# Clinical Investigation

# Serum Antibodies to *Giardia lamblia* by Age in Populations in Colorado and Thailand

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*We measured levels of antibodies to* Giardia lamblia *by age in serum specimens from persons in Denver, Colorado, and Soongnern, Thailand. Serum levels of immunoglobulin (Ig) G, IgM, and IgA* G lamblia*-specific antibodies measured by enzyme-linked immunosorbent assay increased substantially during childhood in both geographic areas, although children in Soongnern showed significantly higher mean levels of each antibody class (*P<.05*). After adolescence, levels of* G lamblia*-specific IgM fell steadily with age in both populations. In contrast, specific IgA levels remained elevated throughout life among the Thai but decreased to low levels among adults in Denver. Similarly, rates of carriage of* G lamblia *were high among children aged 1 to 4 years in Denver and Soongnern (14.3% versus 26.5%, respectively) but were much lower among adults in Denver (0% versus 14%;* P<.01*). These data suggest that levels of* G lamblia*-specific IgM may reflect exposure to the parasite early in life in both areas. Levels of parasite-specific IgA may reflect recurrent exposure to* G lamblia *in Soongnern, where* G lamblia *is endemic, but less frequent exposure to the parasite in Denver, where exposure is often episodic.*

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In developed countries, symptomatic *Giardia lamblia* infections occur most often among persons following episodic exposure to the parasite, such as during waterborne outbreaks,[1-3] while backpacking,[4] visiting Colorado mountain towns,[2,3,5] or traveling.[6] Chronic asymptomatic infections with *G lamblia* also occur among persons with recurrent exposure, such as toddlers in day-care centers,[7,8] long-term residents of Colorado mountain towns,[2] and homosexual men.[9,10] In developing countries, recurrent exposure and endemic infections are most common and are often associated with asymptomatic excretion of *G lamblia.*[11-15] The reasons for the differences in the clinical features of *G lamblia* infections in developed and developing countries are not well understood. One proposed explanation is that recurrent exposure to the parasite leads to protective immunity.[2,3,16] The production of antibody to *G lamblia* may be critical to the development of immunity to the parasite since persons with hypogammaglobulinemia show high rates of symptomatic infection with the organism.[17,18] Therefore, because specific antibodies may be important in protective immunity and may represent markers for infection, we compared levels of immunoglobulin (Ig) G, IgM, and IgA antibodies to *G lamblia* by age in persons in a developing area, Soongnern, Thailand, and in a developed area, Denver, Colorado.

## Subjects and Methods

### *Subjects Studied*

In the Soongnern district, Nakhon Ratchasima, Thailand, serum specimens were collected in March 1980 from 12 infants born at Soongnern Hospital (cord blood); 135 healthy children in well-baby clinics, schools, and health centers; and 60 healthy adults. In Denver, serum specimens were obtained in 1984 and 1985 from the following urban residents: 13 infants (cord blood), 128 healthy children enrolled in several vaccination trials, and 69 healthy adults. Stool specimens and historical information other than age and sex and place of residence were not available for subjects whose serum was tested. All specimens were preserved at −20°C. Informed consent was obtained from each subject or their representatives in Thailand and the United States.

### *Serologic Methods*

Serum was tested for IgG, IgM, and IgA antibodies to *G lamblia* by the enzyme-linked immunosorbent assay as previously described.[19,20] We used solubilized, axenically grown, *G lamblia* WB strain trophozoites (ATTC No. 30957) as the solid-phase antigen (1 μg per well), diluted the serum 1:100, and applied goat antihuman IgG, IgM, and IgA horseradish peroxidase-conjugated antibodies (Tago, Inc, Burlin-

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game, California) to detect *G lamblia*-specific antibodies. Specimen volumes of 100 μl were tested in triplicate. Each plate was divided equally with serum from age-matched persons from Denver and Soongnern. Results were standardized by adjusting values from each plate to the mean of the sum of the optical densities for seven control serum specimens from all plates. The specificity of the assay was established in preliminary competitive inhibition studies in which the adsorption of specimens of both US and Thai serum for 45 minutes at 37°C on a rotator with $6 \times 10^6$ formalin-fixed *G lamblia* WB strain trophozoites resulted in a 50% to 80% decrease in optical density in assays of parasite-specific IgG, IgM, and IgA. In contrast, adsorption with *Cryptosporidium* oocysts and freshly excysted sporozoites ($5 \times 10^8$; generously provided by Michael Arrowood and Charles Sterling, University of Arizona), *Trichomonas vaginalis* ($6 \times 10^6$), *Campylobacter jejuni* ($10^9$), enterotoxigenic *Escherichia coli* ($10^9$), and *Candida albicans* ($10^9$) resulted in less than a 12% decrease in optical density.

