**Giardia intestinalis** Is Unlikely To Be a Major Cause of the Poor Growth of Rural Gambian Infants

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ABSTRACT Parasite-specific plasma immunoglobulins have been used to indicate the presence of *Giardia intestinalis* infection in 60 infants living in a rural area of The Gambia. Infants were studied longitudinally between 2 and 8 mo of age. The median age for first exposure to *G. intestinalis* was between 3 and 4 mo, and by 8 mo all but 3 infants (95%) showed a positive titer on at least one occasion. Raised *Giardia*-specific IgM titers were associated with reduced weight gain in the 2 wk preceding a positive titer, but catch-up growth occurred in the following 2 wk. IgM antibody titers were also positively associated with intestinal permeability (lactulose/mannitol ratio), urinary lactose excretion, plasma concentrations of α1-antichymotrypsin and total IgM, IgA and IgG immunoglobulins. However, infant growth over the whole 6-mo period (i.e., between 2 and 8 mo of age) was not related to mean *Giardia*-specific antibody titers, nor the time of first exposure to the parasite. The data suggest that giardiasis in these very young breast-fed children occurs as a mild, acute disease, and its presence could not explain the marked, long-term growth faltering observed in many of the subjects. J. Nutr. 129: 872–877, 1998.

KEY WORDS: • giardiasis • intestinal parasite • *Giardia* antibodies • infant growth • The Gambia

*Giardia intestinalis* is the most common intestinal protozoan parasite of man and although it is regularly found in most industrialized countries, it is particularly prolific in Third World communities (Meyer 1990). While undoubtedly infection with this parasite can at times result in severe diarrhea and dehydration requiring hospitalization, such cases are rare and infection commonly occurs without overt clinical symptoms (Wolfe 1990). Nevertheless, because giardiasis is associated with malabsorption of fat and reduced mucosal disaccharidase activity in the small intestine (Buret et al. 1992, Farthing 1993, Katelaris and Farthing 1992), there is a general acceptance that infection with this parasite is likely to result in failure to thrive and poor growth in infants and children (Islam 1990, Thompson et al. 1993). Evidence in the literature for such an effect is, however, rather sparse. Gupta and Urrutia (1982) reported increased growth in weight and height of Guatemalan children in whom giardiasis had been virtually eliminated by twice-monthly doses of metronidazole. However, metronidazole is a wide-spectrum antibiotic, and the improvement noted cannot be unequivocally assigned to its anti-giardial properties. In another Guatemalan study (Farthing et al. 1986), *Giardia*-positive children were found to have poorer weight gain than those without this infection (P = 0.03) but only in the second year of life; no effect was seen in the first or third years. Similar small effects on growth were reported from studies in The Gambia (Cole and Parkin 1977) and in Zimpapwe (Loewenson et al. 1986, Mason and Patterson 1987). However, these data do not give a clear guide of the importance of *G. intestinalis* infection as a cause of childhood growth faltering and malnutrition in developing countries.

Poorer than expected growth during the first year of life is a frequent occurrence in infants living in the rural West Kiang area of The Gambia (Lunn et al. 1991). Dietary deficiencies seem unable to explain the poor growth (Prentice 1993), but close associations between gastrointestinal disease and both height and weight growth were described. In particular, repeated intestinal permeability tests indicated the presence of persistent enteroopathy in the small intestine of slow-growing infants (Lunn et al. 1991). *Giardia intestinalis* causes persistent damage to the mucosa of the small intestine (Farthing 1993), and previous work showed the parasite to be common in this part of The Gambia (Cole and Parkin 1977). The aim of this investigation was to determine to what extent *G. intestinalis* infection was associated with the etiology of the observed mucosal enteropathy and the consequence of such involvement on infant growth faltering in this area. In this study, the presence of infection was assessed serologically by the measurement of *Giardia*-specific immunoglobulins (Goka et al. 1986).

