INTESTINAL COLONIZATION OF BREAST-FED CHILDREN IN A RURAL AREA OF LOW SOCIOECONOMIC LEVEL *

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Intestinal colonization of newborns is a natural phenomenon that begins immediately after the rupture of membranes. A great many microbial entities, predominantly bacteria, gain access to the gastrointestinal tract, mainly through the mouth. Experiments with animals and with humans revealed the influence that the flora has on the morphology and physiology of various organs and its importance in the host resistance to a variety of stresses, including infection with pathogenic agents. The flora also has important metabolic capacities that are related to the host nutrition.[1, 2]

In spite of the vast amount of information regarding characteristics and functions of the gastrointestinal flora of animals and man, the phenomenon of natural colonization of human newborns has not been adequately explored. There are no studies on children of preindustrial societies where breast-feeding is a common practice and where there is a continuous exposure to an unsanitary environment. This is the case particularly with the *Escherichia coli* group and its enteropathogenic varieties. Most of the present knowledge is delivered from studies of epidemics in artificial environments such as hospitals or from ill children, usually bottle-fed.[3, 4] There is not enough knowledge regarding the behavior of *E. coli* in breast-fed children of low socioeconomic conditions while they are in their own ecosystem.

This report deals with the intestinal colonization of breast-fed village children studied in their own environment by means of a design that retains at a minimum disturbance of the customs and living conditions of the inhabitants, and concerns the anaerobic and facultative flora, with some emphasis on the *E. coli* group.

Area of Study

The place of study was Santa María Cauqué, a village belonging to the Maya-Cakchiquel cultural group. It is located at 36 km from the Capital City of Guatemala, alongside the Pan-American Highway, at an altitude of 6,100 feet. The village has a population of 1.300 predominantly illiterate Indians. The average birth rate is 50 per 1,000 inhabitants, and the morbidity and mortality of infants and pre-school children are high.[5]

Living Conditions. The homes have one or two rooms where cooking and most of the indoor family life take place. Villagers sleep on mats on the floor

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or on low beds. Water is carried in earthen jars from public fountains and reservoirs to the homes where it is stored in large containers of several gallons' capacity (FIGURE 1) that are not customarily cleaned. Water is removed with smaller jars by hand, which involves the immersion of fingers.

Childbirth. Infants are delivered within the home by folk midwives who lack a knowledge of modern obstetrical practices. Mothers give birth in the squatting or kneeling position. Defecation is common during delivery.

Dietary Practices. Infants are customarily breast-fed immediately after birth by a foster mother, generally a relative, and by the mother as soon as colostrum and milk become available. Children receive maternal milk for prolonged periods, occasionally up to four years of age. Introduction of very small amounts of food to supplement breast milk begins at three to nine months. By their first birthday, most infants are receiving a supplementary diet comparable in composition to that of the adult, but in smaller amounts. At weaning, the child's diet is obviously deficient in calories and in proteins of good biological value. The mode of definitive weaning is 28 months.[5]

The main staple diet of villagers is corn, which is eaten in the form of "tortillas" and "tamales" (flat or bulky-cooked pancakes of lime-treated corn). Beans and diluted coffee supplement the diet. A variety of vegetables, mainly weeds, is also consumed. Meat and eggs are eaten once a week. Children receive proportionally less food than adults, while working men accordingly consume more. In general, diets lack protein of high biological value.[5, 6] It is evident that the feeding practices and available food induce a progressive deterioration of the nutritional state that begins with initiation of weaning.[5]

METHODS

Cohorts of Children

The studies reported here cover two aspects: 1) the development and nature of the fecal flora, and 2) the incidence of infection with serotypes of enteropathogenic *Escherichia coli* (EEC) and its relation to diarrheal disease.

For the study of the fecal flora, 30 consecutive newborns were recruited in 1967–1968 and were studied during their first week of life. Twelve of these infants were observed for at least one year.

For the study of EEC infections, 81 infants born during 1964–1966 were studied prospectively for varying lengths of time. Forty-three of these were observed for at least three years.

Clinical Evaluations

A pediatrician visited the home of every cohort child every week during the first month of life and every two weeks thereafter in order to evaluate the growth and general health condition of the child. The homes were also visited by field personnel once a week. This allowed illnesses to be reported promptly and permitted an accurate determination of their onset, characteristics, and diagnosis.

Diarrheal Disease. Based on familiarity with the syndrome in this region, diarrheal disease was defined as an illness associated with four or more abnormal bowel movements within 24 hours. A liquid or semi-liquid consistency, or the

