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Epidemiologic Patterns of Acute Diarrhea and Endemic *Shigella* Infections in Children in a Poor Periurban Setting in Santiago, Chile

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To prepare a field site for evaluating preventive interventions against endemic shigellosis, the authors followed prospectively a cohort of 360 children (90 each of children aged 0–11, 12–23, 24–35, and 36–47 months) in Santa Julia, a low socioeconomic area in Santiago, Chile, from November 1986 through April 1989 with twice weekly household visits for diarrheal disease; infants replaced children who reached 60 months of age. Coprocultures on 2 consecutive days from children with diarrhea and from age-matched controls within the cohort were cultured for *Shigella*. Bacteriologic surveillance was also maintained in the health center and children's hospital serving Santa Julia. In this community, where all households had access to potable water (68% inside) and all but 3% had access to a toilet, but where there was marked crowding, the overall incidence of diarrheal disease in the cohort was low (2.26 episodes/12 child months of observation in children aged 0–11 months and 2.09 in those aged 12–23 months), yet *Shigella* infections were common. *Shigella* accounted for 10% of diarrheal episodes in the cohort (vs. 3.2% isolation rate in controls, $p < 0.0001$). The incidence of shigellosis in children aged 12–47 months was 0.16 cases per 12 child months of observation; in the first 5 years of life, a child had a 67% chance of experiencing shigellosis. *Shigella sonnei*, *Shigella flexneri* 2a, and *S. flexneri* 6 caused > 79% of the infections. *Shigella* occurred more often in hospitalized cases of diarrhea than in age-matched cases detected in the health center or by household surveillance ($p < 0.0001$). An initial episode of *Shigella* diarrhea did not diminish overall the risk of subsequent shigellosis but did confer 72% protection ($p = 0.05$) against illness due to the homologous serotype. The high rate of both *S. sonnei* and *S. flexneri* shigellosis in a population with a low background rate of diarrhea makes Santa Julia an appropriate site for assessing the efficacy and effectiveness of measures to reduce *Shigella* infections. *Am J Epidemiol* 1991;134:614–27.

diarrhea; *Shigella*

Diarrheal disease and dysentery caused by *Shigella* constitute health problems in industrialized as well as in less-developed countries (1–3). In the less-developed world,

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Shigella accounts for approximately 8–13 percent of cases of diarrhea in children under 5 years of age in clinic-based surveys (4–9) and for approximately 4–8 percent of the episodes of diarrhea detected through household surveillance of cohorts of young children (10–14). With the increasing use of oral rehydration in national diarrheal disease control programs, deaths from acute diarrheal dehydration have markedly declined in many areas (15). One consequence has been an increase in the relative importance of certain other forms of diarrheal disease that are less affected by oral rehydration, such as dysentery (frank blood and mucus in stools) and persistent diarrhea (lasting >14 days) (16).

Since 1968, in addition to endemic *Shigella* disease, one serotype, *Shigella dysenteriae* 1, has been causing extensive outbreaks of severe disease accompanied by high case fatality in much of the developing world (17–20). The overall situation is further complicated by the widespread and increasing resistance of *Shigella* to previously useful antibiotics (such as ampicillin and trimethoprim/sulfamethoxazole) (17–23), which seriously hampers the treatment of severe shigellosis in less-developed countries.

Shigellosis is one bacterial enteric infection that persists in industrialized countries, despite the widespread availability of bacteriologically controlled water supplies and flush toilet sanitation (1, 2). The residual problem in developed settings largely derives from the fact that clinical infection can be transmitted by as few as 10 *Shigella* organisms (24) even without neutralization of gastric acid. Thus, *Shigella* is spread by direct fecal-hand-oral contact wherever personal hygiene is compromised, as among preschool children in day-care centers (25, 26), among patients in custodial institutions for the mentally impaired (27, 28), or among underprivileged preschool children residing under crowded conditions in inner cities (29).

Because *Shigella* infections constitute a refractory problem worldwide, the development of new and improved *Shigella* vaccines has been targeted by the World Health Organization as a high priority to provide an additional control measure (30–32). New *Shigella* vaccine candidates are reaching the stage of clinical trials (33–38). In order to assess their safety, efficacy, and practicality in distinct settings, promising *Shigella* vaccines will have to be evaluated in a variety of populations representing diverse ages, geographic areas, and socioeconomic levels. In Santa Julia, a periurban, low socioeconomic area located within Area Oriente (the Eastern Administrative Area) of Santiago, Chile, we undertook to study intensively and prospectively the epidemiology of *Shigella* infections in a population where crowding is severe but where potable water and some form of sanitation are available to most households. Heretofore, prospective studies of endemic shigellosis have generally involved rural or urban populations in less-developed countries living under conditions without potable water or sanitation (3, 10, 11, 16, 39) or in urban settings in industrialized countries (26). Santa Julia constitutes one appropriate site for assessing potential interventions against endemic shigellosis.

