DIARRHEAL DISEASES OF INFANCY IN CALI, COLOMBIA: STUDY DESIGN AND SUMMARY REPORT ON ISOLATED DISEASE AGENTS [1,2]

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Especially in developing countries, it is important that the etiologic agents of early childhood diarrhea be isolated and identified, and that their routes of transmission be defined. This article describes a study conducted for those purposes in five working-class districts of Cali, Colombia, where environmental conditions appear roughly comparable to those of working-class districts in many other Latin American cities.

Introduction

Diarrhea is often a major symptom of severe illness in developing countries. For example, the 1968-1972 Inter-American Investigation of Mortality in Childhood, a study covering 13 Western Hemisphere areas, found that diarrheal disease was the major underlying cause of death in 28.6 per cent of the 35,095 deaths reported among children under five years of age (*1*). "Diarrheal disease" of course, is a symptom complex, not a recognizable disease entity. The presence or severity of diarrhea often depends as much on nutritional status, food intake, environmental conditions, and chemical factors as it does on the actual disease agent. In fact, the former circumstances may cause noninfectious diarrhea in some cases.

Despite this, at least 25 per cent of all infants and young children with diarrheal illness in developing tropical and subtropical areas are usually found to be excreting bacteria, viruses, or parasites that are considered pathogenic. It is true, however, that the average age at which infants and children are exposed to such agents in these areas is generally well below the age at which they would be exposed in more temperate climates—where judgments as to the agents' pathogenicity have traditionally been made. In such developing areas the risks of initial infection, of simultaneous infection with several pathogenic agents, and of reinfection are all greater, and the proportion of asymptomatic people who excrete the organisms is much greater, than in temperate regions.

In view of these differences, a study was conducted to learn more about infectious agents present in the stools of a cohort of healthy Colombian children studied from birth until the end of their second year of life. The agents were detected by direct examination for parasites and by in vitro culture methods. This article describes the design of

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the study, observed defecation patterns, and laboratory results. Other findings, including observed infection patterns and relationships between the agents isolated and the occurrence of diarrhea as a symptom, will be reported separately.

Materials and Methods

The Setting

Cali, a rapidly growing metropolitan area with nearly a million inhabitants, is located in the fertile Cauca River Valley of western Colombia. This agricultural region, important for production of sugarcane, soybeans, and cotton, lies at an altitude of about 1,000 meters, between two mountain ranges of the northern Andean chain. The mean annual temperature is 24°C, and although there are two wet and two dry seasons, temperature variations are slight.

With regard to childhood mortality in Cali, the forementioned Inter-American Investigation found an overall death rate of 16.1 per 1,000 children under 5 years of age, about 70 per cent of the deaths occurring in the first year of life. Specific infectious and parasitic diseases were judged to be the main underlying cause in over a third of the cases (6.9 deaths per 1,000 children), with deaths caused by diarrheal diseases (5.0 per 1,000) accounting for over two-thirds of these (*1*).

Survey Area and Population

Five adjoining working-class districts with a total population estimated at over 40,000 were chosen for this study. The districts are located southeast of Valle University, less than 15 minutes away by jeep. A recently completed health center in the study area was used as a field office and treatment station.

A pregnancy survey conducted during the summer of 1962 in the study area was used to locate the children being born between July 1962 and February 1963. All such newborns were included in the study if: (1) they were born at home (not in a hospital); (2) the mother was a permanent resident of the study area when her child was born; (3) the child weighed at least 2,500 grams at birth and had no obvious congenital deformity; and (4) the mother agreed to the conditions set for admission into the study, which included a statement of intent to remain in the study area for at least two years. All children admitted were offered free and accessible medical treatment for the duration of the study—including treatment for both nondiarrheal and diarrheal illness.

The home of every child admitted was visited once a week by a field worker, who took down the child's weight and feeding data,[4] as well as information about stool frequency and consistency that had been recorded daily in the course of the week by the child's mother. Each mother was instructed by a health worker and given visual aids to assist in making accurate daily records of stool consistency (on a scale of 1 to 4), frequency of defecation, and presence or absence of mucus, blood, or pus in the stools.

For sample collection purposes, diarrhea was considered present when a child (regardless of age) defecated over three times in one day, or when there was obvious presence of blood, mucus, or pus in any stool. By this definition, if a child had diarrhea on any day during the week, a fecal specimen was taken at the time of the weekly visit and was promptly transported to the laboratory. Also, routine specimens were taken at monthly intervals, regardless of diarrhea symptoms. These procedures were followed from the time a child entered the study group until it reached 104 weeks of age, or until it was inadvertently withdrawn from the study for any of several reasons.

