Immune response to *Giardia lamblia* in a water-borne outbreak of giardiasis in Sweden

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Summary. In one of the largest outbreaks of waterborne giardiasis reported from Europe, more than 3000 persons were exposed to contaminated water and over 1400 cases of giardiasis were diagnosed by microscopy. The outbreak resulted from an overflow of sewage water into the drinking water system of a Swedish ski resort. The period of contamination was about 1 week. Sweden is a non-endemic area for *Giardia lamblia* infection and, for most individuals affected, this was their first contact with the parasite. Few other enteropathogens were isolated from the patients involved. Therefore, an immune response to *Giardia* was unlikely to be biased by other concomitant infections.

Serum samples from 352 exposed persons were collected and analysed for specific IgG and IgA antibodies to *G. lamblia* by indirect immunofluorescence and the results were related to the microscopic examination of faeces and the occurrence of diarrhoea. As controls, sera from 428 healthy persons were analysed at the same time by identical methods. IgG or IgA antibodies, or both, were found in 68% of patients whose diagnosis was made by microscopy, and in 22% of exposed but microscopically *Giardia*-negative persons, but in only 10% of healthy controls. The findings show that patients reported as negative for parasites might be infected. The time between infection and blood sampling influenced the result of the antibody test. The results suggest that stool examination should be the primary means of diagnosis of *G. lamblia* infection and that serological analysis performed at least 3 weeks after infection could contribute to diagnosis in a non-endemic region, when giardiasis is suspected but the parasite has not been detected.

Introduction

Giardia lamblia is one of the most common intestinal pathogenic parasites. It is endemic in many countries, particularly in the Third World, where 30–50% of children may harbour the parasite continuously or intermittently, with or without diarrhoea.^{1,2} Several outbreaks spread by water as well as person to person have been described.^{3–8} Giardiasis is diagnosed primarily by repeated microscopy of faeces and, occasionally, of duodenal aspirate.^{9–11} Since *G. lamblia* is usually a non-invasive parasite and resides within the gut lumen or attaches to the duodenal mucosa,¹² a strong immune response may not be expected. Nevertheless, circulating and local antibodies are produced.¹³ Both enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence have been used for the diagnosis of giardiasis^{14,15} and are frequently employed in epidemiological studies, and where clinical giardiasis is suspected.^{16–19}

Giardiasis is not endemic in Sweden and only a few citizens acquire indigenous infections.^{20,21} At the ski

resort of Sälen, a waterborne outbreak of giardiasis occurred over Christmas 1986, when about 3000 persons were exposed to contaminated water following a sewage overflow into the drinking water system; 1400 cases of giardiasis were diagnosed by microscopy (De Jong *et al.*, personal communication). To investigate the systemic antibody response to *G. lamblia* in a Swedish population exposed during a short and well-defined period to *Giardia* as a single pathogen, sera were collected during the weeks following infection. The sera were analysed for IgG and IgA antibodies to in-vitro cultured *G. lamblia* trophozoites by indirect immunofluorescence and the results were compared to those of the established microscopic diagnostic technique. The presence of specific anti-*Giardia* antibodies was also analysed in two control groups of healthy persons.

Materials and methods

Sera

Persons exposed to Giardia. Following the outbreak of giardiasis in Sälen, blood samples were obtained

from 352 exposed persons. Nearly 50% of the samples were collected within 3 weeks of infection. Sera were analysed from 145 persons whose faeces was shown by microscopic examination to contain *Giardia*, and from 207 persons in whose faeces *Giardia* was not detected. In 158 (76%) of these persons, only a single stool sample was examined. Two or more serum samples were collected from 51 and 40 persons of the respective groups. Details of gastrointestinal symptoms of 229 of the 352 exposed persons were obtained. In less than 9% of these exposed persons, other parasites were found such as *Entamoeba histolytica*, *Endolimax nana* and *Iodamoeba bütschlii*.

Control persons. Serum samples were collected (in 1982) at Malmö General Hospital from 181 native and 47 naturalised Swedes (total 228), who applied for work in community services. In none was *Giardia* detected by microscopic examination of two faecal specimens prepared by formol-ether concentration.⁹ The Epidemiological Department at the National Bacteriological Laboratory kindly provided 200 serum samples obtained during spring 1989 from healthy recruits for military service. Faeces were not examined for the presence of *Giardia* (table I).

Until analysis all sera had been kept frozen at -20°C or -70°C .

