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PREVALENCE OF PATHOGENIC ENTERIC BACTERIA IN CHILDREN OF 31 PANAMANIAN COMMUNITIES*

MIGUEL KOURANY,† MANUEL A. VASQUEZ,† AND LEONARDO J. MATA‡
Gorgas Memorial Laboratory, Panamá, Republic of Panamá, and Institute of Nutrition of Central America and Panamá (INCAP), Guatemala, Central America

ABSTRACT: Bacteriological studies were made on children 10 years old and under from 700 families in Panama City and 30 rural and semirural communities. Culture of rectal swabs from 1,178 children revealed a prevalence rate of 3.7% for enterobacterial pathogens, that for *Shigella* sp. (2.8%) being highest. *Salmonella* sp., enteropathogenic *Escherichia coli*, and *Edwardsiella tarda* were recovered less frequently. Differences in prevalence of *Shigella* infections in age groups were not significant, but *Shigella* was not found in children less than 1 year old. *Shigella* was isolated more frequently from children with diarrhea (4.8%), than from those without diarrhea (1.9%); the difference is significant ($P < 0.05$). There was no positive correlation between isolations of enterobacteria with disposal of excreta, sources of drinking water, and the number of children occupying the same bed. Prevalence rates for pathogens in 31 communities ranged from 0 to 13.3%; in 11, no pathogens were encountered, and *Salmonella* sp. and enteropathogenic *E. coli* were found in 7. *Shigella* infections were found in 17 communities, indicating its relative importance as a pathogen.

(Accepted 12 January 1971)

In Panamá, as in other preindustrial countries, diarrhea is reported as one of the major causes of death among infants and young children. Diarrheal mortality often is underrated since lay officials and school teachers in rural areas do most of the recording. Thus, cause of death is frequently uncertain or inaccurately reported.

Mortality figures of diarrheal disease for Panamá for the period 1960–67 are available from deaths listed as owing to "gastroenteritis" and "colitis." Although the death rate for infants has declined steadily since 1960, it is still about 40 per 10,000 for children less than 1 year old.[1,2]

Information on disease occurrence in Panamá is even more scarce and unreliable because of practical difficulties in registration and inconsistent reporting. Morbidity figures are limited to those from health centers and a few hospitals, with little information from private physicians. Morbidity data, representing medically supervised cases from most of the government-supported health institutions in the country, have been collected since 1963.[3]

Incidence of diarrhea (gastroenteritis and colitis) is greater in infants under 1 year. In this age group, the reported rate rose from 52 cases per 1,000 infants in 1966 to 65 per 1,000 in 1967.[4] In children aged 1 to 4 years, diarrhea was less frequent, but rates were still high; for 1966 the age-specific rate was 14.5 per 1,000 and for 1967, 16.1 per 1,000. Morbidity, per 1,000 children in the 5- to 14-year age group, was 2.0 diarrhea cases in 1966 and 2.6 in 1967.[4] The increase in these rates probably reflects the better medical coverage of the population and improved registration observed in recent years.

Information is scant on the prevalence and distribution of the Enterobacteriaceae that are commonly associated with diarrheal disease in Panamá. Recent studies of infants with diarrhea who attend the outpatient clinic of the Children's Hospital in Panama City revealed that only a low percentage excreted pathogenic Enterobacteriaceae.[5] These studies showed, however, that patho-

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Please address requests for reprints to: Gorgas Memorial Laboratory, P.O. Box 2016, Balboa Heights, Canal Zone.

† Gorgas Memorial Laboratory, Panamá, Republic of Panamá.

‡ Microbiology Division, Institute of Nutrition of Central America and Panamá (INCAP), Guatemala, Central America.