*Parasitology*

In Thailand, we determined the prevalence of *G lamblia* infection among 537 healthy Soongnern villagers from stool specimens collected during random visits to their homes. In the United States, stool specimens and questionnaires were obtained from 44 healthy adults in Denver and from 315 children between 12 and 36 months of age, 236 of whom were randomly selected from day-care centers.[21] Stool specimens were fixed in polyvinyl alcohol and in a 10% formalin solution and examined microscopically for intestinal parasites, both directly and after formalin-ether (or ethyl acetate) concentration. In Denver, saline and iodine wet mounts of fresh stool specimens also were examined. Stool specimens from Thai villagers were evaluated by John Cross, PhD, Uniformed Services University of Health Sciences, and specimens in Denver were examined by the Colorado Department of Health, Denver Veterans Administration Medical Center, University of Colorado Medical Center, and Denver General Hospital. Rates of *G lamblia* infection among symptomatic persons in Denver submitting stool specimens for parasitologic evaluation were calculated by reviewing laboratory notebooks for results between 1981 and 1984 at the institutions listed. Stool and serum specimens were collected in the same geographic areas but not from the same subjects in Denver and Soongnern.

*Statistics*

Mean antibody levels were compared by analysis of variance. Paul F. Smith provided statistical support.

## Results

*Serology*

A pronounced rise in *G lamblia*-specific IgM antibody levels was found during the second through the fifth years of life in both the Denver and Soongnern populations ($P < .001$), but the levels were substantially higher in residents of Soongnern (Figure 1-A). The differences in *G lamblia*-specific IgM levels in residents of Soongnern compared with those in Denver were most pronounced in persons younger than 20 years ($P < .005$), although levels decreased in both populations during the adult years.

Similar to the results for IgM, both populations showed a significant increase in *G lamblia*-specific IgA after the first year of life ($P < .01$ in both groups; Figure 1-B). Although levels subsequently decreased in Denver residents, IgA antibody levels remained elevated in persons in Soongnern throughout adulthood. This persistent elevation in IgA stands in marked contrast to the decline in specific IgM levels in adults in Soongnern, suggesting that *G lamblia*-specific IgA antibodies may reflect recurrent exposure to the parasite. Compared with the Denver residents, Soongnern residents showed a significantly higher level of *G lamblia*-specific IgA