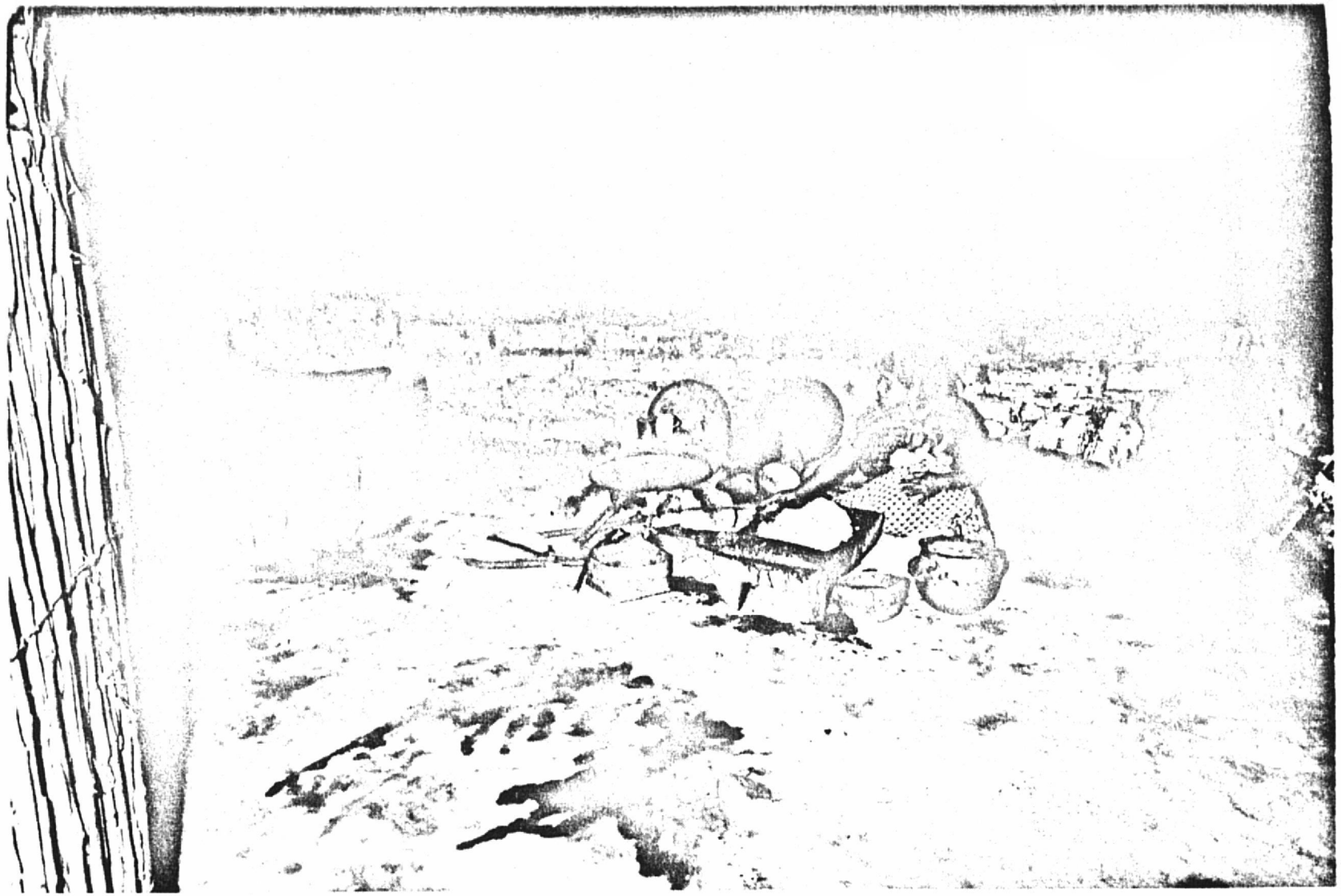

FIGURE 1. Typical one-room adobe home of one of the cohort children. In order to feed her child, the mother interrupted her task of grinding corn for the preparation of "tortillas." To the left is the "tapexco," or bed, and the fire on the dirt floor. Behind her, a large earthen jar contains the all-purpose water available in the dwelling.

presence of blood and mucus in the stools, comprised criteria that independently or jointly characterized them as abnormal. Less than four stools per day was considered indicative of diarrhea if blood and mucus were also present, or if egestions were liquid or semi-liquid, voluminous, or violently expelled.[7] Two bouts of diarrheal disease in the same child were considered independently if they were separated by a span of 15 days or more. A serotype of EEC isolated from seven days before to seven days after onset of diarrhea was assumed to be associated causally with the disease.

Treatment. No attempt was made to modify dietary practices or traditional customs and beliefs. Treatment of diarrheal disease consisted mainly in supportive measures and in providing *per os* or intravenous fluid when necessary. No antibiotics or sulfa drugs were employed in the treatment of diarrheal disease. Intramuscular penicillin was administered when bronchopneumonia became associated with diarrheal disease. In no instance were antimicrobial drugs given *per os* to cohort children.

Collection of Fecal Specimens

Fecal specimens were collected in half-pint cardboard containers by mothers or field personnel. Sterile wooden tongue depressors were used to transfer feces from the diapers or site of defecation to the containers. Specimens were transported to a laboratory installed directly in the village clinic and were processed no later than 30 minutes after egestion. The isolation and identification of cultures were performed directly in the village laboratory.

Whenever possible, meconium was collected for the study of the indigenous fecal flora. Efforts were made to gather several specimens during the infant's first week of life, and weekly samples were collected from all 12 infants during the following 12 months.

For the study of enteropathogenic *E. coli*, weekly specimens were taken from all children during the study period.

Isolation, Identification, and Quantitation of Bacteria

Indigenous Flora. Ten-fold dilutions of feces were prepared in tap water which had been adsorbed with 1% charcoal, filtered, and sterilized. Volumes of 0.01 ml of selected dilutions were streaked onto the agar surface of culture media by calibrated platinum-rhodium loops.[8] The size of the area inoculated, the kind of media used for the various bacterial groups, and the time and conditions of incubation are indicated in TABLE 1. The techniques used for quantitation of bacteria are described elsewhere.[9, 10] Anaerobic cultures were made with the use of Gaspak disposable generators.[11] The criteria employed for identification of anaerobic and facultative bacteria are described elsewhere.[10, 12]

Counts of *E. coli* were obtained in Tergitol 7 with 0.004% of triphenyltetrazolium chloride (T7T), MacConkey and Blood agar media. Counts obtained in blood agar and T7T often were one log higher, on the average, than those in MacConkey. To study the development of the *E. coli* flora, dilutions were plated on quadrants of T7T agar. After incubation (37° C, 24 hours), at least four colonies from each of the highest dilutions were transferred to TSI