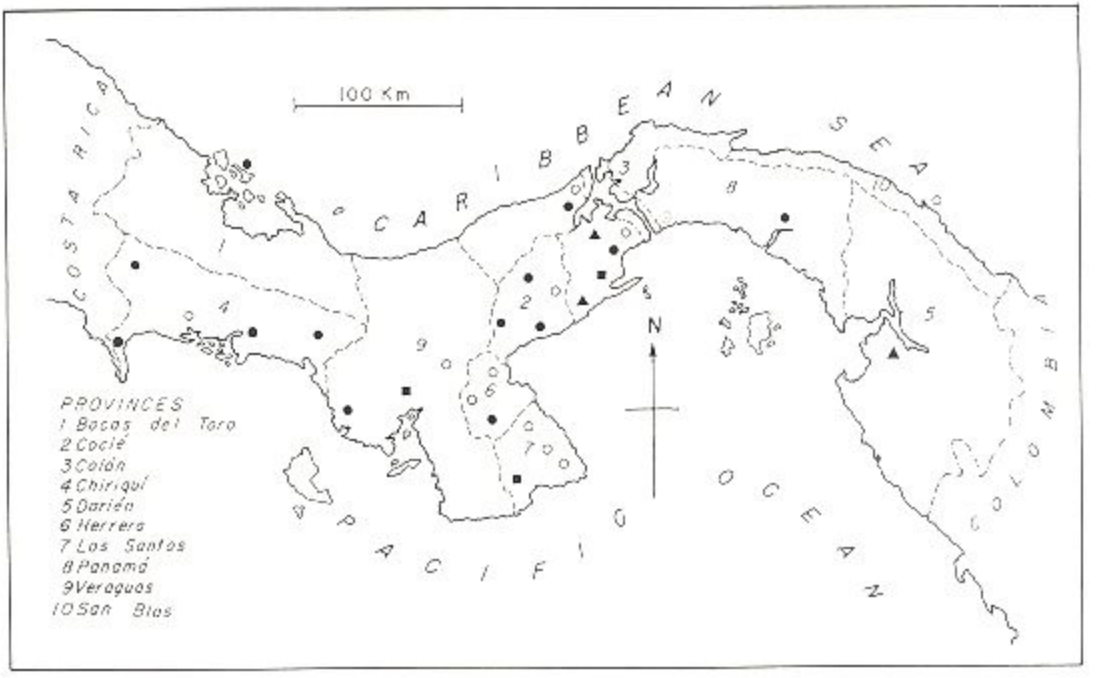

FIGURE 1. Map of the Republic of Panamá, showing the locations of the communities studied in the nationwide survey of pathogenic Enterobacteriaceae. Panama City shown by ⊗ is positive for *Shigella*. For towns and villages, ● is positive for *Shigella*; ■ is positive for *Shigella* and other pathogens;* ▲ is negative for *Shigella* but positive for other pathogens,* and ○ is negative for pathogenic Enterobacteriaceae.

* Enteropathogenic *Escherichia coli*, *Salmonella*, or *Edwardsiella*.

genic enteric bacteria were more prevalent in diarrhea than in nondiarrhea patients. Furthermore, diarrheal disease is related to certain environmental and social conditions that also are known to favor dissemination of enteric organisms.[6]

A nationwide survey of the prevalence of Enterobacteriaceae seemed indicated if we were to learn more about the variety and relative frequency of serotypes in the country and to determine possible differences in prevalence of the various groups of bacteria according to host and environmental characteristics. The feasibility of conducting such a study became practical and desirable with initiation of a nutritional survey of Panamá, conducted by the Institute of Nutrition of Central America and Panamá (INCAP) and the Office of International Research of the National Institutes of Health of the United States of America (OIR).[7]

MATERIALS AND METHODS

Seven hundred families were selected from Panama City and from 30 rural and semirural communities of the Republic of Panamá. The latter, whose locations are shown in Figure 1, were taken at random from the 425 *Cabeceras de Distrito* (districts) and *Cabeceras de Corregimiento* (counties) with less than 25,000 inhabitants. From each of the rural and semirural areas, 20 families with 5 alternates were randomly taken; 100 families from Panama City were likewise selected.[7] Adequate preparation of the participants was made by health personnel the day preceding the visit of the field team.