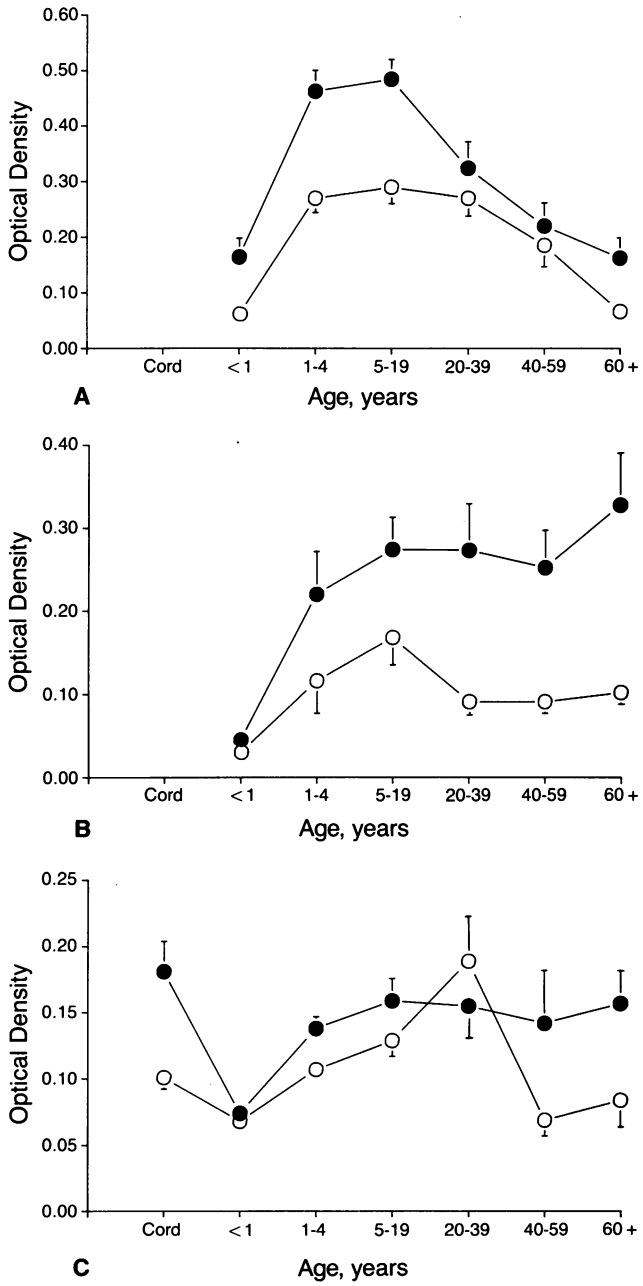


**Figure 1.**—Age-specific serum antibody levels to *Giardia lamblia* are shown for 210 persons in the United States (○) and 207 Thai villagers (●). Points represent the mean (± standard error) optical density values by enzyme-linked immunosorbent assay for **(A)** immunoglobulin (Ig) M, **(B)** IgA, and **(C)** IgG. The number of serum specimens tested in each age group for the US/Thai populations is as follows: cord blood, 13/12; younger than 1 year, 41/41; 1 to 4 years, 60/60; 5 to 19 years, 35/34; 20 to 39 years, 27/28; 40 to 59 years, 21/20; and 60 years or older, 13/12.

TABLE 1.—*Identification of* Giardia lamblia *in Stool Specimens in Denver, Colorado, and Soongnern, Thailand, 1981 Through 1985*

| Location | Age, years | Sample Source | Number Studied | Number With G lamblia (%) |
|---|---|---|---|---|
| Denver | All ages | Clinical specimens* | 10,803 | 799 ( 7.4) |
| Soongnern | All ages | Randomly selected villagers | 537 | 113 (21.0) |
| Denver | 1-4 | Randomly selected toddlers† | 315 | 45 (14.3) |
| Soongnern | 1-4 | Randomly selected villagers | 102 | 27 (26.5)‡ |
| Denver | 20-39 | Healthy adults | 44 | 0 ( 0.0) |
| Soongnern | 20-39 | Randomly selected villagers | 136 | 19 (14.0)‡ |

*Specimens were submitted to the Colorado Department of Health, excluding refugee screening, and to the Clinical Microbiology Laboratories of the University of Colorado, Denver General Hospital, and the Veterans Administration Medical Center, Denver, 1981 to 1984.

†Randomly selected toddlers came from day-care centers (n=236) and non-day-care centers (n=79)—16% and 9% with *G lamblia*, respectively—Denver, 1985 (Novotny et al[23]).

‡Denver population compared with population in Soongnern, $P<.01$.

at all ages ($P<.05$), but the differences were most striking among adults (Figure 1-B).

The levels of *G lamblia*-specific IgG in cord blood specimens from Soongnern, reflecting maternal antibody levels, were significantly higher than from Denver (mean optical density, $0.180 \pm 0.02$ versus $0.10 \pm 0.01$; $P<.004$; Figure 1-C). Similar to those of IgM, specific IgG levels were significantly higher in the 1- to 4-year-old group compared with children younger than 1 year in both Denver and Soongnern ($P<.001$). Similar to those of IgA, levels of specific IgG in subjects in Soongnern remained elevated throughout life. Specific IgG antibody levels were highest in persons aged 20 to 39 years in the Denver population and then fell significantly in later life.

Although all data are presented as mean optical density levels, analysis of these data according to median optical density levels or percent of serum specimens above a threshold value produced similar results. No clear bimodal distribution of values was noted in any group. Using a threshold value as a cutoff, more than 80% of Thai adults (≥20 years of age) were seropositive for *G lamblia*-specific IgA compared with less than 20% of adults in Denver.

