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INCIDENCE AND ETIOLOGY OF INFANTILE DIARRHEA AND MAJOR ROUTES OF TRANSMISSION IN HUASCAR, PERU

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Community-based studies of diarrhea etiology and epidemiology were carried out from July 1982–June 1984 in 153 infants residing in a poor peri-urban community near Lima, Peru. Study infants had nearly 10 episodes of diarrhea in their first year of life. Diarrhea episodes were associated with organisms such as *Campylobacter jejuni*, enterotoxigenic and enteropathogenic *Escherichia coli*, *Shigella*, rotavirus, and *Cryptosporidium*. These organisms appeared to be transmitted to infants in the home through animal feces, through contaminated water and food, and by direct person-to-person contact. A particularly important route of transmission may have been weaning foods, which were often contaminated because of improper preparation and inadequate cleaning of utensils. Improved feeding practices, along with avoidance of animal feces and improved personal and domestic hygiene, should be considered important interventions in reducing the high incidence of diarrhea in infants in developing countries.

Campylobacter* infections; diarrhea, infantile; dysentery, bacillary; *Escherichia coli* infections; food contamination; infant food; rotavirus infections; *Shigella

In community-based studies in developing countries, diarrhea has been found to occur up to 10 times per year among children in the first few years of life (1–3). This

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diarrhea is caused by a variety of infectious microorganisms, some of which have been recognized only recently (2, 3).

The factors contributing to the high incidence of diarrhea among young children in developing countries certainly include overcrowded conditions, poor sanitation, contaminated water, and inadequate food hygiene (4). These conditions are accentuated in urban slums, where recent migrants may also make abrupt changes in traditional practices of child care, including feeding of infants (5).

One frequently noted effect of urbanization is a decrease in the prevalence of breastfeeding and the early introduction of milk formula, too often inappropriately diluted and contaminated, to the diet of young infants (6). Several studies have demonstrated a lower incidence of diarrhea in breastfed infants than in artifically fed infants, especially with exclusive breastfeeding during the first few months of life (7). This may be because of protective factors in breast milk such as leukocytes, specific immunoglobulins, and nonspecific antiinfectious factors (8); however, an equal, if not more important, protective influence of breastfeeding is that breast milk is rarely contaminated with harmful enteric bacteria.

The hazard of contamination of milk or formula given to bottle-fed babies has received a great deal of attention and deserves emphasis particularly in urban and periurban areas of developing countries (9). However, many of the children suffering high rates of diarrhea in developing countries are breastfed and also given traditional weaning foods, such as starchy pastes made with rice, maize, potato, wheat, banana, or other locally available foods. Studies in The Gambia (10) and Bangladesh (11) demonstrated that these foods can be as hazardous bacteriologically as bottle formula when prepared in the usually unhygienic home environments. In addition, these studies showed that the level of contamination was related to the time of storage of the food at ambient household temperatures, presumably because of bacterial multiplication in the food.

While exclusive breastfeeding keeps the infant from being exposed to food with high levels of bacterial contamination, breast milk is not sufficient by itself to satisfy the nutritional requirements of infants beyond three to four months of age (12). Other food must be introduced, even though breastfeeding should continue at least into the second year of life. The need to supplement breast milk with other nutritious foods to maintain optimal growth and the likelihood that these foods will result in diarrhea, which may adversely affect the infant nutritionally, has been called "The Weanling's Dilemma" (10).

To improve the nutritional value and hygienic standard of traditional weaning foods, it is desirable to have an understanding of diarrheal diseases, including their etiology and common routes of transmission. It is expected that this information can then be used to design optimal weaning foods and educational approaches to encourage their adoption.

Materials and Methods

The project was carried out in the underprivileged community of Huascar on the outskirts of Lima, Peru (13). Detailed longitudinal studies of 153 newborns were conducted between July 1982 and June 1984. The community, study population, medical services, and surveillance methodology are described in the accompanying paper (14). In thrice-weekly household surveillance, diarrhea and other illnesses were identified and information such as the number and consistency of stools, the presence or absence of blood, and the occurrence of vomiting, fever, or anorexia was recorded. Children thought to have dehydration on the basis of an examination by a trained field worker were evaluated by a pediatrician and were diagnosed as having dehydration if any objective signs (e.g., sunken fontanel

or eyes, decreased skin turgor) of dehydration were present. All children with diarrhea were provided with oral rehydration solution by the field workers, and the few children with more severe illnesses were taken to a medical center. Infants with dysentery and apparent toxicity were treated immediately with trimethoprim-sulfamethoxazole. Otherwise, treatment was provided only in the case of a positive bacteriologic culture for *Shigella* (trimethoprim-sulfamethoxazole) or *Campylobacter jejuni* (erythromycin), if dysentery was still present when the culture results became available.