All children under 11 years of age, in the families participating in the nutrition survey, were studied for pathogenic Enterobacteriaceae from January through March 1967. The techniques used in the field and in the laboratory were basically the same previously used in prevalence studies.[8,9] Additional information on the sampling procedures and logistics of the nutrition survey is described elsewhere.[7]

A physician collected epidemiological information, including age, sex, number of family members and siblings under 11 years of age, avail-

TABLE 1

Pathogenic Enterobacteriaceae in children 0 to 10 years old from 31 communities of Panamá, 1967

Province	Community	Children examined (*No.*)	Children with pathogens (*No.*)	(%)	Organisms isolated*
Bocas del Toro	Bocas del Toro	32	3	9.4	A2, B2
Chiriquí	Dolega	9	0	—	—
	Cerro Punta	36	2	5.5	A2, B3
	Progreso	46	4	8.7	B2, D
	San Lorenzo	31	2	6.4	A2, B3
	Tolé	38	1	2.6	D
Veraguas	Montijo	33	3	9.1	B1, B6, EEC 055
	Guarumal	41	1	2.4	D
	Raya Santa María	38	0	—	—
Herrera	Chupampa	35	0	—	—
	Santa María	34	0	—	—
	Los Cerritos	34	1	2.9	D
Los Santos	Tonosí	30	4	13.3	D, EEC 0127, E.t.
	Lajamina	31	0	—	—
	La Palma	17	0	—	—
	Tres Quebradas	17	0	—	—
Coclé	Antón	53	1	1.9	B2
	El Cristo	39	2	5.1	A3, C3
	El Retiro	50	0	—	—
	Toabré	45	1	2.2	B3
Panamá	Panama City	126	7	5.5	B2, D
	San José	42	1	2.4	EEC 055
	Buenos Aires	49	3	6.1	B3, *S. anatum*, E.t.
	Zanguenga	35	2	5.7	*S. sundsvall*, E.t.
	Chorrera	37	1	2.7	B3
	Nuevo Arraiján	30	0	—	—
	Chepo	22	2	9.1	B3, D
Colón	Escobal	36	1	2.8	B3
	Piña	29	0	—	—
Darién	Chepigana	37	1	2.7	E.t.
San Blas†	Ailigandi	46	0	—	—
Total		1,178	43	3.7	

* A = *Shigella dysenteriae*; B = *S. flexneri*; C = *S. boydii*; D = *S. sonnei*; EEC = enteropathogenic *Escherichia coli*; S = *Salmonella*; E.t. = *Edwardsiella tarda*. (The number after the letter denotes the serotype.)

† Territorial reservation for the Kuna Indians of Panamá.

ability and quality of water supply, system of disposal of feces and wastes, and number of children sharing the bed with the child studied. The presence or absence of diarrhea during the week, including the day of the survey, was recorded. The presence of blood and mucus and consistency of feces on the day of the examination was also determined. This information was obtained by direct interview with the mother or person responsible for every family.

Rectal swabs were taken on each child, streaked immediately onto Salmonella-Shigella (SS) and MacConkey (MC) agar plates, and placed in a tube of Selenite F broth. All inoculated media were transported overland or by plane to the laboratory in Panama City the day of inoculation and were incubated upon arrival. After incubation for 18 hours, aliquots of the Selenite medium were streaked on bismuth sulfite and SS agar plates and incubated 24 hours.

TABLE 2

Prevalence of pathogenic Enterobacteriaceae in children 0 to 10 years old, by province, Panamá, 1967

Province	Communities surveyed (*No.*)	Children examined (*No.*)	Children with pathogens (*No.*)	Children with pathogens (%)	Organisms isolated*
Bocas del Toro	1	32	3	9.4	Sh
Chiriqui	5	160	9	5.6	Sh
Veraguas	3	112	4	3.6	Sh, EEC
Herrera	3	103	1	1.0	Sh
Los Santos	4	95	4	4.2	Sh, EEC, E.t.
Coclé	4	187	4	2.1	Sh
Panamá†	7	341	16	4.7	Sh, Salm, EEC, E.t.
Darién	1	37	1	2.7	E.t.
Colón	2	65	1	1.5	Sh
San Blas‡	1	46	0	0	
Total	31	1,178	43	3.7	

* Sh = *Shigella*; EEC = enteropathogenic *Escherichia coli*; Salm = *Salmonella*; E.t. = *Edwardsiella tarda*.
† Includes Panama City.
‡ Indian reservation on Atlantic coast of Panamá.