SUBJECTS AND METHODS

A cohort of 60 children living in the village of Keneba in the rural West Kiang region of The Gambia were recruited into the study at 2 mo of age. Recruitment took 12 mo to complete as the number of subjects represents the total number of births per year in Keneba. All

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the infants attended clinics at fortnightly intervals up to the age of 8 mo. At recruitment and at each subsequent clinic, a medical examination was carried out and treatment was provided as necessary. Anthropometric measurements were made and an intestinal permeability test was performed. A finger-prick blood sample (200–300 μL) was collected monthly. In all, 420 blood samples were obtained from 60 children. Weight was recorded (to the nearest 0.01 kg) using electrical scales and length monitored (to the nearest 0.1 cm) with the child lying supine on a Harpenden measuring table (Holtain, Crymmych, UK). Plasma samples were stored at −20°C prior to shipment to Cambridge (UK) and analysis. The prevalence of diarrhea (defined as three or more loose stools per day) and vomiting was assessed by daily questioning of the infants’ mothers throughout the whole 6-mo investigation.

**Anti-Giardia antibodies.** Giardia-specific IgA, IgM and IgG antibodies (GS-IgA, GS-IgM and GS-IgG) in plasma were measured by ELISA as described by Goka et al. (1986). Giardia antigen was prepared as described from Portland I strain trophozoites grown in axenic culture (Keister 1983). Plasma was assayed at 1/200 dilution of the sample in phosphate buffered saline (pH 7.2) containing 10 g/L bovine serum albumin. The assay differed from that described by Goka et al. (1986) only in the substitution of o-phenylenediamine as substrate for the peroxidase reaction. Optical density was read at 492 nm on a Labsystems Multiscan MCC/340 plate reader (Basingstoke, Hants, UK).

Serial blood samples were taken monthly from the age of 2 mo, i.e., at a time when the infants were fully breast-fed and unlikely to have been exposed to the parasite. GS antibody titers at this age would be expected to be low and give a baseline value. In subsequent samples an optical density reading greater than 0.53 (at 492 nm) for GS-IgM was taken as an indication of Giardia infection. This value represented the mean value of the initial, i.e., 8-wk titer plus 1 SD (Al-Tukhi et al. 1993a).

**Plasma proteins.** Plasma α1-antichymotrypsin, albumin and total immunoglobulins IgA, IgM and IgG concentrations were measured by immunoturbidimetric techniques using a Cobas Bio (Roche, Welwyn Garden City, Herts. UK) centrifugal analyzer. The α1-antichymotrypsin was determined according to Calvin and Price (1986) using Dako (High Wycombe, Bucks, UK) antibodies and a Serotec (Kidlington, Oxon, UK) calibrator. The albumin and immunoglobulin assays also used Dako antibodies but were calibrated against Dako standards.

**Intestinal permeability test.** This is a noninvasive technique which provides information about the function and integrity of the mucosa of the small intestine. In this test, a low lactulose/mannitol ratio suggests mucosal normality, whereas a raised level indicates loss of absorptive capacity, breaches of the mucosal barrier, or both (Lunn et al. 1990) and the 5 h excretion rate calculated.

Ethical approval for the study was obtained from the Medical Research Council-Gambian Government Ethical Committee. The study was part of a larger investigation into many aspects of growth, nutrition and infection in Gambian infants. Parents of the subjects were invited to give their consent after the purpose and requirements of the studies had been explained to them and they were free to withdraw their children at any time.

Statistical analyses were performed using SPSS, version 8.0 (SPSS, Chicago, IL). Repeated observations from individual infants were analyzed by analysis of covariance to assess within-subject correlations (Bland and Altman 1995). Other data were assessed by ANOVA, linear regression or chi-square analysis as indicated. Growth data were corrected for age by regression. Intestinal permeability was log transformed to correct for a skewed distribution.