MATERIALS AND METHODS

Study objectives

The general objective of the Santa Julia field project was to provide a defined population of young children in which to conduct applied epidemiologic research of public health importance such as the evaluation of candidate *Shigella* vaccines. Specific objectives included a quantitation of the incidence of all diarrheal disease and of shigellosis by age and by season and an identification of the relative importance of the different *Shigella* serotypes. To accomplish these objectives, we initiated a longitudinal, prospective, community-based study that utilized household visits to detect mild diarrheal illnesses. To complement the information provided by household surveillance and to investigate more severe forms of diarrheal illness, a surveillance system for diarrheal disease was also implemented in the

local health center (Consultorio Santa Julia) and in the pediatric hospital (Hospital Calvo Mackenna) that serve this population.

Study site

Santa Julia is a periurban neighborhood of low socioeconomic level containing 133,909 inhabitants living in mostly ramshackle housing in a geographic area of 12.3 km^2. The birthrate is 19.8 per thousand, and 31 percent of the population is under 15 years of age. A total of 15,525 children less than 5 years of age resided in Santa Julia at the initiation of the study including 2,844 less than 12 months, 2,811 from 12 to 23 months, and 9,870 from 2 to 5 years of age. The Chilean National Health Service provides health care to approximately 96 percent of the population of Santa Julia through a neighborhood health center, Consultorio Santa Julia. The out-migration rate from this community is approximately 8 percent per year and largely involves relocation of residents elsewhere within metropolitan Santiago. The climate is temperate with a mild winter and a warm, rainless summer.

Study subjects

Participants were a stratified sample consisting of 360 Santa Julia children under 5 years of age, 90 falling within each one of four age strata: less than 12 months, 12–23 months, 24–35 months, and 36–47 months of age. When children reached 60 months of age, they were dropped from the study and replaced with children less than 12 months of age.

Participation, which was restricted to pediatric subjects without chronic disease or serious congenital malformations, was at the discretion of parents from whom informed consent was obtained. The protocol was approved by the Ministry of Health of Chile and by ethics committees at the University of Maryland School of Medicine, the World Health Organization, and the US Department of Defense.

Sampling procedure

The population sample was obtained by stratified sampling. Demographic information on every newborn in the community of Santa Julia is contained on a card kept within the Consultorio Santa Julia. These cards are organized according to the three geographic subsectors (of approximately equal population size) that comprise the community of Santa Julia, including Las Torres, Jaime Eyzaguirre, and Chacarillas. The children of each sector were line listed by date of birth. Children falling within the age groups 0–11 months, 12–23 months, 24–35 months, and 36–47 months (the age groups of interest) were consecutively numbered within each age group. From each sector we needed a total of 120 children equally divided among these four age groups (i.e., 30 children per group). Therefore, the number of children in each age stratum of each sector was divided by 30, thereby providing the sampling interval. The first child's card was randomly chosen; the remaining 29 cards were selected by adding the sampling interval to the number of the first card chosen. This sampling procedure was performed for each of the four age groups in each of the three sectors. There is a delay of some weeks until a card containing the demographic information on a newborn is prepared and inserted into the consultorio data base. For this reason, infants in the age group 0–2 months are underrepresented in this universe of cards from which we sampled.

Mothers of selected children were visited to explain the study and to elicit informed consent for their participation. The acceptance rate was approximately 90 percent among mothers of children in all age groups and sections. Infants who replaced children who graduated from the cohort were selected by the stratified sampling procedure described above.

Surveillance for diarrhea in the cohort

Upon inclusion into the cohort, the nutritional status of each child was recorded as expected weight for age (40). The household

of each participating child was visited twice weekly by a trained public health nurse or nurse auxiliary who interviewed the caretaker in order to elicit information about the occurrence of diarrheal illness. Systematic queries focused on the number, consistency, and character (i.e., watery, loose, bloody) of stools that occurred during each 24-hour period since the previous visit; responses were recorded using a precoded questionnaire. Associated symptoms (e.g., lethargy and vomiting) as well as objective signs of dehydration were noted, and the rectal temperature was recorded.

Oral glucose-electrolytes rehydration solution was offered by the nurse when appropriate. Criteria for a child to be seen by the study pediatrician included signs of dehydration, high fever, marked lethargy, or overt dysentery (blood in stools). Children with dysentery or with persistent diarrhea (>14 days) were treated with oral trimethoprim/sulfamethoxazole (41).

Surveillance for cases of diarrheal disease in the consultorio and in the hospital

To accomplish surveillance in the Consultorio Santa Julia (which was open Monday to Friday), a health auxiliary recorded the visits of every child less than 60 months of age with a chief complaint of diarrheal illness. The clinician caring for the child completed a summary of the clinical illness that included a description of the number and type of stools, presence of fever and vomiting, degree of dehydration (if any), and whether antibiotics were used. A single coproculture was obtained from every child with diarrheal illness seen at the consultorio.

