ENTEROPATHOGENIC BACTERIA ASSOCIATED WITH
DIARRHEA AMONG INFANTS IN PANAMÁ

MIGUEL KOURANY AND MANUEL A. VÁSQUEZ

ENTEROPATHOGENIC BACTERIA ASSOCIATED WITH DIARRHEA AMONG INFANTS IN PANAMÁ*

MIGUEL KOURANY AND MANUEL A. VÁSQUEZ

Gorgas Memorial Laboratory, Panamá, Republic of Panamá

ABSTRACT: A total of 1,819 infants with diarrhea were examined in Panamá for the presence of enteropathogenic bacteria. Results of the single rectal-swab cultures from these patients revealed a very low prevalence (7.9%) of all pathogens. Enteropathogenic *Escherichia coli* accounted for 5.3%, *Shigella* for 1.7%, and *Salmonella* for 1.2%. Serotypes of the enteropathogens isolated were determined; *E. coli* 055:B5 was found in 35.4% of the instances of isolation of *E. coli*. Enteropathogenic *E. coli* and *Salmonella* were isolated from infants with diarrhea only, as well as from infants with diarrhea and other symptoms. On the other hand, *Shigella* infections were not encountered in infants with diarrhea alone. Shigellosis occurred in the more severely ill infants, in whom other symptoms accompanied the diarrhea. When the diarrheal cases were grouped by severity of symptoms, the highest rate of infection (12%) was found in the dehydrated infants. Isolations were all from acute, sporadic cases throughout Panamá City, suggesting that the source of infection was not localized.

In the lesser developed countries of the world, including those in Latin America, diarrheal diseases are a major problem in public health. Despite the difficulties and defects in the registration of vital statistics in these countries, it is clear that acute diarrheas are a principal cause of infant mortality and the major cause of morbidity among infants and young children.[1]

Studies on diarrheal disease in México,[2] Guatemala,[3] and Costa Rica[4] have provided better information on the incidence, prevalence, and distribution of *Shigella*, *Salmonella*, and enteropathogenic *Escherichia coli* and the extent to which these pathogens cause acute diarrheas in Central America.

In Panamá, diarrheal disorders likewise are the major health hazard of infants and young children. Although gastroenteritis and colitis have long been reported as the leading causes of death in children during the first few years of life,[5] very little is known about their etiology. We undertook to obtain basic epidemiologic and etiologic data for this country.

Shigella has been demonstrated to be the most common enterobacterial pathogen in the general population of young children with acute diarrheal disease in Guatemala,[3] México,[2] and Egypt.[6] Accordingly, a similar situation was anticipated in Panamá. For this reason these data concern only the bacterial etiology of acute diarrheal disease of Panamanian infants. No attempts were made to investigate enteric viruses or other parasites.

MATERIALS AND METHODS

This investigation was conducted from 1 February 1964 through 30 January 1965 among 1,819 infants 24 months of age or less, residents of Panamá City and surrounding areas, who attended the outpatient clinic at the Children's Hospital in Panamá City. All children were screened through this clinic. Selection of "diarrheal" cases was made throughout the period of study by the same six physicians, who considered a patient to have diarrhea if this was the major complaint for which the infant was brought for medical attention and if the parent reported that five or more stools had been passed within the 24 hours preceding the clinic visit. Presence of mucus or blood, when reported, was considered indicative of diarrhea regardless of the number of bowel movements. Based on these criteria, as many as 15 cases were selected each day except Sundays and holidays.

Rectal temperatures were recorded for all children at the time of the clinical examination; infants with temperatures over 37.5°C were considered to be febrile. Dehydration was judged on physical examination.

On the basis of information supplied by the parents and by the physician's examination, clinical and epidemiologic data for each child

TABLE 1

Infants with diarrhea and those without examined for enteropathogenic bacteria

Age (months)	Group with diarrhea		Control group	
	(No.)	(%)	(No.)	(%)
< 6	681	37.4	133	45.0
6–11	656	36.1	69	23.3
12–17	334	18.4	50	16.8
18–24	148	8.1	44	14.9
Totals	1,819	100.0	296	100.0

TABLE 2

Symptoms in infants with diarrhea

Symptom	Infants	
	(No.)	(%)
Diarrhea only	337	18.5
Diarrhea and:		
Fever > 37.5°C	302	16.6
Vomiting	216	11.9
Fever and vomiting	430	23.6
Dehydration	534	29.4
Totals	1,819	100.0

were recorded at the time of the physical examination. Clinical data included state of dehydration and nutrition, fever, vomiting, and the number of days with diarrhea preceding the clinic visit, determined from the date of onset. Information on medication administered by parents before the hospital visit was not available.

