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Age-Related Rate of Seropositivity of Antibody to *Giardia lamblia* in Four Diverse Populations

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We used a solid-phase enzyme immunoassay to determine the age-specific rates of acquisition of antibody to *Giardia lamblia* in populations living in an inner city area of Baltimore, Md., on an Apache Indian reservation in Arizona, in a rural area of Panama, and in an urban area of Peru (Lima). Antibody to *G. lamblia* was found in a portion of the adults living in all of the study areas. Similar prevalence rates and quantitative levels of antibody were found in the adults living in Arizona (44%), Panama (48%), and Peru (46%). However, a significantly lower ($P < 0.05$) percentage of the adults living in Baltimore (18%) displayed serological evidence of infection. Different patterns of age-associated acquisition of antibody were noted in the study populations. In the United States, children living in Baltimore had low levels of seropositivity throughout childhood, whereas children living on the Arizona Indian reservation showed a progressive acquisition of antibody early in childhood, with adult levels achieved by 8 years of age. In Latin America, children living in Panama attained adult levels of seropositivity between 9 and 20 years of age, whereas children in Peru displayed adult levels of seropositivity in the first 6 months of life. Our findings documented the widespread occurrence of *G. lamblia* infections in diverse populations. Children living in different areas and under different environmental conditions displayed widely differing rates of acquisition of antibody to *G. lamblia*, possibly resulting from different levels of sanitation, water contamination, and person-to-person contact. Our studies indicate that quantitative solid-phase immunoassays can be used to study the epidemiology of parasitic infections such as those caused by *G. lamblia*.

Giardia lamblia is a major cause of intestinal infection throughout the world (1, 17). In both developing countries and the United States, endemic giardiasis is an important cause of morbidity in children and adults (3, 6, 10). However, little is known about the epidemiology of *G. lamblia* infection in different populations. Of particular interest is the determination of the relative rates of giardia infections in infants, children, and adults living in different areas of the world. We thus used an enzyme-linked immunosorbent assay (ELISA) previously developed in our laboratory for the measurement of antibody to *G. lamblia* and applied it to the investigation of the age-specific rates of acquisition of systemic antibody to *G. lamblia* in individuals living in different geographic areas.

MATERIALS AND METHODS

ELISA. A single-antibody noncompetitive ELISA was used to measure immunoglobulin G (IgG) antibody. The performance characteristics and accuracy of the assay were described previously (7, 8). Reaction volumes of 100 μl were used at each step of the assay. In brief, trophozoite preparations were grown in modified TYI-S-33 medium (3a) and, diluted in phosphate-buffered saline (PBS) to a concentration of 2×10^4 organisms per ml, were bound to the wells of U-bottom microtiter plates (Immulon I; Dynatech Laboratories, Inc., Alexandria, Va.) and incubated for 24 h at 4°C. Alternate wells were coated with control fluid preparation from mock-infected cultures in the same fashion as the antigen. After incubation, the plates were washed five times in PBS-Tween 20, and 1% bovine serum albumin diluted in PBS-Tween 20 was added to the wells and incubated for 1 h at 37°C. After another washing, test sera were diluted 1:40 in PBS–Tween 20–1% bovine serum albumin, added to the coated wells, and incubated for 1 h at 37°C. This dilution was chosen because it yielded the highest difference between a positive and a negative control serum in a titration curve. Positive and negative control reagents consisting of sera with known levels of antibody to *G. lamblia*, as determined by immunofluorescence, were included in each test run. After another washing, the reaction was completed by the addition of peroxidase-labeled rabbit anti-human IgG (Copper Biomedical, Inc., West Chester, Pa.) diluted 1:1,000 in PBS–Tween 20–1% bovine serum albumin and incubated for 1 h at 37°C, followed by the addition of a hydrogen peroxide-*o*-phenylenediamine substrate prepared as previously described (17). The color reaction was measured by a microplate colorimeter (Dynatech MR 580) at a wavelength of 450 nm.

For each serum specimen, the specific activity was calculated from duplicate readings by subtracting the mean optical density for the wells coated with control antigen from the mean optical density for the wells coated with giardia antigen. A sample was considered positive if it yielded a specific activity that was at least two standard deviations greater than the mean specific activity of negative control sera.

Serum specimens. Serum samples were obtained from two populations in the United States (Baltimore, Md., and Arizona) and two populations in developing countries (Panama

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TABLE 1. Prevalence and levels of serum antibody to *G. lamblia* in adults

Geographic area	No. of specimens tested	No. (%) of specimens with antibody	ELISA activity[a] [mean (95% confidence interval)]
Baltimore, Md.[b]	41	7 (18)	167 (146–188)
Arizona	62	27 (44)	367 (329–405)
Panama	33	16 (48)	386 (328–446)
Peru	26	12 (46)	414 (375–453)

[a] Expressed as optical density at 450 nm.

