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Epidemiological Analysis of Serologically Determined Rotavirus and Enterotoxigenic *Escherichia coli* Infections in Ecuadorian Children

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The statistical association of rotavirus- and enterotoxigenic *Escherichia coli*-specific serum antibody with demographic and hygienic factors was tested in Ecuadorian children enrolled in a cross-sectional survey. In 7- to 10-month-old children, enterotoxigenic *E. coli*-specific antibody was associated ($P < 0.05$) with poor drinking water quality, lack of a sewage system, and feeding of supplementary food. In 7- to 14-month-old children, rotavirus-specific antibody was associated only with family size but notably not with hygienic factors.

Acute infectious diarrhea is the most important health problem of children in developing areas. Reports from Latin America listed diarrhea as the primary cause of mortality in >20% of all recorded early childhood deaths (12). Prospective community-based investigations showed that enterotoxigenic *Escherichia coli* (ETEC) and rotavirus (RV) were the most common pathogens associated with childhood diarrhea in Latin America (6, 11). a

The transmission mechanisms of the major agents of childhood diarrhea are important considerations in designing water or sanitation projects in developing countries (5, 9, 14). However, in these countries the extent to which transmission of ETEC and RV occurs through waterborne infection, as opposed to other routes of infection, is unclear and controversial (1, 7).

Recently, we have analyzed the age-related prevalence of serum antibody to ETEC in 1,404 Ecuadorian children <6 years old enrolled in a cross-sectional survey (4). Each two-month age interval was represented by about 50 children. One serum sample was obtained from each child. The prevalence of immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) antibody to heat-labile enterotoxin LT and IgM ELISA antibody to pooled lipopolysaccharide antigen from the most common ETEC strains rose from about 12% in 5- to 6-month-old children to about 75% in 11- to 12-month-old children. For the seroepidemiological evaluation, we chose age ranges in which about 50% of the investigated children showed ETEC- or RV-specific antibody. For ETEC infection, 7- to 10-month-old children were chosen. They were classified as ETEC-infected ($n = 37$) or noninfected ($n = 38$) children on the basis of their antibody status. By using the cutoff criteria of our previous seroprevalence study (4), a child showing both antibody specificities was assumed to have been infected with ETEC at some time in the past. A child negative for both antibody specificities was assumed to be noninfected. Children positive for only one antibody specificity were excluded from the analysis. It should be noted that children infected with an ETEC strain elaborating only the heat-stable toxin would not be detected in our serological approach.

Previously, we also evaluated the age-related prevalence of serum antibody to RV in the same children (3). The prevalence of IgG ELISA antibody to RV and of neutralizing antibody rose from about 35% in 5- to 6-month-old children to about 70% in 15- to 16-month-old children. Equal proportions of RV-infected ($n = 77$) and noninfected ($n = 77$) children were observed in the 7- to 14-month-old age group. We classified 7- to 14-month-old children as RV-infected or noninfected on the basis of their antibody status. By using the cutoff criteria of our previous seroprevalence study (4), a child showing both IgG antibody to RV in an ELISA and RV-neutralizing antibody in a focus-reduction test was assumed to have been infected with RV some time in the past. A child negative for both antibody specificities was assumed to be noninfected. Children positive for only one antibody specificity were excluded from the analysis.

In addition to the serological data, a number of demographical, geographical, and hygienic data have been documented for these children (10). Data were collected by trained surveyors who visited the homes of all children for on-site inspection of the living conditions. The children were classified into two complementary subgroups on the basis of sex, population density (urban or rural; urban, ≥20,000 inhabitants per cantón), climate (low-altitude Costa region, ≤1,000 m above sea level; high-altitude Sierra region) and family size (big, ≥7 persons). In addition, children were classified into two subgroups on the basis of drinking water quality (low quality, river, canal, drain, and rain water; better quality, tap water, cistern, piped well water, distribution cart), sanitation system (organized, private or public water closet, bucket or pit latrine; not organized, indiscriminate defecation near the dwelling), refuse system (organized, public collector, burning, dumping; not organized, indiscriminate throwing away of solid waste), and sewage system (organized, public network, digging a hole; not organized, indiscriminate throwing away of liquid waste).