TABLE 1

QUANTITATION OF THE FECAL FLORA OF MAN

Agar Medium	Group of Microorganisms	Dilution Streaked (log 10)	Percentage of Area of 100-mm Plate Streaked Per Dilution	Period of Incubation at 37° C,* hr.	Range of Bacterial Counts
BC-1, BC, and LS	Bacteroides, clostridia, bifidobacteria, lactobacilli, streptococci, and veillonellae	6, 7, 8	33	48 †	10^{8}–10^{12}
L	Microaerophilic lactobacilli, and streptococci	5, 6, 7, 8	25	48 ‡	10^{7}–10^{12}
E	Enterococci	4, 5, 6, 7	25	24	10^{6}–10^{11}
MS	Micrococci, staphylococci, and bacillus	2, 3, 4, 5	25	24	10^{4}–10^{9}
T7T, MacConkey and Blood Agar	*Escherichia coli* and other enterobacteriaceae	2, 3, 4, 5, 6, 7	33	24	10^{4}–10^{11}
SS and MacConkey	Enterobacteriaceae	Undiluted	100	24	10^{3}–10^{4}

* Incubation was aerobic, except where indicated otherwise.
† Incubation was anaerobic, with use of Gaspak disposable generators.
‡ Incubation was in candle jar.

agar slants; and if the reaction was compatible with *E. coli*, the IMViC was made.[13]

Enteropathogenic Escherichia coli. Undiluted feces were streaked on MacConkey agar. Six to ten lactose-fermenting colonies were transferred to nutrient agar and incubated for 18 hours at 37° C. Growth was tested with polyvalent and specific OB commercial sera (BBL). Cultures that agglutinated in any of the specific OB antisera were boiled for one hour and re-tested with the corresponding specific O antiserum. If there was agglutination, the O antigen was tested by tube titration against dilutions of the O antiserum. A positive reaction with a serum dilution of 1:320 or more was confirmatory.[13] The following serotypes were investigated: O111:B4, O55:B5, O127:B8, O26:B6, O86:B7, O119:B14, O124:B17, O125:B15, O126:B16, and O128:B12.

Results

Acquisition of Bacteria by the Breast-Fed Infant

Bacterial concentrations below 10^4 per gram of wet meconium and feces were not detected by the technique for the first week of life of the newborn. Thereafter, bacteria were not identified and their concentrations not estimated below 10^8 per gram for anaerobes, as indicated in Table 1.

The results to be described correspond to all cultures made during diarrheal disease-free periods.

The Neonatal Period. Bacteria were occasionally cultivated from meconium four hours after birth. Six out of nine samples passed from four to seven hours after birth contained facultative bacteria, mainly micrococci, streptococci, and enterobacteriaceae.

The pattern of appearance of bacteria in the neonatal period is seen in Figure 2. Half of the infants showed *E. coli* during their first 24 hours of life in concentrations of 10^8 to 10^{11} per gram. In the second day of life, all infants had *E. coli* with counts ranging from 10^5 to 10^{11} per gram.

In few infants did bifidobacteria appear in their first day of life. Only a third of the infants had these bacteria in their second day of life. However, bifidobacteria increased in proportion to age, and by the end of the first week of life, all infants had these bacteria in concentrations ranging from 10^9 to 10^{11} per gram.

Eighty-three percent of the infants showed anaerobic streptococci in their first day of life in concentrations of 10^6 to 10^{10} per gram of wet feces. By the second day, the counts were of 10^9 to 10^{11} per gram. Streptococci and *E. coli*, then more frequent than bifidobacteria in the first days of life, decreased by the end of the neonatal period to low frequencies; although, when found, their concentrations ranged from 10^7 to 10^{11} per gram.

Clostridia appeared in a few infants from their first day of life. High titers were obtained on the second day, but few infants had these bacteria thereafter.

A small number of infants became colonized by bacteroides and veillonellae in the first weeks of life. Those that were colonized had these bacteria from their second day of life. Bacteroides and veillonellae were not frequent in breast-fed neonates, although their concentrations ranged from 10^8 to 10^{11} when found.

The Postneonatal Period. The frequency of bacteria in 12 breast-fed infants

studied throughout the first year of life is shown in FIGURE 3. Bifidobacteria continued to be the most frequent group, found in concentrations of 10^{10} to 10^{11} per gram. Streptococci, veillonellae, and bacteroides increased progressively with age and were present in more than 75 percent of the infants studied at the end of their first year of life.

