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Infectious Agents in Acute and Chronic Diarrhea of Childhood

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Even today acute diarrheal disease is thought of by many laymen as well as by some medical professionals in developing countries as being a syndrome of alimentary origin. Despite recognition of shigellosis, cholera, salmonellosis, giardiasis, amebiasis, and other enteric infectious diseases, there has been much difficulty in accepting the fact that most of the "nonspecific" diarrheas in the general population were also of an infectious nature. The frequent appearance of diarrhea after onset of weaning in many animal species and in man (1) and the systematic failure in the past to find pathogenic agents in a majority of the diarrheas tended to rule out a microbial etiology.

Epidemiologists, pediatricians, and microbiologists suspected that the nonspecific diarrheas of childhood were also of microbial or viral origin (2) because of their characteristics in poor urban and rural settings. First, in such ecosystems, diarrhea prevails if sanitation and personal hygiene are deficient. Second, infants and preschool children are more frequently and more severely affected than school children, adolescents, and adults, which suggests the acquisition of immunity and host resistance. Third, acute diarrhea in the community follows a pattern similar to that of other infectious diseases in that secondary cases develop after contact with the index case, eventually resulting in self-limiting to extensive outbreaks or even epidemics of great magnitude. It is clear that if personal hygiene and environmental sanitation are deficient, diarrhea is prevalent. This explains the similarity in diarrhea morbidity and mortality between some contemporary developing nations and New York City at the turn of the century, when environmental conditions in New York were as deficient as they are today in some developing nations.

DEFINITION OF DIARRHEA

There are problems in assessing the significance of infectious agents in chronic diarrhea and the malabsorption syndromes (3). Also, there is no universal definition of acute and chronic diarrhea. Generally speaking, acute diarrhea is

described as the expulsion of stools or fluid in excess of normality, usually more than three evacuations per day, which resolves within 5 to 6 days. The presence of fetid stools and blood will also characterize acute diarrhea; dysenteriform diarrhea may last longer. Infectious diarrhea is that in which an enteric pathogen is demonstrated or isolated in the bowel or the stools of the diseased individual. Attribution of etiologic significance to a particular agent would require fulfillment of Koch's postulates or their modification, for instance, demonstration of a rise in specific antibodies. The study of cases within an epidemiological situation is helpful, but absolute proof of causality is not possible or practical in most instances. More than one agent may be present in diarrhea cases, and hitherto unknown agents may also occur, complicating interpretation of etiology. These considerations demand that most or all known etiologic agents at a given time be investigated in the best possible way and also require biomedical and epidemiological insight in interpreting laboratory and clinical findings.

Chronic diarrhea is described as an episode lasting for more than 3 weeks according to some authors or more than 6 weeks according to others. Definitions are established by clinicians working in hospitals who often lack an adequate history of the child's total background. As is illustrated below, the prospective study of the natural history of enteric infection and of diarrhea reveals no clear distinction between repeated acute diarrheal episodes and the "chronic recurrent diarrhea syndrome" observed under poverty and unhygienic conditions. Chronic and chronic recurrent diarrhea are used here interchangeably. The "intractable" chronic diarrhea that establishes itself in the first 3 months of life is not considered in this presentation, because such a syndrome is not seen among children that are exclusively breast fed for several months in rural communities of developing countries.

AGENTS IN ACUTE DIARRHEAL DISEASE

The late 1960s and the decade of the 1970s proved to be fruitful in the field of enteric microbiology in that etiologic participation of rotaviruses and fastidious enteric adenoviruses were discovered (4), and other microorganisms such as enterotoxigenic *Enterobacteriaceae*, *Campylobacter*, *Yersinia* (4), *Aeromonas* (5), and *Cryptosporidium* (6) were rediscovered (Table 1).

Recently, rotavirus-like particles indistinguishable from the rotaviruses were visualized by electron microscopy in cases of human diarrhea. These viruses were not detected by the enzyme-linked immunosorbent assay (ELISA) and have been temporarily designated as pararotaviruses (7); together with calicivirus-like and other particles, they open new possibilities for etiological explanations for some nonspecific diarrheas.

A large plasmid (140 megadaltons) was found related to invasiveness of *Shigella* (8) and *Escherichia coli* (9). *Klebsiella* strains carrying this plasmid were identified in Costa Rica in serious and often fatal infections in "high-risk" hospitalized children (10).