[b] The percentage with antibody and the mean activity of the Baltimore population are significantly different ($P < 0.01$ and $P < 0.001$, respectively) from those of the other three populations.

and Peru). The sera from Baltimore were randomly selected from specimens submitted to the central laboratory of The Johns Hopkins Hospital for routine blood chemistry determinations. The sera from Arizona were prospectively collected as part of an ongoing diarrheal surveillance study in an American Indian population. However, no correlation between *G. lamblia* in stools and serum antibodies to *G. lamblia* was attempted in this study. This population lives on the Fort Apache Indian Reservation in Central Arizona and is characterized by a low level of out-migration. Most of the population lives in modern housing located in a moderate-population-density housing area. The sera from Panama were obtained from rural inhabitants of low socioeconomic status, as previously described (11). The sera from Peru were obtained from children who were admitted to the Instituto de Investigacion Nutricional (Lima) for malnutrition and from adults referred to the Canto Grande Health Center. These populations consisted primarily of individuals living in urban settings in Lima and belonging to a low socioeconomic group.

RESULTS

The prevalence rates of IgG antibody to *G. lamblia* in adult populations (>20 years old) in the different areas are presented in Table 1. Serum antibody to *G. lamblia* was found in adults living in each of the areas studied. The populations of rural areas of Panama and the United States and of urban Peru showed similar prevalence rates, 48, 44, and 46%, respectively. The overall prevalence in the urban population of Baltimore, Md. (18%), was significantly lower ($P < 0.01$ by chi-square analysis) than in any other population that was investigated. The specific activities (optical density units) of IgG antibody to *G. lamblia* were also significantly lower ($P < 0.001$ by Student's t test) in Baltimore than in the other three areas.

The age-specific rates of acquisition of antibody to *G. lamblia* displayed distinct patterns in each of the study populations (Fig. 1). Children living in Baltimore, Md., had low rates of seropositivity throughout childhood. However, children living on the Indian reservation in Arizona had progressively increasing rates of seropositivity until they reached adult rates between 3 and 8 years of age. A slower rate of acquisition of antibody to *G. lamblia* was evident in children living in rural areas in Panama, with adult levels of seropositivity reached between 9 and 20 years of age. The highest antibody prevalence throughout infancy and childhood was displayed by the population of Peruvian children. In this population, the acquisition of adult rates was observed as early as the first 6 months of life.

DISCUSSION

Despite the recognition that *G. lamblia* can cause severe diarrheal disease and malabsorption (2, 4, 16), extensive studies have not been performed to establish the age-related pattern of *G. lamblia* infection in various parts of the world. Such lack of data may in part be attributed to the inadequacy of traditional parasitologic methods, such as stool examination, for accurate population studies of *G. lamblia* infection, because intermittent excretion of *G. lamblia* cysts can occur during disease and asymptomatic infection (5, 15). The immunologic assessment of the extent of prior infection with the parasite in the community has been hindered by the lack of an efficient and objective immunoassay capable of accurately quantitating antibodies in large numbers of samples. Although various methods, such as immunodiffusion (13) and immunofluorescence (14), have been used for the immunologic investigation of giardiasis, such techniques are not easily applicable to large-scale population studies, because of difficulties in standardization and objective quantitation. On the other hand, we recently developed a quantitative enzyme immunoassay for the detection of *G. lamblia* antibody and applied the assay to the quantitation of serum IgG and breast-milk-secretory IgA anti-*G. lamblia* antibody in different populations of lactating women (7). This assay displayed a sensitivity for the measurement of giardia antibody higher than that of analogous immunofluorescence systems. Moreover, the specificity of the assay for giardia was confirmed by a blocking assay using monoclonal antibody directed at giardia antigen.

In the present study we used this assay to determine the age-specific rates of acquisition of *G. lamblia* antibody in four different populations. These studies revealed a high prevalence of antibody to *G. lamblia* in adults living on an Apache Indian reservation in Arizona (44%), in rural areas of Panama (48%), and in urban areas of Lima, Peru (46%). The antibody levels found in these populations of adults living in widely different geographic areas were not significantly different. Such findings may be attributed to a consistently high level of exposure to *G. lamblia* in the populations. On the other hand, significantly lower prevalence rates and levels of antibody were found in adults living in Baltimore, Md. The relative rarity of serum antibody to *G. lamblia* in adults living in Baltimore might be explained by a low overall incidence of infection and low person-to-person transmission. Only 80 of 8,752 (0.9%) stool samples submitted to The Johns Hopkins Hospital parasitology laboratory in the last 5 years have been found to contain giardia organisms (T. Spahr, personal communication). The low prevalence of *G. lamblia* antibody in an urban American population is consistent with the low prevalence of antibody that we documented in lactating women living in Houston, Tex. (24%), compared with age-matched lactating women living in Mexico City, Mexico (77%) (7). Previous studies using immunofluorescence (6) and an ELISA (12) also showed prevalence rates of antibody to *G. lamblia* in adults living in an American city (Washington, D.C.) (14%) very different from those in adults living in an urban area of a less developed country (Dacca, Bangladesh) (45%).