Enterobacteriaceae were always found at every age group. *E. coli*, however,

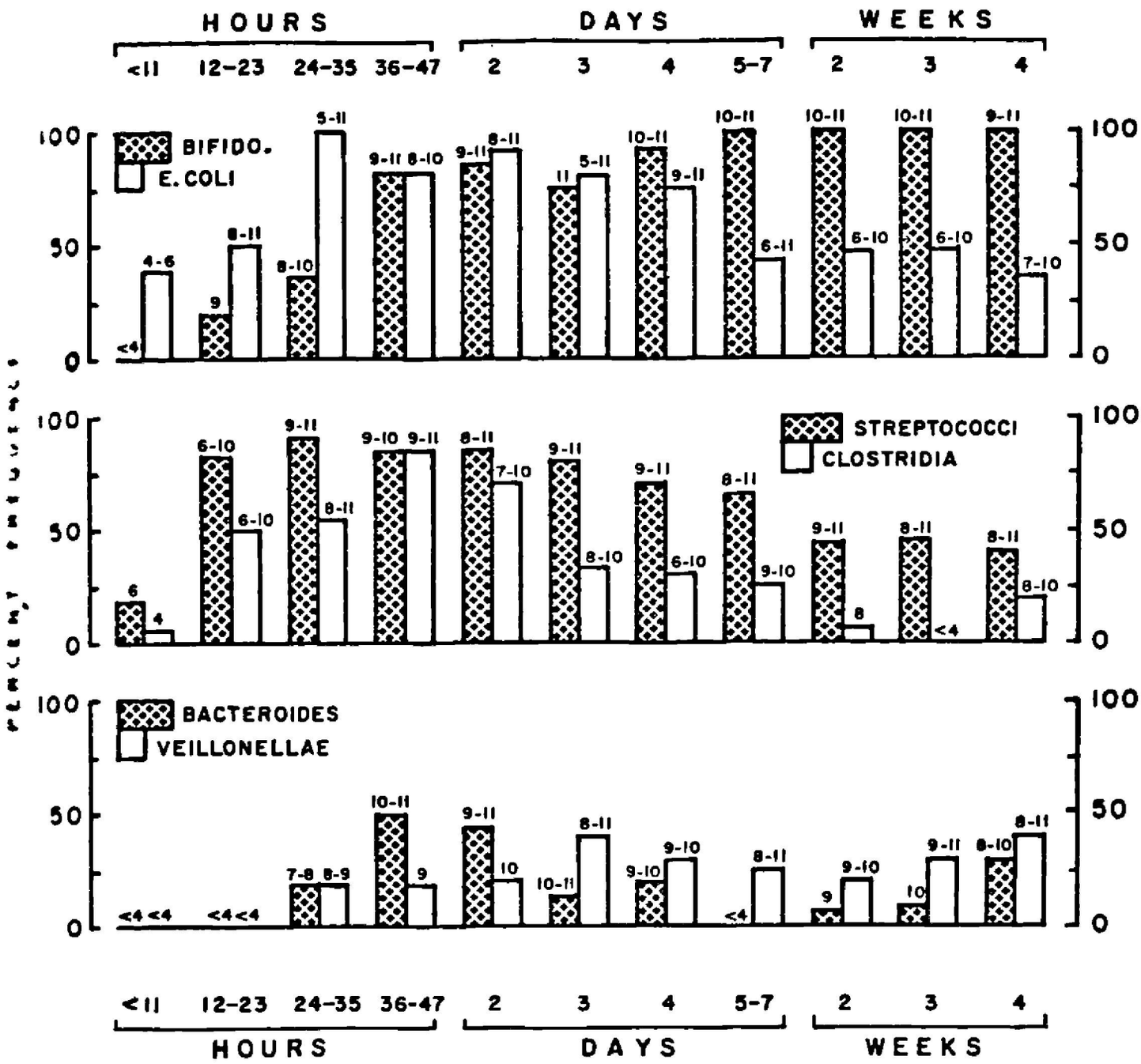

FIGURE 2. Frequency of bacterial groups in meconium and feces collected during the neonatal period, Santa María Cauqué, Guatemala, 1967–1968. Numbers on top of columns indicate the range of bacterial counts expressed as the reciprocal of $\log_{10}$ of concentration per gram of wet meconium or feces.

was not present (in concentrations of 10^6 or more) in all children within the neonatal period, but, rather, increased steadily in proportion to age. At two months of age, this group of bacteria was found in all infants, and, in most instances, where cultures were made. Enterobacteriaceae and other aerobic or facultative bacteria were always present in concentrations two to three logs below the level attained by bifidobacteria.

