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Detection of *Giardia lamblia* Antigen in Children Living in a Peruvian Periurban Shantytown (Pueblo Joven)

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Stool microscopy and an enzyme-linked immunosorbent assay (ELISA) for *Giardia lamblia* antigen detection were compared for detecting *G. lamblia* in 30 Peruvian infants. Of 1,131 fecal specimens, *G. lamblia* was detected by ELISA alone in 44, by microscopy alone in 17, and by both methods in 91. In another group of 17 children negative for *G. lamblia* by stool microscopy, 6 had *G. lamblia* detected by ELISA or duodenal aspiration: 2 only by ELISA, 1 only by duodenal aspirate examination, and 3 by both examinations. The ELISA is useful for the detection of *G. lamblia* in fecal specimens but compared to stool microscopy does not significantly increase the detection of cases.

Giardia lamblia is one of the most common intestinal parasites that infect children in both developed and developing countries (2, 5, 7).

Difficulties are encountered in the detection of *G. lamblia* because of its erratic excretion pattern (3). If many *G. lamblia* infections of children were not detected by stool examination, then prior conclusions concerning diarrhea and growth of the children would be suspect.

There were two objectives of the current study. The first was to determine whether the antigen detection enzyme-linked immunosorbent assay (ELISA) would detect *G. lamblia* infection earlier and more frequently than stool microscopy in children in a single community monitored longitudinally. Both the ELISA and stool microscopy were used to determine the age in months when *G. lamblia* infection was first acquired and the variation in the pattern of parasite excretion in children living in a Peruvian community in which *G. lamblia* is highly endemic. The second objective was to compare results of the antigen detection ELISA with those obtained by examination of duodenal fluid of hospitalized children negative by stool microscopy.

Cohort study. The study subjects came from Canto Grande, a periurban shantytown in Lima, Peru, established approximately 15 years ago (6).

Stools were collected weekly for 2.5 years as part of a cohort study (4, 6) to define the epidemiology of *G. lamblia*. Seventy-two children (the present study group) had stools collected weekly for 1 year after birth. The stools were examined with a light microscope and were then stored frozen for a maximum of 1 year.

To determine whether the ELISA would detect cryptic infections with *G. lamblia* not demonstrated by stool microscopic examination, we retrospectively selected from these 72 children the following two groups of infants for this study: (i) 15 children who had at least one stool sample positive by microscopy for *G. lamblia* during the first year of their lives and (ii) 15 children negative for *G. lamblia* by microscopic examination throughout the same 1-year period. For these 30 children born between December 1985 and July 1986, we examined 1,131 stool samples (mean ± standard deviation, 38 ± 6 stools per infant) by both ELISA and microscopy.

Within hours after collection, stools were examined by direct microscopy. An additional portion of each stool specimen was preserved in Merthiolate-iodine-Formalin solution and processed by both the Formalin-ether sedimentation and zinc sulfate flotation techniques (1). A further portion of each stool was preserved in Merthiolate-iodine-Formalin, and a final portion was frozen at −70°C in 0.15 M phosphate-buffered saline (pH 7.4).

Comparison of duodenal aspirate and stool examination. Seventeen children hospitalized at the Instituto de Investigación Nutricional for either malnutrition (11 children) or as part of a study of chronic diarrhea (6 children, 4 with diarrhea at the time of the study) did not have *G. lamblia* detected in at least two stool microscopic examinations. To determine whether these children had cryptic infections with *G. lamblia*, each child had a duodenal aspiration performed. Also, the initial and additional stool specimens (all negative by microscopy) obtained within a week of the aspiration were examined by ELISA.

All duodenal aspirates were centrifuged, and the pellets were examined microscopically for the presence of trophozoites. All individuals had at least two stools (mean ± standard deviation, 2.3 ± 1.6) examined by ELISA and by stool microscopy of both direct and Formalin-ether-concentrated specimens.

