Anthelmintic treatment of rural Bangladeshi children: effect on host physiology, growth, and biochemical status[1–3]

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ABSTRACT

Background: The effect of helminth infestation on the nutrition, growth, and physiology of the host is still poorly understood. Anthelmintic treatment of children in developing countries has had varying success in terms of growth improvements.

Objective: The objective of this study was to assess the effect of regular deworming on child growth, physiology, and biochemical status.

Design: The study was a 12-mo longitudinal intervention in 123 Bangladeshi children aged 2–5 y. Treatment (mebendazole) or placebo tablets were administered every 2 mo for 8 mo and again at 12 mo. Weight, height, midupper arm circumference, intestinal permeability, plasma albumin, α_1-antichymotrypsin, and total protein concentration were assessed every 2 mo.

Results: Treatment with mebendazole reduced the prevalence of *Ascaris lumbricoides* from 78% to 8%, of *Trichuris trichiura* from 65% to 9%, and of hookworm from 4% to 0%. There was no significant difference in the growth of treated children compared with those given placebo tablets. No changes in intestinal permeability or plasma albumin were observed after deworming. Significant decreases in total protein ($P < 0.001$) and α_1-antichymotrypsin ($P < 0.001$) were observed in the treatment group, indicating possible reductions in inflammation and immunoglobulin concentration after deworming. A significant increase in the prevalence of *Giardia intestinalis* (from 4% to 49%) in the treatment group was associated with a short-term reduction in weight ($P = 0.02$) and higher intestinal permeability ($P < 0.001$) in infected subjects. No long-term effects of *G. intestinalis* on growth were observed.

Conclusion: Low-intensity helminth infections, predominantly of *A. lumbricoides* and *T. trichiura*, do not contribute significantly to the poor growth and biochemical status of rural Bangladeshi children. *Am J Clin Nutr* 2001;73:53–60.

KEY WORDS Malnutrition, helminthiasis, giardiasis, growth, children, anthelmintic treatment

INTRODUCTION

Whether intestinal geohelminth infections play a significant role in the etiology of childhood malnutrition remains to be shown unequivocally (1, 2). Helminth infection is thought to contribute to child malnutrition through subtle reductions in digestion and absorption, chronic inflammation, and loss of nutrients (3–5). Deworming has led to improved growth, increased physical activity, and improved appetite in children (6–11), sometimes as soon as 3–9 wk after treatment (10, 11). Several studies, however, did not show any significant growth improvements after anthelmintic therapy (12–17). The interpretation of these findings is complicated by population differences in growth, nutrition, and the prevalence and intensity of helminth infection. Further understanding of the relation between helminth infection and child health is required. To achieve this, the mechanisms by which helminths affect the growth, nutrition, and physiology of the host must be investigated.

Intestinal helminths can cause injury to the mucosa of the small intestine, causing malabsorption and gastrointestinal losses of nutrients (2, 3). Hypoalbuminemia is reported frequently in trichuriasis and in hookworm disease (3, 18). Moreover, local inflammation at the site of infection appears to provoke a systemic inflammatory response with elevated plasma concentrations of acute phase proteins and cytokines (18). In children, cytokine-induced anorexia may contribute to growth faltering (19, 20).

The present study aimed to assess child growth, intestinal permeability, and biochemical status longitudinally during a 12-mo program of regular deworming. This was an expansion of an earlier investigation in the same population (21, 22). In addition, a larger study was conducted in parallel in 1402 children aged >18 mo. The larger study monitored growth, morbidity, and socioeconomic variables but did not assess biochemical or physiologic status (23, 24).

Dual sugar intestinal permeability, the ratio of lactulose to mannitol (L:M) in urine, has been used to assess small intestinal function and integrity (25, 26). In the Gambia, poor growth of infants is significantly correlated with a greater L:M (27, 28). Experimental studies showed protein-losing enteropathy, increased

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permeability, and reduced plasma albumin concentrations in rats infected with *Nippostrongylus brasiliensis* (29, 30). If helminth infection causes damage to the small intestine, leading to poor nutrient digestion and absorption (5), anthelmintic treatment may lead to improved functional integrity of the gut.

In addition to assessment of intestinal permeability, we set out to measure plasma albumin and α_1-antichymotrypsin (ACT). These markers were used to ascertain whether deworming leads to reduced protein loss from the intestine and reduced inflammation and immune response.