Assay procedure

Antibodies to *Giardia* were measured by indirect immunofluorescence (IFL) as described by Islam *et al.*¹⁶ with a suspension of axenically cultured *G. lamblia* trophozoites as antigen (10^4 parasites/ $20\ \mu\text{l}$ /test). Fluorescein-labelled sheep anti-human-IgG and anti-IgA conjugates were used as reagents for detecting bound anti-*Giardia* IgG and IgA antibodies. Serial two-fold dilutions were tested, starting with a single 10-fold dilution. Detectable fluorescence at titres of ≥ 10 was regarded as a positive result.

Statistical analysis

Data were analysed by the χ^2 test and simple regression analysis.

Results

The age distribution of the population exposed to *Giardia* was 2–77 years. The female: male ratio was 170:182. The female: male ratio of the healthy individuals collected at Malmö General Hospital was 193:35 and the age range 18–64 years (table I).

Controls

To establish the seroreactivity in a Swedish control population, 428 serum samples from healthy individuals were analysed by IFL; 42 (10%) of samples contained IgG or IgA antibodies, or both, with

Table I. Age distribution of individuals exposed to *G. lamblia* (Sälen) and control persons (Malmö and recruits)

Age group (years)	Number (%) of individuals		
	Sälen	Malmö	Recruits
<5	5 (1)	0	0
5–10	10 (3)	0	0
10–14	19 (5)	0	0
15–19	42 (12)	5 (2)	200
20–24	81 (23)	41 (18)	0
25–29	40 (12)	25 (11)	0
30–34	34 (10)	19 (8)	0
35–39	34 (10)	22 (10)	0
40–44	26 (7)	19 (8)	0
45–49	26 (7)	14 (6)	0
50–54	13 (4)	28 (12)	0
55–59	10 (3)	31 (14)	0
60–64	4 (1)	24 (11)	0
>65	8 (2)	0	0
Total	352	228	200

specificity for *Giardia* (table II). Among the recruits, 16 (8%) of 200 had either IgG or IgA antibodies, or both; specific IgA antibodies were demonstrated in three persons, one of whom was also IgG-positive. Of the 228 serum samples collected at Malmö General Hospital from people demonstrated not to be infected with *Giardia*, 26 (11%) had IgG anti-*Giardia* antibodies; in only one person, also IgG-positive, were specific IgA-antibodies detected. The occurrence of *Giardia* antibodies did not differ significantly ($p > 0.05$) between immigrants (6 of 47; 13%) and Swedes (20 of 181; 11%).

None of the healthy individuals had an IgG titre > 20 or an IgA titre > 10 .

Giardia-exposed persons

Amongst the 352 investigated persons who had been exposed to contaminated water at Sälen, 144 (41%) were found to have *Giardia* antibodies at a titre ≥ 10 . The anti-*Giardia* antibody response estimated by IFL was generally at a low titre. Only 13% of sera from individuals in whose faeces *G. lamblia* could be demonstrated by microscopy and no sera from *G. lamblia*-negative persons, had a titre > 40 .

Table II. Specific anti-*G. lamblia* titres (IgG and IgA) in control persons

Titre	Number (%) of persons with			
	IgG		IgA	
	Recruits	Malmö	Recruits	Malmö
<10	186 (93)	202 (89)	197 (99)	227 (99.6)
10	11 (6)	23 (10)	3* (1)	1* (0.4)
20	3 (1)	3 (1)	0	0

*One was also IgG positive.

Giardia-positive patients. Specific IgG was detected in 87 (60%) of 145 of the patients whose faeces were shown to contain *Giardia*. The titres varied from 10 to 320 (table III). Specific IgA antibodies were demonstrated in 40 (28%) patients. Of these patients, 29 were also IgG-positive, but in 11 patients, only IgA antibodies were detected. Thus, 98 (68%) of 145 *Giardia*-infected patients had IgG or IgA antibodies, or both.

Giardia-negative patients. Of 207 individuals whose faeces was not found by microscopy to contain *Giardia*, 42 (20%) had anti-*Giardia* IgG antibodies (table III). Specific IgA antibodies were detected in 8 (4%) patients of whom four were also IgG-positive. Thus, in 46 (22%) patients, specific antibodies of IgG or IgA class, or both, were found. In three seropositive patients the stool examination was performed during or after treatment with nitroimidazoles.

Seroconversion observed in persons exposed to Giardia. Two serum samples were obtained from 51 individuals whose faeces were *Giardia*-positive, and 40 who were *Giardia*-negative. Seroconversion measured by the IgG antibody response was observed in 9 (18%) of 51 and 3 (8%) of 40 patients, respectively.

Kinetics of the specific Giardia IgG antibody response. Most of the sera were obtained within 1 month after

infection and nearly 50% within 3 weeks after infection. Therefore, the serological results might have been affected by the time that elapsed between infection and blood sampling. In 102 sera collected from *Giardia*-positive patients less than 3 weeks after infection, only 49% were IgG positive, but 66% of 97 sera collected more than 3 weeks after infection had an IgG antibody response. The sensitivity had increased significantly ($\chi^2 = 5.84$; $p < 0.02$).