Fecal samples were obtained whenever there was: 1) an increase in stool frequency of two or more in relation to the previous days, along with a decrease in stool consistency; 2) an increase in stool frequency of three or more, independent of consistency; 3) the passage of watery stools; or 4) blood in the stool. The surveillance workers took a sample of the feces (whole stool or rectal swab) at the time of suspected diarrhea and routinely one or two times each month when diarrhea was not present. The definition of a diarrhea episode was formulated during the analysis of surveillance data, as described below.

Samples of feces were placed immediately in vials as follows: Two rectal swabs were placed in Cary-Blair transport medium (one vial with antibiotics [vancomycin (10 mg/liter), polymyxin B (2,500 IU/liter), and trimethoprim (5 mg/liter)] added to transport *Campylobacter*), one stool sample was placed in phosphate-buffered saline (pH 7.4), and one stool sample was placed in thimerosal, iodine, and formalin. The samples in Cary-Blair transport medium were refrigerated in the evening and plated the next morning. One of these samples (from the vial without antibiotics) was plated on thiosulfate-citrate-bile salts-sucrose, *Salmonella-Shigella*, and MacConkey agars, incubated for 18 to 24 hours, and examined for the presence of *Salmonella*, *Shigella*, vibrios, and other enteropathogens by standard methods (15–17).

Five typical *Escherichia coli*-like colonies were picked from MacConkey agar and placed on nutrient agar slants. These colonies were later spotted onto nitrocellulose filters (on the surface of MacConkey agar) and processed for detection of the *E. coli* genetic sequence determining production of heat-stable toxin or heat-labile toxin by the DNA probe method (18, 19). A swab was also spotted directly onto a nitrocellulose filter (on MacConkey agar), and the resulting bacterial growth (stool blot) was evaluated by the DNA probes for heat-stable toxin and heat-labile toxin (18, 19). If positive results were obtained for the stool blot but not for the individual colonies, the colonies were retested. Positive colonies were confirmed by repeat DNA probe assays, and selected colonies were confirmed by assays for enterotoxins (adrenal cell assay for heat-labile toxin and infant mouse assay for heat-stable toxin) (20, 21). The specimen was considered positive only if individual colonies could be demonstrated to be positive. The same five colonies were screened for enteropathogenic *E. coli* by slide agglutination with three pools of OK antisera (pool 1: O86, O127, O55, O111; pool 2: O44, O119, O128, O125; pool 3: O26, O158, O114, O142). The antisera were prepared by inoculating rabbits with formalin-killed *E. coli* (strains used were enteropathogenic *E. coli* serotypes supplied by Dr. F. Orskov, Statens Seruminstitut, Copenhagen, Denmark) (22). All colonies that were positive in the screening were then agglutinated with monovalent OK antiserum. Definitive O:H serotyping was performed at the Centers for Disease Control (Atlanta, GA) or in Lima by tube agglutination of boiled bacteria with O antiserum and subsequent H typing (also by tube agglutination).

The second swab in Cary-Blair medium was plated on *Campylobacter* agar containing 7 per cent sheep blood and antibiotics (bacitracin (25,000 IU/liter), novobiocin (5 mg/liter), cycloheximide (50 mg/liter),

cefazolin (15 mg/liter), and colistin (10,000 U/liter)) and incubated at 42 C, and colonies typical of *C. jejuni* were identified (17). We analyzed the sample in phosphate-buffered saline by means of the enzyme-linked immunosorbent assay for rotavirus (23), using reagents supplied by the Programme for the Control of Diarrhoeal Diseases of the World Health Organization. The thimerosal, iodine, and formalin-preserved stool specimens were examined for parasitic ova and parasites by direct microscopy. A sample of the preserved stool specimens was studied for *Cryptosporidium*. Slides were smeared with fecal material, and after drying they were fixed in methanol for one minute, rinsed in tap water, immersed in 1 N sodium hydroxide for one minute, and rinsed again. The smears were stained using the Kinyoun modification of the Ziehl-Neelsen stain (24). Red-stained oocysts were sought in 10 randomly selected high power fields.

Microbiologic studies of food and water were done for all children at six and nine months of age. Specimens of raw foods, prepared menu items, and water were collected during the household dietary studies, in which infants were observed for 12 hours on each day of study. Specimens were placed in sterile containers and kept refrigerated (<24 hours) until laboratory processing. Food specimens were homogenized in a Stomacher (Cooke Laboratory Products, Dynatech Labs, Inc., Alexandria, VA), serially diluted 10-fold in sterile saline, and spread plated on eosin-methylene blue agar. Counts of *E. coli*-like colonies were determined, and the count per gram or per milliliter of the sample was used as an indicator of fecal contamination. In addition, undiluted samples (1.0 ml) were inoculated into GN and selenite F enrichment broths (9.0 ml) and then plated the next day on xylose-lysine-deoxycholate and Hektoen agars. Undiluted (or 1:10 dilutions for solid or semisolid foods) samples (0.1 ml) were also plated directly on thiosulfate-citrate-bile salts-sucrose and *Campylobacter* agars. A 10^5 dilution (0.1 ml) was plated on sheep blood agar, and the plate was examined for *Bacillus cereus*-like colonies (25). Food specimens (0.1 ml) were spotted onto nitrocellulose filters, which were on the surface of MacConkey agar plates (26). Water specimens (100 ml) were filtered through 0.45-micron nitrocellulose filters, which were then removed from the vacuum manifold and placed on MacConkey agar for overnight incubation (27). In addition, five *E. coli*-like colonies per sample of food or water were picked from the eosin-methylene blue plate and stored on nutrient agar slants until they were inoculated onto nitrocellulose filters and processed. All of the nitrocellulose filters were subjected to the DNA probe assays for heat-labile toxin and heat-stable toxin (18, 19). Food or water specimens were considered positive if either the individual colonies or the direct food or water blot, which has been shown to be more sensitive (26, 27), was positive.