SUBJECTS AND METHODS

The investigation was carried out in a poor rural area in the Jamalpur district of northern Bangladesh. The study area lay on the banks of the Jamuna river and was particularly susceptible to devastating floods, land loss, and food shortages. The work was carried out in collaboration with the Save the Children Fund, United Kingdom, as part of its maternal and child primary health care program. The Save the Children Fund staff recorded all births in the area; consequently, the exact ages of the children were known. The Ministry of Health of Bangladesh granted ethical approval for the study on the basis that all children would be treated at the end of the study.

Sample selection and study design

For the larger 18-mo study, all children aged 2.0–6.0 y within the catchment area of the health center were invited to participate, resulting in a final sample size of 1402 (23). For the present study, a subsample of children aged 2.0–6.0 y were selected from the 8 villages that were within walking distance of the health center [3.2-km (≈2-mile) radius]. These were allocated to the treatment and placebo groups at random (4 villages in each). The 8 villages were uniform in terms of type of housing (bamboo with mud floors), water supply (deep tubewell), mode of subsistence (rice, sugar cane, and jute cultivation), and access to primary school education.

The subsample was selected at random by using household numbers from the clinic records. The target sample size was 120 children, which was the maximum number that could be handled in terms of collection, storage, and processing of stool, blood, and urine samples. In actuality, 123 children were enrolled. All parents were informed of the nature of the study. Informed consent was given and there was the freedom to withdraw at any stage.

The treatment and placebo tablets were given in a double-blind manner; neither the fieldworkers nor the parents were aware of the group to which they belonged. Treatment was randomized by village of residence. Previous fieldwork in the area showed that mothers became anxious if worms appeared in the stools of some children but not in those of their siblings, relatives, or neighbors. This anxiety led to more mothers going to the health center independently to seek deworming treatment. A village design had the advantage that siblings or children in neighboring households experienced the same effects, thus reducing concerns about the efficacy of the tablets. In addition, this design eliminated any possibility of fieldworkers mixing up treatments for children in the same household.

Data collection

Parents were asked to bring their children to the health center every 2 mo for 8 mo. The children were reassessed at 12 mo, after which all the children, including the placebo group, received an anthelmintic. Anthropometric measurements were made with use of the techniques described by the World Health Organization (WHO) (31). Children were weighed with a hanging balance (Camden Medical Supplies, London). Height was measured with a vertical height stick with a sliding headpiece. Midupper arm circumference (MUAC) was measured with an insertion tape after the midpoint of the left upper arm had been marked. At each assessment, an intestinal permeability test was performed and a small blood sample (≈200 μL) was obtained by finger prick with use of a monolet (Monojector; Sherwood Medical Company, Ballymoney, United Kingdom).

Anthelmintic administration

At the first assessment, children in the treatment group were given 10 mg pyrantel pamoate/kg (Combantrin; Pfizer, Sandwich, United Kingdom) and the mothers were provided with plastic containers to collect all stools passed by their children over the next 24 h. This drug was selected for the initial treatment because it causes a more rapid elimination of *Ascaris* worms than does mebendazole. Furthermore, the worms are expelled intact, enabling an estimation of worm burden. Adult worms were extracted from feces with the naked eye and then washed, weighed, and counted. Lack of running water and poor sanitary facilities prevented the sieving of stools to extract small worms. Twenty-four–hour stool collection was also conducted in the placebo group at baseline to ensure that all procedures were the same for both groups and to maintain blinding to treatment status.

A single 500-mg dose of mebendazole (Vermox; Janssen, Wantage, United Kingdom) was used for subsequent bimonthly treatments because of the superior broad spectrum action on *Ascaris*, *Trichuris,* and hookworm. Identical placebo tablets, provided by Janssen, were given in the same dosage and frequency. The fieldworkers and the parents were blinded to the allocation of treatment and placebo tablets throughout the study. At the 12-mo follow-up, all children (treatment and placebo groups) were given pyrantel pamoate, and stools were collected for 24 h to assess worm burdens.

Fecal microscopy and egg counts

The parents were asked to collect a stool sample from their children early in the morning of the assessment day. A portion of this sample (0.5–1.5 g) was placed in a preweighed universal tube of 10% formalin in saline. The samples were transported to Dhaka for analysis at an international research laboratory. Quantitative fecal microscopy was conducted with use of a modification of the ether sedimentation technique (32). The laboratory technician conducting the microscopic analysis was also blinded to the study design and the treatment or placebo status of stool specimens. The number of helminth eggs was counted and the number of eggs per gram of feces was calculated from the volume of fluid examined and the weight of the stool specimen.