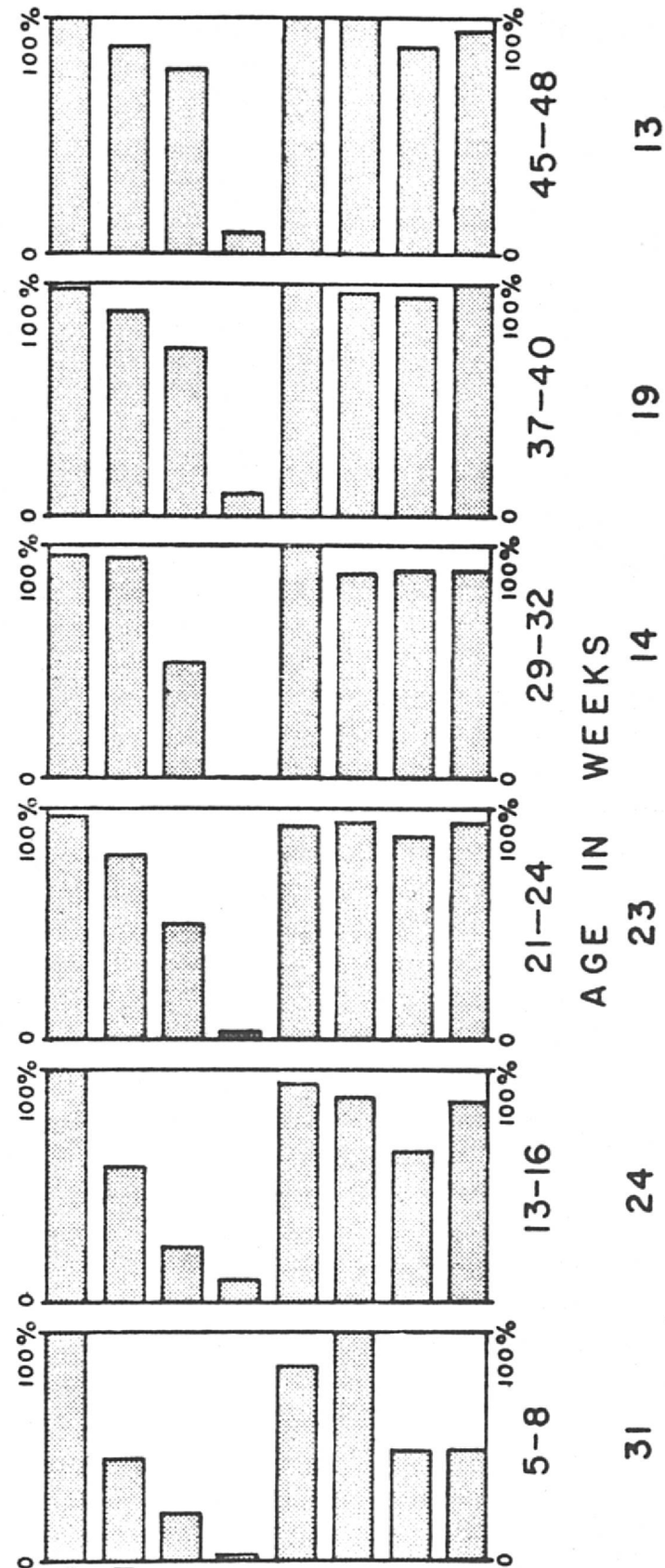

FIGURE 3. Frequency of bacterial groups in feces of 12 breast-fed infants studied from birth to one year of age, Santa María Cauqué, Guatemala, 1967–1968. Minimum bacterial count values detected are: 10^8 for bifidobacteria, streptococci, bacteroides, clostridia and veillonellae; 10^6 for enterococci; and 10^4 for micrococci and *E. coli*. Data correspond to disease-free periods only.

Clostridia was rarely found in the postneonatal period.

The frequency of bacterial groups cultured in concentrations of 10^8 per gram or more during the first nine months of life is shown in TABLE 2. *Eubacterium*, *Catenabacterium* and *Ramibacterium* were sporadically isolated (unpublished).

The same group observations described hold true for individually analyzed infants.

TABLE 2

FREQUENCY OF BACTERIAL GROUPS IN 262 FECAL SAMPLES OF HEALTHY INFANTS DURING THE FIRST NINE MONTHS OF AGE, SANTA MARIA CAUQUE, GUATEMALA 1967–1968

Bacterial Group	Lowest Concentration Detected	Number of Samples Positive	Percentage
Bifidobacteria	8 *	258	98.5
Veillonellae		157	59.9
Streptococci		152	58.0
Bacteroides		75	28.6
Lactobacilli		27	10.3
Clostridia		17	6.5
Micrococci	4	255	97.3
Enterobacteriaceae	4	233	88.9
Enterococci	6	184	70.2

* Log_{10} of bacterial concentration per gram of wet feces.

Shift of Fecal Flora of the Breast-Fed Child to That of the Adult

The fecal flora in the first two days of life consisted of streptococci, clostridia, bifidobacteria and facultative gram-negative bacilli. Shortly thereafter, bifidobacteria proliferated and outnumbered the other bacteria by the end of the first week of life. This phenomenon was accompanied by a decreased frequency in the appearance of clostridia, *E. coli*, and other bacterial groups.

At the end of the first week of life, all breast-fed infants studied had a "simple" flora almost totally formed by bifidobacteria in concentrations of the order of 10^{11} per gram of wet feces. The flora remained in this way during the period of exclusive breast-feeding (from birth to three months of age). During this period, the average concentration of *E. coli* was low (FIGURE 4).

Thereafter, there was a greater occurrence of certain bacterial groups in detectable concentrations (10^8 per gram or more) such as streptococci and bacteroides, as described before and illustrated in FIGURE 3. A proliferation of facultative gram-negative bacilli and enterococci was also detected (FIGURE 4). The relative concentrations of the facultative component particularly increased during the periods 13 to 16 and 21 to 32 weeks of age. This component was formed almost exclusively by enterobacteriaceae, of which *E. coli* was the predominant group. Other gram-negative facultative bacilli such as *Providence* and *Klebsiella-Aerobacter* were also found in high titers. *Proteus* was rare, and

Pseudomonas was not found. The anaerobic component of the flora, however, always outnumbered the aerobic one by two to three logs, although differences became less marked by the end of the first year of age.

Finally, with progressive food supplementation, the establishment of a solid diet, and eventual weaning, the anaerobic, gram-negative, nonsporulated bacilli (bacteroides) became more frequent and numerous and, eventually, outnumbered the other bacterial groups.