Microbiologic contamination of the home environment was studied by culturing selected objects that had contact with food or children. Objects, such as feeding bottles or spoons, were rinsed with 10 ml of sterile saline. Hands were each placed in a sterile plastic bag with 10 ml of saline, massaged, and removed; the saline was saved for culture. The nipples of breastfeeding mothers were cultured with a moistened cotton-tipped swab. The presence of *E. coli*-like colonies was considered evidence of fecal contamination. Fecal samples from animals living in the house were taken and studied for enteric pathogens, such as *Campylobacter* and *Salmonella*. Environmental and animal specimens (five *E. coli*-like colonies per specimen) were studied by DNA probes for heat-stable toxin- or heat-labile toxin-producing *E. coli*.

After analyzing the daily number and characteristics of the stools in relation to the ages of the children, we adopted criteria to define a diarrhea episode. An episode of diarrhea was defined as at least one day with liquid stools totaling at least six for

infants aged less than one month, five for infants aged one month, and four for infants aged more than one month. The duration of a diarrhea episode was considered to be every day that met the main criteria plus all previous or subsequent days with liquid stools if the number of such stools was equal to that of the main criteria or less than that of the main criteria by one. New episodes of diarrhea were diagnosed when infants had at least two illness-free days following recovery from a previous episode.

Enteropathogens were considered to be associated with a diarrhea episode if they were identified from a specimen obtained during the episode or one day before or after the eposide. If more than one specimen was obtained during an episode, all positive results were accepted. Of all episodes with specimens obtained, 76 per cent had one specimen, 16 per cent had two specimens, and 8 per cent had three or more specimens. Enteropathogens identified from other specimens were not considered to be associated with an episode of diarrhea, although some probably represented carriage during early convalescence. Without the use of serotyping or more sophisticated bacterial or viral strain markers, we considered it impossible to separate persistent infection in convalescence after diarrhea from new infection. Furthermore, because of the possibility, by our definition, that a new episode of diarrhea could begin after two illness-free days, it was considered desirable that a specimen be uniquely assigned to one episode. We calculated the age- and pathogen-specific incidences and prevalences of diarrhea using the number of days at risk determined by household surveillance of study children. The pathogenicity of the enteropathogens was assessed from the diarrheal and routine stool specimens from study infants and was defined as the proportion of infections associated with diarrhea divided by the total number of infections (diarrheal and nondiarrheal) identified. An average of 244 total specimens (range, 217–296) were evaluated in each month of age of study infants. Statistical comparisons were done with Student's *t*, chi-square, and Fisher's exact tests, as appropriate.

RESULTS

Diarrhea epidemiology

During the surveillance period, 1,299 episodes of diarrhea were identified. The incidence of diarrhea was 9.8 episodes per child in the first year of life, and it did not differ substantially by month of age (range, 0.64–1.0 episode per child-month).

Of the 1,299 diarrhea episodes, 952 (73 per cent) were studied for enteropathogens. Diarrhea episodes that were not studied were primarily of short duration (77 per cent lasted one to two days) and were not cultured since they were resolved prior to the thrice-weekly surveillance worker's visit. Routine specimens for enteropathogens from non-ill children were obtained on 1,973 occasions. During the diarrhea episodes, *C. jejuni* and enterotoxigenic *E. coli* were most common, but both had a similar frequency of recovery in ill and non-ill children (table 1). However, *C. jejuni* tended to have a higher isolation rate in diarrhea cases (12.8 per cent) than in controls (8.7 per cent) in the first four months of life. Enteropathogenic *E. coli*, rotavirus, and *Shigella* were significantly associated with diarrhea in comparison to controls. Cryptosporidia were identified in seven of 100 specimens from diarrhea episodes and in one of 100 routine samples ($p < 0.05$). Other pathogens were infrequent (table 1). Infection with more than one pathogen was noted in 10.7 per cent of episodes.