Intestinal permeability test

Dual sugar intestinal permeability was used to assess small intestinal function and integrity (25). The test uses lactulose and mannitol, sugars that are taken up from the gut and appear in urine without being metabolized. Monosaccharides, such as mannitol, are excreted in lower quantities in patients with mucosal pathology or damage of the small intestine, whereas the excretion of disaccharides, such as lactulose, is increased (26).

Children were given 4 g lactulose (Duphalac; Duphar, Southampton, United Kingdom) and 1 g mannitol (Sigma; Poole, Dorset, United Kingdom) dissolved in 20 mL water. This makes a sweet-tasting drink that is taken readily (33). Children were asked to refrain from eating 2 h before attending the clinic. In practice, this did not need to be enforced because the children either did not eat breakfast or they ate it very early (≈0600). The test was administered at ≈0930. For the following 2 h, the children refrained from all food but were encouraged to drink water. All urine produced over the next 5 h was collected and stored in plastic bottles containing a few drops of chlorhexidine gluconate (0.2%; wt:vol). Toward the end of the session, the children and their caregivers were provided with a popular local dish of meat, rice, and lentils. Total 5-h urine volume was measured and, after mixing, an aliquot was taken and stored at −20°C until analyzed in Cambridge, United Kingdom. Urinary concentrations of lactulose and mannitol were measured by automated enzymatic procedures on a centrifugal analyzer (Cobas Bio; Roche, Welwyn Garden City, United Kingdom) (33, 34).

Plasma proteins

The finger-prick blood sample was collected into a microtainer with lithium-heparin separator (Becton Dickinson, Oxford, United Kingdom), centrifuged, and stored at −20°C until analyzed in Cambridge, United Kingdom, at 1500 × *g* for 5 min at ambient temperature. Plasma proteins were determined by using immunoturbidometric techniques on the Cobas Bio centrifugal analyzer. Albumin was assayed by using antibodies from Incstar (Winnersh, Buckinghamshire, United Kingdom) and Seronorm standards (Nycomed, Birmingham, United Kingdom). ACT is a marker of the acute phase reaction that can indicate acute, chronic, and subclinical infections (35, 36). ACT was measured by using Dako (High Wycombe, Buckinghamshire, United Kingdom) antibodies with Serotec (Kidlington, Oxon, United Kingdom) calibrators according to the method of Calvin and Price (35). Total protein was measured by using an automated bicinchoninic acid procedure (Pierce and Warriner, Chester, United Kingdom).

Data analysis

z Scores of weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) were calculated by using the growth references of the WHO and the Centers for Disease Control and Prevention (ANTHRO, CDC, Atlanta). Statistical analysis was performed by using SPSS-PC (version 8.0, SPSS UK Ltd, Chertsey, United Kingdom). Growth was assessed by using the bimonthly increments in WAZ, HAZ, and WHZ and the final increases after 8 and 12 mo of deworming. Fecal-egg-count data were transformed to $\log_{10}$ (egg count + 1) to normalize the distribution (3). L:Ms displayed nonnormal distributions and these were transformed to ln L:Ms in line with other studies (28). The geometric mean of L:Ms is also provided in the tables. Changes from baseline to final measurement were examined by using within-subject (paired) *t* tests.

Between-group differences in growth and biochemical status were examined by using repeated-measures analysis of variance. For all variables examined, ϵ values indicated that the assumption of compound symmetry was not met; therefore, the Greenhouse-Geisser correction was made (37). This provides a conservative estimate of within-subject effects by correcting the df and corresponding *P* value while *F* values remain unaltered. The use of a different correction (eg, Huynh-Feldt) did not change the observed level of significance.

RESULTS

A total of 117 of 123 children completed the study. Four children moved out of the study site to live with relatives and 2 children withdrew for no specific reason. Of the 117 who completed the study, 84 (72%) attended all 6 assessments, 25 (21%) attended 5 of 6 assessments, and 8 (7%) attended 4 of 6 assessments. In almost all cases, failure to attend the clinic was due to absences from the village or family commitments and not to ill health. Children who missed their assessment day were given treatment or placebo tablets on a follow-up household visit. Statistical analyses were first conducted by including the 8 children who attended 4 of 6 assessments ($n = 117$) and were then repeated after these subjects were excluded ($n = 109$). Although the results were no different, this article presents the data after the exclusion of the 8 subjects with low attendance. Of the 109 children included in the analysis, some were unable to provide a stool or urine sample on a particular assessment day. In some cases, the blood collection was inadequate for laboratory analysis. The repeated-measures analyses included only the children who provided a sample at every assessment. Sample sizes varied for each measure and are indicated in the tables.