Collection of Specimens

The field nurse provided each mother with sterile screw-cap jars and applicator sticks for

[4] Data on types of milk and solid food received.

the collection of feces. The mother was instructed to place the infant's freshly passed feces into each of four jars once every four weeks in time for the nurse's prearranged monthly visit. Two of these samples, one fresh and one preserved in polyvinyl alcohol, were used for parasitologic examination. The third (another fresh sample) was employed in viral isolations, and the fourth (placed in a buffered glycerol-saline medium) was used in bacteriologic studies. Specimens were refrigerated two to four hours after collection and were transported to the laboratory the same day. If no specimens were available at the time of the nurse's visit, or if their bulk was inadequate, another set was collected during the next 24 hours and was taken to the laboratory by a field worker.

Laboratory Examinations

Parasites. All the specimens were examined directly by the standard method of making a 2 mg smear from a saline suspension of fresh feces. Each smear was examined, using a photoelectric cell and following the technique described by Beaver (*2,3*). Nematode eggs were counted and protozoa, if present, were identified after staining the smears with an aqueous iodine solution. All stools were examined by formalin and other sedimentation. In addition, fluid stools preserved in polyvinyl alcohol were examined after staining with iron hematoxylin.

Bacteria. The specimens were examined for *Salmonella, Shigella,* and certain serotypes of enteropathogenic *Escherichia coli.*

Portions of each glycerol-saline suspension of fecal material were inoculated directly onto MacConkey agar plates and also, after overnight enrichment in tetrathionate broth and selinite F broth, onto SS agar and brilliant green agar plates. Ten coliform colonies were subsequently picked from the MacConkey agar plates and inoculated onto veal brain infusion agar slants.

Bacterial suspensions of selected colonies from the slants were then tested by slide agglutination against pools of *E. coli* "OB" antisera. Cultures showing agglutination were tested against individual "OB" antisera and were retested after heating for 10 minutes at 100°C to determine the O antigen. Antisera were available to identify the following *E. coli* serotypes: O26:B6, O55:B5, O86:B7, O111:B4, O119:B14, O125:B15, O126:B16, O127:B8, O128:B12.

Non-lactose-fermenting colonies from all the agar plates were inoculated onto triple-sugar-iron agar, semisolid agar, Simmons' citrate agar, tryptone water, and Christensen's urea agar. Colonies of *Salmonella* and *Shigella* were then identified by a standard combination of biochemical tests and agglutination tests against antisera prepared in the laboratory.

Viruses. Twenty per cent fecal suspensions were centrifuged at 2,500 rpm for 90 minutes at 4°C. The resulting supernatants were treated with 500 units of penicillin, 500 μg of streptomycin, and 2 μg of amphotericin per ml for one hour, and were then stored at -20°C. Three tubes of primary human embryonic kidney (HEK) cells (*4*) and three tubes of HeLa cells were then inoculated with a small portion (0.25 ml per tube) of the treated supernatant. Both sets of cell cultures were grown in Hanks balanced salt solution containing 0.5 per cent lactalbumin hydrolysate, 0.1 per cent yeast extract, and 1 per cent fetal calf serum (FCS). HeLa cells were observed for seven days and HEK cells for 14 days without changing the medium. Cultures showing cytopathic effects were passed twice after the cytopathic effect was observed. Cultures showing no cytopathic effect were given one blind passage before being classed as negative.

In addition, 25 per cent of the centrifuged and treated specimens, preselected on the basis of a random numbers table, were inoculated into white Swiss mice less than one day old (*5*). One litter of mice was inoculated with each specimen (diluted 1:2), every mouse receiving 0.02 ml subcutaneously and 0.02 ml intracerebrally. The mice were then observed for 12 days. Tissues from those dying or showing signs of illness were harvested and

inoculated into a second litter. No blind passages were performed.

HEK and HeLa tissue culture isolates were tested for hemagglutinating activity with human type O erythrocytes at 4°C and 37°C (*6, 7*). Hemagglutinating isolates were tested by hemagglutination inhibition (HI) with antisera[5] against hemagglutinating echoviruses (types 3, 6, 7, 11, 12, 13, 19, 21, 29) and reovirus type 1. Nonhemagglutinating isolates (found to include many poliovirus isolates) were tested by serum neutralization against pools of enterovirus antisera[5] and were typed against individual sera by means of a standard tube neutralization test. Isolates showing a cytopathic effect typical of adenoviruses were tested in a similar manner against adenovirus antisera.[5] Those isolates which could not be typed with the available antisera were tested by complement fixation (CF) for adenovirus-group antigen.