Among the enterotoxigenic *E. coli*, 207 (56 per cent) produced only heat-labile toxin, 113 (31 per cent) produced only heat-stable toxin, and 48 (13 per cent) produced both. Enteropathogenic *E. coli* were of a number of different serotypes (table 2). Fifteen per cent of the enteropathogenic *E.*

TABLE 1

Enteropathogens identified in routine and diarrheal stool specimens from 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Pathogen	Routine (n = 1,973)		Diarrheal (n = 952)	
	No.	%	No.	%
Campylobacter jejuni	191	9.7	96	10.1
Enterotoxigenic *Escherichia coli*				
LT-producing†	86	4.4	35	3.7
ST-producing	31	1.6	25	2.6
ST- and LT-producing	21	1.1	10	1.1
Enteropathogenic *E. coli*	69	3.5*	58	6.1*
Rotavirus	20	1.0*	24	2.5*
Shigella sp.	15	0.8*	19	2.0*
Giardia lamblia	16	0.8	7	0.7
Salmonella sp.	11	0.6	8	0.8
Aeromonas hydrophila	2	0.1	5	0.5
Plesiomonas shigelloides	3	0.2	3	0.3
Vibrio cholerae non-O group 1	1	0.05	1	0.1
Mixed	73	3.7*	102	10.7*
Negative	1,434	72.8*	559	58.7*

* $p < 0.01$.

† LT, heat-labile toxin; ST, heat-stable toxin.

TABLE 2

Serotypes of enteropathogenic E. coli isolated from stool cultures of 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Serotype*	No. of strains	Serotype*	No. of strains
O86:ND	8	O128:ND	8
O86:H2	2	O128:H21	8
O86:H6	6	O128:H27	1
O86:H10	2	O128:H35	1
O86:H11	4		
O86:H18	1	O44:ND	1
O86:H19	8	O44:H18	17
O86:NM	1		
		O127:ND	1
O119:H6	22	O127:H10	2
O119:NM	2	O127:H18	1
		O127:H21	4
O111:H2	1	O127:H40	5
O111:NM	19		
		O142:ND	2
O114:H10	8	O142:H6	1
O114:H49	5	O142:H21	8
O114:NM	7		
		O55:ND	2
O125:H21	10	O55:H6	2
		O55:H7	1
O125:NM	9	O55:NM	1
		O158:ND	1

* ND, H type not done; NM, nonmotile.

coli were DNA probe-positive for heat-stable toxin or heat-labile toxin or both; the proportion producing enterotoxins was highest for O128 *E. coli* (59 per cent). Rotavirus strains were predominantly of subgroup I (39 of 65 tested), and the remainder were of subgroup II. Nine of the subgroup I strains were electropherotyped; eight were of the short type, which is consistent with subgroup I, and one was of a long type, suggesting an animal strain or a naturally occurring reassortant. Fifty of the rotavirus strains were serotyped; 37 were serotype 2, 10 were serotype 1, and three were serotype 3.

The pathogenicity of the most frequent enteropathogens (single infections) was calculated for infants aged birth to five months and aged six to 11 months (table 3). The pathogenicity of *C. jejuni* and of enterotoxigenic or enteropathogenic *E. coli* ranged from 0.35 to 0.57 and did not differ in younger or older infants for any of these agents. The pathogenicity of rotavirus was higher in older infants than in younger infants ($p = 0.02$). Conversely, the pathogenicity of *Shigella* was higher in younger infants than in older infants ($p = 0.02$). Since mixed infections with two or more enteropathogens were common, we examined the interaction of these infections by comparing the pathogenicity of mixed infections versus that of single infections with selected enteropathogens (table 4). Mixed infections of *C. jejuni* with enterotoxigenic or enteropathogenic *E. coli* or of enterotoxigenic *E. coli* with enteropathogenic *E. coli* did not have a higher association with diarrhea than did the single pathogens alone. Mixed infections of rotavirus and one or more other agents had a pathogenicity of 0.86, which was higher ($p < 0.002$) than that of *C. jejuni*, enterotoxigenic *E. coli*, or enteropathogenic *E.*

TABLE 3

Pathogenicity of enteropathogens, by age group, in 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Enteropathogen	Age 0–5 months			Age 6–11 months		
	No. of infections with diarrhea	Total infections	Pathogenicity	No. of infections with diarrhea	Total infections	Pathogenicity
C. jejuni	73	177	0.41	65	188	0.35
Enterotoxigenic *E. coli*	42	119	0.35	40	103	0.39
Enteropathogenic *E. coli*	43	75	0.57	44	97	0.45
Rotavirus	18	33	0.55*	23	28	0.82*
Shigella sp.	14	17	0.82*	16	34	0.47*

* $p = 0.02$, chi-square test.