*Parasitology*

*Giardia lamblia* organisms were identified more commonly in randomly selected Thai villagers than among symptomatic Denver residents (Table 1). Among healthy persons in both Soongnern and Denver, *G lamblia* was identified more often in the young children than in adults. Age-related differences in rates of carriage were most striking between adults in the two areas (Table 1).

## Discussion

The age-specific levels of antibodies to *G lamblia* in Denver and in Soongnern, Thailand, show similarities in the younger age groups in all antibody classes but notable differences later in life, particularly in specific IgA levels. The early increase in parasite-specific IgM levels in both Denver and Soongnern populations may parallel the intensity of exposure to *G lamblia* early in life. The IgM antibody class is most commonly associated with acute exposure to pathogens, including *G lamblia*.[21] In Denver, the rate of *G lamblia* infection per population is highest in children aged 1 to 4 years,[22] and many urban toddlers excrete *G lamblia* (Table 1).[23] Similarly, in Soongnern, children are more often infected than adults.

The age-related pattern of *G lamblia*-specific IgG and IgA levels is similar in Thais. In contrast to those of parasite-specific IgG and IgM, levels of parasite-specific IgA more clearly distinguish between populations in Soongnern and Denver. Asymptomatic carriage of *G lamblia* is common among adults in developing countries,[14-16,24,25] including in Soongnern where 14% of otherwise healthy adults surveyed excreted the parasite. Rates of excretion of *G lamblia* among healthy adults in the United States are generally much lower (Table 1).[26] During an outbreak of waterborne giardiasis, levels of parasite-specific IgA correlated best with exposure to the parasite,[27] and specific IgA may also best reflect exposure to the parasite in these populations. The parasite-specific IgA detected in serum may be produced systemically or it may originate in part from local intestinal antibody production.[28]

The specific IgG peak in the serum from adults aged 20 to 39 years in Denver corresponds to the peak number of cases of *G lamblia* reported by age in Colorado; 40% of reported cases of *G lamblia* occur in this age group.[22] In the Soongnern population, the IgG pattern by age is comparable to that described in Panama,[29] which suggests a similar pattern of exposure. The degree of protection for infants against *G lamblia* infection from parasite-specific IgG in cord blood is not known. In previous studies, these specimens were tested for antibodies to rotavirus, the heat-labile enterotoxin of *E coli*, Norwalk virus, hepatitis A virus,[30] and *C jejuni*.[31] The results from Soongnern are similar to the patterns for *C jejuni*-specific IgM and IgA in the same Thai population,[31] particularly with the progressive rise in levels of IgA with age, which may reflect ongoing exposure. Similarly, levels of antibody to rotavirus, an infection that causes illness primarily in young children but not in adults, increase early in life in Soongnern residents and remain elevated throughout life.[30]

That *G lamblia* infections in Thai adults are most often asymptomatic whereas in urban US adults, infection often leads to illness may be explained in several ways. Geographic differences may exist in the pathogenicity of *G lamblia* strains.[32,33] High rates of asymptomatic excretion among US day-care-center toddlers[7,8] and homosexual men[9,10] in association with recurrent exposure, however, argue against this hypothesis. Moreover, travelers to developing countries may become ill with *G lamblia* strains acquired in those countries.[6] Alternatively, local intestinal conditions that are conducive to the growth of *G lamblia*[34,35] or to the expression of

virulence factors, similar to those proposed for *Entamoeba histolytica*,[36] may differ in different populations in the two countries. Finally, immunity to the virulence factors causing *G lamblia*-associated illness may develop in persons with recurrent exposure or the strains may mutate on exposure to a specific antibody.[37] In this regard, symptoms were reported in 12 of 14 (86%) Bangladeshi infants newly infected with *G lamblia* but in only 1 of 27 (4%) infected mothers.[25] Moreover, most children in Thailand and Peru with *G lamblia* infection are asymptomatic.[11-13] Antibody to *G lamblia* may be a marker of immunity as parasite-specific levels are elevated in healthy Thai adults and in healthy homosexual men.[19] Antibody to *G lamblia* may be required for immunity as evidenced by the high rates of illness among *G lamblia*-infected persons with hypogammaglobulinemia[17,18] and by the protective effects of *G lamblia*-specific IgA in breast milk.[38]

Levels of parasite-specific IgM may provide a useful tool for determining rates of acute exposure to the organism[19,21,33] in populations. Levels of specific IgA may correlate more closely with recurrent exposure. Continued exposure to the organism is likely required to maintain specific antibody in serum as levels decline steadily over six months following an initial infection (E.N.J., unpublished data, 1986). The ability of antibodies to *G lamblia* to provide protection against *G lamblia*-associated illness by inhibiting adherence,[39] modifying surface antigens,[37] or killing the organism[40,41] remains a stimulating area for investigation.