TABLE 1. *Etiologic agents of acute diarrhea*

Rotaviruses, pararotaviruses	*Arizona, Plesiomonas*
Noncultivatable adenoviruses	*Clostridium difficile*
Cultivatable adenoviruses	*Clostridium perfringens*
27-nm agents (Norwalk, Hawaii, Montgomery)	*Staphylococcus aureus*
	Bacillus cereus
Enteroviruses (ECHO, Coxsackie)	*Giardia lamblia*
Coronaviruses	*Dientamoeba fragilis*
Astroviruses, caliciviruses (?)	*Entamoeba histolytica*
Enterotoxigenic *Escherichia coli* (ST, LT)	*Balantidium coli*
Enterophatogenic *Escherichia coli*	*Cryptosporidium*
Enteroinvasive *Escherichia coli*	*Isospora belli*
Other bacteria carrying 140-MD plasmid (?)	*Plasmodium falciparum*
Shigella	*Trichuris trichiura*
Salmonella	*Strongyloides stercoralis, S. fulleborni*
Vibrio cholerae	*Trichinella spiralis*
Vibrio parahaemolyticus	*Necator americanus, Ancylostoma duodenale*
Campylobacter fetus jejuni	*Capillaria phillippinensis*
Aeromonas hydrophila	*Schistosoma mansoni, S. japonicum, S. intercalatum*
Edwardsiella tarda	
Yersinia enterocolitica	

Often in cases of diarrhea in the general population, the agents involved in classical food poisoning are not mentioned or are not investigated at all. In the last 10 years, village weaning foods have been found to contain large numbers of bacteria originating in food (*Bacillus cereus, Clostridium perfringens*) or in man through direct or indirect contamination (*Escherichia coli, Staphylococcus aureus*) (11,12). These agents proliferate well in weaning foods and are a potential source for diarrhea to which very little attention has been paid. Also, *Clostridium difficile* has been found in association with misuse or abuse of antibiotics and has caused pseudomembranous enterocolitis (4,13).

Most authors do not consider parasites to be important causes of diarrhea since studies in severe hospitalized cases are usually negative for parasites. This concept is being revised (14), since long-term prospective study has shown parasites to be causally related to acute and chronic diarrhea. To the list of classical parasites, *Cryptosporidium* should now be added (6), as should *Plasmodium falciparum* malaria, which has an important diarrhea component, particularly in small children.

The list of potential pathogens is not complete, and probably yeasts, mycoplasma, chlamydia (and viroids?) will be added eventually as causes of acute diarrhea in man.

Relative Prevalence of Etiologic Agents in Acute Diarrhea

Prevalence rates in various studies reflect differences in capability of the laboratories involved, in methods used, and also in geographic, ecologic, and social conditions of human populations. Before one can make conclusive state-

ments about the relative frequency of infection, coordinated international multidisciplinary studies must be done, using the same set of definitions, the same protocol, and comparable field and laboratory methods. Comprehensive laboratory investigation reveals potential pathogens in 60 to 70% of community diarrheas, but many cases remain of unknown etiology and are still designated as "nonspecific diarrheas." Table 2 illustrates findings in two contrasting ecosystems, where similar laboratory techniques were employed to investigate agents in urban and rural children with acute diarrhea over a relatively long period to guarantee representativeness of frequency rates.

It is quite evident that enterotoxigenic *Escherichia coli* (*ETEC*) and rotaviruses stand out as the main agents associated with acute diarrhea. The low rotavirus infection rate in Bangladesh (15,16) contrasts with a higher rate in Costa Rica (17,18); in industrial countries such as the United States, Canada, and Japan, rotaviruses are even more prominent (4) than in traditional Bangladesh or transitional Costa Rica. Aside from geographical peculiarities such as the presence of El Tor and classical cholera in the Indian subcontinent, *ETEC* ranks first in poor traditional societies, followed in importance by *Shigella*, rotaviruses, *Campylobacter*, *Salmonella*, *Entamoeba histolytica*, and *Giardia lamblia* (15,16); *ETEC* is not so important in industrial nations, and rotaviruses rank first, followed by *Shigella*, *Salmonella*, and parasites (4). Countries in transition fall in the middle (18,19). Recently, *Cryptosporidium* was found relatively frequently in Australia and Costa Rica, associated with acute diarrhea in immunologically uncompromised children (6,20).

