Hookworm Infection among School Age Children in Kintampo North Municipality, Ghana: Nutritional Risk Factors and Response to Albendazole Treatment

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Abstract. Children (n = 812) 6–11 years of age attending 16 schools in the Kintampo North Municipality of Ghana were screened for participation in a study on hookworm infection, nutrition, and response to albendazole. The prevalence of Necator americanus hookworm infection (n = 286) was 39.1%, and significant predictors of infection included age, malaria parasitemia, lack of health care, school area, levels of antibodies against hookworm, and low consumption of animal foods. The cure rate after a single dose (400 mg) albendazole was 43%, and the mean fecal egg count reduction rate was 87.3%. Data for an in vitro egg hatch assay showed a trend toward reduced albendazole susceptibility in post-treatment hookworm isolates (P = 0.06).

INTRODUCTION

More than half a billion persons worldwide, including 156 million children, are infected with blood-feeding hookworms. Hookworm infection increases risk of anemia and causes negative effects on growth, iron status, and cognition in children with high intensity infections. Hookworm is most prevalent in resource poor areas where limited access to sanitation, sewage treatment, and use of night soil in agriculture contribute to transmission. Although rarely fatal, the morbidity associated with chronic infection may increase susceptibility to other tropical diseases, including HIV, tuberculosis, and malaria.

Mass drug administration (MDA) of anthelmintics is a cornerstone of the World Health Organization (WHO) strategy for integrated control of seven neglected tropical diseases. With regard to hookworm, long-term follow-up from semi-annual treatment programs of pre-school age and school age children shows a decrease in intensity of infection (≥ 80% in Zanzibar), but smaller effects on prevalence. Similar results from early studies suggested that MDA decreases intensity and prevalence of infection over time, although a recent Cochrane review concluded that there is limited evidence of consistent effects of MDA on nutrition and cognition among school children. This recent suggestion that the benefits of school based deworming may be overstated has generated significant dialogue regarding the potential for MDA to control geohelminth infections globally.

In 2007, a community-based study in the Kintampo North Municipality (KNM) of Ghana identified a hookworm prevalence of 45% (n = 292), most notable for the absence of other geohelminths. Adults with hookworm had a significantly lower average body mass index (BMI), despite the predominance of low intensity infection (< 1,000 eggs per gram [epg] of feces). In children, we noted a hookworm prevalence of 56%, and many were co-infected with Plasmodium falciparum. Among all persons treated with single-dose albendazole (400 mg), we observed a cure rate of 61% and an individual arithmetic mean fecal egg count reduction (FECR) rate of 82%, both of which were lower than anticipated.

We report results of a follow up study conducted in KNM in 2010. The purpose of this study was to validate treatment results from our previous study and test the hypothesis that nutrition predicts infection status and response to single-dose albendazole. The field study also provided an opportunity to implement in vitro testing of hookworm isolates for susceptibility to albendazole, thereby providing baseline data to track emerging resistance in KNM.

METHODS

Ethical approval. This study was approved by the Yale University Human Investigations Committee and the Institutional Review Boards at the Noguchi Memorial Institute for Medical Research, the Ghana Health Service, and the Scientific Review Committee and the Institutional Ethics Committee at the Kintampo Health Research Center. In addition, District Ministry of Health representatives and District Ministry of Education representatives approved the study and assisted in communicating with participating schools. Participant enrollment. In June 2010, 16 schools located along a 90-km stretch of the major highway north of Kintampo were invited to participate in a research study. Each school provided a list of all students 6–11 years of age. Duplicate height and weight measurements were obtained for each child by using a stadiometer and electronic scale. Height for age (HAZ), weight for age (WAZ), and BMI for age (BAZ) z-scores were calculated for each participant by using the WHO anthropometric calculator (Anthro Plus version 1.0.3, http://who-anthropplus.software.informer.com/1.0/). Students with HAZ ≤ −1.80 or HAZ ≥ −0.10 were invited to participate in the study. One child was randomly selected from each household to eliminate the potential for clustering effects at the household level. HAZ cutoffs for inclusion were derived from the screening sample to obtain a final sample size of 300 persons (with an extra 10% included at the initial selection to enable nonparticipation) from the 812 screened. In the selected group of children, approximately half had low HAZ.
scores and half had high or normal HAZ scores. This sampling method was designed to investigate the relationship between chronic undernutrition (stunting) and prevalence of hookworm infection within a geographic region by using a sample size commensurate with the resources of the study team. After receipt of permission from community leaders, teachers were asked to invite the parents of the potential participants for a meeting to explain the study and ask for consent. The final study population consisted of 286 participants from 16 communities (Figure 1).

**Sample collection and processing.** Fecal collection containers and directions for use were distributed to individual students, and collected the following day. After receipt of the first fecal sample, students were asked to provide a second fecal sample the following day. Microscopy was used to identify and count parasite ova by using the Kato-Katz method (Vestergaard-Frandsen, Lausanne, Switzerland) as outlined by WHO.22 Hookworm eggs were purified from positive fecal samples by serial suspension and centrifugation in 0.9% NaCl, 0.015% Brij-35, and 2.18 M NaNO3 (specific gravity = 1.185), followed by filtration through an 80-μm filter, using the method reported by Reiss and colleagues.23 Egg concentrations were adjusted to 50 eggs/100 μL for use in the egg hatch assay (EHA; see below); remaining eggs were frozen for DNA analysis. Each hookworm infected child was referred for single dose of albendazole treatment (400 mg) (Wormzap; GR Industries Limited, Accra, Ghana) under direct observation. Ten to fourteen days after treatment, fecal samples were collected for two consecutive days and analyzed as described above. The FECR rates were calculated as individual and group arithmetic means by using the methods recommended by Vercruysse and others.24