Controls for this study were chosen from patients attending the clinic for illnesses other than diarrheal disease and who were reported to have been without diarrhea for more than 7 days.

Both groups, diarrheal and control, were characterized by age, sex, place of residence, and a number of socioenvironmental characteristics.

Rectal swabs were collected from all patients and control children by the physicians and sent to the laboratory in screw-capped tubes containing a buffered glycerol-saline solution.

Plates of MacConkey's and SS agars (Baltimore Biological Laboratory, Baltimore, Maryland) were streaked with each rectal swab, and the swab was then placed in a tube of the enrichment medium Selenite F within 3 hours after the samples were taken in the clinic. After incubation of the selenite at 37°C for 18 hours, SS agar and bismuth sulfite agar plates were streaked with aliquots and incubated. All plates were examined after incubation, following the procedures recommended by Edwards and Ewing.[12]

Ten lactose-fermenting colonies from each MacConkey's agar were individually transferred to heart-infusion slants. After incubation, viable and heat-killed bacterial suspensions from each slant were tested against enteropathogenic E. coli-specific OB and O antiserum (Difco Laboratories, Detroit, Michigan). Blood-agar plates were not used in this study. Polyvalent and OB antiserum were employed in the slide agglutination tests for screening and presumptive identification.

Cultures agglutinating with OB antiserum were then tested against O antiserum in the tube agglutination test, and agglutinating titers of 1:320 or greater were considered positive. Enteropathogenic E. coli cultures thus identified were confirmed biochemically. These procedures, however, did not permit a complete characterization of O antigenic components and of H antigens.

From each streaked plate, four to six colonies not fermenting lactose were transferred to triple-sugar iron-agar slants and after incubation were examined biochemically and serologically for the presence of Salmonella, Shigella, and the Arizona group of organisms by conventional procedures.[13] Slide agglutination tests with polyvalent grouping antiserum samples (Difco Laboratories) were performed on all colonies giving typical Shigella and Salmonella biochemical reactions. This permitted preliminary identification and grouping of all except the rarer shigellae and salmonellae serotypes. Final examination was performed at the Enteric Bacteriology Unit, National Communicable Disease Center, Atlanta, Georgia.

RESULTS

A total of 1,819 patients, varying in age from 28 hours to 24 months, was examined for the presence of enteropathogenic bacteria (Table 1). About 74% were under 1 year of age. Because controls were selected from children within the same age group as the study sample, living in the same communities, and, as far as could be determined, under similar socioenvironmental conditions, only 296 infants without diarrhea were available for examination during the course of the investigation. About 68% of these were less than 1 year old.

Table 1 shows the age distribution of all the children examined. The two groups, study and control, were not completely representative of the

TABLE 3

Results of single rectal-swab cultures from infants with diarrhea, grouped by symptoms at time of examination

Symptom	No. of infants examined	Infants with pathogens		No. harboring particular bacterial pathogens					
				Enteropathogenic E. coli (EEC)		Shigella		Salmonella	
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Diarrhea only	337	21*	6.2	20	6.0	0	—	2	0.6
Diarrhea and:									
Fever > 37°C	302	22†	7.3	14	4.6	6	2.0	3	1.0
Vomiting	216	13	6.0	11	5.1	0	—	2	1.0
Fever and vomiting	430	24	5.6	14	3.3	5	1.2	5	1.2
Dehydration	534	64‡	12.0	37	6.9	20	3.7	9	1.7
Total	1,819	144	7.9	96	5.3	31	1.7	21	1.2

* One infant had EEC and Salmonella.
† One infant had EEC and Shigella.
‡ Two infants had EEC and Salmonella.

general population because patients of the upper socioeconomic group are seen by private physicians.

Diarrhea unaccompanied by fever, vomiting, or dehydration was observed in 18.5% of the infants studied. The association of diarrhea with certain other symptoms is summarized in Table 2. Dehydration was associated with diarrhea in 29.4% of the cases, and in these children more than 70% had histories of fever and vomiting along with the diarrhea. Hospitalization was required for 223 of the 534 dehydrated infants. This represented 41.8% of this group and 12.3% of the total study cases.

The outpatient status of those with diarrhea precluded determination of the total duration of diarrheal illness; however, the number of days from onset until the first clinic visit was recorded. Fifty-eight percent (1,059 infants) of the cases examined were ill 1 to 3 days and 40% (732 infants) 4 or more days before attention was sought. Information for the remaining 2% was not available. In the group of infants ill 1 to 3 days, 70 specimens (6.6%) were culturally positive for enteropathogenic bacteria; in those ill 4 or more days, 71 specimens (9.7%) were positive.

