

Seroepidemiology of Heat-Labile Enterotoxigenic *Escherichia coli* and Norwalk Virus Infections in Panamanians, Canal Zone Residents, Apache Indians, and United States Peace Corps Volunteers

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Serum antibody titrations against the heat-labile enterotoxin (LT) of *Escherichia coli* were carried out on Panamanians, U.S. citizens resident in the Panama Canal Zone, Apache Indians living on the reservation in Whiteriver, Arizona, and Peace Corps volunteers before they traveled overseas. Antibody titers to Norwalk virus were also carried out on serum from Panamanian and Canal Zone residents. A high prevalence of low-titer LT antibodies was found in infants and adults from Panama, the Canal Zone, and Whiteriver. Panamanian children aged 1 to 5 years had the highest LT antibody titers. Peace Corps volunteers had a low prevalence and titer of LT antibodies. Prevalence and titer of antibodies to Norwalk virus were generally higher in Panamanians compared with Canal Zone residents of the same age. In the populations we studied, various modes of transmission and mechanisms of immunity likely explain the differences which we observed in antibody prevalence and titer to these two enteric pathogens.

Enteric disease pathogens, both viral and bacterial, have wide differences in infection rates throughout the world. Enterotoxigenic *Escherichia coli* infections, endemic in many less-developed countries, are a rare cause of diarrhea in the developed world (2, 5, 8). Norwalk virus, an important cause of diarrhea throughout the world, infects younger populations in less-developed countries (3, 6). The reasons for the variation in age and geographic distribution of these agents are unexplained. Enterotoxigenic *E. coli* infections in the normal host result from ingestion of a large inoculum of organisms often encountered only in highly contaminated reservoirs of food or water (4). Enterotoxigenic *E. coli*-induced diarrhea may thus occur more frequently in less-developed countries where sub-optimal food and water hygienic practices are more common than in developed countries. In contrast, Norwalk virus with its lower infectious dose appears to be easily transmitted via the person-to-person route (1). The lack of an absolute requirement for a contaminated vehicle (e.g., water or food) may further explain why Norwalk virus is an important cause of diarrhea in both developed and less-developed countries. Seeking to more precisely define the reasons

for the widely different pathogen-specific infection rates, we took advantage of a unique situation in the Republic of Panama—the homogeneous group of United States citizens who worked and resided in the former Canal Zone and the Panamanian nationals who also worked in the Canal Zone but who lived in the adjacent Republic of Panama. Our data suggest that residence in the Republic of Panama provides a different pattern of exposure to enterotoxigenic *E. coli* and to a less degree Norwalk virus.

Background. At the time this study was carried out, only United States citizens employed by the Panama Canal Co. could live in the Canal Zone. These people had lived in the Canal Zone for various periods of time, from less than 1 year to a lifetime (average duration of residence was 4 years). Living conditions in the Canal Zone were very adequate with good housing, safe drinking water, and optimal hygienic handling and storage of food which was largely imported from the United States. Day-to-day living and recreational activities of the 40,000 Canal Zone residents were carried out almost exclusively within the confines of the 12- by 60-mile band of the Pan-American isthmus making up the Canal Zone. Panamanian nationals could not live in the Canal

Zone but did comprise a major segment of the work force which maintained the Panama Canal. These individuals, living for the most part with their families in nearby urban Panama City, commuted freely into the Canal Zone for each work day. Because of their employment in the Canal Zone, most Panamanian employees lived in conditions somewhat better than those of the average Panamanian but considerably worse off than those of the average worker in most developed countries. By virtue of their Canal Zone employment, these Panamanian workers, their families, and all Canal Zone residents were entitled to receive low-cost, high-quality health care at medical clinics located in the Canal Zone. The diverse background of the patients seeking care at these clinics provided us with an excellent population in which we could determine levels of exposure and immunity to enterotoxigenic *E. coli* and Norwalk virus.

MATERIALS AND METHODS

Each week from November 1978 to January 1980 we selected the first two patients with diarrhea with onset in the previous 48 h who presented to the pediatric outpatient clinic located at Gorgas Hospital, the large inpatient facility in the Canal Zone. Informed consent was obtained from the responsible parent or guardian. Stool and venipuncture samples were obtained from each patient and from all of his or her household family contacts within 48 h of the time the child was brought to the clinic. The etiological agents that we detected, the degree of secondary spread, and the identification of risk factors associated with the acquisition of particular enteropathogens will be described in a separate communication. Because of the possibility that intrafamilial spread of enterotoxigenic *E. coli* or Norwalk virus might have occurred in the family contacts of

study children with enterotoxigenic *E. coli* or Norwalk virus-induced diarrhea, we excluded these children and their family contacts from the present investigation.

