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Changes in Serum Immunoglobulin G Levels as a Marker for *Cryptosporidium* sp. Infection in Peruvian Children

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In a retrospective analysis, we assessed the usefulness of two serologic enzyme-linked immunosorbent assays as epidemiologic tools for the detection of cryptosporidiosis episodes in children from a Peruvian community. The incidence rate determined by the serologic assay was higher than the rate determined by stool microscopy (0.77 versus 0.41 infection/child-year of surveillance).

Most studies designed to characterize the epidemiology of *Cryptosporidium* sp. infection have relied on stool microscopy to identify infection episodes. However, because microscopy is relatively insensitive when small numbers of oocysts are excreted (acid-fast stool microscopy is 83.7% sensitive and 98.9% specific relative to PCR) and because the period of oocyst shedding can be short, many infections may escape microscopic detection (2, 5, 9). Earlier work demonstrated that serum immunoglobulin G antibody responses to two immunodominant sporozoite surface antigens develop upon infection and can be detected for some time after resolution (half-life of 12 weeks) (4, 6, 7, 8). To assess the usefulness of these antibody assays as epidemiologic tools, we assayed longitudinally collected serum samples from children who participated in a birth cohort study of diarrheal disease in Lima, Peru, between 1995 and 1998 (1, 3, 10).

Data were assessed using multiple linear and Poisson regression, pooled *t* tests, chi-square test, and Wilcoxon test. When applicable, the generalized estimating equation procedure was used to adjust for correlation among multiple responses from the same child. Analyses were performed using SAS version 8.0, Sudaan version 8.0.2, or SigmaStat version 2.03.0 (SPSS, Inc.). Statistical significance was set at 0.05.

As previously described, stool specimens were collected from study participants at weekly intervals (more frequently when diarrhea occurred or when enteric protozoa were detected) and examined by microscopy for *Giardia* sp., *Cryptosporidium* sp., and *Cyclospora* sp. (1). The current study included all cohort children with more than one microscopy-confirmed cryptosporidiosis episode who donated multiple serum samples ($n = 28$); all those with one cryptosporidiosis episode who donated ≥7 sera ($n = 29$); and 17 randomly selected children with no microscopic evidence of *Cryptosporidium* infection who donated ≥7 sera. Using the infection episode definition of Bern et al. (1), the selected children had 92 *Cryptosporidium* infections detected by microscopy during 224.3 child-years of stool surveillance (0.41 infection/child-year of follow-up). They were similar to the excluded cohort participants ($n = 158$) in terms of mean age at enrollment (16 days versus 15 days, respectively; $P = 0.67$), sex (54% male versus 58% male; $P = 0.55$), and incidence of diarrhea (6.2 episodes/year of follow-up versus 7.3 episodes/year of follow-up; $P = 0.22$) but had more days of follow-up (mean of 1,091 days versus mean of 667 days; $P < 0.0001$) and a higher proportion of stools that were positive for *Cryptosporidium* (0.019 versus 0.010; $P = 0.0005$), *Giardia* (0.187 versus 0.127; $P = 0.0035$), and *Cyclospora* (0.021 versus 0.010; $P = 0.0272$). Because of the shared fecal-oral route of transmission, the overselection of children with *Cryptosporidium* infection may have captured a greater proportion of children who were also infected with *Cyclospora* and/or *Giardia*.