Bacterial agents of possible etiologic significance were recovered from 144 (7.9%) of the 1,819 diarrheic infants examined. Of these agents, 64.9% were enteropathogenic E. coli, 20.9% were Shigella, and 14.2% were Salmonella. Arizona organisms were not found. Of the control group, four of the 296 infants (1.4%) harbored entero-

pathogenic bacteria. E. coli 055:B5 was isolated from two infants, E. coli 0111:B4 from one infant, and E. coli 0119:B14 from another.

An over-all infection rate of 5.3% was observed for enteropathogenic E. coli in the diarrheic infants (Table 3) as compared with 1.4% in the control group; Shigella and Salmonella were 1.7% and 1.2% respectively, as opposed to 0% for the controls.

In Table 3, results of the single rectal-swab cultures from the infants with diarrhea are summarized by symptoms at the time of examination. Enteropathogenic E. coli and Salmonella were isolated from infants with diarrhea only, as well as from infants with diarrhea and other symptoms. On the other hand, Shigella infections were not encountered in infants with diarrhea alone. Shigellosis occurred in the more severely ill children in whom other symptoms accompanied the diarrhea. When the three genera of bacteria isolated were considered together, the highest rate of infection, 12%, was found in infants with diarrhea and dehydration. This group experienced significantly more enterobacterial infections (p. 0.002) than any of the others.

Infection rates for the three pathogens in both sexes were equal. Prevalence rates by age (Table 4) for the enteropathogenic E. coli varied little between age groups. Rates for Shigella were considerably lower and generally uniform; those for Salmonella were the lowest. The differences were not significant.

Escherichia coli. Nine of the 11 most common enteropathogenic types of E. coli associated with

TABLE 4

Enteropathogenic bacteria in infants with diarrhea, by age

Age (months)	No. of infants examined	No. of infants with pathogens	Entero-pathogenic E. coli (EEC)		Shigella		Salmonella	
			(No.)	(%)	(No.)	(%)	(No.)	(%)
< 6	681	51	35	5.1	10	1.5	6	0.9
6–11	656	54*	34	5.2	10	1.5	12	1.8
12–17	334	26†	17	5.1	9	2.7	2	0.6
18–24	148	13	10	6.8	2	1.4	1	0.7
Total	1,819	144	96	5.3	31	1.7	21	1.2

* Two infants had *EEC* and *Salmonella*.
† One infant had *EEC* and *Shigella*; another had *EEC* and *Salmonella*.

diarrhea in infants were isolated during the course of this investigation (Table 5). *E. coli* 055:B5 was most frequent (35.4%), with *E. coli* 0128: B12 (27.0%) and *E. coli* 0111:B4 (18.8%) next in order. These three serotypes accounted for 81.2% of the pathogenic *E. coli* recovered and

were also the only types encountered in every age group. *E. coli* 0112:B11 and 0124:B17 were not found.

Shigella. The distribution of the serotypes isolated is shown in Table 5. A total of 31 shigellae representing two species were recovered.

TABLE 5

Enteropathogenic bacteria isolated from infants with diarrhea, by serologic type and age

Organism		Age (months)				Strains isolated	
		< 6	6–11	12–17	18–24	(No.)	(%)
E. coli:	026:B6		2		1	3	3.1
	055:B5	16	11	5	2	34	35.4
	086:B7	1	1			2	2.1
	0111:B4	5	5	4	4	18	18.8
	0119:B14	3	1			4	4.2
	0125:B15	1	1			2	2.1
	0126:B16	2	3	1	6	6	6.3
	0127:B8			1		1	1.0
	0128:B12	7	10	6	3	26	27.0
Total		35	34	17	10	96	100.0
Sh. flexneri:	1a			1		1	3.2
	1b	6	8	2	1	17	54.7
	2a	1		2		3	9.7
	3a	1	1			2	6.5
	3c				1	1	3.2
	4a		1	1		2	6.5
	6	1		2		3	9.7
Sh. sonnei		1		1		2	6.5
Total		10	10	9	2	31	100.0
S. cubana			1			1	4.8
S. infantis			3			3	14.2
S. javiana		1				1	4.8
S. newport		1	1			2	9.5
S. oslo		1				1	4.8
S. panama		1	1	1		3	14.2
S. poona		1				1	4.8
S. rubislaw			1			1	4.8
S. saint paul		1	4	1	1	7	33.3
S. typhimurium			1			1	4.8
Total		6	12	2	1	21	100.0

Sh. flexneri 1b, the most frequent, accounted for 54.7% of *Shigella*. It was also the only serotype found in all age groups. *Sh. sonnei* was isolated only twice, but *Sh. dysenteriae* and *Sh. boydii* were not encountered.