Correlation between diarrhoea and antibody response. Occurrence or absence of diarrhoea was reported in 229 persons. Of 184 patients who had diarrhoea, specific *Giardia* IgG antibodies were detected in serum collected from 81 (44%). Of 45 non-diarrhoeic individuals, 12 (27%) were IgG positive ($\chi^2 = 4.52$; $p < 0.02$). Specific *Giardia* IgA antibodies were found in 30 (16%) patients presenting with diarrhoea, compared to 8 (18%) patients without diarrhoea (n.s.; table IV).

Distribution of Giardia seropositivity with age

In healthy individuals, the percentage of specific IgG or IgA responses was twice as high in the age-group 20–39 years than in other age groups. In *Giardia*-exposed persons, this pattern was not seen. High titres (≥ 80) were observed only in *Giardia*-positive patients (table V).

Statistical analysis

Patients found to be *Giardia*-positive by microscopic examination of the faeces had *Giardia* antibodies significantly more frequently than patients in whose faeces *Giardia* could not be detected ($\chi^2 = 75.72$; $p < 0.001$). The same finding was obtained when the two antibody classes were evaluated separately (IgG, $\chi^2 = 57.91$; $p < 0.001$; IgA, $\chi^2 = 40.73$, $p < 0.001$). There was no statistical correlation between the findings of IgG and IgA antibodies (table VI).

Comparison of the antibody response between *Giardia*-negative patients and healthy individuals revealed a statistically significant higher number of seropositives among the patients ($\chi^2 = 18.00$; $p < 0.001$), suggesting that some of the patients reported as *Giardia*-negative were infected with the parasite.

Table III. Titres of anti-*Giardia* antibodies, IgG and IgA, in *Giardia* (microscopy)-positive (145) and -negative (207) exposed persons

Antibody titre	Number (%) of exposed persons who were			
	<i>Giardia</i> -positive		<i>Giardia</i> -negative	
	IgG*	IgA*	IgG	IgA
< 10	58 (40)	105 (73)	165 (80)	199 (96)
10	18 (13)	15 (10)	19 (9)	7 (3.5)
20	46 (32)	12 (8)	18 (9)	0
40	12 (8)	7 (5)	5 (2)	1 (0.5)
80	5 (3)	4 (3)		
160	3 (2)	2 (1)		
320	3 (2)			

* Antibodies of either category may co-exist.

Table IV. Correlation between specific IgG and IgA antibodies to *G. lamblia* and microscopically diagnosed giardiasis in exposed persons with or without diarrhoea

Clinical status	Faeces microscopy	Number	Number of exposed persons					
			IgG-positive			IgG-negative		
			Total	IgA + ve	IgA - ve	Total	IgA + ve	IgA - ve
Diarrhoeic	+	99	61	22	39	38	6	32
	-	85	20	1	19	65	1	64
Non-diarrhoeic	+	13	9	4	5	4	2	2
	-	32	3	1	2	29	1	28

Table V. The distribution of anti-*G. lamblia* titres with age

Age groups	Number with anti- <i>Giardia</i> titres							Total (%) with anti- <i>Giardia</i> antibodies
	< 10	10	20	40	80	160	320	
Controls								
< 20 yrs	191	11	3					205 (7)
20-39	92	14	1					107 (14)
40-59	86	4	2					92 (7)
≥ 60	19	5						24 (21)
<i>Giardia</i> microscopy positive (exposed)								
< 20 yrs	12	3	6	4	2			27 (56)
20-39	30	11	24	5	2	1	2	75 (60)
40-59	14	2	13	3	1	2	1	36 (61)
≥ 60	2	2	3					7 (71)
<i>Giardia</i> microscopy negative (exposed)								
< 20 yrs	40	5	3	1				49 (18)
20-39	90	10	11	3				114 (21)
40-59	32	4	3					39 (18)
≥ 60	3	0	1	1				5 (40)

Table VI. Correlation between IgG and IgA antibody responses in *Giardia* faeces microscopy-positive patients

IgA titre	Number of patients	Number of patients with IgG titres						
		< 10	10	20	40	80	160	320
< 10	105	47	15	34	6	1	2	0
10	18	5	1	6	3	2	1	0
20	11	4	2	3	1	0	0	1
40	7	2	0	2	1	1	0	1
80	2	0	0	1	0	0	0	1
160	2	0	0	0	1	1	0	0
Total	145	58	18	46	12	5	3	3

Specificity of the IFL calculated on *Giardia*-exposed, but stool-negative persons, was 78%. The same calculation on 228 healthy stool-negative individuals showed a specificity of 89%. The sensitivity of the assay was estimated to be 68% as judged by detection of *Giardia*-specific IgG or IgA antibodies, or both, in patients demonstrably infected with *Giardia* by microscopic examination of faeces.