TABLE 2. *Percent prevalence rates of infectious agents in acute urban and rural diarrheas in two contrasting ecosystems*

Agent, alone or combined with other agent	Dacca, Bangladesh, 1979–1980[a]	San José, Costa Rica, 1976–1979[b]	Matlab, Bangladesh, 1978–1979[c]	Puriscal, Costa Rica, 1979–1981[d]
Enterotoxigenic *E. coli*	20.0	14.3	26.9	9.0
Rotaviruses	19.4	45.3	4.7	15.0
Shigella	11.6	8.1	15.8	2.0
C. fetus jejuni	11.6	8.0[e]	n.i.[f]	6.0
V. cholerae	5.5	n.i.	0.3	n.i.
Non-group 0:1 *Vibrio*	1.1	n.i.	1.1	n.i.
Salmonella	0.6	7.3	0	1.0
E. histolytica	6.1	0	0.2	0
G. lamblia	5.6	4.5	0.5	0.3
One or more pathogens	66.0	63.2	50.5	42.0

[a] Data from Stoll et al. (16).
[b] Data from Mata et al. (18).
[c] Data from Black et al. (15).
[d] Data from Vives et al. (17).
[e] December 1980 through June 1981 only.
[f] Not investigated.

MAGNITUDE OF SPECIFIC ACUTE DIARRHEAS IN LATIN AMERICA

Based on good estimates of the child population of Latin America in 1976, and according to present knowledge on the relative frequency of the various diarrheas, the overall specific diarrhea morbidity was calculated for that particular year (Table 3). It was necessary to extrapolate from incidence figures of either two cases per child per year (21) or 7 to 8 cases per child per year (22). In using the information in Table 3, the lower estimate of rotavirus diarrhea is recommended when dealing with poor traditional populations, where rotaviruses are not as prominent as in industrial nations. On the other hand, the highest estimate should be considered for *ETEC, Shigella, Campylobacter, Giardia, E. histolytica,* and probably *Cryptosporidium.* The inverse seems the case in industrial countries.

RELATIVE IMPORTANCE OF CHRONIC DIARRHEA

An unquestionable decrease in diarrheal disease mortality in Latin America became evident even before the advent of oral rehydration (23). The improvement is attributable to steady socioeconomic development and an emphasis placed by governments and international organizations on health education, water supplies, and sanitation. Furthermore, broad application of oral rehydration resulted in a sharp decrease in diarrhea deaths in the field and in the hospitals (24,25). As a result of the compounded effect of such improvements, chronic diarrhea is now emerging as a very important health problem and cause of death in nations in rapid transition, often surpassing other infectious diseases. For

TABLE 3. *Estimated prevalence of acute specific diarrheas in children 0–4 years old, Latin America, 1976**

Specific diarrhea	Prevalence rate (%)	Millions of cases	
		2 attacks per child per year	8 attacks per child per year
Rotavirus	10–40	10–40	40–160
ETEC	10–30	10–30	40–120
Shigella	5–20	5–20	20–80
Giardia	5–20	5–20	20–80
Campylobacter	8–15	5–15	20–60
Adenovirus	5–10	5–10	20–40
Cryptosporidium	4–5	4–5	16–20
Salmonella	2–4	2–4	8–16
E. histolytica	1–3	1–3	4–12
Total		100	400

* Estimated for 50 million children 0–4 years old.

instance, in the National Children's Hospital in Costa Rica, chronic diarrhea is now the main cause of death among all the diarrhea syndromes (E. Mohs, *personal communication*), and it is the main associated factor in severe protein–energy malnutrition (PEM) (Table 4). This phenomenon is being noted with increasing frequency in other developing nations. The present interest in chronic diarrhea derives from its confusing etiologic picture, the difficulty in handling the disease in hospital and field, and the high fatality rate observed in hospitalized individuals.

INFECTIOUS AGENTS OF CHRONIC DIARRHEA

The agents found in chronic diarrhea generally are the same as those diagnosed in acute diarrhea, but their relative frequency varies (Table 5) (14). *Shigella* and parasites, especially *Giardia* and *E. histolytica*, are more prominent in poor urban and rural children living with deficient hygiene and environmental sanitation. *Dientamoeba, Cryptosporidium, Isospora,* and *Strongyloides* probably are more frequent than usually described, and the reported low incidence is likely attributable to incompleteness in diagnosing these parasites. *Dientamoeba* requires careful examination of stained preparations, *Isospora* often requires biopsy of the small intestine, and *Cryptosporidium* is just being recognized in a few countries. On the other hand *Strongyloides* larvae generally require concentration.