A neutralization test with *E. coli* heat-labile toxin (LT) and cholera antitoxin as previously described was used for determining LT antibody titers in the sera of all patients and their family contacts (10). All LT antibody titrations carried out in the two different participating laboratories were standardized each time against the same Swiss Serum Institute cholera antiserum, which had previously been arbitrarily assigned the value of 1,000 U of anti-*E. coli* LT per ml. This standardization assured us of the validity of comparing LT titers done at separate times in the two laboratories. Antibody titrations to Norwalk virus were carried out in one laboratory on serum from patients without Norwalk virus-induced diarrhea and selected family contacts. A radioimmunoassay was used to measure antibody titers against the Norwalk virus (7). All antibody titrations for LT and Norwalk virus were carried out in duplicate with appropriate positive and negative controls.

LT antibody titers in sera previously collected from asymptomatic individuals in earlier studies involving Apache Indians living in the only area in the continental United States where enterotoxigenic *E. coli* have been shown to be endemic (10) and from United States Peace Corps volunteers (9) before they traveled overseas were also included in the present study for comparison purposes.

RESULTS

Acute sera were collected from: 47 Panamanian patients with diarrhea and their 215 family contacts, all living outside the Canal Zone in the Republic of Panama; 38 Canal Zone patients (United States citizens) and their 88 family contacts, all living in the Canal Zone; 213 Apache

TABLE 1. Prevalence and geometric mean titer of antibodies to LT of *E. coli* in age groups in various populations.

Age group (yr)	Panama						United States					
	Panamanians			Canal Zone residents			Apache Indians			Peace Corps volunteers		
	No. tested	% with detectable antibody	GMT ^a	No. tested	% with detectable antibody	GMT ^a	No. tested	% with detectable antibody	GMT ^a	No. tested	% with detectable antibody	GMT ^a
>0.25 to <1	24	42 ^b	10.1	17	88	11.5	77	78	21.8 ^c	0		
1 to <5	55	98	74.1 ^{d,e}	28	86	24.2 ^d	65	91	37.0 ^d	0		
5 to <25	112	98	34.9	49	92	15.6	13	77	49.2	19	5	0.8
>25	71	97	19.1	32	84	16.3	58	90	23.1	17	24	2.5
Total	262	94	37.4	126	88	17.1	213	85	28.4	36	14	2.2

^a GMT, Geometric mean titer. Values are reciprocals and were calculated only for positive sera.

^b Antibody prevalence in children aged 3 months to 1 year was significantly ($P < 0.01$) lower in Panamanian children than in Canal Zone or Apache Indian children.

^c Antibody titers in children aged 3 months to 1 year were significantly higher ($P < 0.05$) in Apache children than in Panamanian or Canal Zone children.

^d Antibody titers in children aged 1 to 5 years from a particular population group were significantly higher ($P < 0.01$) than titers in any of the other age groups in that same population group.

^e Antibody titers in children aged 1 to 5 years were significantly higher ($P < 0.001$) in Panamanian children than in Canal Zone or Apache children.

Indians living on the White River reservation in rural Arizona; and 36 United States Peace Corps volunteers before they had begun their overseas assignment.

Enterotoxigenic *E. coli* LT antibodies. Panamanian children aged 3 months to 1 year had a significantly lower ($P < 0.05$) prevalence of antibodies to LT than either Canal Zone or Apache infants (Table 1). After 1 year of age, the majority of all subjects in these three ethnic groups had acquired antibodies to LT. Peace Corps volunteers consistently had a low prevalence of LT antibodies.

Apache infants had nearly twofold-greater LT antibody titers than did Panamanian or Canal Zone infants. Children aged 1 to 5 years in all three ethnic groups had the highest antibody titers of any age group. These high titers were most striking in Panamanian children. LT titers in Panamanian and Canal Zone subjects aged 5 to 25 years were approximately two times lower than the titers in subjects from the same respective ethnic group aged 1 to 5 years. These lower titers were not seen in Apaches aged 5 to 25 years. All three groups more than 25 years old had similar low titers. Peace Corps volunteers of all ages had far lower titers than those observed in any of the other groups.

We were unable to demonstrate in Canal Zone subjects a correlation between duration of residence in the Canal Zone and LT antibody titers. Familial clustering of high or low LT antibody titers did not occur in any population group.

Norwalk virus antibodies. The prevalence of antibodies to the Norwalk virus increased with age in Panamanians but not in Canal Zone residents (Table 2). Norwalk virus antibodies were present in nearly one-half of the Panamanian subjects 5 or more years old but in only one-quarter of similarly aged Canal Zone residents. Norwalk virus antibody titers were also consistently higher in Panamanians than in Canal Zone

residents. Duration of residence in the Canal Zone did not correlate with an increased likelihood of having antibodies to the Norwalk virus.