TABLE 4

Pathogenicity of single infections versus mixed infections with selected enteropathogens in 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Enteropathogen	No. of infections with diarrhea	Total infections	Pathogenicity
C. jejuni	138	365	0.38
Enterotoxigenic *E. coli*	82	222	0.37
Enteropathogenic *E. coli*	87	172	0.51
Rotavirus	41	61	0.67
C. jejuni/ETEC†	18	41	0.44
C. jejuni/EPEC	8	14	0.57
ETEC/EPEC	17	34	0.50
Rotavirus/*C. jejuni*, ETEC, or EPEC	18	21	0.86*

* $p < 0.002$, chi-square test, versus single infections with *C. jejuni*, enterotoxigenic *E. coli*, or enteropathogenic *E. coli*.

† ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*.

coli, but not significantly higher than that of single infection with rotavirus.

Most episodes of diarrhea lasted between one and seven days (table 5). Episodes of shigellosis tended to have a longer median duration. Overall, 7.1 per cent of diarrhea episodes lasted longer than two weeks. Ninety-one of the 92 episodes of greater than 14 days duration were studied for enteropathogens. Sixty-two (68 per cent) had at least one pathogen identified; 26 (29 per cent) had more than one pathogen. The prolonged episodes were associated with the following organisms: enterotoxigenic *E. coli* (37 per cent), *C. jejuni* (24 per cent), enteropathogenic *E. coli* (21 per cent), rotavirus (9 per cent), *Shigella* (5 per cent), *Giardia lamblia* (3 per cent), *Aeromonas hydrophila* (3 per cent), and *Salmonella* (2 per cent).

The proportions of episodes associated with specific pathogens in which fever was present are indicated in table 6. Twenty-eight episodes were associated with clinically detectable dehydration (2.2 per cent); 18 of 26 episodes studied were associated with one or more enteropathogens. The proportion of episodes with dehydration was analyzed for single pathogen groups of greater than 10 episodes and for episodes with more than one identified pathogen or no identified pathogen. The percentage of episodes with dehydration for these selected groups was 10.5 per cent for *Shigella*, 8.3 per cent for rotavirus, 2.9 per cent for enterotoxigenic *E. coli*, 2.1 per cent for *C. jejuni*, and none for enteropathogenic *E. coli*. The percentage of episodes with dehydration was 7.8 per cent for episodes with more than one pathogen (higher than the percentage for enterotoxigenic or enteropathogenic *E. coli* (all $p < 0.001$) but not different from the percentage with rotavirus alone) and 1.4 per cent for episodes in which no pathogen was found.

Enteropathogens isolated during diarrhea episodes had a similar distribution in the different seasons of the year, with the exception of *C. jejuni*, which had the high-

TABLE 5

Duration of episodes of diarrhea, by pathogen identified in stool specimens, in 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Pathogen	No. of episodes	Mean duration (days)	% distribution (days)				Median duration (days)
			1–7	8–14	15–21	22+	
Shigella sp.	19	9.0	53	32	11	5	7.0
Enteropathogenic *E. coli*	58	7.1	74	10	10	5	5.0
Enterotoxigenic *E. coli*	70	8.9	60	21	7	11	4.5
Rotavirus	24	6.0	79	8	8	4	4.0
C. jejuni	96	5.7	75	20	3	1	4.0
None identified	558	5.6	78	17	3	2	4.0

TABLE 6

Fever occurring during episodes of diarrhea associated with specific pathogens in 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Pathogen	No. of episodes	Fever not present		Fever reported present, but not documented		Fever confirmed (≥38 C)	
		No.	%	No.	%	No.	%
Rotavirus	24	4	17	7	29	13	54
C. jejuni	91	21	22	24	25	46	48
Enterotoxigenic *E. coli*	69	17	25	19	28	33	48
Shigella sp.	18	3	17	7	39	8	44
Enteropathogenic *E. coli*	58	22	38	20	34	16	28
None identified	547	161	29	150	27	236	43

est occurrence during the summer months (December–March).

Food, water, and environmental contamination

Samples taken from raw foods (table 7) indicated that cereals, dairy products, and meats were the items most frequently contaminated with *E. coli*, an indicator of fecal contamination. The samples of evaporated canned milk taken within one hour of opening the can ($n = 66$) had a lower frequency of contamination (3 per cent) than those taken after one hour or more of storage ($n = 115$) at ambient household temperatures (43 per cent, $p < 0.001$). Furthermore, after one hour, some of the samples had a very high *E. coli* colony count (20 per cent had $>10^3$ per ml), indicating extensive multiplication of bacteria in the can.

Menu items given to infants were also studied at the time of consumption (table 8). Milk and food specially prepared for infants (cereals or purees) were the most often contaminated. Foods eaten by the entire family, such as soups, stews, and fried foods, were less often contaminated. However, for most food items, the frequency of contamination was related to the amount of time since initial preparation. Six (13 per cent) of 47 cereals or purees consumed within one hour of preparation had evidence of fecal contamination, compared with eight (42 per cent) of 19 samples of those items eaten more than one hour after preparation ($p < 0.05$). Likewise, 12 (7 per cent) of 167 samples of soups or stews eaten within one hour of preparation were contaminated, compared with 13 (24 per cent) of 54 of those menu items eaten more than one hour after preparation ($p < 0.005$).