Characteristics of the treatment and placebo groups

At recruitment, the mean (±SD) ages of the children were 3.12 ± 0.1 y in the treatment group and 3.24 ± 0.63 y in the placebo group (NS). The groups had similar socioeconomic profiles, with nearly half of all heads of households being landless laborers (49% in the treatment group and 42% in the placebo group; $\chi^2 = 0.48$, NS). Among landowning households, the mean area of land owned was not significanlty different between the 2 groups. The proportion of boys and girls was significantly different in the 2 groups (35 boys and 19 girls in the treatment group; 20 boys and 35 girls in the placebo group; $\chi^2 = 9.51$, $P = 0.02$).

Parasites

The prevalence of *Ascaris lumbricoides*, *Trichuris trichiura,* hookworm, and *Giardia intestinalis* infection in the treatment and placebo groups as assessed by fecal microscopy is shown in **Table 1**. At the beginning of the study, *T. trichiura* infection was more common in the treatment group ($\chi^2 = 23.27$, $P < 0.001$). The initial prevalence of *A. lumbricoides* and hookworm infection was not significantly different between the groups. Mean egg counts, for infected individuals only, were as follows: *A. lumbricoides*, 1007 eggs/g of stool (epg); *T. trichiura*, 121 epg; and hookworm, 66 epg. These counts did not differ significantly between the treatment and placebo groups. The mean intensity of infection for all helminths was low by the WHO classification (cutoffs: *Ascaris,* <5000 epg; *Trichuris,* <1000 epg; and hookworm, <5000 epg; 38). Therefore, no treatment of children in the placebo group was required on medical grounds. The mean *A. lumbricoides* worm burden in the treatment group was 4.3 worms/child (maximum: 25).

In the treatment group, the prevalence of *A. lumbricoides* infection decreased to 8% after a single dose of anthelmintic (Table 1). The prevalence of *T. trichiura* and hookworm infections decreased more gradually. However, infection with *G. intestinalis* increased from 4% to 49% after 4 mo ($\chi^2 = 25.16$, $P < 0.001$).

TABLE 1
Percentage prevalence of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and *Giardia intestinalis* in the treatment and placebo groups at each assessment

	Treatment group[1]						Placebo group[2]					
	0 mo[3] (n = 46)	2 mo (n = 50)	4 mo (n = 51)	6 mo (n = 52)	8 mo (n = 52)	12 mo (n = 53)	0 mo (n = 45)	2 mo (n = 55)	4 mo (n = 53)	6 mo (n = 53)	8 mo (n = 52)	12 mo (n = 53)
	%						%					
Ascaris	78 [36]	8 [4]	2 [1]	4 [2]	4 [2]	17 [9]	71 [32]	74 [41]	73 [39]	57 [30]	67 [35]	75 [40]
Trichuris	65 [30]	37 [19]	25 [13]	14 [7]	10 [5]	8 [4]	16 [7]	11 [6]	27 [12]	11 [6]	15 [8]	15 [8]
Hookworm	4 [2]	0 [0]	4 [2]	2 [1]	0 [0]	4 [2]	11 [5]	13 [7]	17 [9]	13 [7]	11 [11]	21 [11]
Giardia	4 [2]	20 [10]	49 [25]	38 [20]	29 [14]	24 [13]	16 [7]	9 [5]	19 [10]	36 [19]	17 [9]	19 [10]

[1] The treatment group received a 500-mg dose of mebendazole at 0, 2, 4, 6, 8, and 12 mo.
[2] The placebo group received identical placebo tablets at 0, 2, 4, 6, and 8 mo followed by a single 500-mg dose of mebendazole after the 12-mo assessment.
[3] All stool samples were examined before administration of mebendazole or placebo.
[4] n in brackets.

In the placebo group, *Ascaris*, *Trichuris,* and hookworm infection remained unchanged and the prevalence of *Giardia* increased less dramatically to 19% after 4 mo (compared with 49% in the treatment group; $\chi^2 = 10.42$, $P < 0.01$). *Giardia* infection remained higher in the treatment group than in the placebo group for the remaining months of the study.

Twelve months after the first deworming, both groups were given anthelmintic treatment and *A. lumbricoides* worm loads were assessed by 24-h stool collection. The mean worm burden in the placebo group was 4.1 (maximum: 40) for infected cases. Of the 9 infected cases in the treatment group, 5 yielded small immature worms and 4 produced only 1 adult worm, representing recent and very light reinfections.