Among bacteria, Shigellae are agents of chronic diarrhea because they tend to persist in the intestine. Enteroinvasive *Escherichia coli* and other bacteria carrying the 140-MD plasmid probably are more important than currently suspected. Other potentially invasive bacteria such as *Campylobacter* and *Yersinia* need more attention as possible agents of chronic diarrhea.

Overgrowth of the small intestine with facultative and anaerobic bacteria

TABLE 4. *Pathology in 42 children with severe protein–energy malnutrition, 4 months of 1981, National Children's Hospital, Costa Rica*[a]

Pathology	Number (%)
Chronic diarrhea	9 (21)
Congenital defects, metabolic alterations	9 (21)
Massive parasitosis and chronic diarrhea	8 (19)
Child abuse	5 (12)
Mental retardation	4 (9)
Prematurity	2 (5)
Respiratory infection	2 (5)
Other infections	2 (5)
Abdominal surgery	1 (2)
Total with chronic diarrhea	17 (40)
Total with infectious agents	12 (29)

[a] From E. Mohs (*personal communication*).

TABLE 5. *Etiologic agents of chronic diarrhea*

Giardia lamblia	*Shigella*
Entamoeba histolytica	*Escherichia coli*, enteroinvasive
Dientamoeba fragilis	Other bacteria carrying 140-MD plasmid (?)
Isospora belli	*Campylobacter fetus jejuni*
Cryptosporidium	*Yersinia enterocolytica*
Strongyloides stercoralis	Bacterial overgrowth of small intestine
Trichuris trichiura	Coronavirus
Necator americanus, Ancylostoma duodenale	Adenovirus (?)
Schistosoma mansoni	Rotavirus (?)

is commonly found in children and adults suffering from chronic diarrhea, malabsorption, and malnutrition in many tropical and subtropical countries (26–29).

Finally, coronaviruses have been recognized in India in chronic diarrhea (4,30), and fastidious and culturable adenoviruses also deserve consideration because they can persist in the human host. Recent evidence in Costa Rica indicates that rotaviruses are frequently found in hospitalized children with chronic diarrhea and malnutrition (see below).

PERSISTENCE OF *SHIGELLA* INFECTION IN SMALL CHILDREN

Studies conducted in a convalescent home for deprived children in Guatemala where sanitary conditions were deplorable revealed that persistence of *Shigella* and multiple infections were rather common (31). *Shigella* infections were adequately monitored in 20 children by daily rectal swabs for 7 months. The persistence of the same serotype over prolonged periods and the finding of two or three different serotypes on the same day were not rare events. One-third of the children were chronically infected, and all harbored shigellae at least once during the study period. Two children excreted *S. sonnei* for at least 7 months, and another two for 5 (Table 6).

Persistence of *Shigella* and of *Giardia* was also recorded in rural children observed prospectively in their own ecosystem (32). At the time of the study

TABLE 6. *Duration of* Shigella *infection in children with chronic malnutrition and chronic recurrent diarrhea, convalescent home, Guatemala, 1965*

Number of children	Duration (months)	Serotype
2	7	*S. sonnei*
2	5	*S. sonnei*
4	1	*S. flexneri*
12	1	*S. flexneri*
7	2	*S. sonnei*
5	2	*S. flexneri*

From Catalán et al. (31), with permission.

(1964–1969), the specimens had to await bacteriologic cultivation and examination with trichrome staining; furthermore, the prevailing knowledge was that *Shigella* infections were short-lived, resolving with the disappearance of symptoms, and *Giardia* was not considered an important pathogen. The persistence of *Shigella* and *Giardia* became clear in these children, who did not receive drugs, when the data were graphically displayed as in Fig. 1. Arbitrarily, two *Shigella* infections were considered independent if separated by 3 or more negative weeks (Table 7) (32). Among 132 distinct episodes of *Shigella* in the cohort, 46 (35%) lasted for only 1 week, and 37 (28%) for 2 to 4 weeks. Thirty-seven percent of the infections lasted from 5 to 38 weeks, and almost one-third of these lasted 13 weeks or more.