To accomplish hospital surveillance, each day (except Saturday and Sunday) a nurse from the team visited Calvo MacKenna Children's Hospital to review all hospital admissions due to diarrheal illness in all services including emergency room, infectious diseases unit, and infant/toddlers ward. Every child who came from the community of Santa Julia was cultured (once, as described), and clinical data were systematically recorded until discharge.

Clinical definitions

Diarrhea is defined as an overt change in the child's normal stool pattern, characterized by an increase in the frequency (to at least three stools per 24-hour period) and a decrease in the consistency of stools to an unformed state. *Dysentery* refers to loose stools that contain gross blood, with or without mucus.

An *episode of diarrhea or dysentery* is considered to have commenced after 7 consecutive days without diarrhea and to have ended on the first day that was followed by 7 consecutive days without diarrhea. *Episodes of Shigella diarrhea or dysentery* are defined as above but accompanied by the isolation of *Shigella* from coprocultures taken at the time of illness. *Asymptomatic Shigella infection* refers to isolation of *Shigella* from a child in the absence of diarrheal illness.

Selection of control children

After the cohort had been assembled, the 30 children within each sector and age stratum were separated by sex and again line listed. One by one, each male on the list was matched by simple random method with another male in his group as a "one-way" control. For example, in the process of selecting controls, child 2 could not be a control for child 8 if child 8 was already selected to be the control for child 2. This process of selection of controls was repeated for the females in the group in each age stratum and sector.

Clinical specimens

When an episode of diarrhea or dysentery was detected, stool specimens or rectal swabs were obtained for bacteriologic culture on 2 consecutive days from the ill child; analogous specimens for culture were also obtained on the same 2 consecutive days from a predetermined, age-matched asymptomatic control child in the cohort.

Weekly prevalence of *Shigella*

A subsample of 120 children were cultured weekly for *Shigella*. They were selected by random sampling of 10 children from each age stratum within each of the three subsectors (total = 120 children). This bacteriologic surveillance was instituted to provide information on the magnitude of the reservoir of *Shigella* throughout the calendar year. When a child in the subsample graduated from the cohort, he was replaced with another randomly selected child in order to maintain circa 120 prevalence cultures per week.

Laboratory methods

Stool samples were transported in glycerol phosphate-buffered saline (42) and inoculated onto plates of MacConkey's, xylose lysine desoxycholate, and *Salmonella-Shigella* agar (42). Plates were incubated at 35–37°C for 18–24 hours. Suspicious colonies were subcultured to slants of Kligler's iron agar. Those giving a typical *Shigella* pattern were confirmed by standard biochemical tests and serogrouped by agglutination with specific antisera (43). Serotyping of *Shigella flexneri* isolates was performed by the method of Carlin et al. (44) using monoclonal antibodies kindly provided by Nils Carlin of the Swedish Bacteriological Laboratory and Alf Lindberg of Huddinge Hospital, Stockholm, Sweden.

Assessment of sociodemographic factors and levels of sanitation and water supply

Information on sociodemographic characteristics, water quality, sanitation, and hygiene levels was obtained at baseline through a questionnaire that focused on information about the mother's education, the occupation of the head of the household, the type of housing and ownership, the degree of household crowding, the type of water supply and waste disposal, and the presence of selected possessions (e.g., a refrigerator). The density of houseflies was not quantitated.

Epidemiologic measures

The mean incidence of diarrheal or dysenteric episodes per child per 12 months of observation in each age group was calculated by dividing the total number of episodes detected by the total child months of observation, for children within that age group, and multiplying by 12. The analogous incidence rates for each age group were calculated for *Shigella*-positive diarrhea and dysentery. To calculate the cumulative percentage of children who experienced diarrhea in 1 year, the number of children within each age bracket who were observed for at least 12 consecutive months and who experienced at least one bout of diarrhea was divided by the total number of children of that age followed for at least 12 consecutive months and expressed as percentage.

Statistical methods

Rates were compared by χ^2 or Fisher's exact test (two tails). The Wilcoxon rank sum test was used where a nonparametric test was indicated.

RESULTS

Characteristics of the Santa Julia participants

Between November 1, 1986, and April 30, 1989, 504 children entered the study, 249 males and 255 females; only two children selected by the sampling method were ineligible due to chronic disease (Down's syndrome and cerebral palsy). A total of 360 children were in the cohort at the beginning of the study, while at study termination the cohort numbered 306. Despite living in substandard housing and otherwise representing a pediatric population of low socioeconomic level, 95 percent of the children were well nourished; only 5 percent suffered from mild (first degree) malnutrition, while just a single child presented moderate malnutrition. The median duration of breast feeding was 7 months, ranging from 0 to 48 months.