## REFERENCES


1. Craun GF: Waterborne giardiasis in the United States: A review. Am J Public Health 1979; 69:817-819
2. Istre GR, Dunlop TS, Gaspard B, et al: Waterborne giardiasis at a mountain resort: Evidence for acquired immunity. Am J Public Health 1984; 74:602-604
3. Moore GT, Cross WM, McGuire D, et al: Epidemic giardiasis at a ski resort. N Engl J Med 1969; 281:402-407
4. Barbour AG, Nichols CR, Fukushima T: An outbreak of giardiasis in a group of campers. Am J Trop Med Hyg 1976; 25:384-389
5. Wright RA, Spencer HC, Brodsky RE, et al: Giardiasis in Colorado: An epidemiologic study. Am J Epidemiol 1977; 105:330-336
6. Steffen R, Rickenbach M, Wilhelm V, et al: Health problems after travel to developing countries. J Infect Dis 1977; 156:84-91
7. Black RE, Dykes AC, Sinclair SP, et al: Giardiasis in day-care centers: Evidence of person-to-person transmission. Pediatrics 1977; 60:486-491
8. Pickering LK, Woodward WE, DuPont HL, et al: Occurrence of *Giardia lamblia* in children in day-care centers. J Pediatr 1984; 104:522-526
9. Kean BH, William DC, Luminais SK: Epidemic of amoebiasis and giardiasis in a biased population. Br J Vener Dis 1979; 55:375-378
10. William DC, Shookhoff WB, Feldman M, et al: High rates of enteric protozoal infections in selected homosexual men attending a venereal disease clinic. Sex Transm Dis 1978; 5:155-157
11. Nacapunchai D, Tepmonkol M, Tharavanij S, et al: A comparative study of four methods for detecting antibody in asymptomatic giardiasis. Southeast Asian J Trop Med Public Health 1986; 17:96-100
12. Areekul S, Viravan C: Prevalence of *Giardia lamblia* and its effect on hematologic profile in asymptomatic school children. Southeast Asian J Trop Med Public Health 1986; 17:96-100
13. Gilman RH, Miranda E, Marquis GS, et al: Rapid reinfection by *Giardia lamblia* after treatment in a hyperendemic third world community. Lancet 1988; 1:343-345
14. Gilman RH, Brown KH, Visvesvara GS, et al: Epidemiology and serology of *Giardia lamblia* in a developing country: Bangladesh. Trans R Soc Trop Med Hyg 1985; 79:469-473
15. Zaki AM, DuPont HL, Elalamy MA, et al: The detection of enteropathogens in acute diarrhea in a family cohort population in rural Egypt. Am J Trop Med Hyg 1986; 35:1013-1022
16. Knight R: Epidemiology and transmission of giardiasis. Trans R Soc Trop Med Hyg 1980; 74:433-436
17. Ament ME, Ochs HD, Davis DD: Structure and function of the gastrointestinal tract in primary immunodeficiency syndromes: A study of 39 patients. Medicine (Baltimore) 1973; 52:227-248
18. Hermans PE, Diaz-Buxo JA, Stobo JD: Idiopathic late-onset immunoglobulin deficiency—Clinical observations in 50 patients. Am J Med 1976; 61:221-237
19. Janoff EN, Smith PD, Blaser MJ: Acute antibody responses to *Giardia lamblia* are depressed in patients with the acquired immunodeficiency syndrome. J Infect Dis 1988; 157:798-804
20. Smith PD, Gillin FD, Brown WR, et al: IgG antibody to *Giardia lamblia* detected by enzyme-linked immunosorbent assay. Gastroenterology 1981; 80:1476-1480
21. Goka AKJ, Rolston DDK, Mathan VI, et al: Diagnosis of giardiasis by specific IgM antibody enzyme-linked immunosorbent assay. Lancet 1986; 2:184-186
22. Hopkins RS, Olmstead RN: *Campylobacter jejuni* infection in Colorado: Unexplained excess of cases in males. Public Health Rep 1985; 100:333-336
