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Elevated Levels of Immunoglobulin A to *Giardia lamblia* during a Waterborne Outbreak of Gastroenteritis

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During an outbreak of diarrheal illness among residents of a trailer park in rural Vermont, 37 (30%) of 122 residents met the case definition of outbreak-related giardiasis. Convalescent-phase sera from 24 residents and 20 nonresident control subjects were tested by enzyme-linked immunosorbent assay for immunoglobulin G (IgG), IgM, and IgA antibodies to *Giardia lamblia*. Residents showed higher levels of parasite-specific antibody than did nonresident controls for IgG and IgA but not IgM. Nine residents with giardiasis had a higher median level of *G. lamblia*-specific IgA but not IgG or IgM than 15 healthy residents (0.61 versus 0.16 optical density units; $P = 0.004$). Moreover, parasite-specific IgA levels were higher in those consuming tap water than in those who did not (0.31 versus 0.08 optical density units; $P = 0.03$) and increased with increasing water consumption. Levels of serum antibody to *G. lamblia*, particularly IgA, may be useful in determining exposure to *G. lamblia*-contaminated water and illness from *G. lamblia* during waterborne outbreaks of diarrheal illness.

Waterborne outbreaks of gastrointestinal illness are important public health problems in the United States and are frequently caused by *Giardia lamblia* (3, 5). Diagnosis of giardiasis by microscopic examination of stool specimens may be limited by the sensitivity of the test (18) and by infection having cleared in persons investigated late in the course of outbreaks. Serologic tests specific for *G. lamblia* may supplement stool examination in epidemiologic investigations since most people with *G. lamblia* infection develop serum antibodies to the parasite (8, 11, 15, 17). We report the first use of levels of *G. lamblia*-specific IgA antibodies in sera of people in an outbreak of waterborne giardiasis.

MATERIALS AND METHODS

Epidemiology. The Epidemiology Division, Vermont Department of Health, Burlington, investigated an outbreak of diarrheal illness in 1986 in a residential trailer park consisting of 44 trailers and 122 residents located in rural Vermont. The investigation began after three park residents with laboratory-confirmed *G. lamblia* infection were reported within 2 days to the state health department; only one case of giardiasis had been reported from the same town during the previous 3 years.

All park residents were interviewed in detail between 28 and 30 May 1986 regarding food and water consumption and gastrointestinal symptoms and again between 16 and 26 June to ensure that all ill persons had been identified. For respondents with gastrointestinal symptoms, exposure to park tap water was quantitated by the number of glasses of unboiled tap water usually consumed per day during the week before the onset of symptoms. For those without symptoms, it was estimated as usual daily water consumption.

A case of outbreak-related giardiasis was defined as a trailer park resident who had *G. lamblia* cysts identified in stool or who reported three or more loose watery stools per day lasting 5 or more days between 1 April and 28 June 1986. Five days of diarrhea was required to meet the case definition to exclude persons with sporadic illness caused by common bacterial or viral pathogens, which is usually of shorter duration (2).

Blood specimens were requested from all adult residents during both interviews. Since the investigation was conducted as the outbreak was waning, serologic analysis was limited to the convalescent-phase blood samples drawn 3 to 6 weeks after the onset of symptoms; the sera tested for noncase residents were those obtained at the second interview. Control blood specimens were obtained from 20 healthy, asymptomatic state health department employees who had no history of acute or chronic diarrhea, giardiasis, or recent foreign travel and who lived in and around Burlington but were not residents of the park.

Stool specimens collected in 10% Formalin were examined for *G. lamblia* cysts by the Formalin-ether concentration method (14). Stool specimens were not examined for cryptosporidia. The residue obtained by pumping 150 gallons (ca. 568 liters) of untreated park water through a wound Orlon filter (minimum pore size, 1 μm) was kindly examined for *G. lamblia* cysts by Judith F. Sauch at the U.S. Environmental Protection Agency Laboratory, Cincinnati, Ohio, using sequential immunofluorescence–phase-contrast microscopy (13). Park water also was tested for coliform bacteria by standard methods (1).

