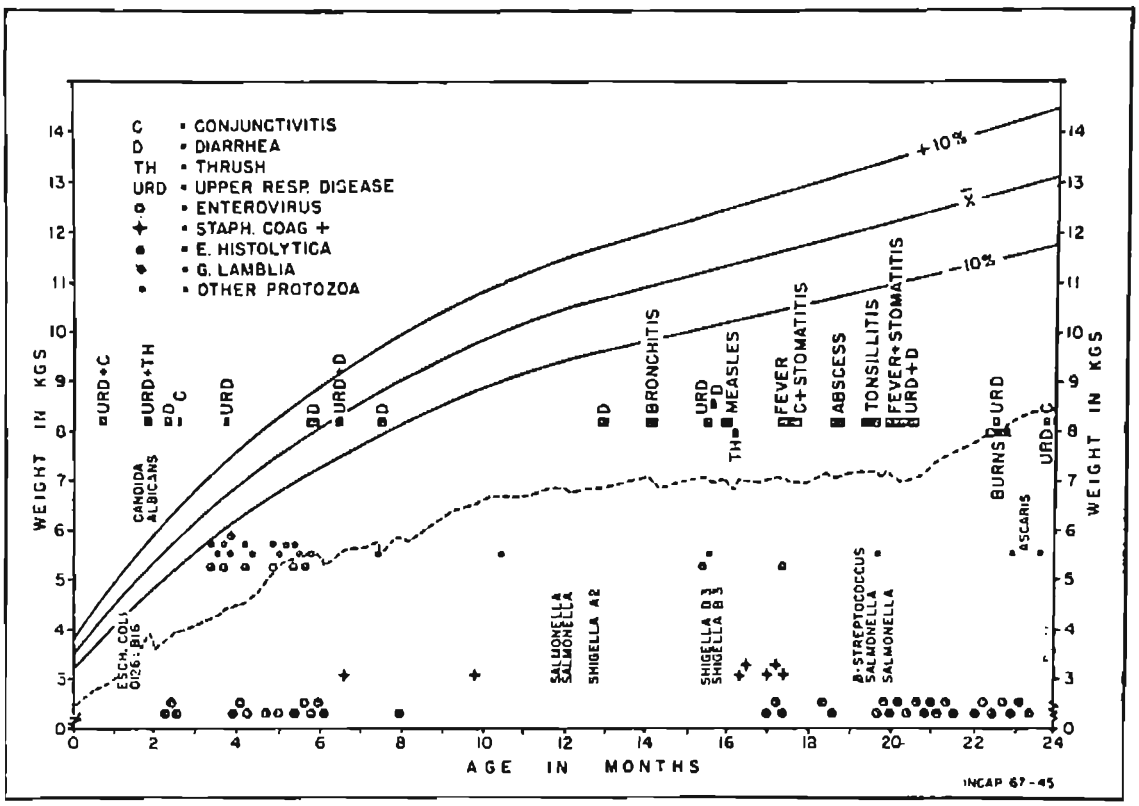

Fig. 3. Infections, infectious diseases, and weight by months in a Guatemalan village child.

Shigella and enteroviruses becomes progressively more frequent. The history of a representative child is presented in *Fig. 3*. Clinical diarrhoeal disease also is more frequent. The behaviour of shigellosis is informative. Clinical attack was essentially absent in the first half of the first year. Incidence rose to 3.9 cases per 100 person-weeks of exposure by the end of the second year. The ratio of undifferentiated diarrhoeal disease (diarrhoea unassociated with *Shigella*) to shigellosis was 40:1 in the first quarter year; 23:1 by the end of the first year; 4:1 during the first quarter of the second year; and, by the fourth quarter of the second year, it was close to parity, 1.3:1. The ratios apply only to clinical disease. The design of the study also permitted differentiation of transient or persistent colonizations without symptoms (inapparent infection or the carrier state) from clinical diarrhoeal disease. What happens in the third, fourth and fifth years of early childhood is still to be determined.

Information on the role of viruses in causation of diarrhoeal disease is incomplete. Studies of an aging cohort during the past three years have not yet progressed

to type identification of virus isolates. Their significance, and that of other agents, is determinable because cases of clinical diarrhoea have been matched by age and locality to other children of the cohort, having no diarrhoea at the time. The first 262 cases of diarrhoeal disease observed in this population are presented in *Tables III and IV*.