23. Novotny T, Hopkins R, Shillam P, et al: Prevalence of *Giardia lamblia* and *Cryptosporidium* and risk factors for infection among children attending day care facilities in Denver. Public Health Rep 1990; 105:72-75
24. Hossain MM, Ljungstrom I, Glass RI, et al: Amoebiasis and giardiasis in Bangladesh: Parasitological and serological studies. Trans R Soc Trop Med Hyg 1983; 77:552-554
25. Islam A, Stoll BJ, Ljungstrom I, et al: *Giardia lamblia* infections in a cohort of Bangladeshi mothers and infants followed for one year. J Pediatr 1983; 103:996-1000
26. Healy GR: The presence and absence of *Giardia lamblia* in studies on parasite prevalence in the USA, *In* Jakubowski W, Hoff JC (Eds): Waterborne Transmission of Giardiasis. Cincinnati, Ohio, US Environmental Protection Agency, 1979, pp 92-103
27. Birkhead G, Janoff EN, Vogt RL, et al: Elevated levels of immunoglobulin A to *Giardia lamblia* during a waterborne outbreak of gastroenteritis. J Clin Microbiol 1989; 27:1707-1710
28. Conley ME, Delacroix DL: Intravascular and mucosal immunoglobulin A: Two separate but related systems of immune defense? Ann Intern Med 1987; 106:892-899
29. Miotti PG, Gilman RN, Santosham M, et al: Age-related rate of seropositivity of antibody to *Giardia lamblia* in four diverse populations. J Clin Microbiol 1986; 24:972-975
30. Echeverria P, Burke DS, Blacklow NR, et al: Age-specific prevalence of antibody to rotavirus, *Escherichia coli* heat-labile enterotoxin, Norwalk virus, and hepatitis A virus in a rural community in Thailand. J Clin Microbiol 1983; 17:923-925
31. Blaser MJ, Taylor DN, Echeverria P: Immune response to *Campylobacter jejuni* in a rural community in Thailand. J Infect Dis 1986; 153:249-254
32. Aggarwal A, Nash TE: Comparison of two antigenically distinct *Giardia lamblia* isolates in gerbils. Am J Trop Med Hyg 1987; 36:325-332
33. Nash TE, Herrington DA, Losonsky GA, et al: Experimental human infections with *Giardia lamblia*. J Infect Dis 1987; 156:974-984
34. Farthing MJG, Keusch GT, Carey MC: Effects of bile and bile salts on growth and membrane lipid uptake by *Giardia lamblia*—Possible implications for pathogenesis of intestinal disease. J Clin Invest 1985; 76:1727-1732
35. Gault MJ, Gillin FD, Zenian AJ: *Giardia lamblia* stimulation of growth by human intestinal mucus and epithelial cells in serum-free medium. Exp Parasitol 1987; 64:29-37
36. Mirelman D: Ameba-bacterium relationship in amebiasis. Microbiol Rev 1987; 51:272-284
37. Adam RD, Aggarwal A, Lal AA, et al: Antigenic variation of a cysteine-rich protein in *Giardia lamblia*. J Exp Med 1988; 167:109-118
38. Nayak N, Ganguly NK, Walia B, et al: Specific secretory IgA in the milk of *Giardia lamblia*-infected and uninfected women. J Infect Dis 1987; 155:724-727
39. Lev B, Ward H, Keusch GT, et al: Lectin activation in *Giardia lamblia* by host protease—A novel host-parasite interaction. Science 1986; 232:71-73
40. Hill DR, Burge JJ, Pearson RD: Susceptibility of *Giardia lamblia* trophozoites to the lethal effect of human serum. J Immunol 1984; 132:2046-2052
41. Deguchi M, Gillin FD, Gigli I: Mechanism of killing of *Giardia lamblia* trophozoites by complement. J Clin Invest 1987; 79:1296-1302