G. lamblia antigen was detected by a double antibody ELISA previously described by Ungar et al. (11).

For the cohort study, an episode of *G. lamblia* infection was defined as starting when the first *G. lamblia*-positive specimen was obtained and continued until three negative examinations occurred within a 4-week period (6). The numbers of episodes of *G. lamblia* detected by ELISA alone, by stool microscopy alone, and by both methods were tabulated. The duration of episodes that continued beyond the last study day was calculated from the beginning of the episode to the last study day. The median durations were compared by the Kendall test. Duodenal aspiration results were compared by Fisher's test with SPSS software (Microsoft Corp., Redmond, Wash.).

G. lamblia was detected either by microscopy or by

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TABLE 1. Detection of *G. lamblia* antigen by microscopy and ELISA from 1,131 stool samples from 30 Peruvian children monitored during the first 12 months of life

ELISA result[a]	No. of results by stool microscopy examination	
	Positive	Negative
Positive	91	44
Negative	17	979

[a] For the ELISA, the sensitivity was 84%, the specificity was 96%, the predictive value of a positive test was 67%, and the predictive value of a negative test was 98%.

ELISA in 152 of 1,131 (13%) stool samples (Table 1). In 91 of 152 (60%) cases, both assays were positive; in 44 (29%), *G. lamblia* was detected only by ELISA; and in 17 (11%), *G. lamblia* was detected only by microscopy. There were no significant differences ($P < 0.2$) between the numbers of episodes of *G. lamblia* infection detected by the two methods.

Of 17 stool samples positive by microscopy and negative by ELISA, 2 had both cysts and trophozoites, 8 had only cysts, and 7 had only trophozoites.

Two of the 15 children classified in the *G. lamblia*-negative group by microscopy were positive by ELISA, both during the last trimester of the first year of observation. There were 33 episodes of *G. lamblia* demonstrated in 17 children. Fifteen episodes were detected by both methods. Ten were detected only by ELISA, compared with eight episodes detected only by microscopic examination.

G. lamblia was excreted by the 17 *G. lamblia*-positive infants (as determined by ELISA, microscopy, or both methods) in two distinct patterns, one continuous and the other intermittent. In 11 children there were 11 episodes in which *G. lamblia* was excreted weekly for 1 or more months. In 3 of these 11 children *G. lamblia* was excreted in nearly every stool examined for periods up to 9 months. Intermittent short episodes of *G. lamblia* excretion occurred with six children (22 episodes). Recurrent infection was frequent.

The age at which *G. lamblia* was first detected (mean ± standard deviation, 8 ± 4 months) and the median duration (4 weeks) of fecal excretion of *G. lamblia* per episode did not vary with respect to the assay that was used to detect the parasite.

Six of 17 hospitalized children (35%) who were negative by stool microscopy had *G. lamblia* detected either by ELISA or in duodenal aspirates. The sensitivities of the two assays were similar. Of the six positive children, two (33%) were found to be positive only by ELISA, one (17%) was found positive by examination of the duodenal aspirate alone, and three (50%) were found positive by both assays.

Nearly 50% of infants living in the periurban shantytowns of developing countries are infected one or more times with *G. lamblia* during their first year of life, yet the effect of this parasite on a child's health is not clear (5, 8).

Children in this study excreted *G. lamblia* in a highly variable manner. Similar variability in the pattern of *G. lamblia* excretion is observed in adults (9). Also, subjects who are infected with *G. lamblia* may have stool examinations that remain consistently negative for long periods (3, 9).

Although the ELISA is somewhat more sensitive than a single microscopic stool examination, when multiple sequential stool examinations are performed, there is no difference in the number of episodes of *G. lamblia* infection found by ELISA and by microscopy. Certainly, for children negative for *G. lamblia* by stool microscopy, the ELISA does not detect a high percentage of cryptic *G. lamblia* infections.

The ELISA, when used for North American patients, had a sensitivity of 92% (11), compared with 84% in our study. Our lower sensitivity may be due to the long period for which specimens were stored frozen. In this study and another study (11), nearly all *G. lamblia* aspirate-positive patients were detected by ELISA.

The sensitivity of the ELISA for the detection of *G. lamblia* in stools might be increased by using antibody to the stable cyst wall antigen rather than antibody to the surface membranes of the trophozoite, which frequently change their antigenic structure (10).

The antigen detection ELISA will serve as a useful and reliable technique for the detection of *G. lamblia* in therapeutic and epidemiological studies.

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