DISCUSSION

By the age of 5 years nearly all subjects, excluding Peace Corps volunteers, had acquired antibodies to *E. coli* LT. However, infection in Panamanian children appeared to occur later than in Canal Zone or Apache children: fewer than 50% of Panamanian children less than 1 year of age had detectable antibodies to LT. However, after age 5 years, LT antibody prevalence was the same in the three ethnic groups. The consistently higher LT titers in children 1 to 5 years of age compared with infants from the same ethnic group suggest that in the populations we studied, LT *E. coli* infection occurred most often in young children. In particular, Panamanian children aged 1 to 5 years appeared to be most frequently exposed to enterotoxigenic *E. coli* or other organisms which produced LT or an immunologically similar toxin. The precise source of infection with these organisms was not determined in this study. The lower LT antibody titers we measured in adults from all ethnic groups likely reflects a lack of recent exposure.

We have no explanation for the relatively lower prevalence of LT antibodies in Panamanian infants. The prevalence of breast feeding in infants is certainly much higher in Panama than in the Canal Zone. It is unclear whether this practice alone could account for the delayed rate of acquisition of infection with LT-producing *E. coli* in young Panamanian children. Between the ages of 1 and 5 years, Panamanian children experienced relatively more infection with enterotoxigenic *E. coli*; by age 5 years, the gap in prevalence rates for enterotoxigenic *E. coli* infection which had previously existed between

TABLE 2. Prevalence and geometric mean titer of antibodies to Norwalk virus in age groups of subjects residing in Panama

Age group (yr)	Panamanians			Canal Zone residents		
	No. tested	% with detectable antibody	GMT ^a	No. tested	% with detectable antibody	GMT ^a
>0.25 to <1	12	0	0	11	18	114
1 to <5	23	13	121	14	7	117
≥5	20	45	447 ^b	21	24	136
Total	55	21.8 ^c	222	46	17	125

^a GMT, Geometric mean titer. Values are reciprocals and were calculated only for positive sera.

^b Antibody titers in subjects aged 5 or more years were significantly higher ($P < 0.05$) in Panamanians than in Canal Zone residents.

^c The trend of increasing prevalence of antibody with increasing age was significant ($P < 0.05$) in Panamanians.

Panamanian infants and the other groups was no longer present. The time between age 1 and 5 years, particularly for the cohort of Panamanian children, would represent a time of increasing consumption of foods and liquids which are more likely to be contaminated than breast milk. Perhaps Canal Zone and Apache children, less likely to be breast fed, were therefore more likely to consume at an early age prepared foods and drinks which may have acted as vehicles for transmission. Panamanian children may have been relatively protected from these potentially contaminated sources as long as they were exclusively breast fed. This protection, however, rapidly waned after weaning.

The age dynamics of LT antibodies in Panamanians in our study are similar to the age changes of anti-cholera toxin antibodies seen in cholera endemic areas such as India and Bangladesh (1a). *Vibrio cholerae* and enterotoxigenic *E. coli* are immunologically similar, and it is likely that the epidemiology of the two diseases may also be similar. In cholera-endemic areas, adults have relative immunity against cholera despite relatively low anti-cholera toxin antibodies. An analogous situation may be present in adults in areas where LT *E. coli* infection is endemic.

The higher LT antibody titers in adult Canal Zone residents when compared with titers in Peace Corps volunteers implies that residence in the Canal Zone does increase the risk of acquiring enterotoxigenic *E. coli* when compared with residence in the continental United States. The source of this low-level exposure of Canal Zone residents was not determined in this study. Person-to-person transmission of these organisms between Canal Zone residents and Panamanians at a shared workplace is unlikely for reasons mentioned above. LT antibody titers in adult male Canal Zone residents were similar to those in adult female Canal Zone residents despite male Canal Zone residents having had much more frequent exposure at work to Panamanians. A plausible transmission source for Canal Zone residents would be the intermittent exposure to contaminated food and water sources located either in the Canal Zone, or more likely in the Republic of Panama.

Panamanians but not Canal Zone residents had an increase with increasing age in the likelihood of acquiring antibodies to Norwalk virus. These findings concur with those of Greenberg et al. (6) and Cuckor (3). We also confirmed these groups' earlier observations demonstrating that acquisition of Norwalk virus antibodies occurs at a younger age in subjects from less

developed areas of the world. Less-developed areas likely provide more conducive conditions for transmission.

As the development of vaccines against these enteric agents progresses, it will be important to better define the various modes of transmission and mechanisms of immunity which may be responsible for the contrasts in age-related serum immunity that we have demonstrated for LT *E. coli* and Norwalk virus.

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