Discussion

The persons exposed to *Giardia* in this study were mainly adults. Only 34 persons were younger than 15 years. As controls, serum samples were collected from healthy individuals. The occurrence of specific *Giardia* IgG and IgA antibody responses of 352 persons exposed to contaminated drinking water was studied by IFL. Specific IgA or IgG antibodies, or both, were detected in 68% of all *Giardia*-positive patients tested. This figure is low compared to earlier studies showing a sensitivity of over 80%^{14, 15, 19, 22} and similar to the results of some other studies.^{18, 23, 24} Methods used were IFL^{14, 15, 19, 22, 23} and ELISA.^{15, 18, 24} In most

earlier reports, the patients presented with clinical giardiasis. The purpose of this study was to evaluate serological diagnosis during an outbreak of giardiasis including both symptomatic (presenting with diarrhoea) and asymptomatic infections.

In 32% of proven *Giardia*-positive patients neither specific IgA nor IgG antibodies could be detected and, generally, the antibody titres were low. There may be several reasons for a poor or non-detectable antibody response, including low infection dose, rapid recovery without treatment, early treatment, and a short time-interval between infection and blood sampling. Nearly 50% of the sera were obtained within 3 weeks after infection and most of them within 2 weeks. Our data suggest that antibodies are more often and more readily detected in sera drawn more than 3 weeks after infection (49% vs 66% IgG positive). In experimental human infections with *G. lamblia* (including 10 volunteers), Nash *et al.*²⁴ found that 50% were IgG-positive at 3 weeks and 70% at more than 3 weeks, figures in agreement with our findings during natural infection.

Birkhead *et al.*²⁵ suggested that "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 diarrhoeal illness." However, our study shows that in a natural infection of a naive population, specific IgA antibodies measured by IFL could be demonstrated in only 28% of *Giardia* stool-positive patients and 4% of *Giardia* stool-negative patients. Low incidences of specific IgA response—48%,¹⁸ 36% and 29%²⁶—have also been reported in clinical giardiasis when ELISA methods have been used. Specific IgA for serodiagnosis seems to be too insensitive to complement or replace diagnosis by microscopy, although 82% of the IgA- and *Giardia* (microscopy)-positive patients had diarrhoea.

The specificity of serodiagnosis was only 78% in comparison with faecal microscopy and 22% of 207 *Giardia*-negative patients had IgG or IgA antibodies, or both. False-positive antibody results due to cross-reactivity with other intestinal parasites or pathogens are not likely, as very few other pathogens were isolated from patients involved in the Sälen outbreak. It seemed to be an outbreak caused by one pathogen, *G. lamblia* (DeJong *et al.*, personal communication). False-negative results following microscopic examination of faeces are, however, possible and, in this context, it is important to note that 158 exposed persons were considered to be *Giardia*-negative on the basis of a single faecal examination. Farthing *et al.*¹³ reported that even after three faecal specimens had been examined from patients with proven giardiasis, up to 15% of these individuals had a negative stool examination. The diagnosis may be missed on account of intermittent excretion of *Giardia* cysts. In liquid stools only trophozoites may be excreted. These parasitic forms are very fragile and soon disintegrate

and can escape detection unless stool samples are analysed immediately after collection.

In the three seropositive but microscopy-negative patients identified, a *Giardia* infection could have been eradicated by treatment given before stool samples were collected. Additional patients may have cleared their infection spontaneously. The statistically significant higher number of seropositive patients among individuals exposed to *Giardia*, but negative as judged by stool examination, than among healthy individuals ($p < 0.001$) also indicates some false-negative diagnoses by faecal examination.

The specificity of the IFL calculated on the healthy, microscopy-negative individuals was 89% and also suggests that some false-negative results were obtained by microscopy or that antibodies remained after a cleared infection.

When giardiasis is suspected, repeated microscopic examination of faeces remains the method of choice for diagnosis. In a population of low endemicity for *Giardia* and where antibodies are seldom found in healthy persons, a positive antibody test indicates contact with the parasite and motivates treatment in symptomatic persons even when faecal microscopy is negative. Serological diagnosis is most reliable when samples are analysed more than 3 weeks after infection. However, even in this situation, about one-third of infected patients have no detectable antibodies. The combination of repeated stool examination and serological assays may yield the highest sensitivity of diagnosis of giardiasis in populations of low endemicity.

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