TABLE III

INFECTIOUS AGENTS IN DIARRHOEAL DISEASE, BY AGE OF PATIENT

Santa María Cauqué, 1964–1966

*Infectious agent**	*Age in months*					
	0–11		*12–23*		*Total*	
	Cases $n = 128$	*Controls* $n = 128$	*Cases* $n = 134$	*Controls* $n = 134$	*Cases* $n = 262$	*Controls* $n = 262$
Enteroviruses (all except Coxsackie)**	47(37)	35(27)	65(49)	69(52)	112(43)	104(40)
Coxsackie viruses***	13(10)	7(6)	16(12)††	7(5)	29(11)†	10(4)
Double virus infections	25(20)	17(13)	31(23)	51(38)	56(21)	68(26)
Shigella	3	0	23(17)†	9(7)	26(10)†	9(3)
Salmonella	3	1	0	0	3	1
Enteropathogenic *Escherichia coli*	1	1	0	0	1	1
E. histolytica	4	0	6(5)	4(3)	10(4)	4(2)
G. lamblia	1	3	13(10)	15(11)	14(5)	18(7)
D. fragilis	0	0	1	0	1	0
Other parasites	16(13)	11(9)	35(26)	33(25)	51(20)	44(17)

* Infectious agent alone or in association with one another.
** Cytopathogenic agents recovered in one or more than one of the following systems: primary human amnion, primary human kidney, and HEp-2 cells.
*** Agents recovered in newborn mice.
† Significant; $P < 0.01$.
†† Highly significant; $P < 0.001$.
Numbers in parenthesis are rounded percentages.

Infectious agents, considered as broad groups, were slightly more frequent in clinical cases than in controls, *Table III*, the difference being significant for coxsackie viruses and *Shigella* ($P < 0.01$ and < 0.001 respectively).

Double virus infections were relatively common, and equally frequent in cases and controls. No multiple bacterial infections were observed. All diarrhoeal episodes described in this report were among children less than two years old, all of whom were breast fed. There was less probability, therefore, of multiple bacterial infections for these subjects than for older children. Enteropathogenic *Escherichia coli* was essentially absent in cases of diarrhoeal disease and in healthy children, as is true of other primitive populations where children are breast fed during the first years of life.

Table IV summarizes combinations of viruses, bacteria and parasites in cases of diarrhoeal disease and in matched controls. Most infectious agents were recovered with increasing frequency as age advanced. The differences in frequency of agents in cases and controls of the first and second year of life were significant, when coxsackie viruses, enteric pathogenic bacteria and enteric pathogenic parasites were considered alone or in association with one or more of the others. Despite these differences, the prevalence of viruses, bacteria and parasites at any particular time was essentially

TABLE IV

INFECTIOUS AGENTS IN DIARRHOEAL DISEASE, BY AGE OF PATIENT
Santa María Cauqué, 1964–1966

Infectious agent	*Age in months*					
	0–11		*12–23*		*Total*	
	Cases $n = 128$	*Controls* $n = 128$	*Cases* $n = 134$	*Controls* $n = 134$	*Cases* $n = 262$	*Controls* $n = 262$
Coxsackie viruses, Bacteria* and Parasites**, alone or in association with one another	25(20)†	12(9)	58(43)†††	35(26)	83(32)††††	47(18)
Enteroviruses, Bacteria and Parasites**, alone or in association with one another	59(46)††	40(31)	107(80)	97(72)	166(63)††	137(52)
Viruses***, Bacteria and Parasites****, alone or in association with one another	63(49)	48(38)	86(64)	88(66)	149(57)	136(52)
No Viruses, Bacteria or Parasites present	65(51)	80(62)	48(36)	46(34)	113(43)	126(48)

* *Shigella*, Enteropathogenic *Escherichia coli* and *Salmonella*.
** *E. histolytica*, *D. fragilis*, *G. lamblia*.
*** Enteroviruses and Adenoviruses.
**** Any parasite.
† Significant; <0.05.
†† Significant; <0.02.
††† Significant; <0.01.
†††† Highly significant; $P < 0.001$.
Numbers in parenthesis are rounded percentages.

the same in cases and in controls. The prevalence of infectious agents among young children of this area is regularly high, a circumstance which makes comparison of cases and controls difficult and requires large numbers. The eventual identification of virus isolates may permit a more definite answer to the part these agents have in causation of diarrhoea.

Satisfactory controls are always difficult from an epidemiological standpoint. The present ones were stringent, because some of the children serving as controls were known to harbor infectious agents, particularly *Giardia*, *Shigella* or Coxsackie viruses, because of a persisting convalescent carrier state after a preceding clinical attack. Controls also expectedly included healthy carriers of infectious agents currently prevailing in the community, the shifting nature of which is well recognized[10,11]. Sound interpretation of the role of a particular bacterium or virus in causality of diarrhoea thus requires information on the current identity of these variants.

COMMENT

Observations in Guatemala among young children have identified distinctive features in the community behaviour of diarrhoeal disease. Deaths among childhood populations in rural areas confirmed the importance of diarrhoeal disease. They

demonstrated a considerable contribution by malnutrition. Field studies in India and Guatemala showed a peak incidence of childhood diarrhoeal disease during the weaning period, enhanced by a progressively developing malnutrition and greater exposure to environmental risks of infection.

Those studies also showed the practical need of a more precise definition of what constitutes a case of diarrhoea, and a more consistent practice in counting days of diarrhoea in a particular attack. The standards presented here admittedly are arbitrary, but they are based on the behaviour of diarrhoeal disease as observed under field conditions in an area where the disease is highly prevalent. Failure to define terms has resulted repeatedly in inability of different workers to compare results.