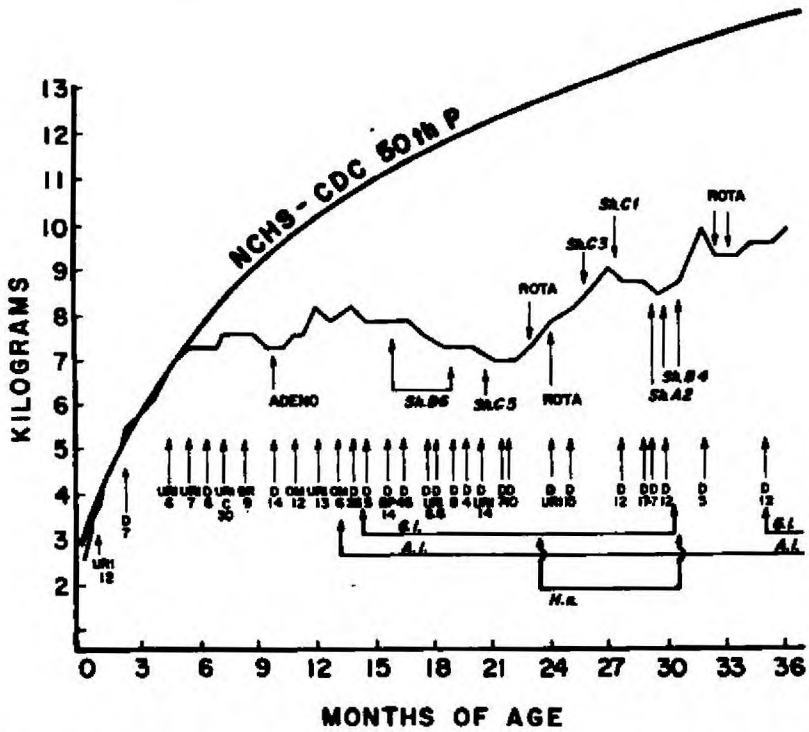

FIG. 1. Enteric infections, diarrheal episodes, and weight curve of cohort male child No. 34. Key: URI, upper respiratory infection; D7, diarrhea of 7 days duration; OM, otitis media; BR, bronchitis; BP, bronchopneumonia; *G.l., Giardia lamblia; A.l., Ascaris lumbricoides; H.n., Hymenolepis nana; Sh.A., Shigella dysenteriae; Sh.B., Sh. flexneri; Sh.C., Sh. boydii;* rota, rotavirus; ADENO, cultivatable adenovirus. This low-birth-weight child was exclusively breast fed for several months. Growth was adequate during the first 5 months of life as compared to the 50th percentile of the NCHS–CDC growth curves. Thereafter, a sequence of periods of growth deceleration, growth arrest, and weight loss in conjunction with diarrhea episodes led to progressive deterioration of the nutritional status and to stunting. Diarrhea episodes were associated, more often than not, with pathogenic enteric infectious agents.

Whereas in the convalescent home *S. sonnei* was the most persistent, in rural children it was ephemeral. *Shigella dysenteriae 2* persisted longer than any other serotype, with 10% lasting 17 to 38 weeks; *S. boydii* serotypes were intermediate between *S. dysenteriae 2* and *S. sonnei*. The display of longitudinal data showed that most infectious diarrheas were associated with pathogenic enteric agents (22,23,32). It must be kept in mind that at the time of the study (1964–1969) rotaviruses, *ETEC, Campylobacter,* and *Cryptosporidium* were not investigated; when an ELISA became available for rotaviruses, they were retrospectively investigated in frozen fecal extracts but were not found to persist or to be associated with chronic infection. The tendency of agents such as *Shigella* and *Giardia* to persist was evident (Table 7) in association with chronic recurrent diarrhea in rural children and in the convalescent home. Since observations extended over the entire first 3 years of life of a cohort of 45 children, alterations in physical growth could be recorded, and most inflections in the weight curve were found to be related to episodes of acute and chronic diarrhea, although growth alterations were also observed in connection with some apparently asymptomatic infections (32).