Anthropometry

The results of repeated-measures analysis of variance based on the bimonthly anthropometry from 0 to 8 mo and the final assessment at 12 mo are shown in **Table 2**. Between-group differences in growth were examined by testing for interaction between the groups over time (group × time). No such significant interaction was observed for WAZ or HAZ, indicating that growth patterns were the same in the treatment and placebo groups. A significant group effect was observed for height-for-age, with the treatment group having significantly greater height-for-age than the placebo group. The magnitude of this difference stayed constant from baseline to the final assessment (parameter estimates on all assessments: $0.05 > P > 0.01$) and was unrelated to treatment effects.

A significant interaction between groups over time was observed for MUAC (Table 2; $P = 0.01$). Arm circumference was significantly greater in the treatment group than in the placebo group at 2 and 4 mo but showed no significant difference between groups at the last 3 assessments. Hence, MUAC worsened in the placebo group from months 2 to 4 but improved subsequently.

Analysis of covariance was performed by using the general linear model to overcome any confounding effect of the initial differences in z scores in the treatment and placebo groups. z Score increments (ΔHAZ, ΔWAZ, and ΔWHZ) were examined by using baseline z scores as a covariate. All of the analyses showed that group (treatment compared with placebo) had no significant effect on ΔWAZ, ΔHAZ, or ΔWHZ after 8 or 12 mo. Furthermore, baseline nutritional status had no significant effect on growth increments over 8 or 12 mo. Therefore, better baseline nutritional status was not associated with significantly better or worse growth over the study period. In sum, there were no significant improvements in growth in children receiving a regular anthelmintic compared with those receiving a placebo.

Parasite infection and anthropometry

The relation between parasite infestation and growth was examined irrespective of treatment grouping. At the beginning and the end of the study, no significant differences in anthropometry were observed between helminth-infected and uninfected children, and there was no association between the intensity of

TABLE 2
Repeated-measures analysis of anthropometric status in the treatment and placebo groups

	Weight-for-age		Height-for-age[1]		MUAC[2]	
Month	Treatment (n = 46)	Placebo (n = 43)	Treatment (n = 43)	Placebo (n = 42)	Treatment (n = 49)	Placebo (n = 48)
0	−2.44 ± 0.11[3]	−2.69 ± 0.12	−2.31 ± 0.21	−3.03 ± 0.21	15.1 ± 0.13	14.8 ± 0.13
2	−2.41 ± 0.12	−2.57 ± 0.12	−2.29 ± 0.20	−3.04 ± 0.20[4]	14.8 ± 0.12	14.4 ± 0.13[4]
4	−2.43 ± 0.11	−2.69 ± 0.11	−2.37 ± 0.20	−2.99 ± 0.21[4]	14.8 ± 0.12	14.3 ± 0.13[4]
6	−2.44 ± 0.11	−2.65 ± 0.11	−2.31 ± 0.19	−2.99 ± 0.19[4]	14.7 ± 0.13	14.5 ± 0.13
8	−2.40 ± 0.11	−2.70 ± 0.11	−2.37 ± 0.19	−3.03 ± 0.19[4]	14.8 ± 0.14	14.7 ± 0.14
12	−2.27 ± 0.12	−2.39 ± 0.11	−2.28 ± 0.18	−2.89 ± 0.18[4]	15.3 ± 0.13	14.9 ± 0.14

[1] Significant group effect, $P = 0.016$.
[2] Group × time interaction, $P = 0.01$ (Greenhouse-Geisser correction). MUAC, midupper arm circumference.
[3] $\bar{x}$ ± SE.
[4] Significantly different from treatment, $P < 0.05$.

TABLE 3
Longitudinal analysis of hookworm infection and change in weight-for-age *z* score (ΔWAZ)

No. hookworm-positive stools	ΔWAZ			
	8 mo	*P*	12 mo	*P*
0 (n = 74)	0.004	0.05	0.22	0.03
1 (n = 10)	0.14		0.37[1]	
2 (n = 3)	0.10		0.40	
3–4 (n = 3)	−0.51		−0.27[1]	

[1] Significantly different from 8 mo, $P < 0.05$.

infection and nutritional status at baseline. However, longitudinal analysis of the effect of infection on growth performance did show some relations. Each subject was given a score between 0 and 4 for each parasite, corresponding to the number of assessments (excluding the initial visit) at which parasite eggs or cysts were detected in stools. Few children tested positive at all 4 examinations; therefore, children with 3 or 4 positive stools were grouped together. The parasite scores were then compared with anthropometric changes in each subject at 8 and 12 mo. With both groups pooled, children with ≥3 hookworm-positive stools had a smaller ΔWAZ at 8 and 12 mo (**Table 3**; $P = 0.05$ and $P = 0.03$, respectively). However, the number of children in this category was small and the relation was not linear (children with no hookworm infection showed smaller WAZ increments than did those with one positive stool). *A. lumbricoides* and *T. trichiura* showed no association with longitudinal growth, even when the children with giardiasis were excluded from the analysis.