Isolates obtained only from mouse passages were identified by complement-fixation tests with antisera against Coxsackievirus A, types 1-8 and 10-19.[5]

No special effort was made to isolate more than one virus type from each specimen. The presence of additional types was detected first by typing "breakthroughs" in neutralization tests and subsequently by confirming the identity of agents isolated in more than one system from a single specimen.

[5]Aside from the exceptions noted below, all the enterovirus antisera were prepared in rabbits at the authors' laboratories.

Antiserum against coxsackievirus A-11 was prepared in mice.

Rabbit antisera vs. echovirus types 2, 9, 14, 15, 18, 21, 22, 23, 25, and 26 were obtained from a commercial source.

Coxsackievirus A-20a antiserum was obtained from the Research Resources Branch of the National Institute of Allergy and Infectious Diseases.

Antisera vs. adenovirus types 1-18, prepared in horses, were obtained through the courtesy of Dr. R. G. Robinson, U.S. Center for Disease Control, Atlanta, Georgia. Antisera against adenovirus types 19-22 and 25-27 were prepared in rabbits at the authors' laboratories. Serum for the adenovirus group CF test was obtained from a commercial source.

All the antisera were titrated and tested for heterologous neutralizing activity in the authors' laboratories before being used in identification tests.

Results

Demographic Information

A total of 296 children from 294 households were ultimately enrolled in the study. Demographic data were obtained from interviews in 279 of these households, which contained 1,825 people and 281 study children. Most members of these households (55 per cent) were children under 15 years of age; very few persons (5 per cent) had reached the age of 45.

Males were only slightly outnumbered by females until about age 10, but the sex ratio thereupon became increasingly disproportionate until age 24, there being over twice as many women of that age as men. After that the situation reversed itself, there being more men than women from age 25 to age 49. These differences in the adult sex ratios at specific ages were not due to disproportionate numbers of one sex among relatives, visitors, or servants. Rather, the disparities were caused by differences in the age of parents in the primary family units. That is, the mothers of study children were generally 5 to 10 years younger than the fathers.

The households ranged in size from three to 19 persons, the mean number of inhabitants being 7.5. The houses of these groups varied in size from one to seven rooms, the mean number of rooms being 2.3. Ninety-three per cent of the houses had four rooms or less, and the average number of people living in each room was 3.4. Some households had more than 12 persons per room. Only one study child slept in a room by himself, and most shared a room with two, three, or four other persons.

In the 264 households where fathers were present, 257 (97 per cent) of the fathers were employed. This high rate of employment may well have been related to financial conditions required for building and occupying a house in the study areas. Most of the men had unskilled or semiskilled jobs and very few were self-employed. Thirty-two of

the 279 mothers were employed. Twenty-six fathers and 20 mothers had never attended school; both mothers and fathers had spent an average of 4.5 years in school.

There was a wide range of household incomes, but there were few families that could be described as anything but poor by Colombian standards. The mean household income reported was 153 pesos (US$15) per week, and 96 per cent of the households earned less than either 500 pesos total or 50 pesos per person in a week.

The neighborhoods included in the study were either recently developed or under development at the time. Most housing units were made with brick (72 per cent), but some were built with dried earth over slate (7 per cent), bamboo (1 per cent), multiple or mixed materials (18 per cent), or other ingredients (2 per cent). Their floors were made of mixed materials (51 per cent), tile (27 per cent), cement (6 per cent), or packed earth (18 per cent). Most of the roofs were of tile. A majority of the houses had access to a piped public water supply, 99 having water piped to a toilet and kitchen, and 69 having a tap on the premises; but 107 had no piped water, obtaining water instead from containers carried to the house. Effluents were discharged into a closed sewage system at 71 per cent of the homes, into an open ditch at 8 per cent, and into the road at 21 per cent. Most residences (60 per cent) had a toilet inside the house, 38 per cent had one outside on the premises, and five (2 per cent) had none.

Defecation Patterns

The 296 newborn children enrolled in the study were observed weekly from July 1962 to February 1965. Of the total, 173 (58 per cent) were observed for the full scheduled two-year interval. Over all, the observation period ranged from four to 108 weeks, the average weeks of observation per subject being 84. In the 123 cases where a child left the study, reasons cited for the withdrawal were departure from the study area (106), refusal to participate further (10), and death of the subject (7). Of the seven deaths, four were attributed to diarrheal disease, one to meningitis, one to purpura, and one to unknown causes.