A RETROSPECTIVE ANALYSIS OF AGENTS IN ACUTE AND CHRONIC DIARRHEA

The availability of data files with longitudinal information on etiologic agents, infectious morbidity, and physical growth permitted a retrospective analysis to explore causal associations (32). An analysis was made on the complete histories of the first 22 cohort children from birth to 3 years of age. The presence of an agent the week before, during the week, or the week after occurrence of diarrheal disease was considered causally related. Table 8 shows that *Giardia* and *Shigella* were the agents most frequently related to diarrhea (about 20% each), followed by rotaviruses and *E. histolytica* (about 10% each). Although 59.6% of the diarrhea cases harbored at least one potential pathogen, *Shigella* and *Giardia*

TABLE 7. *Duration of* Shigella *infection (cases and carriers), 45 cohort children observed from birth to 3 years, Santa Maria Cauque, 1964–1969*

Group	Number of infections	Duration in weeks[a]					
		1	2–4	5–8	9–12	13–66	17–38
S. dysenteriae	29	7 (24)[b]	10 (34)	4 (14)	3 (10)	2 (7)	3 (10)
S. flexneri	75	18 (24)	21 (28)	18 (24)	9 (12)	5 (7)	4 (5)
S. boydii	21	14 (67)	6 (28)	1 (5)			
S. sonnei	7	7 (100)					
Total	132	46 (35)	37 (28)	23 (17)	12 (9)	7 (5)	7 (5)

[a] Two infections were considered independent if separated by more than 2 weeks.
[b] Number of cases (row percentage).
From Mata (32), with permission.

TABLE 8. *Etiologic agents in 381 cases of diarrhea in 22 children observed from birth to 3 years of age, Santa Maria Cauque, Guatemala, 1964–1969*

Agent[a]	Number positive (%)
Giardia lamblia	92 (24.1)
Shigella species	89 (23.3)
Rotavirus	44 (11.5)
Entamoeba histolytica	44 (11.5)
Adenovirus (cultivatable)	29 (7.6)
Salmonella species	11 (2.9)
Enteropathogenic *Escherichia coli*	6 (1.6)
Dientamoeba fragilis	3 (0.8)
One or more of the above	227 (59.6)

[a] Enterotoxigenic bacteria, *Campylobacter*, and *Cryptosporidium* not investigated.

appeared singly in only 10 to 11% of instances. Only 41% of the positive cases had single infections; 14% had double, and 3.7% had triple infections. In one episode four different agents were diagnosed; multiple infections would have been more prominent had *ETEC*, *Campylobacter*, and *Cryptosporidium* been investigated.

One of the advantages of the retrospective analysis was the display of longitudinal data as shown in Fig. 1 for child 34 of the cohort. This low-birth-weight infant grew satisfactorily with exclusive breastfeeding during the first 5 months of life if compared with the 50th percentile of the National Center for Health Statistics (NCHS) growth curves. At about 5 months of age, two episodes of upper respiratory infection and one of diarrhea (without a recognized agent) were related to marked deceleration of growth while the child was exclusively breast fed. Other respiratory infections were observed, and at 10 months of age, one attack of acute diarrhea with a cultivatable adenovirus resulted in pronounced weight loss. After several acute respiratory infections, and at age 14 months, the child initiated a series of recurrent episodes of diarrhea that extended into the third year of life. This series of diarrhea attacks was initiated by infection and colonization with *G. lamblia*, which continued at least until age 3 years, except for 4.6 months of apparent negativity. At 16 months of age, colonization with *S. flexneri 6* lasting for at least 12 weeks was detected, but this particular child suffered from at least five additional episodes with varying *Shigella* serotypes.

AGENTS IN CHILDREN CHRONIC PROTEIN-ENERGY MALNUTRITION AND DIARRHEA

Without knowledge of the past history of the child, no definitive conclusions can be drawn on the etiology of chronic diarrhea in children admitted with PEM. It is clear that in ecosystems such as Santa María Cauqué, malnutrition results from repetitive insults by infectious diseases, particularly diarrhea, measles, and whooping cough (22,32). In Guatemalan rural children admitted to the clinic with severe PEM and diarrhea (PEM-D), the striking finding was the

TABLE 9. *Etiologic agents in feces of 13 children with severe protein–energy malnutrition and chronic diarrhea, Guatemala, 1969–1971*

PC-215	Negative
PC-215r	Negative
PC-253	Negative
PC-223	*Shigella B2*
PC-249	*Giardia*
PC-252	*Giardia*
PC-213	*Giardia*[a]
PC-226	*Giardia, Trichomonas*
PC-241	*Giardia, Trichomonas, Salmonella*
PC-222	*Giardia*, hookworm, adenovirus
PC-237	*Giardia, Trichuris*, hookworm
PC-235	*Giardia, Trichuris, Strongyloides*
PC-250	*Giardia, E. histolytica, Trichuris*, hookworm, *Strongyloides, Edwardsiella*[a]