Intestinal permeability

At the baseline assessment (pretreatment), there was no significant difference in intestinal permeability (ln L:M) between groups (**Table 4**). Repeated-measures analysis of variance was performed to examine changes over time. The group × time interaction was nonsignificant, as was the group effect alone. Seasonal variation in intestinal permeability was apparent; poorest intestinal status followed the monsoon period (month 8; Table 4). Within-subject analysis of permeability also showed no significant improvement of permeability values in either group from 0 to 12 mo (treatment group $n = 46$, NS; placebo group: $n = 47$, NS).

At the beginning of the study, the children with *Ascaris* infection (irrespective of grouping) had poorer permeability values than did those without *Ascaris* infection, but the difference was not significant (mean ln L:M: −1.43 in *Ascaris*-infected children compared with −1.62 in uninfected children; $t = -1.4$, NS). The permeability values for children with and without *Trichuris* and hookworm infections were very similar.

Plasma protein concentrations

The repeated-measures analysis of plasma albumin, ACT, and total protein concentrations in the treatment and placebo groups are shown in **Table 5**. Because of a centrifuge failure on the study site, samples taken from the first assessment (month 0) were lost.

No significant group × time interaction was observed for plasma albumin concentration. In the placebo group, plasma albumin increased significantly from month 2 to month 12 (mean difference = 2.14 g/L; $n = 54$, $P < 0.001$). No significant increase was observed in the treatment group. Both groups showed a seasonal decrease in albumin concentration at 6 and 8 mo but, on the whole, values were well maintained.

There were no significant relations between plasma albumin and any helminth infection except at month 4 when, with both groups pooled, *Ascaris*-infected children had significantly lower plasma albumin concentrations than did uninfected children (33.7 and 36.1 g/L, respectively; $t = 2.51$, $P < 0.05$).

The mean value of ACT at each assessment decreased to within the normal range of 0.2–0.6 g/L (32). Again, there was no significant interaction between ACT concentrations in each group over time (Table 5). Within-subject analysis showed a decrease in ACT concentration over the study period. This was significant in the treatment group (mean difference = 0.12 g/L; $n = 36$; $P < 0.001$) but not in the placebo group (mean difference = 0.04 g/L; $n = 54$; $P = 0.06$). No relation was observed between this acute phase protein and any parasitic infection at any time.

A significant group × time interaction was observed for total plasma protein concentration. Total protein was significantly higher in the treatment group at months 2 and 4 (Table 5) and decreased thereafter relative to the placebo group. From month 2 to month 12, total protein decreased significantly in the treatment group (mean difference = 5.51 g/L; $n = 37$, $P < 0.001$). There was no similar reduction in children receiving the placebo. However, total protein concentration showed no association with the presence or absence of any parasite.

Giardia infection

The rapid increase in *Giardia* infection in the treatment group soon after the initial deworming was examined for possible effects on growth and intestinal permeability. The treatment group was divided into children with and without *Giardia* infection in month 4 of the study. The children with *Giardia* had significantly poorer growth in WAZ and WHZ from 2 to 4 mo and significantly poorer permeability in month 4 than did the uninfected children (**Table 6**). Significantly worse permeability was again observed in *Giardia*-infected and -uninfected children within the treatment group at month 8 (ln L:M = −1.19 and −1.44, respectively; $P < 0.05$). *Giardia* infection was not associated with growth, permeability, or plasma proteins at other assessments.