Salmonella. As shown in Table 5, *S. saint paul*, one of the 10 serotypes identified, predominated, with *S. infantis* and *S. panama* next in frequency of occurrence. *S. saint paul* accounted for seven of the 21 salmonellae isolated, and was the only species appearing in all age groups.

Double infections. In four cases of diarrhea, three infants less than 1 year old were infected with both *E. coli* and *Salmonella* (*E. coli* 0111:B4 and *S. panama*; *E. coli* 0128:B12 and *S. panama*; and *E. coli* 0128:B12 and *S. saint paul*). In the fourth case, in a child 14 months old, *E. coli* 0111:B4 and *Sh. flexneri* 6 were found.

DISCUSSION

This study was limited to a search for bacterial pathogens. Other agents such as viruses, parasites, and certain noninfectious conditions such as food allergies, metabolic disorders, and emotional stress were not investigated.

Clinic procedures did not allow for methods of investigation that might be considered ideal, such as immediate plating of specimens on selective media, serial specimens from each patient, or use of stools rather than rectal swabs. Use of a single specimen in a transport medium could be expected to give a lower count than immediate, direct plating. However, all specimens were plated within 3 hours, and we believed that this did not materially reduce the yield of organisms.

On the basis of our data, 7.9% of the cases of diarrhea examined were associated with entero-bacterial pathogens. The predominance of entero-pathogenic *E. coli* over *Shigella* and *Salmonella* infections suggests that pathogenic *E. coli* plays a greater role in diarrhea in infants in this area than *Shigella* or *Salmonella*. The percentage of isolations for enteropathogenic *E. coli* was, respectively, three and four and one-half times greater than those for *Shigella* and *Salmonella* (Table 4). All isolations were made from sporadic cases of diarrhea in infants, pointing to the existence of a generalized reservoir of infection. No epidemic came to the attention of the clinic during this study.

Asymptomatic carriers of these organisms were

found in four of the 296 controls. At the time of the study, diarrhea was the single most common complaint in infants seen in the clinic. The 296 control infants, as opposed to 1,819 cases of diarrhea, represent the ratio of nondiarrheic to diarrheic infants seen during the clinic in this study.

Not unexpectedly, infants manifesting the more severe form of diarrhea yielded a higher percentage of bacterial pathogens than those with the mild type (Table 3). Also, shigellosis, unlike salmonellosis and pathogenic *E. coli* infections, was associated only with the more severe diarrheas.

The frequent occurrence of *E. coli* 055:B5 (one-third of all *E. coli* isolates) in these infants is similar to the findings in the United States and other parts of the world where it is the most frequently isolated serotype from sporadic and epidemic outbreaks of diarrhea in infants.

In comparable investigations elsewhere, such as Puerto Rico,[5] Thailand,[7] Egypt,[16] and Indonesia,[11] enteropathogenic *E. coli* was reported to be the most frequently found bacterial agent in diarrheic patients under 2 years of age. On the other hand, they were inconsequential among infants and children with acute diarrheal disease in Guatemala City[12] and in Costa Rica.[10]

The extremely low prevalence rates of *Shigella* and *Salmonella* found in this investigation are not in conflict with findings in other areas. Hardy and Watt,[13] Floyd *et al.*,[9] and Pierce *et al.*[17] have already established that incidence of infection with *Shigella* varies among different age groups of children and that this organism is not an important factor in diarrheal diseases of early infancy, but becomes conspicuous in those of early childhood. The importance of *Shigella* in the diarrhea of older children in Panamá remains to be determined.

Sh. flexneri 1b was the most common serologic strain recovered in this study. This differed from the findings in Guatemala[10] and Costa Rica[10] where other serotypes were more frequently isolated. We believe that differences in serotype distribution may be accounted for by the particular epidemiologic conditions prevailing and are reflections of the variation in interplays between infectious agent, host, and environment.

The infrequent occurrence of salmonellae in the infants studied is not uncommon. These

agents had no appreciable part in childhood diarrhea in Guatemala,[12] Puerto Rico,[8] and Thailand.[9] The importance of *Salmonella* in the endemic diarrheal disorders of older children in Panamá is now the subject of another investigation.