Between 5 and 10 nonlactose-fermenting colonies of all types and sizes from each plate were transferred to triple-sugar-iron agar (TSI) and lysine-iron agar (LI) slants. Lactose-fermenting colonies from MC agar were subcultured in heart infusion (HI) agar. TSI, LI, and HI agar slants were incubated 24 hours.

Preliminary screening and identification was carried out by inoculation of Christensen's urea, Simmon's citrate, and semisolid agar, and by agglutination with polyvalent and type-specific *Shigella* and *Salmonella* antisera. Only the 11 most common enteropathogenic *Escherichia coli* serotypes were investigated, namely: 026:B6, 055:B5, 086:B7, 0111:B11, 0119:B14, 0124:B17, 0125:B15, 0126:B16, 0127:B8, and 0128:B12. Cultures were confirmed by biochemical and serological tests.[10] The media and sera used were from Baltimore Biological Laboratory, Baltimore, Maryland and Difco Laboratories, Detroit, Michigan, respectively.

RESULTS

Over-all prevalence

A total of 1,178 children under 11 years of age were studied (Table 1). Of these, 43 (3.7%) were found excreting pathogenic enterobacteriaceae. Prevalence of pathogens by locality ranged from 0 to 13.3%, although differences were not significant. *Shigella* infections were present in 17 of the 31 communities surveyed, while *Salmonella*, enteropathogenic *E. coli*, and *Edwardsiella tarda* (a newly recognized species apparently associated with diarrhea)[11] were detected in six of the villages. No pathogens were isolated in 11 of the 31 communities.

TABLE 3

Prevalence of pathogenic Enterobacteriaceae in children from 31 Panamanian communities, by age

Age	Children examined (*No.*)	Children with pathogens (*No.*)	Children with pathogens (%)	No. harboring particular pathogens: *Shigella* (*No.*)	*Shigella* (%)	*Salmonella* (*No.*)	*Salmonella* (%)	Enteropathogenic *E. coli* (*No.*)	Enteropathogenic *E. coli* (%)	*Edwardsiella* (*No.*)	*Edwardsiella* (%)
<6 mo.	43	1	2.3	0	—	0	—	0	—	1	2.3
6–11 mo.	65	2	3.1	0	—	0	—	0	—	2	3.1
1–4 yrs.	511	16	3.1	13	2.5	0	—	2	0.4	1	0.2
5–10 yrs.	550	24	4.4	20	3.6	2	0.4	1	0.2	1	0.2
Not determined	9	0	—	0	—	0	—	0	—	0	—
Total	1,178	43	3.7	33	2.8	2	0.2	3	0.3	5	0.4

TABLE 4

Prevalence of pathogenic Enterobacteriaceae in children, according to selected sanitary facilities and social conditions in 31 communities of Panamá, 1967

Sanitary and social conditions	Children examined		Children with pathogens		No. harboring particular pathogens			
					Shigella		Others*	
	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Excreta disposal site								
Ground	189	16.0	5	2.6	2	1.0	3	1.6
Pit privy	727	61.7	28	3.8	21	2.9	7	1.0
Flush toilet	161	13.7	8	5.0	8	5.0	0	—
River or sea	88	7.5	1	1.1	1	1.1	0	—
No information	13	1.1	1	—	1	—	0	—
Total	1,178	100.0	43	3.7	33	2.8	10	0.8
Source of drinking water								
Piped	372	31.6	18	4.8	16	4.3	2	0.5
Well	640	54.3	19	3.0	13	2.0	6	0.9
Stream	129	11.0	4	3.1	2	1.5	2	1.5
Rain	27	2.3	2	7.4	2	7.4	0	—
No information	10	0.8	0	—	0	—	0	—
Total	1,178	100.0	43	3.7	33	2.8	10	0.8
No. persons sharing bed with child								
None	349	29.7	10	2.9	9	2.6	1	0.3
1 or 2	692	58.7	28	4.0	19	2.7	9	1.3
3 or more	124	10.5	5	4.0	5	4.0	0	—
No information	13	1.1	0	—	0	—	0	—
Total	1,178	100.0	43	3.7	33	2.8	10	0.8

* *Salmonella*, enteropathogenic *Escherichia coli* or *Edwardsiella tarda*.