Relations with growth

Although small intestinal permeability and plasma protein concentrations showed no relation with parasitic infections, these variables were significantly associated with growth during the study. ΔHAZ was significantly correlated with ln L:M ($r = -0.19$, $P < 0.05$ and $r = -0.22$, $P < 0.02$ at 8 and 12 mo, respectively). Similarly, greater increases in WAZ were associated with better intestinal permeability values ($r = -0.21$, $P < 0.02$ and $r = -0.21$, $P < 0.05$ at 8 and 12 mo, respectively). Plasma

TABLE 4
Repeated-measures analysis of the ratio of lactulose to mannitol (L:M) in the treatment and placebo groups

Month	Treatment (n = 34)		Placebo (n = 32)	
	ln L:M ± SE	L:M	ln L:M ± SE	L:M
0	−1.53 ± 0.09	0.22	−1.40 ± 0.09	0.25
2	−1.48 ± 0.09	0.23	−1.56 ± 0.09	0.21
4	−1.36 ± 0.07	0.26	−1.31 ± 0.08	0.27
6	−1.49 ± 0.08	0.22	−1.38 ± 0.09	0.25
8	−1.34 ± 0.08	0.26	−1.01 ± 0.09	0.36
12	−1.77 ± 0.08	0.17	−1.48 ± 0.09	0.22

TABLE 5
Repeated-measures analysis of plasma protein concentrations in the treatment and placebo groups[1]

	Albumin		ACT		Total protein	
Month	Treatment (n = 36)	Placebo (n = 35)	Treatment (n = 35)	Placebo (n = 35)	Treatment (n = 36)	Placebo (n = 35)
2	38.15 ± 0.62	37.61 ± 0.63	0.58 ± 0.027	0.57 ± 0.027	75.4 ± 0.082	70.6 ± 0.83[2]
4	36.61 ± 0.67	34.54 ± 0.68	0.53 ± 0.023	0.51 ± 0.023	74.7 ± 1.17	70.9 ± 1.19[3]
6	35.63 ± 0.52	35.17 ± 0.52	0.55 ± 0.024	0.54 ± 0.024	65.8 ± 0.90	66.3 ± 0.92
8	34.78 ± 0.74	34.33 ± 0.75	0.54 ± 0.019	0.54 ± 0.019	64.5 ± 1.42	63.9 ± 1.43
12	39.14 ± 0.45	39.56 ± 0.45	0.46 ± 0.014	0.50 ± 0.014	69.9 ± 0.77[4]	69.9 ± 0.78

[1] $\bar{x}$ ± SE. ACT, α_1-antichymotrypsin.
[2,3] Significantly different from treatment: [2] $P < 0.001$, [3] $P < 0.05$.
[4] Group × time interaction, $P < 0.05$ (Greenhouse-Geisser correction).

albumin concentration was positively correlated with HAZ values at 8 and 12 mo ($r = 0.212$, $P < 0.05$; $r = 0.177$, $P < 0.05$, respectively) but not with ΔHAZ.

DISCUSSION

This is the first longitudinal study to examine physiologic and biochemical responses to anthelmintic treatment among children in a community setting. In contrast with some earlier studies (13, 15), anthelmintic administration successfully reduced the prevalence of *A. lumbricoides*, *T. trichiura,* and hookworm infection to very low rates. Despite this, treated children did not show any significant improvements in growth. This result is in keeping with the concurrent study of 1402 children that did not detect any growth improvements, even after 18 mo of regular deworming (23).

Deworming treatment was randomized by village rather than by individual, representing a necessary compromise between scientific, ethical, and logistic needs; this is a recognized problem of research in this field (39). The same design was used in Myanmar, where significant growth improvements were observed (9). In fact, in the Myanmar study, placebo tablets were not administered because of the negative response induced by ineffective tablets and fears of withdrawal from the study. Therefore, the negative findings of the present study cannot be attributed to study design alone. The village design did result in significant differences in HAZ. However, these differences were not associated with better or worse growth over the study period. The baseline prevalence of *Trichuris* infection was also significantly different between the 2 groups. However, even when treatment is randomized by individual, highly significant differences between groups can arise; this was the case for hookworm prevalence and intensity in a Kenyan study (8).

The sudden increase in *Giardia* infection in the treatment group was an unexpected outcome that, arguably, may have negated any benefit of deworming (40). This finding is not explained readily because there are no previous reports of an increase in *Giardia* infection after anthelmintic treatment. The treatment villages were interspersed with placebo villages and it is therefore unlikely that this was an outbreak affecting only the treatment villages. Some of the increase may be attributable to the monsoon (June–September), but this would have affected all the villages equally. Furthermore, *Giardia* infection remained high in the treatment group long after the monsoon. *Giardia* infection appears to have had short-term effects on weight gain and permeability in the treatment group but did not result in any long-term growth retardation. Intestinal colonization by other pathogens after deworming is an area worthy of future investigation

Higher intestinal permeability was associated with poorer growth, in accord with results from the Gambia (27). In addition, poorer permeability was significantly associated with reported episodes of diarrhea (41) and thus sensitive to short-term changes in gut function. Seasonal changes in permeability were apparent, but no change in permeability was linked to treatment. The elevated permeability ratios of both groups indicate that mucosal status was poor compared with United Kingdom values of ≈0.15 (25) but were better than the values from an earlier survey of children in the same area ($\bar{x}$: 0.43) (22).