Serology. Serum samples were assayed blindly for immunoglobulin G (IgG), IgM, and IgA to *G. lamblia* by enzyme-linked immunosorbent assay as previously described (6, 13). Briefly, 1 μg of soluble protein from axenically grown *G. lamblia* trophozoites (WB strain, ATTC 30957) passed three

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times through a French pressure cell (American Instrument, Urbana, Ill.) at 15,000 lb/in^2 was added to each well of flat-bottomed microtiter plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.) in 100 μl of 0.1 M carbonate buffer (pH 9.6). After overnight incubation at 4°C, the wells were washed with 0.01 M phosphate-buffered saline with 0.05% Tween 20 and 0.01% thimerosal (PBS-TT), and the plates were incubated overnight with 200 μl of PBS-TT with 0.1% gelatin (PBS-TTG) to limit nonspecific reactivity. Each test serum was diluted 1:100 in PBS-TTG with 5 mg of bovine gamma globulin, and 100 μl was added to each of three wells and incubated for 1 h at 37°C. Horseradish peroxidase-conjugated goat antibody to human IgG, IgM, and IgA (Tago, Burlingame, Calif.), diluted in PBS-TT with 1% bovine serum albumin and 0.1% bovine gamma globulin, was added for 1 h at 37°C. Following a final wash, the optical density (OD) produced at 405 nm using 2,2′-azino-bis(3-ethylbenzthiazolinesulfonic acid) (Sigma Chemical Co., St. Louis, Mo.) as the developer was read on a microELISA plate reader (MR600; Dynatech).

Control wells included diluent only, three known negative serum samples, and four known positive serum samples. The values for each sample were standardized by adjusting the values from each plate to the mean of the sum for the seven control serum samples for all plates tested. Variation between replicate wells was consistently within 10%. The IgG, IgM, and IgA antibodies detected were specific for *G. lamblia* as determined previously (7, 8) by adsorption of immune sera with *G. lamblia* trophozoites, *Trichomonas vaginalis*, and *Cryptosporidium* oocysts and freshly excysted sporozoites provided by Charles Sterling and Michael Arrowood; and *Campylobacter jejuni*, enterotoxigenic *Escherichia coli*, and *Candida albicans*. The results are presented in OD units since previous assays showed that OD declined linearly with the serum dilution between 1:100 and 1:1,600.

RESULTS

Epidemiology. All 122 park residents completed questionnaires. One or more stool specimens were obtained from 71 (58%) residents; two or more were obtained from 18 (25%) of these. Thirty-seven (30%) residents had a case of outbreak-related giardiasis: 23 residents had *G. lamblia* cysts detected in stool, and an additional 14 residents had diarrhea lasting ≥5 days but either their stools were not tested or cysts were not identified. The peak number of cases occurred 2 weeks before the initial interviews and extended over a period of 2 months (Fig. 1). Case residents resided in 23 (52%) of the 44 trailers, and there was no apparent clustering of cases in any portion of the park. The attack rates for males and females were similar. Eight (21%) of 38 residents aged <20 years and 29 (35%) of 84 residents aged ≥20 years met the case definition.

Consumption of park water increased the risk of having giardiasis: the attack rate was 33% (37/113) among residents who drank ≥1 glasses per day of tap water compared with 0% (0/9) among residents who reported no daily water consumption (relative risk = infinity; $P = 0.06$, Fisher exact test, two-tailed). The attack rate increased with increasing water consumption (Table 1). Neither food nor other exposure was incriminated.

Drinking water pumped to the park from a stream was chlorinated, although contact times were estimated to be only a few minutes during periods of peak water use. Two *G. lamblia* cysts were identified in the residue of filtered water. Untreated park water had ≥20,000 coliforms per ml. A sanitary survey revealed a beaver dam and numerous homes with septic field sewage systems within 5 km of the park. A recent release of a large volume of water into the stream following destruction of a beaver dam was suspected to have led to the outbreak.

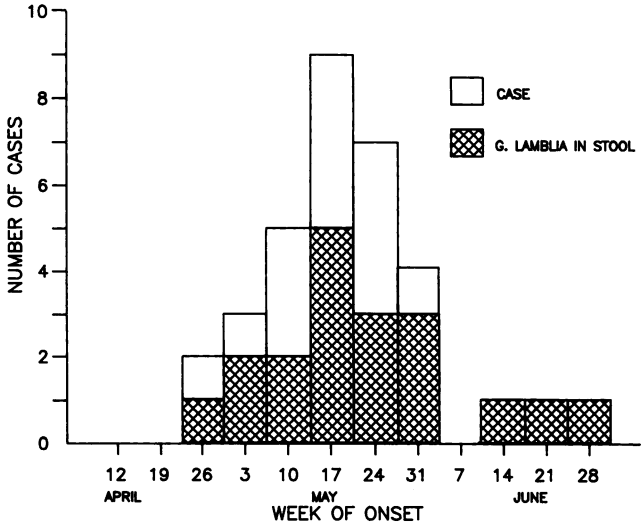

FIG. 1. Cases of giardiasis by week of symptom onset for the 33 symptomatic cases, Vermont, 1986.