Records were kept of the defecation frequency, stool consistency, and presence of blood, mucus, or pus in the stools of the study children. Figure 1 shows the average daily defecation rates observed every four weeks, as well as standard deviations, for the total study population. The same information, grouped into 26-week periods, is presented in Table 1.

As it can be seen, the rates decreased progressively from birth to the end of the second year of life. This decrease was most pronounced in the first 20 weeks of life, when over half the observed drop occurred. After that the decline proceeded in a more gradual and regular manner.

The observed average male and female defecation rates were found to differ. Although the differences were small, males tended to defecate more often than females at all ages. Moreover, the sharp drop in frequency that came soon after birth occurred earlier and more abruptly in females and may have ended even before the eighth week of life. Variations around the mean values were similar for both males and females and decreased with age.

The defecation frequency of the average child varied a good deal during the first eight weeks of life. After that, the subjects could be grouped into the following three categories: (1) those who showed distinct and regular patterns; (2) those whose patterns were of a cyclical type; and (3) those with continuing pronounced variations (no set pattern). Of the 250 children who were observed long enough to distinguish patterns, more than 70 per cent had what could be described as regular patterns, 13 per cent had cyclical patterns, and 17 per cent had grossly irregular patterns.

The prevalence of "liquid days" (days on which at least one liquid stool was passed) is shown by age and sex in Table 2. Both the

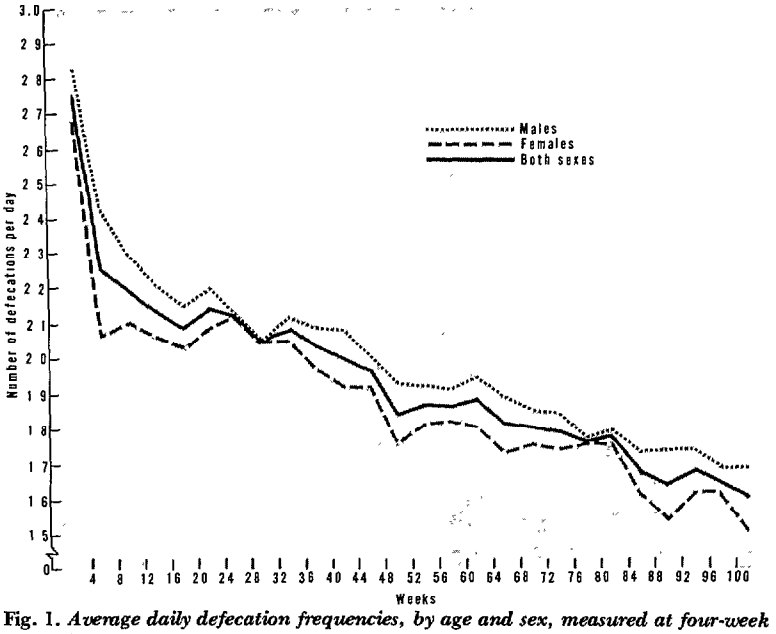

Fig. 1. ***Average daily defecation frequencies, by age and sex, measured at four-week intervals.***

Table 1. Variations in the average number of defecations per day in four 26-week intervals, by sex.

Age of subject (in weeks)	Average number ($\bar{x}$) of daily defecations and the standard deviation (SD) from this average					
	Males		Females		Total	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1 - 26	2.32	1.52	2.15	1.46	2.24	1.50
27 - 52	2.06	1.43	1.96	1.40	2.01	1.42
53 - 78	1.89	1.39	1.78	1.33	1.83	1.35
79 - 104	1.74	1.32	1.62	1.27	1.68	1.30
Total	2.03	1.42	1.90	1.38	1.96	1.40

total number of liquid days reported and the ratio between liquid days and observed days were found to vary in accord with the children's age. For example, in the first 28 weeks of life liquid days accounted for 10 per cent of all days the children were observed. But by the 85-108 week period, the figure had fallen to 4.4 per cent. This decrease occurred in a regular manner; unlike the age-related changes in defecation frequency, it was not especially rapid in the first few weeks of life or at any other portion of the period observed.

As previously noted, "diarrhea" may be defined in terms of either defecation frequency or stool consistency. As the data in Table 3 show, our study found a clear relationship between the prevalence of liquid days and the number of defecations per day. The

Table 2. Prevalence of reported "liquid days" (days with one or more liquid stools), by age and sex.