Unlike in the study by Northrop et al (22), albumin concentration did not increase significantly after treatment. However, children without *Ascaris* infection did have significantly higher albumin concentrations in month 4 (both groups pooled), indicating that short-term improvement may have occurred. The prevalence of helminths was similar in the 2 studies, as were pretreatment albumin concentrations (22), but the earlier study looked at changes only 9–14 d after deworming, and it is not known whether this increase was sustained.

Anthelmintic treatment did result in small improvements in the plasma concentrations of both ACT and total protein. Only the decrease in total protein, however, was significant in the repeated-measures analysis. Reduced concentrations of acute phase protein would be in keeping with a reduction in systemic inflammatory stress after deworming. Given that albumin concentrations did not change, the decrease in total plasma protein concentration may have reflected a decrease in immunoglobulin concentration. This would also be in keeping with a decreased antigenic challenge after worm expulsion. However, it was sur-

TABLE 6
Effect of *Giardia intestinalis* infection on children in the treatment (mebendazole) group in month 4 of the study[1]

Treatment group only	*Giardia* absent (n = 26)	*Giardia* present (n = 25)	P
Weight gain (kg)	0.281 ± 0.31[2]	−0.095 ± 0.30	0.038
ΔWAZ	−0.050 ± 0.19	−0.081 ± 0.19	0.02
Height gain (cm)	0.73 ± 0.71	0.965 ± 0.70	NS
ΔHAZ	−0.110 ± 0.185	−0.040 ± 0.189	NS
ΔWHZ	0.11 ± 0.281	−0.093 ± 0.27	0.016
Intestinal permeability (ln lactulose: mannitol)	−1.60 ± 0.40	−1.16 ± 0.48	0.001

[1] WAZ, weight-for-age z score; HAZ, height-for-age z score; WHZ, weight-for-height z score.
[2] Growth increment (±SD) over months 2–4 by t test.

prising that neither of these measurements could be related to the presence or absence of any parasite.

In sum, helminth infection appears to play a minor role in the poor growth and nutritional status of children in this population of northern Bangladesh. The lack of improvement may be attributed to a low intensity of helminth infection. This contrasts with the high intensities reported in studies that observed growth improvements (8, 9). In the present study, hookworm had a low prevalence and intensity, yet continuous infection was weakly associated with poorer weight gain. *Ascaris* and *Trichuris*, the most common helminths, were not associated with impaired growth. Elsewhere, improvements in growth after deworming have been attributed largely to the removal of hookworm (8, 10) and fewer studies showed health improvements in children with only *Ascaris* and *Trichuris* infection (9, 11). One study with extremely high prevalence and intensity rates of *Ascaris* infection showed only marginal improvements in child weight after deworming (16). A lack of improvement was also reported after anthelmintic treatment and iron supplementation were given to Indonesian children with *Ascaris* and *Trichuris* infection (17).

The null effect of deworming in this study could perhaps have stemmed from an inadequate sample size or duration of follow-up. However, significant improvements were observed in studies with smaller samples (eg, $n = 23$, $n = 55$, and $n = 72$; 42, 10, 11) and of much shorter duration (7 and 9 wk; 42, 10, 11). One might also argue that the benefits of deworming would have been greater in school-aged children, who are often reported to have the highest prevalence and intensity of infection (8–11). However, an earlier survey in northern Bangladesh found no increase in the prevalence or intensity of helminth infection from 4 to 15 y of age (21).

In contrast with the null effect of deworming, weight gain, MUAC, albumin concentration, ACT concentration, and intestinal permeability were all significantly associated with child morbidity and socioeconomic variables (24, 41). This suggests that these measures are highly sensitive to short-term changes in health status, leaving little doubt that improvements from deworming, had there been any, would have been detected.

In conclusion, anthelmintic treatment with successful worm expulsion resulted in improvements in ACT and total protein but no improvements in growth. The question to be answered is not whether these parasites have the potential to retard growth but whether routine anthelmintic prophylaxis substantially improves growth and nutritional status of malnourished children under the prevailing conditions. On the basis of this study, the answer is "No," and other factors must be sought to explain the poor growth performance of these preschool children.

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