A total of 638 serum samples (median, 8 samples/child; range, 3 to 14 samples/child) were assayed for antibodies to *Cryptosporidium* as previously described (7). The median interval between serum collection dates was 115 days (mean, 115 days; range, 17 to 849 days). A serologically determined episode of *Cryptosporidium* infection was identified when the following conditions were met: (i) the interval between two consecutive serum samples was ≤180 days; (ii) antibody levels in the second serum sample were >160 and >57 arbitrary units for the 27-kDa and 17-kDa antigens, respectively; (iii) antibody levels for both antigens were elevated in the second sample relative to the first; and (iv) at least one of the antibody levels increased ≥50% during the interval. The 160 and 57 arbitrary

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TABLE 1. Stool microscopy assay and ELISA results for 74 children

Serology result	Microscopy result	n^a	Mean antibody level[b]/median (range)	
			27-kDa	17-kDa
Positive	Positive	48	1,339[c]/1,396 (232–6,400)	533[d]/386 (59–6,400)
Positive	Negative	60	727[c]/759 (163–5,211)	286[d]/270 (59–4,668)
Negative	Positive	25	221[e]/178 (63–2,579)	60[f]/54 (0–1,314)
Negative	Negative	376	162[e]/143 (0–5,618)	38[f]/43 (0–3,619)

[a] Number of serologic intervals in each category. Five consecutive serologic positive episodes (four microscopy positive episodes and one microscopy negative episode) were not included.
[b] Geometric means were calculated by exponentiating the log mean of the antibody values. One was added to each antibody value in order to derive logs of zero values.
[c] Significant difference ($P = 0.0025$).
[d] Significant difference ($P = 0.0031$).
[e] Difference not significant ($P = 0.2058$).
[f] Difference not significant ($P = 0.5837$).

unit cutoff values were based upon the mean plus 3 standard deviations of the values of a group of Western-blot-negative controls (4). Consecutive serologically determined episodes were considered separate events if the interval between them was >90 days. A total of 514 intervals, representing 139.9 child-years of surveillance, satisfied part i of our definition. The antibody assays identified 108 separate cryptosporidiosis episodes in 56 of the 72 study children (0.77 infection/child-year of serologic surveillance). If only the randomly selected, stool-negative children were considered, 6 of 17 (35%) had evidence of infection by a serologic assay (10 infections, 29.1 child-years of serologic surveillance; 0.34 infection/child-year of serologic surveillance).

A serologic antibody episode was considered to be associated with *Cryptosporidium* oocyst shedding if the second serum sample of the interval was collected within a window of 7 days before the first oocyst detection to 90 days after the last oocyst detection. We excluded a serologic cryptosporidiosis episode from analysis if both samples in the interval were collected >14 days after the last oocyst detection. Of 92 microscopy-confirmed cryptosporidiosis episodes, 19 (21%) did not have sera collected in the appropriate time window for analysis (Table 1). Of 73 episodes with serum coverage, 48 (66%) were associated with a serologic response (Table 1). The mean duration of oocyst excretion was significantly shorter for the 25 seronegative episodes than for the 48 episodes with an antibody response (mean of 4.8 days and median of 1 day versus mean of 9.7 days and median of 8.5 days, respectively; $P = 0.0107$). In addition, the interval between oocyst detection and serum collection was significantly longer for episodes that lacked a response than for those with a response (mean of 38 days and median of 32 days versus mean of 27 days and median of 14.5 days, respectively; $P = 0.036$).

Sixty of the 108 total serologic responses (56%) were not associated with oocyst excretion (Table 1). Antibody levels to the 27- and 17-kDa antigens were lower in these 60 serologic episodes than in the 48 episodes associated with oocyst excretion ($P = 0.0025$ and $P = 0.0031$, respectively). This difference may reflect the fact that the time interval between infection and serum collection is unknown for the 60 serology-only episodes, but by our definition, it is less than 90 days for the 48 stool-confirmed episodes with serologic responses. Alternatively, the magnitude of the antibody response could be related to the infection intensity, and oocyst-negative infections may have been less intense.

Although neither acid-fast stool microscopy nor the serum enzyme-linked immunosorbent assays (ELISAs) captured all of the infections, the ELISAs do reveal that cryptosporidiosis is much more common in the children of the Peruvian study community than was previously appreciated. With properly spaced serum samples, the ELISA should prove to be a valuable tool for epidemiologic study, especially in communities where cryptosporidiosis is endemic and asymptomatic infection is common.

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