Baseline sociodemographic information

The median family size was six, and the majority of heads of household held no jobs or worked only sporadically. Sixty-two percent of the families lived in houses that they neither owned nor rented. Extremely crowded living conditions were the general rule; 58 percent lived in cramped dwellings in which the number of beds was less than the number of household occupants minus the number of couples. All dwellings had access to potable water, although 32 percent of families had to go outside the home to collect water. All households had garbage removal twice each week. Sanitary facilities for disposing of human waste were quite variable; 64 percent had a toilet inside the home, 34 percent had access to toilets outside the home, and 3 percent of families had only latrines. Less than one-half of the families (48 percent) had a refrigerator. Two percent of mothers had no formal education. Twenty percent of mothers work outside the home; in general, the children of working mothers are cared for by an older sibling or a grandmother.

Retention of the cohort

During the 30 months that the study lasted, the cohort was followed prospectively with an average observation period of 20 months per child (range, 1–30 months). Of the total of 504 children who entered the study during these 2½ years, 82 (16.2 percent) left before study termination, and one died before the end of the study. Sixty-seven of the 82 children lost to follow-up were the consequence of migration out of the study area, while only 15 of 504 children (3.0 percent) dropped out because of refusal to continue participation.

This pediatric cohort provided 9,951 child months of observation, equivalent to 332 children observed during 30 months (table 1). Ten percent of this total observation was contributed by infants under 12 months of age and 18 percent by children aged 12–23 months; 72 percent of the total observation was provided by children aged 24–35, 36–47, or 48–59 months of age (table 1).

Incidence and seasonality of diarrhea

A total of 1,218 episodes of diarrhea were detected in the cohort through household surveillance. In 1,137 (93.3 percent) of these episodes, stool samples were obtained from the cases and, in 1,129 (92.6 percent) of the episodes, stool samples were obtained from both the cases and the matched controls.

The incidence of diarrhea showed a marked seasonality in all age groups with significantly higher rates being recorded in the warm summer months of December through February (866 episodes in 5,947 summer months of observation) than in the cool winter months of June through August (352 episodes per 4,004 winter months of observation) ($p = 0.000001$).

As summarized in table 1, all measures of diarrheal disease were highest in infants less than 12 months of age and decreased thereafter in the older age groups. These age differences remained when corrected for season.

Hospitalization

Over the course of the study period (30 months), 257 of the 15,525 children less than 5 years of age in Santa Julia had a total of 280 hospitalizations due to diarrhea, giving an annual rate of 7.2 hospitalizations due to diarrheal disease per 1,000 children under 5 years of age. Rates of hospitalization decreased with age from 30.5 hospitalizations per 1,000 infants under 12 months to 0.2 hospitalizations per 1,000 children over 47 months of age.

Seven children (1.4 percent) of the 504 who participated in the cohort study over the 30 months of surveillance were hospitalized because of acute diarrheal illness during the surveillance period, giving a yearly rate of 8.4 hospitalizations per 1,000 children under 5 years of age; age-specific hospitalization rates ranged from 62.5 per 1,000 infants under 12 months of age to 0.0 per 1,000 children above 47 months of age.

Clinical features of cases detected through the three tiers of surveillance

Clinical features of the cases of diarrheal disease detected through the three tiers of

TABLE 1. Incidence of diarrhea by age and percentage of children followed at least 1 year who developed diarrhea during prospective surveillance of a cohort of children: Santa Julia, Santiago, Chile, November 1986 through April 1989

Age group (months)	No. of children by age at admission to cohort	Total child months of observation	Total no. of episodes of diarrhea	Incidence of all episodes/12 months	% of children who experienced diarrhea in 1 year
0–11	219	959	181	2.26	79
12–23	95	1,760	306	2.09	64
24–35	99	2,360	287	1.46	61
36–47	91	2,469	264	1.28	56
≥48	0	2,403	180	0.90	50
Total	504	9,951	1,218	1.47	

TABLE 2. Comparison of clinical features of cases of diarrhea seen at the three levels of surveillance: Santa Julia, Santiago, Chile, November 1986 through April 1989

Site of surveillance	Total no. of diarrheal cases	% <12 months of age	Male: female ratio	% with fever	% with dysentery	% with dehydration
Hospital	280	77 (a)*	1.35	73 (b)	21 (c)	85 (d)
Consultorio	1,655	37 (e)	1.10	40 (f)	9 (g)	11 (h)
Household surveillance	1,218	15 (i)	0.90	36 (j)	7 (k)	3 (l)

* a vs. e, $p < 0.00001$; b vs. f, $p < 0.00001$; c vs. g, $p < 0.0001$; d vs. h, $p < 0.0001$; e vs. i, $p < 0.00001$; f vs. j, $p = 0.031$; g vs. k, p = not significant; h vs. l, $p < 0.00001$; a vs. i, $p < 0.00001$, b vs. j, $p < 0.00001$; c vs. k, $p < 0.0001$; d vs. l, $p < 0.00001$.

surveillance are summarized in table 2 where a clear-cut gradient is seen with hospitalized cases being the most severely ill but also the youngest.

Duration of diarrhea

The average duration of diarrheal illness detected by household surveillance was 7.3 days. Twenty-four percent of episodes lasted less than 4 days, and 40 percent had durations between 4 and 7 days; 10.1 percent of episodes continued for more than 14 days to reach the criterion for persistent diarrhea (16). There was no difference in duration by age.