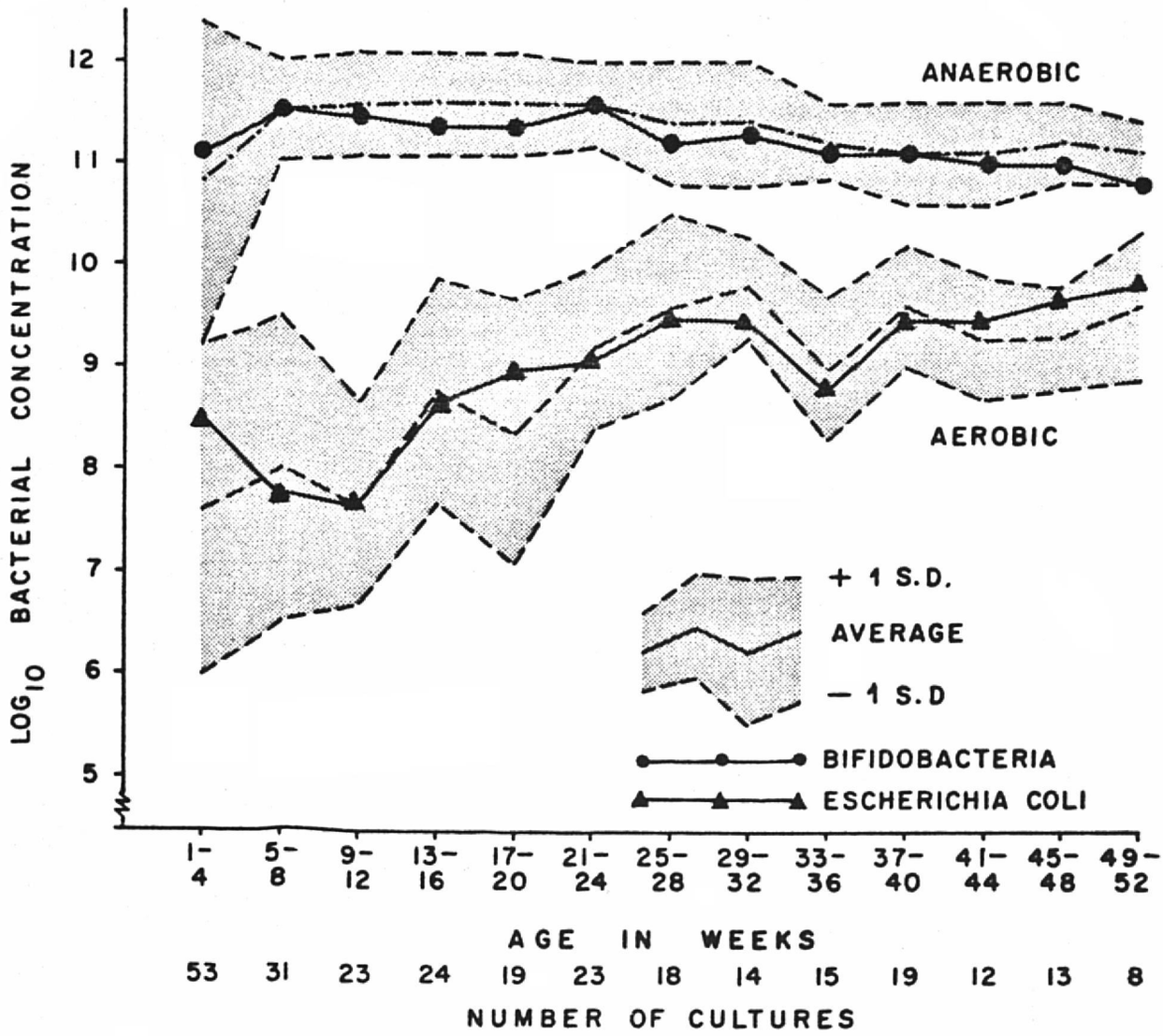

FIGURE 4. Anaerobic and facultative fecal flora of 12 breast-fed infants studied from birth to one year of age, Santa María Cauqué, Guatemala, 1967–1968. Figures were calculated from data of cultures obtained in disease-free periods.

The flora in breast-fed infants, weaned children, and adults of the area is compared in TABLE 3. Nearly 100% of all culturable bacteria in breast-fed infants was bifidobacteria. During weaning, there was a decrease by one log in the concentration of anaerobes and a proliferation of bacteroides. In the adult, these were more frequently encountered than bifidobacteria and outnumbered all other bacterial groups. The characteristic feature in the transition of one type of flora to another is a slow and subtle process that begins with the supplementation of maternal milk and continues throughout the prolonged weaning period observed in this region. The weaning period is characterized by higher rates of diarrheal disease in this population, as described subsequently.

TABLE 3

FECAL BACTERIAL FLORA IN BREAST-FED CHILDREN AND IN ADULTS, SANTA MARIA CAUQUE, GUATEMALA

Bacterial Group	12 Breast-Fed Children Age in Weeks				12 Weanlings 2 to 3 Years Old	12 Adults 13 to 37 Years Old
	5–8	13–16	21–24	45–48		
Bifidobacteria	11.1* (31/31)†	11.4 (24/24)	11.6 (22/23)	11.0 (13/13)	10.6 (12/12)	9.4 (9/12)
Bacteroides	9.6 (6/31)	10.3 (6/24)	10.2 (11/23)	9.9 (10/13)	9.2 (10/12)	10.3 (12/12)
Total Anaerobes	11.5	11.6	11.6	11.2	11.0	10.5
Total Aerobes	8.0	8.8	9.2	9.3	9.0	8.8
Ratio Anaerobes / Aerobes	3160 / 1	630 / 1	250 / 1	79 / 1	100 / 1	50 / 1
% Anaerobes in Total	>99.9	>99.8	99.7	98.8	99	98

* Average $\log_{10}$ of bacterial counts per gram of wet feces.
† Number of cultures with 10^8 or more of the bacterial groups in total number of cultures.

Infection with Enteropathogenic Escherichia coli

A total of 7,792 weekly cultures were made in the cohort of children, as shown in TABLE 4. Only 40 infections with enteropathogenic *E. coli* (EEC) were demonstrated, resulting in an overall infection rate of 0.51 per 100 child-weeks of experience. In view of the low frequency of EEC encountered, the data will be presented as incidence rates. Infection with EEC was lowest in the

TABLE 4

ENTEROPATHOGENIC *Escherichia coli* IN CHILDREN STUDIED FROM BIRTH TO THREE YEARS OF AGE, SANTA MARIA CAUQUE, GUATEMALA, 1964–1969

Age Weeks	Number of Children	Weeks at Risk *	Number of Infections †	Rate per 100 Child-Weeks
0–25	81	1783	6	0.34
26–51	65	1546	7	0.45
52–77	52	1192	7	0.59
78–103	46	1096	12	1.1
104–129	44	1100	3	0.27
130–155	43	1075	5	0.46
Total:		7792	40	0.51

* Weeks in which children were examined clinically and bacteriologically.
† Two infections were considered independent if separated by two weeks or more.

first and fifth semesters of life (0.3 per 100 child-weeks), and highest in the second half of the second year of life (1.1 infections per 100 child-weeks), that is, when weaning is at its peak.