The number of ETEC-infected children divided by the total number of children in a given subgroup was calculated and compared to the ratio in the complementary subgroup. Statistical analysis was done by the Pearson chi-square test unless specified otherwise in the text. The proportion of ETEC-infected children (Table 1) was higher for females than for males and was higher for rural areas than for urban areas, but the difference was not statistically significant ($P = 0.13$). This proportion was higher in children from the low-altitude Costa region than in children from the high-altitude Sierra region of Ecuador, but the difference was not statistically significant. The proportion of ETEC-infected children was higher in children living with poor-quality

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TABLE 1. Epidemiological analysis of serologically identified infection with ETEC in 7- to 10-month-old Ecuadorian children

Epidemiological parameter	Total no. of children	No. of infected children (% of total)	P^a
Sex			
Male	37	15 (41)	0.20
Female	38	22 (58)	
Zone			
Urban	43	18 (42)	0.20
Rural	32	19 (59)	
Altitude			
>1,000 m	28	10 (36)	0.11
≤1,000 m	47	27 (57)	
Drinking water			
Low quality	23	16 (70)	0.04
Better quality	51	21 (41)	
Sanitation system			
Not organized	25	15 (60)	0.32
Organized	49	22 (45)	
Refuse system			
Not organized	34	18 (53)	0.81
Organized	40	19 (48)	
Sewage system			
Not organized	29	19 (65)	0.05
Organized	45	18 (40)	
Family size			
≤6 persons	44	25 (57)	0.15
≥7 persons	30	12 (40)	
Supplementary food intake			
<100 kcal/day	27	9 (33)	0.05[b]
≥100 kcal/day	48	28 (58)	

[a] Yates' corrected chi-square test.
[b] Fisher exact test, two-tailed.

TABLE 2. Epidemiological analysis of serologically identified infection with RV in 7- to 14-month-old Ecuadorian children

Epidemiological parameter	Total no. of children	No. of infected children (% of total)	P^a
Sex			
Male	72	39 (54)	0.41
Female	82	38 (46)	
Zone			
Urban	81	42 (52)	0.74
Rural	73	35 (48)	
Altitude			
>1,000 m	53	30 (57)	0.30
<1,000 m	101	47 (47)	
Drinking water			
Low quality	57	26 (46)	0.54
Better quality	96	50 (52)	
Sanitation system			
Not organized	62	29 (47)	0.66
Organized	91	47 (52)	
Refuse system			
Not organized	65	27 (42)	0.11
Organized	88	49 (56)	
Sewage system			
Not organized	73	35 (48)	0.80
Organized	80	41 (51)	
Family size			
≤6 persons	85	36 (42)	0.04
≥7 persons	68	40 (59)	
Supplementary food intake			
<100 kcal/day	41	22 (54)	0.68
>100 kcal/day	112	54 (48)	

[a] Yates' corrected chi-square test.

drinking water and without a sanitation or sewage system than in children living under better hygienic conditions; the difference was statistically significant for drinking water quality and presence of sewage system (Table 1). Family size was not associated with the proportion of ETEC-infected children. ETEC infections were more frequently observed in children who received more than 100 kcal of supplementary food per day than in children receiving less than 100 kcal/day of supplementary food in addition to breast-feeding (1 cal = 4.184 J) ($P = 0.05$, Table 1). Note that the subgroup of children living with low-quality drinking water showed a higher proportion of children fed less than 100 kcal of supplementary food per day than the subgroup of children living with better-quality drinking water (45 versus 29%, respectively).

Our seroepidemiological analysis points consistently to a waterborne transmission of ETEC infection in Ecuadorian children. This concurs with the few reports in which ETEC transmission has been studied (2, 7, 8, 13). With the only exception being family size, none of the demographic or hygienic conditions investigated showed a statistically significant association with the proportion of RV-infected children at the 5% significance level (Table 2). This proportion was higher in children living in big families than in children from relatively small families ($P = 0.04$; Table 2). Notably, there was not even a trend towards higher proportions of RV-infected children in children living under poor hygienic conditions. Large waterborne outbreaks of RV have been reported, but they involved adults and non-group A RV and thus might not be typical of group A RV infections in young children (1). The effect of family size on RV infection status implicates person-to-person spread of RV. Water and sanitation improvements are usually very expensive and beyond the economic ability of most developing countries (14, 15). Therefore, a greater understanding of the transmission mechanisms of the different enteric pathogens is required before optimal and cost-efficient measures against diarrheal diseases can be designed.

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