Isolation of *Shigella* in cases and in matched controls

Overall, *Shigella* was isolated in 10.0 percent of the 1,137 cultured episodes of diarrheal illness in the cohort under prospective household surveillance but from only 3.2 percent of 1,126 matched controls without diarrhea (table 3), a highly significant difference ($p < 0.000001$). In every age group except infants less than 12 months, the isolation rate of *Shigella* was significantly higher in cases than in controls. The relative risk was highest in the second and third years of life.

TABLE 3. Isolation of *Shigella* from diarrheal cases and controls, by age, in a cohort of children under active household surveillance: Santa Julia, Santiago, Chile, November 1986 through April 1989

Age group (months)	Clinical status	Diarrheal episodes	Episodes with *Shigella*		*p* value	RR*
			No.	%		
0–11	Diarrhea	171	8	4.7	0.4	2.0
	Controls	167	4	2.4		
12–23	Diarrhea	287	23	8.0	0.00014	7.3
	Controls	285	3	1.0		
24–35	Diarrhea	274	30	10.9	0.00043	3.8
	Controls	273	8	2.9		
36–47	Diarrhea	245	35	14.3	0.0053	2.3
	Controls	242	15	6.2		
≥48	Diarrhea	160	18	11.3	0.029	3.0
	Controls	159	6	3.8		
Totals	Diarrhea	1,137	114	10.0	<0.000001	3.1
	Controls	1,126	36	3.2		

* RR, relative risk.

Age-specific incidence of *Shigella* diarrhea and dysentery

Rates of shigellosis were lowest in infants less than 12 months of age and in children over 4 years of age; the incidence in children 12–47 months of age was impressively consistent, ranging from 0.15 to 0.17 episodes per 12 child months of observation (table 4). Overall, approximately one-fourth of the cases of *Shigella* diarrheal infection (27 of 114 cases, 23.7 percent) manifested overt dysentery. The data in table 4 suggest that, during the first 5 years of life, a child in Santa Julia has a 67 percent likelihood of experiencing an episode of diarrheal illness due to *Shigella* and a 15 percent chance of having *Shigella* dysentery.

Seasonality of *Shigella* infections

The isolation of *Shigella* showed a marked seasonality with a notable increase in the warm months of the year (figure 1). Ninety-six (84.2 percent) of the 114 isolations of *Shigella* in the cohort occurred during the 5,947 summer child months of observation versus only 18 isolations during the 4,004 winter child months of observation ($p < 0.000001$).

Rate of isolation of *Shigella* in relation to surveillance method, sampling site, and clinical syndrome

The rate of isolation of *Shigella* by age varied in relation to the site of sampling (home, active surveillance; consultorio or hospital, passive surveillance) (table 5). Based on data from single coprocultures (in order to standardize methods used at the different sites), a gradient was observed in the rate of isolation of *Shigella*, being highest in hospitalized cases and lowest in cases detected during household visits. In age groups 12–23 and 24–35 months, these differences were highly significant (table 5). The rate of isolation from cases of overt dysentery (31.5 percent) was more than four times higher than that from cases of nondysenteric illness (7.4 percent) ($p < 0.000001$) (table 6).

Serotypes of *Shigella* in Santa Julia

Three serotypes, *Shigella sonnei*, *S. flexneri* 2a, and *S. flexneri* 6, accounted for 79.3 percent of the cases of shigellosis in Santa Julia detected through household surveillance and 85.7 percent of the cases detected at treatment facilities. *S. flexneri* was found more often in hospitalized children than in cases of diarrhea detected otherwise (table 7).

Clinical features of shigellosis

The median age of symptomatic children from whom *Shigella* was isolated was 34 months (range, 1–65 months), while the median age of culture-positive asymptomatic children was 40 months (range, 9–60 months) (p = not significant, Wilcoxon rank sum). A total of 53 of 114 symptomatic children (46.5 percent) with *Shigella* infection detected through household visits had fever; 34 percent had vomiting; 27 percent had dysentery. The mean duration of diarrhea was 9 days (range, 1–48 days).

TABLE 4. Number of episodes and incidence of all *Shigella* diarrheal illness, nondysenteric *Shigella* diarrhea, and *Shigella* dysentery in a cohort of children followed prospectively: Santa Julia, Santiago, Chile, November 1986 through April 1989

Age group (months)	Child months of observation	Episodes of *Shigella* diarrheal illness					
		All clinical types		Nondysenteric diarrhea		Dysentery	
		No.	Incidence/12 child months	No.	Incidence/12 child months	No.	Incidence/12 child months
0–11	959	8	0.10	8	0.1	0	0.0
12–23	1,760	23	0.16	16	0.1	7	0.05
24–35	2,360	30	0.15	21	0.1	9	0.05
36–47	2,469	35	0.17	28	0.1	7	0.03
≥48	2,403	18	0.09	14	0.07	4	0.02