Weeks of life	Males			Females			Total		
	No. of subject-days[a]	No. of liquid days	% liquid days	No. of subject-days[a]	No. of liquid days	% liquid days	No. of subject-days[a]	No. of liquid days	% liquid days
1 - 28	24,209	2,692	11.1	23,147	2,057	8.9	47,356	4,749	10.0
29 - 56	22,755	1,967	8.6	22,766	1,696	7.4	45,521	3,663	8.0
57 - 84	18,673	1,363	7.3	20,025	1,171	5.8	38,698	2,534	6.5
85 - 108	12,228	658	5.4	13,502	472	3.5	25,730	1,130	4.4
Total	77,865	6,680	8.6	79,440	5,396	6.8	157,305	12,076	7.7

[a]Observation of one subject for one day constituted one subject-day.

Table 3. Defecations per day and liquid days, showing how the proportion of liquid days varied as defecation frequency rose.

No. of daily defecations	First 52 weeks			Second 52 weeks			Total (104 weeks)		
	Total days	No. of liquid days	% liquid days	Total days	No. of liquid days	% liquid days	Total days	No. of liquid days	% liquid days
1	30,851	767	2.5	34,292	251	0.7	65,143	1,018	1.6
2	30,401	1,579	5.2	23,998	668	2.8	54,399	2,247	4.1
3	16,329	1,973	12.1	7,400	1,014	13.7	23,729	2,987	12.6
4	5,192	1,586	30.5	2,021	934	46.2	7,213	2,520	34.9
5	1,978	940	47.5	721	469	65.0	2,699	1,409	52.2
6	1,419	778	54.8	566	378	66.8	1,985	1,156	58.2
7	177	121	68.4	81	66	81.5	258	187	72.5
8	142	107	75.4	87	81	93.1	229	188	82.1
9	220	174	79.1	133	123	92.5	353	297	84.1

average prevalence of liquid days rose from 1.6 per cent among children of both sexes who defecated once a day to more than 84 per cent among children who defecated nine times a day. That is, the overall prevalence of liquid days increased by roughly 10 per cent, on the average, per additional daily defecation.

This relationship varied with the age and sex of the child. Children under one year old with a low defecation frequency (one or two defecations per day) had a higher prevalence of liquid days than older children with the same defecation frequency.

Also, males were found to have a higher average proportion of "liquid days" than females at all ages and at all defecation frequencies. The sex difference appears to have been greater when defecation rates were very low (1-2 per day) or very high (7-8 per day).

The data collected on the presence of blood, mucus, and pus showed that pus was rarely reported, and that when it was reported, blood and mucus were reported as well. Categories used in our tabulations were therefore the presence of blood, of mucus, or of blood, pus, and mucus together (BPM). The frequency of days when one or more of these were present is shown in Table 4. The frequency with which blood was reported did not show marked sex or age variations and was low at all times. The prevalence of mucus changed with age, and the rate was higher in males than in females at all ages. However, the frequencies of blood, of mucus, and of BPM all increased as the number of defecations per day rose. That is, a child appeared more likely to pass blood, mucus, or BPM when the frequency of his defecations increased. There was also a clear relationship between the BPM rate and the presence of a liquid stool, the extent of this relationship varying with age. In addition, the data indicate that children with BPM had a greater percentage of liquid days at all ages throughout the observation period—including periods before, during, and after BPM days.

Laboratory Results

In the course of the study 8,895 monthly and "diarrheal" stool specimens were collected and processed, an average of 0.4 specimens per patient-week of observation. Table 5 shows the number of specimens

Table 4. Prevalence of blood, mucus, and pus in the feces of study children, by age.

Weeks of life	Subject-days[a]	Blood		Mucus		Blood, pus, and mucus	
		Days observed	%	Days observed	%	Days observed	%
1 - 28	47,356	122	0.3	342	0.7	425	0.9
29 - 56	45,521	153	0.3	683	1.5	723	1.6
57 - 84	38,698	153	0.4	950	2.5	998	2.6
85 - 108	25,730	41	0.2	401	1.6	411	1.6

[a]Observation of one subject for one day constituted one subject-day.

submitted for each type of laboratory examination and the overall findings. The specific types of bacteria, parasites, and viruses found are listed in Tables 6, 7, and 8. It should be noted that live poliovirus vaccine was not being used in this community at the time of the study, and so we believe that all the poliovirus strains isolated were wild.