Teas, which were often used beginning in the first month of life, had a low frequency of contamination after preparation by heating (3 per cent of 87 specimens). If served in a cup, teas also had low levels of contamination at the time of consumption (2 per cent of 49 specimens). However, if served in baby bottles, 31 per cent (74 spec-

TABLE 7

E. coli contamination (per cent) of raw foods used to prepare weaning foods for 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Food	No. of specimens	*E. coli* colony count per gram or per milliliter					
		0	10^0–10^1	10^2	10^3	10^4	10^{5+}
Cereals	48	69	6	6	6	2	10
Milk products	181	72	7	6	3	3	10
Meats	158	72	0	3	2	10	12
Miscellaneous	20	80	0	0	10	0	10
Roots and tubers	287	82	0	3	5	3	8
Legumes	36	86	3	0	3	6	3
Fruits	16	88	0	0	0	0	12
Vegetables	508	89	<1	2	2	3	4

TABLE 8

E. coli contamination (per cent) of menu items for 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Menu item	No. of specimens	*E. coli* colony count per gram or per milliliter					
		0	10^0–10^1	10^2	10^3	10^4	10^{5+}
Milk	266	77	2	2	2	6	12
Infant foods	66	79	2	3	0	6	11
Fruit or juice	55	82	0	0	7	9	2
Soup or stew	221	89	1	3	2	1	4
Other	21	95	0	0	5	0	0

imens) were contaminated ($p < 0.01$), and 20 per cent had counts of 10^4 or more.

Specific pathogens found in food included *Salmonella* (two samples), *A. hydrophila* (10 samples), *Vibrio cholerae* non-O group 1 (two samples), and enterotoxigenic *E. coli* (six producing heat-stable toxin, two producing heat-labile toxin, and one producing both). No *B. cereus*, *Campylobacter*, or *Shigella* were isolated.

Water present in homes for direct consumption or preparation of menu items was analyzed; 33 per cent (252 specimens) of the samples of unboiled water had fecal contamination, whereas only 4 per cent (185 specimens) of the previously boiled water did. Water contamination frequency did not vary by the environmental temperature, but levels of *E. coli* were higher in the hottest season. The geometric mean *E. coli* colony count of positive specimens was $10^{1.33}$ for days on which the ambient temperature was <25 C, $10^{2.06}$ for days of 25–29.9 C, and $10^{3.38}$ for days ≥30 C ($p < 0.001$ vs. either lower temperature category). Specific pathogens identified in water included *A. hydrophila* (nine samples), *V. cholerae* non-O group 1 (one sample), and enterotoxigenic *E. coli* (eight producing heat-labile toxin and two producing heat-stable toxin). Nine of the 10 enterotoxigenic *E. coli* were from samples collected between September and November 1983.

In the studies of environmental contamination, a high proportion of baby bottles and bottle nipples were contaminated (table 9). Other potential sources of food contamination were the utensils used and the hands of the mothers or other persons responsible for food preparation. Enterotoxigenic *E. coli* were identified on the hands of one of 39 mothers of study infants.

Forty-two per cent of the rectal samples taken from the animals living in the houses were positive for *C. jejuni*, and 5 per cent were positive for *Plesiomonas shigelloides* (table 10). Enterotoxigenic *E. coli* were identified by the DNA probe technique in stools of two chickens and a dog (four positive strains); two strains produced heat-

TABLE 9

Selected items with positive cultures for E. coli, indicating fecal contamination, in the households of 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Item	No. of items cultured	No. positive	% positive
Bottle nipples	26	9	35
Feeding bottles	26	6	23
Spoons	75	12	16
Hands of mother	78	11	14
Can openers	18	1	6
Nipples of mother	64	2	3

TABLE 10

Animals with positive cultures for C. jejuni or P. shigelloides in the homes of 153 infants aged birth to one year in Huascar, Peru, July 1982 to June 1984

Animal	No. of animals cultured	Positive for *C. jejuni*		Positive for *P. shigelloides*	
		No.	%	No.	%
Chickens	23	18	78	0	
Cats	13	7	54	2	15
Dogs	4	1	25	1	25
Other*	22	0		0	
Total	62	26	42	3	5

* Seven rabbits, seven ducks, four guinea pigs, two pigeons, one goat, and one pig.

stable toxin only, one produced heat-labile toxin only, and one produced both.

DISCUSSION

Infants in this community had nearly 10 episodes of diarrhea before their first birthday. These infants had high rates of diarrhea from birth, unlike infants in some other developing countries who have been found to be relatively protected until their second six months of life (28). It may be hypothesized that the early supplementation of breastfeeding with teas, milks, and other foods found in this community may contribute to the high incidence of diarrhea in young infants (3, 29). Indeed, we found that infants in the first six months of life who consumed water, milk, or other food in addition to breast milk had a risk of diarrhea 40 per cent higher than exclusively breastfed infants. Furthermore, nonbreastfed infants in this age group had an incidence of diarrhea 260 per cent higher than exclusively breastfed infants (30).