The low percentage of pathogenic bacteria recovered from cases of diarrhea in our study parallels findings elsewhere,[10, 15, 16] and poses the question of the actual participation of these agents in diarrheal disease. That they are recovered more frequently from diarrheic than from control cases suggests a certain significance to their role in this disease. Other factors should be considered in future studies. An account of preceding treatment—empirical, specific, or nonspecific—should be obtained in order to give adequate weight to the sample upon analysis. A minimum number of samples should be required before a case can be considered not caused by pathogenic bacteria. Finally, consideration should be given to devising media and techniques that would allow the isolation, if present, of spheroplasts or the L forms of *S. typhosa*[16] and other enteric bacteria. It would then be possible to study the significance of such forms in relation to diarrheal disease.

REFERENCES

1. Armijo, R., 1964. Epidemiología de las diarreas agudas de la infancia. *Bol. Ofic. San. Pan.*, 57: 444–453.

2. Olarte, J., Ramos-Alvarez, M., and Galindo, E., 1957. Aislamiento de *Shigella*, *Salmonella* y colis enteropatógenos de los hisopos rectales de 802 casos esporádicos de diarrea. *Bol. Med. Hospital Infantil* (Mexico), 14: 257–262.

3. Gordon, J. E., Pierce, Virginia, Ascoli, Werner, and Scrimshaw, N. S., 1962. Studies of diarrheal disease in Central America. II. Community prevalence of *Shigella* and *Salmonella* infections in childhood populations of Guatemala. *Am. J. Trop. Med. & Hyg.*, 11: 389–394.

4. Moore, H. A., De La Cruz, E., and Perez, F. I., 1964. Prevalencia de bacterias enteropatógenas en Costa Rica. *Bol. Ofic. San. Pan.*, 56: 564–573.

5. Panamá, Dirección de Estadística y Censo. *Estadísticas Vitales: 1961*. Contraloria General, Serie B; Año 21, p. 64.

6. Floyd, T. M., Higgins, A. R., and Kader, M. A., 1956. Studies in shigellosis. V. The relationship of age to the incidence of *Shigella* infections in Egyptian children, with special reference to shigellosis in the newborn and in infants in the first six months of life. *Am. J. Trop. Med. & Hyg.*, 5: 119–130.

7. Edwards, P. R., and Ewing, W. H., 1962. *Identification of Enterobacteriaceae*. Burgess Publishing Co., Minneapolis, Minnesota.

8. Guardiola-Rotger, A., Figueroa de González, E., Kauder, E., Muñoz, A., López, V. A., Gadea, D. E., and Funkenbush, M. J., 1964. Studies on diarrheal diseases. The multiplicity of infectious agents in the intestinal flora of Puerto Rican children with gastroenteritis. *J. Pediat.*, 65: 81–91.

9. Gaines, Sidney, Achavasmith, Yupin, Tharessawat, Malinee, and Duangmani, Chiraphun, 1964. Types and distribution of enteropathogenic *Escherichia coli* in Bangkok, Thailand. *Am. J. Hyg.*, 80: 388–394.

10. Brownlow, W. J., Saba, S., and Radcliffe, F., 1962. A study of infantile diarrhoea in Cairo, Egypt. *J. Trop. Med. & Hyg.*, 65: 117–119.

11. Joe, L. K., Sahab, K., Yauw, G. S., and Makaliwy, C., 1960. Diarrhea among infants and children in Djakarta, Indonesia, with special reference to pathogenic *Escherichia coli*. *Am. J. Trop. Med. & Hyg.*, 9: 626–632.

12. Pierce, Virginia, Ascoli, Werner, de León, Romeo, and Gordon, J. E., 1962. Studies of diarrheal disease in Central America. III. Specific etiology of endemic diarrhea and dysentery in Guatemalan children. *Am. J. Trop. Med. & Hyg.*, 11: 395–400.

13. Moore, H. A., de la Cruz, Enrique, and Vargas-Mendez, Oscar, 1966. Diarrheal disease studies in Costa Rica. II. The prevalence of certain enteric organisms and their relationship to diarrhea. *Am. J. Pub. Health*, 56: 442–451.

14. Hardy, A. V., and Watt, James, 1944. The acute diarrheal diseases. *J. Am. Med. Assoc.*, 124: 1173–1179.

15. Gordon, J. E., Béhar, Moisés, and Scrimshaw, N. S., 1964. Acute diarrhoeal disease in less developed countries. I. An epidemiological basis for control. *Bull. World Health Organ.*, 31: 1–7.

16. Kourany, Miguel, and Kendrick, P. L., 1966. Interaction between a human monocytic cell line and *Salmonella typhosa*. *J. Infec. Dis.*, 116: 495–513.