The specific agents most commonly identified in the study population were *Giardia lamblia* and *Chilosmastix mesnili* among the parasites, echovirus types 6 and 11 among the viruses, and *Escherichia coli* serotype O26:B6 among the bacteria. Of the three groups, viruses were identified far more often than parasites or pathogenic bacteria. On the basis of only initial isolations of a specific agent from a particular child, there were .08 viral isolations per child-week of observation, or .23 initial viral isolations per lab specimen; the comparable figures for pathogenic bacteria were .04 and .10, and for parasites .03 and .09. On the average, children observed for the full two-year period yielded a total of 8.9 initial virus isolations during that period, the number of isolations from any given child ranging from four to 18. The number of initial isolations per child did not appear significantly affected by the day or month in which the subject was born and entered the study.

Initial isolation of more than one agent from the same specimen was a common occurrence. In general, combinations of viruses, bacteria, and parasites—as well as several viruses—were often isolated from the same specimens. Simultaneous first isolations of one virus and one bacterial agent began to be recorded during the subjects' first month of life, and an average of 12 such pairings per month were observed during the first year of life, after which they declined. In contrast, simultaneous observation of a virus and a parasitic agent was uncommon before the subjects' tenth month of life, and pairings of bacterial and parasitic agents were rare. Simultaneous first isolation of two, three, or even four parasitic agents from a single specimen became common around the end of the first year of life.

Discussion

From a public health standpoint, the etiologic agents of infant diarrhea and their probable routes of transmission need to be

Table 5. Infectious agents found in fecal specimens from study children 0-2 years of age.

Classes of agents	No. of specimens examined	No. of agents identified		No. of children infected with identified agents[a]
Bacteria	8,883	Enteropathogenic *E. coli*:	9 serotypes	669
		Salmonella:	21 serotypes	157
		Shigella:	5 serotypes	11
		All bacteria:	35 serotypes	837
Parasites	8,704	Amoebae:	5 species	167
		Flagellates:	3 species	440
		Ciliates:	1 species	1
		Helminths:	6 species	213
		All parasites:	15 species	821
Viruses	8,895	Enteroviruses:	49 serotypes	1,668
		Adenoviruses:	21 serotypes	217
		Reoviruses:	2 serotypes	38
		All viruses:	72 serotypes	1,923

[a]The number of children from whom the agent was isolated at least once.

Table 6. Bacterial pathogens found in fecal specimens from 298 study children 0-2 years of age.

Enteropathogenic E. coli		Salmonella		Shigella	
Serotype	No. of children infected[a]	Serotype	No. of children infected[a]	Serotype	No. of children infected[a]
026:B6	138	S. derby	29	S. boydii	5
0125:B15	89	S. anatum	16	S. sonnei	2
055:B5	85	S. london	16	S. flexneri 3	2
0126:B16	82	S. newport	16	S. flexneri 2	1
0128:B12	63	S. jariana	12	S. dysenteriae	1
0119:B14	62	S. give	11	Total	11
0127:B4	57	S. typhimun	10		
0111:B4	54	S. new brunswick	9		
086:B7	39	S. muenchen	8		
Total	669	S. litchfield	7		
		S. oranienburg	5		
		S. tennessee	4		
		S. uganda	3		
		S. typhimurium var. copenhagen	2		
		S. saint paul	2		
		S. bedford	1		
		S. birmingham	1		
		S. cubana	1		
		S. kaapstad	1		
		S. paratyphi B	1		
		S. tilburg	1		
		S. typhi	1		
		Total	157		

[a]The number of children from whom the agent was isolated at least once.

Table 7. Parasites found in fecal specimens from 296 study children 0-2 years of age.

Amoebae		Flagellates		Helminths		Ciliates	
Species	No. of children infected[a]	Species	No. of children infected[a]	Species	No. of children infected[a]	Species	No. of children infected[a]
E. coli	104	G. lamblia	222	T. trichuria	101	B. coli	1
E. histolytica	36	C. mesnili	123	A. lumbricoides	99		
E. nana	17	T. hominis	95	S. stercoralis	6		
I. butschili	7			H. diminuta	4		
D. fragilis	3			H. nana	2		
				hookworm	1		
Total	167	Total	440	Total	213	Total	1

[a]The number of children from whom the agent was isolated at least once.

Table 8. Viruses found in fecal specimens from 296 study children 0-2 years of age.