### RESULTS

Mean Giardia-specific plasma antibody titers increased slowly with age in all three of the Ig classes examined (Table 1), but the largest increase was seen in GS-IgM. Titers of GS-IgM and GS-IgA in 2-mo-old children were close to United Kingdom control values. In contrast, GS-IgG levels were frequently elevated, probably as a result of absorption of maternal IgG antibodies from breast milk. GS-IgA titers varied in a similar way to GS-IgM, but appeared to be less sensitive: the latter were invariably increased when IgA antibodies rose, but GS-IgA was elevated only on 67% of the occasions when GS-IgM was high. Nevertheless there was a good correlation between titers of all three classes of antibody in individual samples, (GS-IgM vs. GS-IgA, r = 0.44; GS-IgM vs. GS-IgG, r = 0.50; GS-IgA vs. GS-IgG, r = 0.41; P < 0.001 at all times).

The age at which each infant first showed a positive GS-IgM titer was recorded and Figure 1 shows the age distribution of this occurrence together with the cumulative total of infants having had at least one positive titer. Clearly the data show that infants are exposed to Giardia from an early age with peak seroconversion occurring at 3–4 mo. By 6 mo of age, more than 80% of the subjects had experienced at least one GS-IgM positive titer, and all but three infants gave a positive titer before the end of the study, when the infants were 8-mo old. The months of April, May and June represented the most likely time for the first appearance of positive titers (Fig. 2A) and GS-IgM titers were also at their highest during May and June (Fig. 2B). GS-IgG and GS-IgA titers also peaked during these 3 mo (data not shown).

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>GS-IgA</th>
<th>GS-IgM</th>
<th>GS-IgG</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>0.29 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>0.31 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>0.31 ± 0.01</td>
<td>0.53 ± 0.02</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>0.34 ± 0.02</td>
<td>0.61 ± 0.03</td>
<td>0.97 ± 0.07</td>
</tr>
<tr>
<td>6</td>
<td>0.33 ± 0.02</td>
<td>0.60 ± 0.02</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.35 ± 0.02</td>
<td>0.60 ± 0.03</td>
<td>1.05 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.38 ± 0.03</td>
<td>0.70 ± 0.03</td>
<td>1.18 ± 0.05</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 60.
2 Statistical significance of the b coefficient was tested against a null hypothesis of b = 0.

Abbreviation used: GS-Ig, Giardia-specific immunoglobulin.

![Table 1](https://example.com/)
Predictive value of raised GS-IgM antibody titers. Elevated GS-IgM titers were associated with poor weight gain during the 2-wk period before the sample was obtained, (Fig. 3A). Moreover, the higher the antibody titer, the more severe the weight deficit. In contrast, in the 2 wk following elevated antibody titers (Fig. 3B), weight gain increased, the greatest gain being in infants who had exhibited the highest GS-IgM antibody titers. Weight-for age Z-scores showed similar relationships and statistical significance.

Raised GS-IgM titers were associated with elevated intestinal permeability values (Table 2) and with decreased mannitol absorption. No correlation was observed with lactulose uptake, but urinary lactose excretion rose with increased titers. High antibody titers were not associated with episodes of diarrhea or vomiting in these children. Infants (18.6%) had an episode of diarrhea and 6% vomited in the 2-wk period before a positive titer was found, but this did not differ significantly from corresponding figures of 20.3 and 7.1% before a negative titer.

Plasma α1-antichymotrypsin concentrations became elevated with increasing GS-IgM titers (Table 2), suggesting that production of GS antibodies was associated with a systemic inflammatory response. Such a mechanism may explain part of the poor weight gain seen in infants with high GS-IgM titers as high concentrations of plasma α1-antichymotrypsin were overall related to poor weight gain ($r = 0.34$, $P < 0.001$). No changes occurred in plasma albumin concentrations during infection.

Close relationships were also observed between plasma GS IgG titers of each class and the respective total Ig concentration: for GS-IgM and total IgM, $r = 0.784$; for GS-IgA and total IgA, $r = 0.603$; and for GS-IgG and total IgG, $r = 0.420$; $P < 0.001$ for each, ($n = 60$).