Blood specimens were obtained from 24 (29%) of 84 adult residents in 21 (48%) of 44 trailers. The median age of the 24 residents (33.5 years; range, 20 to 57 years) and their sex distribution (9 [38%] male) were similar to those of the control subjects (median age, 35.5 years; range, 28 to 55 years; 11 [55%] male).

Serology. The results of the *G. lamblia*-specific antibody measurements are shown in Fig. 2. Median OD levels of both IgG and IgA *G. lamblia*-specific antibodies were significantly higher among park residents than among the nonresident control subjects (Fig. 2 and Table 1; $P < 0.01$ by Wilcoxon rank sum test for both IgG and IgA). The median specific IgM levels were similar in the two groups. Among the park residents, in contrast, specific IgA antibody levels but not IgG or IgM levels were significantly higher in those with giardiasis than in noncase subjects: among the 24 residents tested, the 9 meeting the case definition (5 with *G. lamblia* cysts in stool, 4 with diarrhea for ≥5 days) had a median specific IgA level significantly greater than that of the 15 noncase residents (0.61 versus 0.16 OD; $P = 0.004$). The median IgG and IgM levels were similar between case and noncase residents (IgG, 0.29 versus 0.25 OD; IgM, 0.22 versus 0.33 OD). Using a more specific case definition based only on stool examination yielded similar results (median IgA OD, 0.61 for stool positive versus 0.33 for stool negative). Median IgG and IgM levels were similar in the two groups.

Specific IgA antibody levels were also substantially greater in residents who consumed tap water than in those who did not (0.31 versus 0.08 OD; $P = 0.03$). Moreover, among the noncase residents, IgA levels increased with increasing water consumption (Fig. 2 and Table 1; $P = 0.06$, Spearman peak correlation test). IgA levels in noncase subjects were lower than in subjects consuming the same amount of water. Neither *G. lamblia*-specific IgG nor IgM levels correlated with water consumption. No trend in IgA levels was seen with increasing age or duration of residence in the park.

TABLE 1. Attack rate for *G. lamblia* infection by water consumption status and median levels of *G. lamblia*-specific IgA in serum for controls and residents by case and water consumption status, Vermont, 1986

Water consumption (no. of glasses/day)	Total no. of subjects interviewed	No. of cases (%)	IgA level (enzyme-linked immunosorbent assay OD)							
			Trailer park residents						Nonresident controls	
			Total		Noncases		Cases			
			No.	Median level	No.	Median level	No.	Median level	No.	Median level
0	9	0 (0)	3	0.08	3	0.08	0			
1–3	46	9 (20)	8	0.17	6	0.13	2	1.10		
>3	67	28 (42)	13	0.43	6	0.20	7	0.61		
Total	122	37 (30)[a]	24	0.20	15	0.16	9	0.61	20	0.09

[a] Chi-square test for trend = 10.2; $P = 0.001$. (Test for linear trend of increasing proportion meeting case definition with increasing water consumption.)

DISCUSSION

This is the first report comparing *G. lamblia*-specific IgA levels with IgG and IgM levels in the setting of a waterborne giardiasis outbreak. Although the serologic study was limited by the availability of blood samples, the data suggest that specific IgA levels may be a good indicator of both illness and exposure to *G. lamblia*-contaminated water in outbreak settings. IgG levels also correlated positively with case status, although less strongly than IgA, but were not correlated with water consumption among noncase subjects. These findings should be confirmed by future studies.

Several factors suggest that the elevated IgA levels in the study subjects were the result of the recent outbreak. Specific IgA levels were similar among people with different durations of residence in the trailer park, indicating that the higher IgA levels were not the result of previous exposures to *G. lamblia* in the trailer park water supply. Also, a dose-response effect was seen with water consumption and antibody level. It is unlikely that the high IgA levels seen in case residents was the result of factors other than drinking trailer park water during this time, since non-water-drinking residents had antibody levels similar to those of controls. Moreover, although other enteric pathogens, such as cryptosporidia, were not sought, the antibodies measured correlated well with both microscopically confirmed and clinically defined cases of giardiasis. Previous adsorption studies showed that these antibodies, including *G. lamblia*-specific IgA, are specific for the parasite (7, 8). Illness caused by *Cryptosporidium* species might not have been excluded by the case definition, but if it were occurring it would have been expected to diminish the correlation between illness and *G. lamblia*-specific serology that was found. Similarly, failure to diagnose giardiasis because stool testing was limited to one sample per person in most cases would be expected to bias the serologic results towards a finding of no difference in antibody levels between persons classified as cases or noncases.