Polioviruses		Echoviruses		Coxsackie A viruses		Coxsackie B viruses		Adenoviruses		Reoviruses	
Types	No. of children infected[a]	Types	No. of children infected[a]	Types	No. of children infected[a]	Types	No. of children infected[a]	Types	No. of children infected[a]	Types	No. of children infected[a]
1	128	11	166	21	101	5	50	2	45	1	36
3	114	6'	124	20[b]	90	3	28	1	38	2	2
2	45	12	101	4	46	2	16	5	21	Total	38
		7	97	10	27	4	5	3	19		
Total	287	8	78	8	25	1	1	12	16		
		13	77	6	21			16	14		
		29	60	9	13	Total	100	15	11		
		19	46	2	8			7	10		
		21	43	12	8			17	6		
		14	25	5	7			9	5		
		24	24	14	7			18	5		
		3	17	13	4			10	4		
		20	17	7	2			11	4		
		25	16	20	2			21	4		
		1	6	20[b]	2			26	4		
		15	5	1	1			27	3		
		9	4					20	2		
		5	3	Total	364			22	2		
		6	2					25	2		
		2	1					4	1		
		4	1					13	1		
		17	1					Total	217		
		18	1								
		22	1								
		26	1								
		Total	917								

[a]The number of children from whom the virus was isolated at least once.
[b]Isolated only by inoculation of specimen material into suckling mice (only 25 per cent of the specimens, selected at random, were used in making these inoculations).

defined so as to provide a basis for appropriate preventive measures. This study was conducted in a working-class neighborhood which for the most part enjoyed the advantages of an assured safe water supply and adequate sewage and refuse disposal. In general, we believe that this community found itself in environmental circumstances similar to those of many other "average" working-class communities in Latin America.

As already noted, "diarrhea" can be defined in terms of defecation frequency or the passing of liquid stools. The observations recorded during this study (regardless of whether microbiologic agents were isolated) demonstrate that "passage of over three stools per 24-hour period, irrespective of the subject's age" constitutes a reasonable operational definition of diarrhea. (The defecation frequency cited exceeded the average observed for the group under study by more than one standard deviation after the first month of life.) This definition, which has been employed in other surveys, is reasonably selective in separating normal from sick children, and is a variant of that proposed by the World Health Organization: "three or more soft or liquid stools within 12 hours, or a single soft or liquid stool containing blood, pus, or mucus" (*8*).

It has already been shown that average males in each age group of our study population defecated more frequently than average females of the same age group (see

Figure 1) and had more "liquid days" (Table 4). And even though a correlation was observed for both sexes between frequency of defecation and the prevalence of liquid days, the higher prevalence of liquid days found for males at all defecation frequencies strongly suggests the presence of some physiological or other factors involved in male defecation that tend to influence stool frequency in one way and stool consistency in another.

Operationally, this study proceeded smoothly from the time that prospective subjects were registered by the pregnancy survey through the second year of observation of the last-enrolled subject. There were no interruptions, nor was there any need to change the study design during the course of the observation period.

With regard to the collection of data, several procedures and circumstances should be explained. In order to avoid possible bias in the handling of specimens by the various laboratories, no distinction of any sort was made between specimens collected routinely and ones collected during diarrheal episodes. This lack of distinction extended to labeling and delivery of specimens, a procedure which precluded subsequent comparison of the results obtained from examination of the two groups of specimens. Also, in some 448 instances during the course of the study, two specimens were received by the laboratory for the same patient during a single week. There were several reasons for this unintended double collection of specimens. Most duplications occurred because of disturbances in the field workers' schedule of weekly home visits caused by multiple holidays, vacations, or transportation problems. Others occurred because in some cases a well-intentioned mother would collect extra specimens when she thought her child was ill. Generally, the results obtained with the two specimens were in agreement; over all, if these extra specimens influenced the results in any way, it would be in the direction of more complete identification of agents present in the stool.

Other noteworthy features were the frequency of stool sample collections and the thoroughness of laboratory examinations. As previously described, routine monthly and special "diarrheal episode" specimens, the latter collected as often as once a week, were subjected to triple laboratory examinations for all known infectious agents identifiable in human stools at the time the project was conducted. Use of human embryo kidney cell cultures and inoculation of suckling mice with material from 25 per cent of the specimens chosen at random added further sensitivity to the process of isolating and identifying viral agents.

But despite identification of an impressive number and variety of infectious agents in most children during the observation period, the potentially identifiable numbers and varieties of agents tended to be considerably reduced by several inherent features of the study design. For example, serologic evidence of infection was not incorporated into the study. Also, if the initially identified virus in a specimen had been inhibited with specific antiserum, this would probably have led to discovery of many more multiple virus infections (*9*). Also, the sampling frequency (despite weekly collections during diarrheal episodes) may have been insufficient for identification of agents excreted only briefly (i.e., between weekly home visits). Finally, the immune electron microscopy technique used to demonstrate agents which may be responsible for a major portion of infant diarrheal disease was not available at the time of our study.