[a] Enterovirus was also present in small intestine.
From Mata et al. (26), with permission.

multiple pathogenic microbiota in the stools (Table 9); the predominant agent among 13 children was *Giardia*. Most cases, including three negative for pathogens, had bacterial overgrowth ($\geq 10^5$ bacteria per ml) in the jejunum (33). Bacterial overgrowth decreased to very low levels during recovery with an appropriate diet and under hygienic conditions (Table 10), suggesting that the abnormal flora resulted from intestinal malfunction favored by environmental contamination. The phenomenon has been recognized in other populations living under marked deprivation (26–29).

With use of the string capsule (Enterotest®) in recent studies in Costa Rica,

TABLE 10. *Jejunal flora of three children with severe malnutrition and chronic diarrhea, on admission and during recovery, Guatemala, 1969–1971*

Child No.	Anaerobes	Facultatives
Admission		
Pc-22	7[a]	7
Pc-226	7	5
Pc-241	6	6
Stabilization (10 days)		
Pc-222	7	7
Pc-226	6	6
Pc-241	<2	<2
Recuperation, phase I (3–4 weeks)		
Pc-222	<2	3
Pc-226	6	6
Pc-241	<2	<2
Recuperation, phase II (8–9 weeks)		
Pc-222	3	2
Pc-226	5	4
Pc-241	<2	2

[a] Logarithm$_{10}$ of colony count per ml of fluid.
From Mata *et al.* (26), with permission.

Giardia appeared to be the predominant agent in the small intestine of children with chronic PEM-D, followed by enteropathogenic bacteria and bacterial overgrowth (Table 11) (34). This study was expanded into another series of 19 children in which rotaviruses were recognized by the ELISA in the small intestine of eight cases (J. Cockerell, L. Reyes, and L. Mata, *unpublished data*).

GENESIS OF CHRONIC DIARRHEA IN TROPICAL COUNTRIES

The prospective observation of Cauqué children indicated that chronic diarrhea does not occur in infants that are exclusively breast fed from birth even if they live in deplorably unhygienic conditions; acute diarrhea may occur in wholly breast-fed infants, but it is generally of mild character (32). Diarrhea begins with weaning as a consequence of repetitive enteric infections, particularly with *Giardia* and *Shigella*, which increase with augmented opportunities for contamination. Other agents are particularly important in acute diarrheas such as *ETEC*, rotaviruses, *Campylobacter*, *E. histolytica*, and probably *Cryptosporidium*. For establishment of chronic diarrhea, the following situations must occur: (1) early weaning coupled with administration of allergenic foods and/or foods contaminated with enteric pathogenic agents; (b) failure of the host to cope with infection and to heal the lesion; and (c) failure to accurately diagnose and inhibit or suppress the causal agent or agents if conditions a and/or b occur. Inhibition or suppression can be affected by feeding colostrum and breast milk to neonates (including high-risk neonates) and continuing with several months of breast feeding. In fact, neonatal diarrhea has virtually disappeared from a large Costa Rican maternity (9,000 deliveries per year) after colostrum administration and promotion of breast-feeding programs were implemented (35,36).

Shigella inflicts serious damage to the child, particularly if the episodes continue as chronic recurrent diarrhea. Even if anthropometric measurements fail to demonstrate an impact on the growth curve, clinical appraisal will reveal, more often than not, apathy, anorexia, flatulence, abdominal distension, and in general a poor health condition. Over a prolonged period, children with the syndrome become wasted and markedly stunted. Superimposed acute infections may precipitate kwashiorkor and cause death.

TABLE 11. *Agents in the proximal small intestine of 58 children with severe protein–energy malnutrition and chronic diarrhea, Costa Rica, 1976*

Agent[a]	Positives (%)
Giardia lamblia	22 (38)
Shigella, Salmonella, EEC	11 (19)
Overgrowth $\geq 10^5$ [b]	8 (14)
One or more of the above	36 (62)

[a] Enteric viruses, *ETEC*, *Campylobacter*, and *Cryptosporidium* were not investigated.
[b] *E. coli* (4 cases), *Klebsiella* (3), *C. albicans* (1).
From López et al. (34), with permission.