The numerous episodes of diarrhea, although believed to be infectious in nearly all cases, were associated with known enteropathogens in only 41 per cent of cases cultured. If the sample of episodes studied for *Cryptosporidium* was representative of all episodes, another 7 per cent could be due to this parasite. In the populations of other developing countries, *Cryptosporidium* has been associated with 4 to 11 per cent of episodes of diarrhea (31–33). At best, only about half of the episodes could be associated with a possible etiologic agent, a proportion similar to that found in community-based studies in other developing countries (2, 3). The failure to find an agent during an episode of diarrhea may be due to a number of factors, including the relatively insensitive methods available with which to identify some pathogens, such as enterotoxigenic or enteropathogenic *E. coli*, and the fact that assays for several known enteropathogens, such as adenoviruses or 27-nm viruses, were not used. These viral agents would be expected to account for at least several per cent of the unexplained episodes of diarrhea (34–36).

The high frequency of stool examination for enteropathogens in this study made it possible to detect numerous infections with a wide variety of agents. Less than 40 per cent of infections with *C. jejuni* or enterotoxigenic *E. coli* and half of infections with enteropathogenic *E. coli* were associated with diarrheal illnesses. This suggests either that frequent subclinical infection occurred or that infection was persistent. Persistent infection seems unlikely since a prolonged carrier state with these organisms is unusual. Furthermore, serial cultures made it possible to observe that cultures would usually become negative within several weeks, but would then become positive again at a later point. Since the risk

of illness with these enteropathogens is known to be directly related to the number of organisms ingested and to buffering of the stomach acid barrier, it is possible that many subclinical infections resulted from ingestion of an insufficient number of organisms to cause disease. It is also possible that passive immunity from antibodies in breast milk or acquired immunity from previous symptomatic or asymptomatic infections may have reduced the apparent pathogenicity of these agents. However, the similar association with diarrhea in younger or older infants for some pathogens provides no clear evidence for an important role of either passive or active immunity. Given the very large number of serotypes of each of these enteropathogens and the largely serotype-specific nature of immunity to them, one may not expect to see a striking effect of immunity in this age group. On the other hand, breast milk antibodies may provide partial protection against diarrhea in younger infants who are infected with rotavirus in the first six months of life (37). Thus, the reduced pathogenicity of rotavirus in younger infants compared with older infants may be due to this passive protection. However, in these infants, *Shigella* appeared to have higher pathogenicity in younger infants.

Mixed infections with any two of the three most common enteropathogens (*C. jejuni* or enterotoxigenic or enteropathogenic *E. coli*) had slightly, but not significantly, higher associations with illness than did the single infections. Mixed infections with rotavirus had pathogenicities similar to that of rotavirus alone. Thus, there was no evidence for additive or greater pathogenicity with mixed infections, as has been suggested from some animal studies (38).

A small fraction of the diarrhea episodes were particularly severe; 2 per cent were associated with dehydration and 7 per cent with durations of longer than two weeks. A higher proportion (69 per cent) of the episodes with dehydration than of milder episodes was associated with known enteropathogens, such as rotavirus or enterotoxigenic *E. coli*, as has been noted previously (28). Likewise, two thirds of the prolonged episodes were associated with at least one enteropathogen. Of particular interest is the high fraction of prolonged episodes, in comparison with shorter episodes, associated with enterotoxigenic and enteropathogenic *E. coli* and *C. jejuni*. Enterotoxigenic *E. coli* were previously found in the stool during 24 per cent of episodes of at least three weeks duration in a similar community-based study in Bangladesh (39). Enteropathogenic *E. coli* (40–42) and *C. jejuni* have also been associated with prolonged diarrhea (43), as has *Shigella* (2, 39).

The most frequently detected agent in this study was *C. jejuni*, which is known to be transmitted to humans by contact with animal feces (44, 45), as well as by contaminated food and water (44, 46, 47). Ten per cent of both diarrheal and nondiarrheal stool cultures contained *C. jejuni*. This high rate of apparently asymptomatic infection has been documented in other developing countries (48, 49). The high background rate of infection suggests that infants are frequently exposed to the organism. One likely source of this exposure is animals living in or around the house. In a special survey in the first year of this study, we found that 69 per cent of the study infants had fowl, mostly chickens, in their homes; 22 per cent had cats, and 36 per cent had dogs (unpublished data). Since we found more than half of the chickens and cats and 25 per cent of the dogs to be infected with *C. jejuni*, animal feces is likely to be an important source of infection for infants, transmitted either through direct contact or by family members, objects, or food in the house. In addition, study infants in homes with fowl or cats were significantly more likely to acquire *C. jejuni* infection than infants in homes without these animals (unpublished data). Furthermore, a separate study in another Lima periurban area demonstrated that the most im-

portant risk factor for pediatric *Campylobacter* diarrhea was exposure to *C. jejuni*-infected chickens in the home (50).