Predictive value of raised anti-Giardia antibody titers on long-term growth. Long-term effects of raised anti-Giardia antibodies on the growth of individual infants were examined by taking the mean of each measurement made over the whole of the 6-mo study period for each child. The mean monthly weight gain over this period was $375 \pm 13$ g (SEM) with a range from 143 to 552 g. Corresponding values for length growth were a mean of $1.43 \pm 0.07$ cm and a range from 0.44 to 2.65 cm.

FIGURE 1 Age-related appearance of elevated Giardia-specific IgM antibodies (GS-IgM) in rural Gambian infants. (A) Age distribution of first occurrence of positive GS-IgM titer and (B) cumulative percentage of infants having had at least one positive GS-IgM titer by age, ($n = 60$).

FIGURE 2 Seasonal variation in Giardia-specific IgM antibodies (GS-IgM) in Gambian infants. (A) Distribution by month of first positive GS-IgM titer. Chi-square analysis shows the monthly variation to be significant, $P < 0.01$ (B) Variation of GS-IgM titers by month, measured as absorbance at 492 nm ($A_{492}$) of a 1:200 plasma dilution. Values are means $\pm$ SEM, total $n = 420$, average $n = 36 \pm 8$ (SD). The variation between months is significant, $P < 0.01$ by ANOVA.
Corresponding coefficients for IgA and IgG were found to predict greater than 60% of the variation in total IgM (n and 0.543, respectively, for IgM, in this way. The regression coefficient for GS-IgM and total antibody titer and intestinal permeability. Also no relation-

ship existed between any of these parameters and the age at which infants first showed a positive GS-IgM titer.

Comparing the mean growth rate for each child with its mean GS-IgM titer showed no long-term relationship with either weight or length gain, (r = 0.13 and 0.07; P = 0.3 and 0.6, respectively). Similarly, no significant relationship was seen with GS-IgA or GS-IgG. Weak associations between mean GS-IgA titer and α1-antichymotrypsin (r = 0.26, P < 0.05) and intestinal lactulose uptake (r = 0.28, P < 0.05) were present, but a correlation no longer existed between antibody titer and intestinal permeability. Also no relation-

ship existed between any of these parameters and the age at which infants first showed a positive GS-IgM titer.

The association between GS antibodies and their respective total Ig type was still strong when the data was analyzed in this way. The regression coefficient for GS-IgM and total IgM, r = 0.780, indicated that the GS antibody titer could predict greater than 60% of the variation in total IgM (Fig. 4). Corresponding coefficients for IgA and IgG were r = 0.740 and 0.543, respectively, (n = 60, P < 0.001 in all cases).

**FIGURE 3** Relationship between Giardia-specific IgM antibody (GS-IgM) titer and weight gain of rural Gambian infants before (panel A) and following (B) blood sampling. Values are means ± SEM after correction for between-subject variation. Correlations within individuals were assessed by analysis of covariance for 60 subjects measured on 420 occasions, r = 0.15, P < 0.01 and r = 0.12, P < 0.05 for before and after blood sampling, respectively).