The establishment of chronic diarrhea is also related to feeding practices. In non-breast-fed infants or in prematurely weaned infants exposed to frequent contamination (i.e., in large urban centers of Latin America), chronic diarrhea may develop during infancy. In Cauqué, where breast feeding is intense and prolonged, chronic diarrhea is not seen in the first months, but it may occur later on.

There is no explanation for the host failure to spontaneously repel infection. Many infants in deprived environments are born with fetal growth retardation or prematurely, conditions in which immunological deficiencies have been described (37). On the other hand, the onset of acute diarrhea shortly after initiation of supplementary feedings (often contaminated with diarrhea agents) marks the beginning of deterioration of intestinal function and nutrition. It is not surprising that in chronic diarrhea the commonest agents are those with capacity to damage the mucosal tissue and impair function.

Chronic diarrhea is attributed to the continued negative action of parasites, bacteria, and viruses on the anatomy and physiology of the mucosa, but the pathogenesis must be elucidated in the context of dietary, immunologic, genetic, and other host and environmental factors. In children in the poor rural and convalescent settings, some analogy with the "gay bowel syndrome" (38) seems too evident. In this syndrome, many pathogenic and nonpathogenic infectious agents are found in excess of expectation as a consequence of life styles that favor oroanal contact and coprophagy. Rural children in poverty frequently ingest food and water contaminated with feces or are in intimate contact with other persons, thus favoring person-to-person transmission, a comparable situation to that of the gay bowel syndrome. Investigation of the pathophysiology and immunology of this and of the "contaminated small bowel" syndromes (27) may also shed light on the pathogenesis of chronic diarrhea of rural children.

METHODOLOGICAL CONSIDERATIONS

Collection of adequate specimens is fundamental, for instance, in *Giardia,* as stool examinations often fail to reveal the parasite whereas the string capsule easily yields positives (34). The number of parasitic forms excreted in feces is several orders of magnitude less than that of bacteria or virions. Furthermore, for diagnostic procedures, bacteria and some viruses are cultured and enriched in order to make the diagnosis, whereas parasites are generally diagnosed as they are excreted in stools. Also, diagnostic techniques for parasites are less sensitive than those employed for bacteria and viruses. Diagnosis for *Strongyloides, Isospora belli,* and other agents may require serial biopsies, proper staining, and thorough microscopic search (39). *Cryptosporidium* can be overlooked if Giemsa stain is not done, because parasitic forms in the mucosa may not be easily seen with hematoxylin–eosin.

The importance of conducting daily bacterial cultures for 3 to 4 days is fundamental to insure that an enterobacterial infection is not missed by culturing

TABLE 12. *Ten desirable features of study of infectious etiology of acute and chronic diarrhea*

I. Rural and urban populations examined
II. Long-term prospective observation
III. Multiple samples examined
IV. Specimens collected in home or at bedside
V. Agents investigated in stools and in small bowel aspirate (string capsule)
VI. Parasites studied in
  a. smear of fresh feces
  b. Giemsa-stained smear
  c. concentrate of feces
  d. trichrome- or iron hematoxylin-stained smear
  e. small bowel aspirate
VII. Bacteria studied in
  a. fresh feces (dark field or phase contrast)
  b. Giemsa-stained smear
  c. cultures of small bowel aspirate in various media and conditions, and quantitation
VIII. Viruses studied by
  a. electron microscopy
  b. immunodiagnostic techniques
  c. cell culture
IX. Exudative cells studied in Giemsa-stained smears
X. Histopathology and etiology studied in small bowel biopsy

on a particular day on which bacilli are few, as observed in the convalescent home study (31). Specimens must be inoculated onto various media, for instance, Tergitol 7® with triphenyltetrazolium chloride for *Shigella* (40), blood agar with ampicillin for *Aeromonas hydrophila* (5), or thioglycolate agar with antibiotics for *Campylobacter* (41). The recent recognition of the 140-MD plasmid related to invasiveness makes the use of the Sereny test compulsory for screening bacteria in diarrhea stools (9).

Ten recommendations to conduct studies on the etiology of acute and chronic diarrhea are proposed on the basis of past experience (Table 12) in the hope that some of them will be applied in future multinational collaborative studies on the infectious etiology of acute and chronic diarrhea.

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