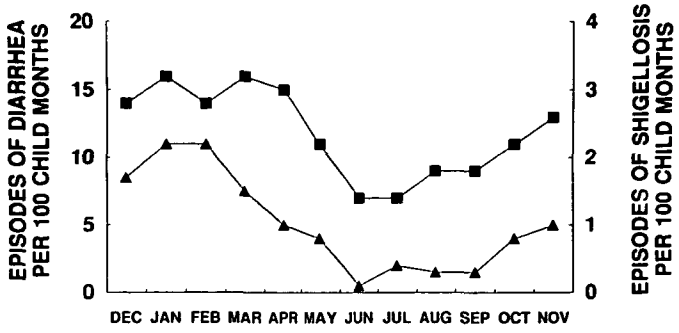

FIGURE 1. The monthly variation in the incidence of diarrheal disease (■) and of shigellosis (▲), expressed as episodes per 100 child months of observation, is shown for a cohort of children <60 months of age in Santa Julia, Santiago, Chile, who were followed for 30 consecutive months between November 1986 and April 1989 with twice weekly household visits to detect episodes of diarrhea. The incidences of both diarrhea and shigellosis were highest in the summer months of December through February.

TABLE 5. A comparison of the relative frequency of isolation of *Shigella* by sampling site and by age group: Santa Julia, Santiago, Chile, November 1986 through April 1989

Sampling site	Age group (months)	No. of diarrheal episodes cultured†	No. of episodes due to *Shigella*
Active surveillance			
Cohort	0–11	171	6 (3.5)‡ (a)*
	12–23	287	19 (6.6) (b)
	24–35	274	23 (8.4) (c)
	36–47	245	31 (12.7)
	≥48	160	13 (8.1)
Passive surveillance			
Consultorio	0–11	605	30 (5.0) (d)
	12–23	585	72 (12.3) (e)
	24–35	229	36 (15.7) (f)
	36–47	119	18 (15.1)
	≥48	117	12 (10.3)
Hospital	0–11	215	17 (7.9) (g)
	12–23	39	12 (30.8) (h)
	24–35	19	7 (36.8) (i)
	36–47	5	0 (0.0)
	≥48	2	2 (100.0)

* a vs. d, $p = 0.55$; b vs. e, $p = 0.014$; c vs. f, $p = 0.016$; d vs. e, $p = 0.15$; e vs. h, $p = 0.0025$; f vs. i, $p = 0.043$; a vs. g, $p = 0.11$; b vs. h, $p = 0.000006$; c vs. i, $p = 0.0036$.

† Only one culture per child per episode was obtained for children seen at the consultorio and at the hospital. Therefore, for purposes of comparison, only the first culture from the active surveillance cohort was included in the analysis.

‡ *Numbers in parentheses, percentage.*

Reinfections by *Shigella*

A total of 85 children in the cohort experienced just a single episode of diarrhea in which *Shigella* was isolated, while 14 others suffered from more than one bout of shigellosis (13 children experienced two separate episodes of *Shigella* diarrheal illness and one child had three). The specific serotypes were determined in the repeat infections suffered by 13 of the 14 children; the remaining child experienced two episodes of diarrhea due to *Shigella boydii*, and the *boydii* isolates were not serotyped. In only three of the 13 instances were repeat infections due to the same serotype: two children each had repeat *S. sonnei* infections, while one other child had shigellosis twice due to *S. flexneri* 2a. One of the two children who had repeat *sonnei* diarrheal infections subsequently de-

TABLE 6. Comparison of the isolation rate of *Shigella* from cases of diarrhea versus cases of dysentery at three sampling sites: Santa Julia, Santiago, Chile, November 1986 through April 1989

Site of sampling	Nondysenteric diarrhea				Dysentery		
	Total cases	No. studied*	No. with *Shigella*	%	Total cases	No. with *Shigella*	%
Active surveillance							
Cohort	1,126	1,048†	68	6.5	89	24†	27.0
Passive surveillance							
Consultorio	1,541	1,508	124	8.2	144	44	30.6
Hospital	221	220	14	6.4	59	24	40.7
Total	2,888	2,774	206	7.4	292	92	31.5

* A coproculture was obtained.
† For purposes of comparison, only the first stool sample was considered, although in this group two cultures were obtained on consecutive days.