G. lamblia-specific IgA may be a better indicator of *G. lamblia* infection than IgG because giardiasis is a noninflammatory enteric infection and IgA is the predominant immunoglobulin produced in the intestinal tract. Serum IgA originates predominantly in the bone marrow. Although the origin of *G. lamblia*-specific IgA in the serum is not known, these antibodies may share the same antigenic specificity as intestinal IgA (4). Parasite-specific serum IgA is increased in residents of Thailand (E. N. Janoff, D. N. Taylor, P. Echeverria, M. P. Glode, and M. J. Blaser, submitted for publication) and subjects with acute giardiasis (8, 11), and IgA is elevated in the breast milk of women in Mexico and

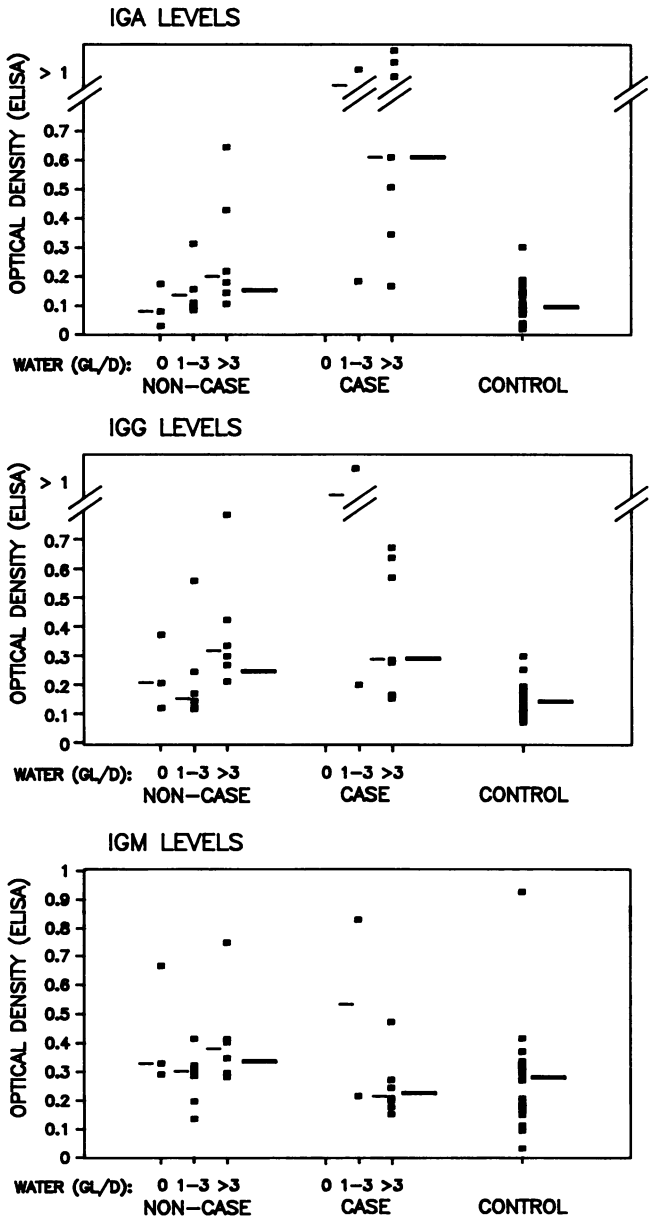

FIG. 2. Levels in serum of *G. lamblia*-specific IgA, IgG, and IgM for controls and residents by case and water consumption status (GL/D, glasses per day), Vermont, 1986. —, Median; ▬, group median.

India (9, 12). It is not yet known whether these IgA antibodies are monomeric or dimeric or whether they are of the IgA1 or IgA2 subclass.

Elevated levels of *G. lamblia*-specific serum IgG have been reported in infected persons (8, 15), Indochinese refugees (16), sewage treatment workers (16), homosexual men (8), and persons in developing countries (9, 10). Parasite-specific IgM may be elevated during acute *G. lamblia* infections (6, 8, 11). However, in our study parasite-specific IgM levels were not predictive of exposure to the organism, perhaps because the study was done several weeks after the peak of illness with subjects who had a spectrum of clinical manifestations. Other studies have examined the parasite-specific IgM response of acutely infected volunteers (11) or involved very symptomatic subjects seeking medical attention (8).

Detection of *G. lamblia*-specific IgA may be a useful epidemiologic tool to supplement stool examination during investigations of waterborne outbreaks of enteric illness. Further studies are necessary to determine the role of specific antibody levels in defining the extent of giardiasis outbreaks in communities, in conducting seroprevalence surveys, and for clinical diagnosis.

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