The study populations revealed great variation in the age-specific rates of acquisition of giardia antibody. The population of children living in Baltimore, Md., displayed a low degree of prevalence at all ages, a finding which is consistent with the low degree of prevalence found in adults in that population. On the other hand, high levels of giardia antibody were found in all age groups in the urban Peru

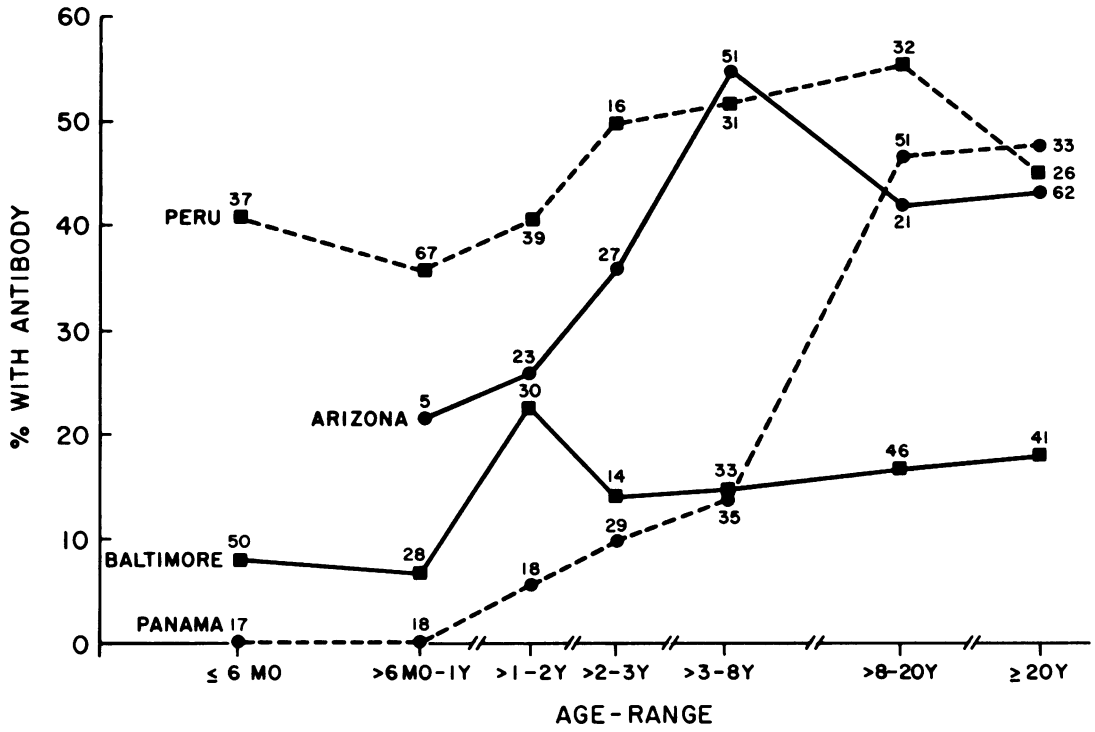

FIG. 1. Prevalence of antibody to *G. lamblia*. The *y* axis indicates the percentage of individuals with measurable levels of IgG antibody to *G. lamblia* in the indicated age periods. The numbers along the points are the numbers of serum specimens tested in each age period.

population, with adult degrees of antibody prevalence apparent as early as 6 months of age. This finding indicates a high prevalence of giardia infection at all ages in this population. Unpublished data from ongoing studies of R. H. Gilman (obtained as part of the Collaborative Research Program on Acute Diarrheal Disease and Nutrition, Johns Hopkins University and Universidad Peruana Cayetano Heredia) have documented prevalence rates of 27 to 62% in routine stool samples obtained from Peruvian infants and young children. Unlike in the Peru population, low degrees of giardia antibody prevalence were noted in infants from the Panama and Arizona study populations. However, a high rate of antibody acquisition and prevalence rates similar to those of the adults were noted after 8 years of age in the Panama population and after 3 years of age in the Arizona population. These findings suggest that infections resulting in the development of serum antibody tend to occur earlier in children living in Peru than in those of the other populations. Although the reasons for the differing epidemiology of giardia infection cannot be determined with certainty by these methods, it might be related to differing levels of sanitation and person-to-person contact or to differing breast feeding practices and nutritional status (5, 9, 10). In particular, widely different sanitation levels in the populations examined are likely to have a direct impact on the spread of giardia infection. In the case of nutrition, it is of note that the Peru population was drawn from children being treated for malnutrition, suggesting that there is an association between a high prevalence of giardia infection and environmental or biological factors related to malnutrition. The possible role of these and other factors should be investigated by environmental and epidemiological studies. This study indicated that quantitative serological assays can be used to detect different rates of exposure to *G. lamblia* in diverse populations. The elucidation of the epidemiology of *G. lamblia* infection in diverse populations might allow for the development of effective strategies for the prevention of giardia infections in highly susceptible individuals.

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