TABLE 7. Serogroups of *Shigella* and serotypes of *Shigella flexneri among isolates obtained from different sampling sites: Santa Julia, Santiago, Chile, November 1986 through April 1989**

Site of sampling	*Shigella sonnei*	*Shigella boydii*	*Shigella dysenteriae*	*Shigella flexneri*	*S. flexneri* serotypes							
					1a	1b	2a	2b	3a	3b	6	1b + 2a
Active surveillance												
Cohort by age group (months)												
0–11	6	1	0	1	0	0	0	0	0	0	1	
12–23	13	2	0	8	0	2	4	0	0	1	1	
24–35	11	3	1	15	0	1	8	0	0	0	3	1
36–47	12	2	0	21	0	2	9	2	3	1	4	
≥48	5	1	0	12	0	0	4	0	2	0	6	
Total	47 (41)†	9 (7.9)	1 (0.9)	57 (50)	0	5	25	2	5	2	15	
Passive surveillance												
Consultorio by age group (months)												
0–11	9	2	0	19	0	1	8	0	2	0	6	
12–23	36	1	1	34	1	5	11	0	0	0	10	
24–35	19	3	0	14	0	3	10	0	0	0	1	
36–47	10	1	0	7	0	0	4	0	0	0	3	
≥48	6	0	0	6	0	0	4	0	1	0	1	
Total	80 (47.6)	7 (4.2)	1 (0.6)	80 (47.6)	1	9	37	0	3	0	21	
Hospital by age group (months)												
0–11	6	0	0	11	0	2	7	0	0	0	1	
12–23	2	0	0	10	1	0	6	0	0	0	2	
24–35	2	0	0	5	0	2	0	0	1	0	2	
36–47	0	0	0	0	0	0	0	0	0	0	0	
≥48	0	0	0	2	0	0	2	0	0	0	0	
Total	10 (26.3)	0	0	28 (73.7)	1	4	15	0	1	0	5	

* Isolates from 152 of the 165 children infected with *S. flexneri* (92%) were available for serotyping.
† Numbers in parentheses, percentage of isolates.

veloped diarrhea due to *S. boydii*. In the other 10 children with repeat infections, the second episode of *Shigella* diarrhea was due to a different serogroup.

The serotyping data proved extremely helpful in allowing us to examine infection-derived immunity. A total of 99 children experienced an initial episode of shigellosis during 8,381 child months of observation for a rate of 1.2 infections per 100 child

months. Since three serotypes, *S. sonnei*, *S. flexneri* 2a, and *S. flexneri* 6, accounted for 78 of these initial episodes (79 percent) during 8,609 child months of observation (0.96 episodes/100 child months), we attempted to quantitate the degree of homologous and heterologous immunity that is conferred by an initial clinical infection due to *Shigella* by concentrating on these three common serotypes. Over a further 1,200 child months of observation thereafter, 10 of these 78 children experienced a second episode of shigellosis (0.83 episodes/100 child months) due to one of these three serotypes. Thus, an initial episode of shigellosis due to *S. sonnei*, *S. flexneri* 2a, or *S. flexneri* 6 conferred only 13.5 percent protection overall against subsequent shigellosis due to one of these three serotypes ($p = 0.93$). Nevertheless, among these 10 children who suffered a second clinical infection, repeat infections due to the identical serotype in the same child occurred only three times during 1,279 months of further observation (0.23 episodes/100 child months). This serotype-specific reinfection rate (0.23/100) is significantly lower than the rate for second shigellosis episodes overall due to these three serotypes (0.83 episodes/100 child months) ($p = 0.05$) and represents 72 percent serotype-specific protection against repeat shigellosis.

Weekly prevalence cultures and the magnitude of the reservoir of *Shigella*

During the 30-month study, a total of 12,622 stool samples were obtained from the children who were routinely sampled each week in the field, representing 12,622 child weeks of bacteriologic surveillance for carriage of *Shigella*. *Shigella* was prevalent during 1.80 percent of the total 12,622 child weeks of observation; excretion was subclinical during 1.56 percent of the child weeks of observation. Seventy-seven (49 percent) of the 158 children who contributed these weekly coprocultures excreted *Shigella* on at least one occasion.

During the total 120 calendar weeks of surveillance of this subcohort, one or more children had positive cultures for *Shigella* during 99 (82.5 percent) of the weeks, demonstrating that a detectable reservoir of *Shigella* was present within the pediatric population in Santa Julia during most of the calendar year. A definite seasonality was observed. From November to April, the rate of isolation of *Shigella* was 2.5 per 100 surveillance coprocultures, while it was 0.97 per 100 cultures from May to October ($p = 0.000001$).

DISCUSSION

Surveillance for diarrheal disease and for shigellosis was initiated to prepare a site where preventive measures against diarrheal diseases and *Shigella*, in particular, could be evaluated in pediatric populations at risk under conditions of natural transmission. While Santa Julia is an underprivileged periurban community characterized by marked crowding, it is nevertheless notably more developed than most other communities in developing countries where pediatric cohorts have been prospectively studied to quantitate the incidence of diarrheal disease (3, 10–14, 16, 45). For example, all households had access to bacteriologically monitored, treated water supplies (although 32 percent had to go outside to collect the water), and two-thirds had a flush toilet within the dwelling. Based on these factors, one might expect the overall incidence of diarrheal disease in young children to be lower than in analogous studies that have been carried out elsewhere in developing countries. Caution must be exercised in comparing results of longitudinal cohort studies of diarrheal disease because of differences in methodology (e.g., in definitions used, in the frequency of household surveillance visits, and in age composition and size of the cohort). Nevertheless, the rates of 2.26 episodes of diarrhea per 12 child months of observation among infants and of 2.09 episodes per 12 child months of observation among children aged 12–23 months in Santa Julia are notably less than the 4–8 episodes per 12 child months