Enterotoxigenic *E. coli* were relatively frequently isolated enteropathogens, although they appeared to be less important than in several studies in other developing countries (2, 3). In previous studies in Bangladesh, the occurrence of enterotoxigenic *E. coli* diarrhea was found to be related to the degree of contamination of weaning foods (11). The identification of enterotoxigenic *E. coli* by the sensitive DNA probe technique in several food and water samples in this study further supports the belief that this organism is foodborne or waterborne (11, 51).

Among the other identified etiologic agents, enteropathogenic *E. coli* were associated with diarrhea. These organisms may have been transmitted like enterotoxigenic *E. coli* in food or water, although person-to-person transmission, especially in hospital nurseries, has also been well documented (41). *Salmonella* is a well known foodborne pathogen; it was recovered from two foods given to infants in this study. Shigellae may be transmitted by food or water, but are easily passed from person to person by direct contact. It is likely that the limited amount of available water and the poor personal hygiene in the study community facilitate the transmission of *Shigella*, and perhaps other organisms such as rotavirus. *A. hydrophila* and *V. cholerae* non-O group 1 were found in both food and water, and it is presumed that they were transmitted via these routes.

Water in this community is delivered by tanker trucks and placed in small household tanks or cylinders. The water available for household use is often contaminated (one third of samples were positive for *E. coli*), either because it is contaminated at the time of delivery or because it is contaminated in the storage container. Since the usual method of obtaining water from the household container is dipping a bucket into the open top, the introduction of fecal material via this route is also likely. Nevertheless, water given to infants is most commonly boiled before it is mixed with evaporated milk or before it is used in the preparation of teas, which are often given to very young infants. Not surprisingly, boiled water and teas immediately after preparation rarely had evidence of contaminating bacteria. Serving these teas in a cup appeared to result in no additional contamination, but giving them to the infant from a feeding bottle resulted in contamination in 30 per cent of feedings. This is consistent with our findings that 23 per cent of feeding bottles and 35 per cent of bottle nipples in these households were positive for *E. coli*, an indicator of fecal contamination. This also illustrates the potential for secondary contamination of food and water because of unhygienic serving utensils or food handling practices. Domestic and personal hygiene are especially difficult to maintain in areas such as this study community, where water is limited in availability and expensive for the impoverished residents, but they may have an important effect in reducing diarrhea (52–54).

Evaporated milk mixed with an equal volume of boiled water was the most frequently used weaning food. The milk was not contaminated at the time the can was first opened; however, bacteria were likely introduced by the can opener itself, and then multiplied in milk left for hours at ambient household temperatures. Furthermore, the milk was given to the infant in a feeding bottle on nearly all occasions observed (266 out of 268), which likely resulted in further contamination from the unhygienic bottle or nipple. The milk was often left in the feeding bottle for hours and the infant fed intermittently from the same bottle, allowing time for further bacterial multiplication.

After milk, the most common foods given to infants were soups and stews. Although the raw foods from which they were made were frequently contaminated, these menu items were usually boiled, resulting in in-

frequent isolation of fecal indicator bacteria immediately after preparation. However, like milk, these items appear to have often been secondarily contaminated, perhaps by kitchen utensils, and to have had increasing levels of fecal bacteria with increasing duration of storage in the home. These foods were usually eaten later in the day after heating, but not boiling, a second time. Specially made weaning foods, such as cereals, were even more often contaminated and demonstrated an even greater increase in bacterial contamination if consumption was delayed more than one hour. These food handling practices resulted in ingestion of fecal bacteria, including enterotoxigenic *E. coli*, *A. hydrophila*, *V. cholerae* non-O group 1, and *Salmonella*, all of which were demonstrated to be present in foods consumed by infants.

The information obtained in this study should be useful for improving child care practices to reduce diarrhea due to the most frequent enteropathogens. Control of zoonotic infections like *C. jejuni* should be possible by reducing contact with animal feces and by improving domestic hygiene. Better domestic and personal hygiene, particularly handwashing, would also decrease the possibility of direct human-to-human transmission of agents like *Shigella*, rotavirus, or *G. lamblia*, as well as reduce the secondary contamination of menu items (52, 54). Improved feeding practices would include a longer (three- to four-month) period of exclusive breastfeeding, longer continuation of breastfeeding, and the use of less-contaminated weaning foods. This could be accomplished by elimination of feeding bottles, better cleaning of utensils, improved food preparation techniques, and reduced duration or improved conditions of food storage after cooking. Boiling of water and food may play a part, but may be constrained by expense in some developing countries (55). Reduced food contamination should decrease transmission of a variety of enteropathogens, especially enterotoxigenic *E. coli* (11). These practices—avoidance of animal feces, improved personal and domestic hygiene, and improved feeding practices—should be considered important interventions in reducing diarrheal morbidity in infants in developing countries.

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