By these criteria most diarrhoeas in the present study were acute, but chronic recurrent diarrhoeal disease was much commoner than ordinarily recognized. The significance of that clinical form in transmission of the disease has been reported for shigellosis.

The behaviour of diarrhoeal disease, in duration, severity, mortality and other characteristics, was essentially uniform among two-year-old children during the time of this study. Whether in the acute or the chronic recurrent forms, cases were clinically indistinguishable whether associated with *Shigella* or "undifferentiated", which could suggest a single common cause as yet unidentified.

This study suggests that the likelihood of finding a particular agent depends on the age of the child, on the season of the year and especially on the prevailing infectious agents of the local region, which change from time to time. *Shigella* and coxsackie viruses were more frequent in cases than in matched controls, a difference statistically significant. Coxsackie viruses, enteric pathogenic bacteria and enteric pathogenic parasites, alone or in association with one or more of the others, also were significantly more common among patients than in matched controls. However, in a large proportion of cases (43 per cent) none of the conceivably probable infectious agents were identified. The finding casts further doubt on the significance of any one in specific causal relationship to the disease. The indication is for investigation of microbial entities not ordinarily considered in relation to diarrhoeal disease, the search for an undetermined agent, and for more knowledge of variations in the microbiota of the intestinal tract, and the factors responsible for them. There remains, as the present working hypothesis, that *Shigella*, *Salmonella*, enteropathogenic *Escherichia coli*, *Entamoeba histolytica*, *Giardia* and some enteroviruses are indeed causally related, but that they account for only part of the totality of diarrhoeas. That leaves diarrhoeal disease as a syndrome and not an entity; the bulk of cases of undetermined origin.

Future investigations of diarrhoeal disease seemingly point in three directions: (a) longitudinal studies where successive annual cohorts of newborn babies are studied, instead of a single cohort, in order to assure that children of different ages are exposed to the same prevailing forces of infectious agents, food supply, and economic and social conditions, all of which change with time; (b) careful study of host characteristics, mainly nutritional and immunological, as they interact with potential infectious agents; and (c) detailed microbiological studies in a limited number of subjects, where all reasonably pertinent infectious agents are studied simultaneaously.

SUMMARY

A cohort of children in a Mayan village of Guatemala was followed from birth to age two years, to study the frequency of diarrhoeal disease and of viruses, bacteria and parasites as determined in faecal specimens collected every week. Attack rates of diarrhoeal disease were high in the first trimester of life, increasing progressively to reach the highest level at the end of the first year and throughout the second year of life. The rise was coincidental with the loss of maternal immunity, the increased exposure to the unsanitary environment and the weaning process. Most diarrhoeas were acute, but chronic recurrent diarrhoea was unexpectedly frequent: about 20 per cent of all episodes. No difference in frequency of infectious agents was observed in the acute or the chronic form. Potentially pathogenic agents (Coxsackie viruses, other enteroviruses, *Shigella* and pathogenic parasites) were more frequent in cases than in matched controls. No agents were found in about 40 per cent of all episodes, a finding that casts doubt on the significance of these agents and calling for an investigation of microbial entities not ordinarily considered in relation to diarrhoeal disease. The main hypothesis advanced, however, is that the agents investigated are causally related but that they account for only part of the totality of diarrhoeas.

RESUMEN

Enfermedad Diarreica en una Cohorte de Niños Guatemaltecos Observados desde el Nacimiento hasta los Dos Años de Edad. Se llevó a cabo un estudio longitudinal en un grupo de niños de una aldea Maya de Guatemala, con el objeto de determinar: la frecuencia de la enfermedad diarreica así como la de virus, bacterias y parásitos en muestras fecales recolectadas semanalmente de todos los niños. La incidencia de enfermedad diarreica fue alta desde el primer trimestre de vida, aumentando progresivamente hasta alcanzar los niveles más altos al final del primer año y durante el segundo año de vida. La elevación coincidió con la pérdida de la inmunidad materna, con el aumento en la exposición al medio ambiente insalubre y con el proceso del destete. La mayoría de las diarreas fueron agudas, pero la forma crónica recurrente fue alta, en contra de lo esperado, constituyendo cerca del 20 por ciento de todos los episodios. No hubo diferencia en la frecuencia de agentes infecciosos entre la diarrea aguda y la crónica. Los agentes infecciosos potencialmente patógenos (virus Coxsackie, otros enterovirus, *Shigella* y parásitos patógenos), fueron más frecuentes en los casos que en los testigos equiparados por edad y estación. No se demostraron agentes en aproximadamente el 40 por ciento de los episodios, un hallazgo que pone en duda la significación de estos agentes, y que justifica la búsqueda de entidades infecciosas que ordinariamente no se investigan en las diarreas. La hipótesis principal, sin embargo, sigue siendo que todos los agentes investigados están relacionados, causalmente, pero que ellos son responsables sólo de una parte de la totalidad de las diarreas.

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