recorded in children of the same age elsewhere in Latin America, including a "favella" in Fortaleza, Brazil (10), a "pueblo nuevo" in Lima, Peru (45), a Mayan highland village in Guatemala (3), and a village in Mexico (12). Only the rural cohort in Puriscal, Costa Rica, of Vives et al. (46), the well-to-do urban population followed by Guerrant et al. (10) in Fortaleza, and an urban cohort followed (once weekly) in Buenos Aires (47) show comparable low incidence rates during the first few years of life. These data support the expectation that provision of potable water and means of disposing of human waste decrease the transmission of many enteric pathogens, resulting in lower diarrhea rates in young children.

Shigella differs from many other bacterial enteropathogens in that its transmission is more closely correlated with practices of hygiene than with levels of sanitation; thus, shigellosis can remain endemic in the face of modern sanitation if hygiene is compromised. In this regard, the marked crowding characteristic of Santa Julia fosters conditions compatible with the transmission of *Shigella*. One might therefore expect shigellosis to be endemic in Santa Julia, despite the low overall incidence of diarrhea in children. The prospective surveillance data confirm this. Approximately 10 percent of episodes of diarrhea in young children in Santa Julia detected by household visits are associated with *Shigella*, while in summer months the figure rises to circa 20 percent of cases. Indeed, during the first 5 years of life in Santa Julia, a child has approximately a 67 percent chance of experiencing a clinical illness due to *Shigella* (table 4).

The prominent rates of *Shigella* infection in Santa Julia, the use of three tiers of prospective bacteriologic surveillance (household, consultorio, and hospital), and the serotyping of *S. flexneri* isolates provided an opportunity to investigate certain aspects of the epidemiology of *Shigella* infections that have been alluded to in the past but that generally have not been well documented. These include the relative importance of different serotypes and the association of *Shigella* with distinct clinical syndromes of diarrheal illness. In this study, we confirm that *Shigella* is much more frequently isolated from cases of overt dysentery than from cases of diarrhea and show that *Shigella* has a propensity to cause severe diarrheal illness (its isolation rate paralleled the severity of cases, being highest in hospitalized children).

This prospective study shows that only a few serotypes (*S. sonnei*, *S. flexneri* 2a, and *S. flexneri* 6) account for a large proportion (>79 percent) of the cases and implies that an efficacious vaccine directed against just a few serotypes could have a notable impact in diminishing *Shigella* disease. In Santa Julia, as in most areas of the developing world, *S. flexneri* 2a is the most prevalent *flexneri* serotype (3, 7, 14, 39, 48, 49).

S. dysenteriae and *S. flexneri* are common in less-developed countries (2, 7, 50), while *S. sonnei*, by far the major serotype found in industrialized countries (1, 51), is relatively uncommon in situations of underdevelopment (2, 7, 50). Enigmatically, despite the relative paucity of *S. sonnei* disease among indigenous persons living in less-developed countries (7, 50–52), *S. sonnei* commonly causes shigellosis among travelers to these same less-developed areas (52–54). It is thus of interest that Santa Julia shows a somewhat unusual, intermediate pattern wherein both *S. sonnei* and *S. flexneri* isolates are common. This likely reflects a community that is undergoing active development, transforming from a less-developed toward an industrialized situation (51). Paradoxically, the universal availability of monitored, potable water in Santa Julia may account for the high frequency of *S. sonnei* infections. The possible explanation is that some strains of *Plesiomonas shigelloides*, an autochthonous bacterial species of surface waters (55), express a polysaccharide O antigen identical to that of *S. sonnei* (56). Under less-developed conditions, repeated ingestion of *Plesiomonas* bacteria through consumption of untreated surface waters may stimulate cross-protection against *S. sonnei*, since O antibodies are believed to mediate protection (57). Whatever the explanation, the unexpectedly frequent occur-

rence of *S. sonnei* infections among children in Santa Julia makes this a particularly interesting site because the efficacy and effectiveness of interventions can be measured against *S. sonnei* as well as against *S. flexneri*.

This prospective study also provides some insights into the acquisition of immunity to *Shigella* in that prior infection with one serotype of *Shigella* appeared to protect against subsequent clinical infection with the identical serotype but not against diarrheal illness due to other serotypes. Formal et al. (58) have recently documented this lack of cross-protection in monkeys. Monkeys that were experimentally infected with *S. flexneri* 2a were completely protected against homologous challenge 5 weeks later with *S. flexneri* 2a but not against challenge with *S. sonnei*.

Shigella vaccine candidates of different varieties are reaching the stage of clinical testing (31- 38). Santa Julia offers an unusually attractive site to undertake evaluations of preventive measures against *Shigella* infections because of its relatively high rate of seasonal, endemic shigellosis and dysentery in a background where the overall incidence of diarrhea is not high.

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