Shigella prevalence rates by province ranged from 0 to 9.4% and appeared highest in the western region of Panamá (Bocas del Toro and Chiriqui); these differences were not significant (Table 2).

The children examined belonged to 424 families, of which 39 (9.2%) were found with only one child excreting enteric pathogens; in 4 families 2 children excreted pathogens. The children of 3 of the latter were excreting *Shigella sonnei*; in the fourth family, one child had *Shigella flexneri* 1 and the other *S. flexneri* 6.

Age-specific prevalence

None of the commonly recognized pathogens (*Shigella*, *Salmonella*, and enteropathogenic *E. coli*) were found in children under 1 year old. Thereafter, infection with *Shigella* became progressively more frequent, but the differences by age were not significant (Table 3). The over-all infection rate for *Shigella* was 2.8%, while *Salmonella* and enteropathogenic *E. coli* were identified in only 0.2% and 0.3%, respectively, of the 1,169 children whose age was determined. *E. tarda*, also rare, was isolated from all age groups.

Prevalence according to certain social and environmental conditions

Results of interviews to determine predominant defecation sites, sources of drinking water, and number of individuals sharing the same bed are summarized in Table 4. Prevalence rates of pathogenic Enterobacteriaceae in children did not vary according to the categories within each of the three variables studied.

Prevalence according to diarrhea

Information on the presence of diarrhea the day of the survey, or on any day of the preceding week, was collected for 1,055 of the children attending the survey. Diarrhea prevalence decreased from 30.8% in children less than 6 months

TABLE 5

Prevalence of Shigella *in stools in relation to diarrhea*

Age (years)	Children examined		With diarrhea			Without diarrhea		
				with *Shigella*			with *Shigella*	
	(No.)	with *Shigella* (No.)	(No.)	(No.)	(%)	(No.)	(No.)	(%)
<1	108	0	29	0		79	0	
1 to 4	455	11	97	4	4.1	358	7	2.0
	56*	2						
5 to 10	492	15	62	5	8.0	430	10	2.3
	58*	5						
Unknown	9*	0						
Total	1,178	33	188	9	4.8	867	17	1.9

* No history obtainable with respect to diarrhea.

old to 12% in children 5 to 10 years old. The over-all diarrhea prevalence rate was 17.8%.

The distribution of *Shigella* isolates by age and by presence of diarrhea (Table 5) revealed more isolations in diarrhea cases (4.8%) than in children without diarrhea (1.9%), a significant difference ($P < 0.05$). More *Shigella* were found in diarrhea than in nondiarrhea cases when patients were divided into age groups.

TABLE 6

Serotypes of Enterobacteriaceae in children from 31 Panamanian communities, 1967

Serotype	Isolations (No.)	Relative proportion (%)
Shigella		
S. dysenteriae 2	3	7.0
3	1	—
S. flexneri 1	1	—
2	6	14.0
3	7	16.3
6	1	—
S. boydii 3	1	—
S. sonnei	13	30.2
Salmonella		
S. sundsvall	1	—
S. anatum	1	—
Escherichia coli		
E. coli 055:B5	1	—
0125:B15	1	—
0127:B8	1	—
Edwardsiella		
E. tarda	5	11.6
Total	43	100.0

Serotypes found

Serotypes of all organisms isolated are listed in Table 6. All four *Shigella* serogroups were represented in the isolations. *S. sonnei* was the most frequent serotype, particularly in children from Panama City, although it was also found in six rural communities. *S. flexneri* 3, *S. flexneri* 2, and *S. dysenteriae* 2 were next in frequency.