Diarrheal disease occurred in the first semester of life at a rate of 4.9 attacks per 100 child-weeks (TABLE 4) and increased with age until it reached a peak of 8.8 per 100 child-weeks in the second year of age. Only one-third of all EEC infections was shown to be associated with diarrheal disease; the rest were asymptomatic.

In infants under six months of age, only 1.1% of diarrheal episodes had an associated EEC infection (TABLE 5). With initiation of weaning, the percentage increased to 3.3 in the second semester of life, and to 3.8 in the third semester. The association of diarrheal disease with EEC infection declined thereafter. In

TABLE 5

ENTEROPATHOGENIC *Escherichia coli* (EEC) AND DIARRHEAL DISEASE IN CHILDREN STUDIED FROM BIRTH TO TWO YEARS OF AGE, SANTA MARIA CAUQUE, GUATEMALA, 1964–1969

Age Weeks	Number of Children	Weeks at Risk	Diarrheal Disease *			Percentage of Diarrheal Disease Associated with EEC
			With EEC	Without EEC	Total	
0–25	81	1783	1(0.06)	86(4.8)	87(4.9)	1.1
26–51	65	1546	4(0.3)	116(7.5)	120(7.8)	3.3
52–77	52	1192	4(0.3)	101(8.5)	105(8.8)	3.8
78–103	46	1096	2(0.2)	93(8.5)	95(8.7)	2.1
Total:		5617	11(0.2)	396(7.1)	407(7.2)	2.7

* Two episodes were considered independent if separated by two weeks or more.

general, the role of EEC in diarrheal disease of children of the area appears considerably less significant than that of *Shigella*. The incidence of this group of organisms was also low during the period of exclusive breast-feeding, but increased markedly in the second and third years of life. The significance of *Shigella* in diarrheal disease in the same cohort of children [14] is shown in TABLE 6 in comparison with that of EEC described above. It is evident that *Shigella* has a greater importance than EEC in the etiology of diarrhea in the first years of life.

Of the ten serotypes investigated, only one (O119:B14) was not found; O55:B5, O111:B4, and O125:B15 accounted for two-thirds of all isolations (TABLE 7), and for nine out of the 11 instances in which EEC was associated with diarrheal disease. Regarding duration of infection, 33 of the 40 infections were detected only in one of the weekly cultures, five were found in two consecutive weeks, and two in three- or four-week periods. *E. coli* O55:B5 persisted more than the other serotypes.

TABLE 6

Shigella AND ENTEROPATHOGENIC *Escherichia coli* (EEC) IN RELATION TO DIARRHEAL DISEASE IN CHILDREN STUDIED FROM BIRTH TO TWO YEARS OF AGE, SANTA MARIA CAUQUE, GUATEMALA, 1964–1969

Age Weeks	Number of Children	Weeks at Risk	Number of Attacks of Diarrheal Disease (DD)			Percentage of DD Associated with:	
			Total	With *Shigella*	With EEC	*Shigella*	EEC
0–25	81	1783	87	1	1	1.1	1.1
26–51	65	1546	120	11	4	9.2	3.3
52–77	52	1192	105	20	4	19.0	3.8
78–103	46	1096	95	37	2	38.9	2.1
Total:		5617	407	69	11	16.9	2.7

DISCUSSION

The significance of the present study is derived from: 1) its long-term prospective nature; 2) its being conducted in a somewhat primitive population not remarkably influenced by customs and practices of modern technology; and 3) the experimental design, which reduces perturbance of the ecosystem to a minimum.

The data show that colonization of the infant's intestinal tract occurred early in life in accordance with observations in industrial societies.[15] A variety of bacterial groups indigenous to the adult of the area was established in the

TABLE 7

SEROTYPES OF ENTEROPATHOGENIC *Escherichia coli* IN A COHORT OF CHILDREN STUDIED FROM BIRTH TO THREE YEARS OF AGE, SANTA MARIA CAUQUE, GUATEMALA, 1964–1969

Serotype	Number of Strains	Number Associated with Diarrheal Disease	Duration of Infections in Weeks			
			1	2	3	4
0111:B4	9	3	8	1	0	0
055:B5	10	4	*5*	*3*	*1*	*1*
0127:B8	4	0	4	0	0	0
026:B6	3	1	3	0	0	0
086:B7	1	0	1	0	0	0
0124:B17	1	0	1	0	0	0
0125:B15	7	2	6	1	0	0
0216:B16	4	1	4	0	0	0
0128:B12	1	0	1	0	0	0
Total:	40	11	33	5	1	1

infant's intestine during the first few days of life. The bacterial groups that appeared early in feces did not necessarily form the bulk of the flora in later life. For example, streptococci, enterobacteriaceae, and clostridia were first to proliferate, appearing in feces in the first two days of life, but declined and remained at a low level of frequency for several weeks thereafter. Bifidobacteria appeared later than other groups but, within one week, constituted the predominant bacteria in feces. During the period of exclusive breast-feeding, *E. coli* and streptococci were not always cultured.

The onset of weaning, together with the introduction of small amounts of fluids (at three months), gruels, and solids (from six to 12 months), marked an increase in the frequency of appearance of streptococci, veillonellae, and bacteroides. As weaning progressed, all children were effectively colonized with *E. coli*, and the concentrations attained were high.