DISCUSSION

Infants in this region of The Gambia are exclusively breast-

fed for the first 2–3 mo of life during which time they grow well and tend to catch up on their slightly low birth-weight and length. Although they continue to receive substantial amounts of breast milk for up to 2 y (Prentice and Paul 1990), the infants are gradually introduced to weaning foods from 2–3 mo coinciding with the onset of growth faltering. In this study, 3–4 mo was the peak age for the appearance of GS IgM antibodies in the plasma of the infants, suggesting that exposure to G. intestinalis occurred at about the same time or soon after weaning foods were first given. Exposure occurring so early in life demonstrates not only the high endemicity of the parasite in this area of The Gambia, but also a high prevalence of fecal contamination of the infants’ food or water. Elevated GS-IgM concentrations in the blood were associated with poor weight gain of infants; previous to the blood sample, the higher the GS-IgM titer the poorer the weight gain. The raised antibody titer was also associated with an elevated intestinal permeability due to reduced mannitol uptake and a small but significant increase in the plasma concentration of the acute-phase protein, α1-antichymotrypsin. Although increased Giardia antibodies were not associated with diarrhea and vomiting, the reduced weight gain, raised intestinal permeability and small systemic inflammatory response are in keeping with a mild episode of giardiasis. Malabsorption of lactose occurs in giardiasis (Farthing 1993) and could raise urinary excretion of this sugar with increasing titers. The impact of the infection on growth however seems to be short-lived. In the weeks following high titers, weight gain was greater than average with infants making up much of the previous weight loss. The effectiveness of recovery was such that when weight or height growth over the whole 6 mo of life was considered, no rela-

tion between elevated GS antibody titers and growth makes it unlikely that this infection could account for a significant part of the progressive growth faltering observed in these infants. Given the high potential for infection and re-infection in the region, the pathogenic nature of the parasite and the relatively immature immune system of infants of this age, this result is rather surprising. It is however in keeping with other reports of the impact of Giardia on this age group (Farthing et al. 1986), and probably the infection may have been limited by protective agents present in breast milk. Human milk was shown to have both cytotoxic (Gillin 1987) and antiadherent activity (Crouch et al. 1991), and GS secretory IgA was demonstrated (Walterspiel et al. 1994). In field studies, Morrow et al. (1992) reported a greater incidence of diarrhea and vomiting in in-

fants and young children who were not receiving breast milk. However their studies also showed that there were no differ-

ences in the prevalence of G. intestinalis cysts in the feces, i.e., the number of children carrying the infection did not differ between breast-fed and nonbreast-fed children. The lack of symptoms observed in the present study is clearly in agreement with such data.

The relevance of the close correlation between the titers of GS-Ig classes, and respective total plasma Ig is not clear. Statistically, the 6-mo mean GS-IgM titer could predict greater than 60% of the 6-mo total IgM mean. Corresponding figures for IgA and IgG were 54 and 29%. One interpretation is that this represents the proportion of respective total Ig that is GS. However a more likely explanation is that mucosal
damage associated with the parasite allowed translocation of various antigenic macromolecules which elicited a polyclonal Ig response (Suskind et al. 1976).

Several groups used the presence of GS Ig in plasma to diagnose infection with G. intestinalis. Goka et al. (1986) reported a good correlation between specific anti-Giardia IgM titers and the presence of giardiasis assessed by standard techniques. GS IgG appeared less useful as such antibodies persisted long after the infection was over in contrast to IgM antibodies which fell sharply after the infection was cleared. More recently the use of raised antibody titers as a reliable indicator of G. intestinalis infection was confirmed in studies of Gambian children (Sullivan et al. 1991) and in Saudi Arabian subjects (Al-Tukhi et al. 1993b). In both the latter studies, the specificity of the technique was greater than 93% though sensitivity was a little lower. In the current study, the availability of serial samples minimizes the likelihood of false positive and negative values. The first samples were taken at the age of 2 mo, a time when the infants were fully breast fed and thus unlikely to have become infected, and were found to have low titers of GS IgM and IgA. Increased titers, signifying the first G. intestinalis infection, could be clearly observed as a sharp increase from this early, baseline value.

The results do not suggest that giardiasis is of little importance in the etiology of malnutrition and growth faltering of children in general. The episodes seen in these particular infants were very mild; they were not associated with diarrhea or vomiting and resolved quickly. In older children or those receiving little or no breast milk, the disease appears to be more severe and consequently may compromise growth (Farthing 1993). However, further studies in such children are required to determine the full impact of this parasite on growth.

**LITERATURE CITED**


Lunn, P. G., Northrop-Clewes, C. A. & Downes, R. M. (1991) Intestinal per-