DISCUSSION

The prevalence of diarrheal disease in the Republic of Panamá resembles that existing in European countries and in the United States at the beginning of this century. In the United States the infant mortality rate owing to diarrheal disease varied between 35 and 56 per 1,000 during the first decade of this century; it was 29 per 1,000 by the end of the second decade, and about 8 per 1,000 by 1930.[12] For children 1 to 4 years old, reported fatalities in the United States fluctuated between 3.3 and 1.7 per 1,000 during the first two decades, but by 1930 the rate had dropped to 0.9 per 1,000.[12]

With allowances for the unreliability of statistics in Panamá, the current rates for diarrheal deaths described in this paper attests to the importance of diarrheal disease as a major cause of death of Panamanian children.

Throughout the world, most diarrheal diseases are thought to have an infectious origin.[13] However, laboratory findings show that only about 20% of the diarrheas can be related to the usually recognized bacterial and parasitic agents.[14] The Enterobacteriaceae are the most important agents,

and shigellae are consistently associated with a significant proportion of diarrheas. When serial cultures are made, this association becomes more obvious.[15]

In our study, the over-all infection rate of enteropathogens in children under 11 years was 3.7%, of which *Shigella* was the commonest. Several serotypes were represented, and various foci appeared throughout the country, indicating the ubiquitous nature of the *Shigella* group.

In Costa Rica, a comparable study revealed a lower prevalence (2.6%) of enterobacterial pathogens in 1,329 nondiarrheic preschool children, and only 1.4% of the infections were due to *Shigella*.[16] On the other hand, infections with *Shigella* averaged 7.7% in 712 children less than 10 years old in Guatemalan villages.[9] This rate is significantly higher than that of Panamá and could be due to different host-parasite-environment interactions in this region.

Salmonella and enteropathogenic *E. coli* were rare. We believe that more *Salmonella* would have been isolated had feces been collected instead of rectal swabs. The rate of enteropathogenic *E. coli*, on the other hand, was comparable to that observed in a previous study of nondiarrheic infants.[5]

The most frequently encountered pathogen in this study was *S. sonnei*. This differed from the findings in Costa Rica and Guatemala where *S. flexneri* was the predominant organism.[8, 9, 16]

Shigella were not found in children under 1 year old. In other preindustrial countries, shigellosis was also rare in the first months of life,[8, 17] suggesting that breast-feeding and the special intestinal milieu that develops with it protects young children from this type infection.[18]

The frequency of infection was slightly greater among children who drank water from faucets (piped water) and had access to flush toilets for the disposal of excreta than among those who did not have or use such sanitary facilities. We had expected fewer infections among the former group. However, analysis of the data and visits to the children's houses disclosed that the majority of children who reported using piped water and flush toilets were from Panama City and lived in substandard dwellings or multifamily tenement slums where these facilities, although present, are notoriously inadequate and must be shared communally.[6] Also, the general lack of knowledge regarding personal hygiene among the study population likely masked any possible beneficial effect that could be derived from these facilities.

Shigella was far more common in children with diarrhea, as defined in this study, than in those without diarrhea ($P < 0.05$), as shown in a similar Guatemalan study.[9]

Diarrheal disease has been well characterized as a dominant public health problem in developing countries where *Shigella* infections have an increased frequency.[14, 19, 20]

The ubiquitous presence of etiological agents invariably leads to the dominant position of acute diarrheal disease in these countries. *Shigella*, the greater risk of exposure in rural and some urban areas because of inadequate sanitary conditions, ignorance, and a deteriorated nutritional state are particularly noted in this regard. Long-term prospective studies have indicated that a considerable proportion of diarrhea cases are related to recent *Shigella* infections,[15] a situation not revealed by the usual prevalence study. This observation should be considered in analyzing the rates obtained in Panamanian children, which in all probability were underestimated.

Among other pathogens, revealed by techniques that differ from those we used, *Salmonella* was found to be common in certain isolated communities of Panamá. In these communities, *Shigella* appears less frequently than *Salmonella*, while enteropathogenic *E. coli*, rare in rural communities, is more frequent in the urban areas where weaning is established at an earlier age.*

Via a long-term prospective study of fixed populations, we are investigating the association of enteropathogenic agents and diarrheal disease. This study will probably give certain epidemiological constants as influenced by variations in host-parasite-environment interactions in Panamá.

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