The anaerobic component of the flora of healthy children outnumbered the aerobic, as demonstrated in other regions of the world.[16–18] The predominance of the anaerobes was accentuated during the period of exclusive breast-feeding. A gradual and subtle proliferation of facultative bacteria began with onset of weaning, and eventually, these bacteria formed up to 10%, but no more, of the total flora under conditions of good health.

Proliferation of *E. coli* is slow during the first months of breast-feeding. This is probably due to the overgrowth of bifidobacteria and, perhaps, to the presence of secretory IgA and other immunoglobulins in milk. These immunoglobulins have been found in significant concentrations in the milk of village women during the first weeks of lactation (unpublished).

The indigenous *E. coli* strains may have a dynastic behavior,[19] remaining for varying lengths of time in the intestinal tract. Establishment of invading *E. coli* varieties may not be easy. As already mentioned, no infections with EEC were discovered in neonates in this study, and few were detected in the first year of life.

This phenomenon is not due to lack of opportunities for infection, in view of the prevailing poor sanitary conditions. For example, significant fecal contamination occurs during delivery, explaining the appearance of early infection with pathogenic agents.[5] On the other hand, fecal bacteria have been demonstrated in maternal milk as it is suckled by the baby in a significant number of cases.[20] The phenomenon of negligible infection of the rural breast-fed infant with EEC also is not due to absence of carriers, since a variety of serotypes of EEC circulate regularly in the village.

It appears, therefore, that the breast-fed infant from rural areas has a particular resistance to colonization with EEC. On the other hand, EEC is found frequently in infants with diarrhea from the city suburbs who are partly or wholly artificially fed and who are similar in ethnic background to village infants.[21]

Effective colonization of breast-fed infants may occur, but when it does, it is transient and often asymptomatic. A probable explanation of the resistance presented by the breast-fed infant to infection with EEC is the predominance of the bifidus flora. This flora is stimulated by maternal milk.[22, 23] It consists of anaerobic gram-positive nonsporulated bacilli capable of producing large amounts of acetic and lactic acid *in vitro* and *in vivo*.[24, 25] Considerable amounts of these substances have been detected in feces of breast-fed infants,[26, 27] accounting for the low pH values of feces attained at the end of the first week of life.[28] Studies *in vitro* have shown that acetic and formic acids, in conjunction with a

low pH, a condition resembling that of the intestine of breast-fed infants, inhibit the growth of *Shigella* and *E. coli*.[29, 30] On the other hand, artificial feeding induces changes in flora composition, consisting of a relative decrease in the number of gram-positive bacilli and a proliferation of coliforms, changes that are accompanied by a rise in the pH of feces.[16] Similar alterations were observed throughout the present study, although they were more protracted due to the prolonged nature of the weaning process. Variations in the conditions of the intestinal milieu were offered to explain the characteristic short-lived and, usually, asymptomatic *Shigella* infection of the breast-fed neonate[31] and the increasing frequency of *Shigella* in village children as they entered into the weaning period.[14]

Regarding infection with EEC, Svirsky-Gross[32] demonstrated that feeding human milk to infants during an outbreak of *E. coli* O111 resulted in the elimination of this serotype from carriers and ill infants after other measures had repeatedly failed. Such observations are supported by reports on the greater resistance to gastroenteritis of the breast-fed, as compared to the bottle-fed, infant.[33, 34] In village infants, the lowest concentrations of *E. coli* were detected during the period of exclusive breast-feeding, and EEC infections were rare.

It appears, therefore, that there are certain conditions in the intestine of the breast-fed infant unfavorable for the proliferation of enteropathogenic bacteria. Wholly breast-fed infants, however, are not free from diarrhea, which appears in low frequency and is usually of a mild nature. Other possible etiologies should be explored, such as *Shigella*-like and *Salmonella*-like varieties of *E. coli* and new enterotoxic *E. coli* serotypes.[35–37]

The oncoming influence of urbanization and industrialization will force a substitution for the traditional ways of nursing. Unless these changes are accompanied by improvements in the environment and proper sanitary education, this transformation will most likely be accompanied by a greater occurrence of early infection and disease by known enteric pathogens.

Summary

The development of the indigenous intestinal flora was studied in breast-fed children of low socioeconomic status, without removing them from their village environment. Efforts were made to minimize disturbance of the ecosystem.

The fecal flora in the first few days of life showed marked differences among infants in regard to kinds and numbers of bacteria. *Escherichia coli* established early in all infants. However, at the end of the first week of life, all infants had a predominant flora of bifidobacteria in concentrations of 10^{11} per gram of wet feces. Clostridia, streptococci, and *E. coli* decreased in the first few weeks of life, paralleling the proliferation of bifidobacteria. Clostridia, bacteroides, and veillonellae were rare in exclusively breast-fed infants.

The flora evolved in a very subtle manner during the prolonged weaning process (which begins at three to six months of age), becoming similar or identical to that of the adult once weaning was fully implemented. During this slow process, the characteristic gram-positive anaerobic component (bifidobacteria) decreased as the gram-negative anaerobic component (bacteroides) increased.

E. coli proliferated to levels of 10^9 to 10^{10} per gram with initiation of weaning. No infections with any of ten serotypes of EEC were discovered in neo-

nates. Enteropathogenic *E. coli* infections appeared later and were short-lived. The rate of infection increased from 0.34 per 100 child-weeks in the first semester of life to 1.1 in the fourth semester. Only 1.1% of the diarrheas recorded in the first semester of life were found associated with EEC. In the second and third semesters, relatively more diarrheas were found causally related to EEC (3.3 and 3.8% respectively).

Therefore, breast-fed village children experienced few infections with enteropathogenic *E. coli*, a phenomenon apparently related to the peculiar characteristics of the intestinal milieu brought about by the predominance of bifidobacteria.

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