In 1959 the World Health Organization set up a study group on diarrheal diseases, and later (following the report of the group) it established a team of personnel in related health sciences to visit countries which had begun or which contemplated surveys of diarrheal disease (*11*). Many subsequent studies of infantile diarrheal disease were conducted throughout the world during the mid-1960's. These were generally of two sorts: (1) longitudinal studies during the first two years of life, including treatment and

nutritional factors and measurement of growth, and (2) case-control studies—that is, attempts at isolation of some or all of the known agents associated with diarrhea during episodes of acute diarrhea and comparison with an asymptomatic control group. The Cali study was really of the first type, but most of the other published studies were of the second. And since episodes of illness were not defined (except for purposes of telling when specimens should be collected in the field) and because no effort was made to relate illness episodes to laboratory isolations, precise comparisons with these other studies are difficult to make.

Perhaps the most ambitious of the longitudinal studies undertaken was that of Scrimshaw, Gordon, and co-workers in rural Guatemala (*12*). Their microbiologic results were primarily concerned with the incidence and prevalence of potentially pathogenic bacteria (*E. coli, Shigella* spp., and *Salmonella* spp.). Shigellae were found in 7.5 per cent of the members of a normal population from one to 10 years of age, while salmonellae were rather uncommon (*13*).

In a comparison of these findings with our initial isolation rates, shigellae in Cali appear to have been relatively more scarce than other groups of pathogens. Additional studies (nonlongitudinal ones) have shown shigellae to be among the most commonly identified bacterial pathogens in the Southwestern United States (*14*), Guatemala (*15*), Puerto Rico (*16*), Pakistan (*17*), and Indonesia (*18*). In contrast, a case-control study of children with diarrhea in Medellín, another large Colombian city, revealed an incidence of shigellae similar to that found in Cali (*19*).

This strikingly low incidence of shigellae in our Cali children could be partly explained by several technical factors (*20*). The methods used to transport and isolate shigellae were not ideal. For one thing, it would have been preferable to use rectal swabs instead of stools. For another, the specimens should have been plated within an hour of passage, because if the stool remains longer at room temperature the chances of recovering shigellae diminish rapidly. It would also have been better to inoculate specimens promptly into a solid agar medium, rather than using buffered glycerol-saline for transport. Finally, for optimal isolation of shigellae, employment of XLD with MacConkey and/or SS agar would have yielded better results than the media which were actually used. However, it is interesting to note that the case-control study of diarrhea in Medellín did employ all these procedures, and yet shigellae were isolated in relatively few cases.

Isolation of viruses from feces of healthy or sick infants depends on many factors—laboratory transport, culture methods, serointerference, and epidemiology. The vast array of viral agents isolated during the present study attests to the ample transmission of these agents within the population at hand, but such transmission may have little to do with causation of diarrhea. Further presentations will provide a more detailed examination of this latter point.

SUMMARY

For public health reasons, it is important that the etiologic agents of early childhood diarrhea be isolated and identified, and that their routes of transmission be defined. This is especially true in tropical and subtropical developing countries, where childhood patterns of exposure to diarrheal disease agents usually differ from those in developed countries, and where diarrheal illness is a frequent harbinger of death among children under five years of age.

This artical describes a study designed to identify diarrheal disease agents and transmission patterns in Cali, a large city of western Colombia's fertile Cauca River Valley. The study area, composed of five working-class districts with a total population of some 40,000, appeared to provide an

environment fairly similar to those of many other "average" working-class communities in Latin America.

Beginning in July 1962, a cohort of 296 children being born in these districts was studied, the period of investigation starting with the date of birth and continuing until each child's second birthday or its premature withdrawal from the study. Weekly home visits were made to establish defecation patterns, feeding practices, and anthropometry. The resulting data were then analyzed in terms of defecation frequencies, occurrence of liquid stools, and the presence of blood, mucus, or pus in the stools. Differences were noted in male and female defecation patterns and in the defecation frequencies of different age groups.

Stool specimens for bacteriologic, virologic, and parasitologic examination were collected monthly on a regular basis and weekly when diarrhea occurred. Numerically, viruses were isolated and identified more often than other agents. The most commonly isolated parasite species and viral and bacterial serotypes were *G. lamblia* (from 222 subjects), echovirus 11 (from 166 subjects), and enteropathogenic *Escherichia coli* 026:B6 (from 138 subjects). Compared with the findings of several studies in other countries, isolations of shigellae were relatively rare.

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