**Egg hatch assay: analysis of in vitro anthelmintic activity of albendazole.** The susceptibility of human hookworm isolates to albendazole was assessed before and after treatment using an in vitro EHA.25,26 Stock solutions of albendazole (Sigma, St. Louis, MO) were prepared in methanol (5 mg/mL) and further diluted in distilled water. Approximately 50 eggs isolated from individual study participants (n = 71) were suspended in a final volume of 100 μL and added to individual wells of a 96 well microtiter plate containing 100 μL of albendazole solution (final concentrations = 0, 0.1, 1, 2, and 5 μg/mL). Plates were incubated at ambient temperature, and after 48 hours the number of hatched first-stage hookworm larvae was counted using light microscopy. When post-treatment fecal egg counts showed a sufficient numbers of eggs, susceptibility was also evaluated from samples collected after albendazole treatment (n = 14). Samples in which the positive control wells (no albendazole) showed a hatch rate < 75% rate were excluded from analysis.

**Speciation of hookworm isolates using polymerase chain reaction.** Genomic DNA was extracted from frozen hookworm eggs collected from 103 persons using the QIAamp DNA stool kit (QIAGEN, Carlsbad, CA). A nested polymerase chain reaction (PCR) amplification method27 was used to amplify species-specific sequences within the internal transcribed spacer of ribosomal DNA. Reaction products were subjected to agarose gel electrophoresis, and successful amplification was indicated by an 870-base pair product for *Ancylostoma duodenale*. The PCR products were excised from the gel and the DNA extracted by using QIAGEN gel extraction kit reagents and protocols (QIAGEN). The DNA sequence was confirmed in a subset of PCR products.

**Blood and serum analysis.** Approximately 1 mL of blood was aliquoted for automated complete blood count analysis conducted at the Kintampo Health Research Center. A malaria rapid diagnostic test kit (First Response Malaria Ag HRP-2; Premier Medical Corporation Ltd., Watchung, NJ) was used to test for *P. falciparum* antigens. Among children who had a positive result for the rapid diagnostic test, thick and thin blood smears were prepared to verify the presence of *Plasmodium* spp. by using light microscopy according to WHO recommendations.22 All study participants were asymptomatic for malaria at the time of screening. Antigen-specific IgG responses against *A. ceylanicum* adult worm excretory–secretory (ES) proteins were measured using a described enzyme-linked immunosorbent assay (ELISA).21,28 Levels of serum reactivity (optical density at 405 nm) for the study population were categorized by quartile for statistical analysis. One community chose not to provide blood samples and was excluded from analysis.

**Household questionnaire.** The survey instrument was adapted from the Demographic and Health Surveys, the Household Dietary Diversity Score for Measurement of Household Food Access: Indicator Guide, and the Escala Latinoamericana y Caribena de Seguridad Alimentaria Household Food Insecurity scale.29-31 The survey instrument was translated into the local

![Figure 1](image-url) Participation selection, recruitment and data collection, Ghana. HAZ = height for age.
language (Twi) and back-translated into English by native speakers to confirm accuracy. Questions included birth date, household socioeconomic characteristics, parental education and occupation, water and sanitation access, access to health care and vaccinations, history of anthelmintic treatment, bed net use, food insecurity, hunger, and dietary diversity. Interviewers from the Rural Health Training School in Kintampo administered the surveys in the local language. A list of household assets was used to construct an absolute wealth index based on the number of assets, including a tile floor, use of advanced cooking fuel (not charcoal, straw or wood), electricity, radio, TV, phone, refrigerator, bike, car or motorcycle, land, cow, horse or donkey, goat or sheep, pig, poultry, bank or savings account, improved water source, improved toilet). The wealth index was tested in the models as a continuous variable and a categorical variable (tertiles of wealth).

Determination of anthropometric status. Child birth dates were confirmed with a household member as part of the household survey. In cases of discordance between dates provided by households and schools, analyses were based on the household birth date when available, and the school birth date if no household birth date was known. A categorical age variable was used to minimize the effect of potential misclassification. An examination of the effects of age on HAZ, WAZ, and BAZ showed that BAZ was less affected by age variation in children 6–11 years of age, and thus was the most robust measure of nutritional status given the known error in the age data. Therefore, HAZ and WAZ were not included in the analysis.

Dietary diversity, household food insecurity, and consumption of animal source foods. Children and caregivers were asked about consumption of foods the previous day and the previous week. Eleven nutrient-rich food groups (excluding condiments and sugar) were transformed into a binary dietary diversity variable, with participants either above or below the group mean. Consumption of animal source foods (ASF) were extracted into a subscale, and transformed into a binary ASF variable with participants above or below the group mean. Household food insecurity was categorized according to standard methods for the Latin American and Caribbean Food Insecurity Scale, which has been used in Ghana. A hunger subscale was extracted from the Escala Latinoamericana y Caribena de Seguridad Alimentaria questions, which included five questions pertaining to reductions in food quantity.

Statistical analysis. All data generated from this study were stored in Microsoft (Redmond, WA) Excel (2007), and statistical analysis was conducted with SPSS (Chicago, IL) versions 17.0 and 19.0. An initial review of descriptive statistics preceded bivariate analysis of factors to determine their relationship to baseline hookworm infection and response to single-dose albendazole. Initial variables tested were derived from a theoretical framework of factors supported by the literature to affect exposure and susceptibility to hookworm infection. Variables representing key constructs from the theoretical framework were analyzed to identify a subset for analysis that minimized the effects of collinearity. Salient variables from the univariate analysis were used in a multivariate analysis adjusting for clustering within schools by applying logistic regression analyses within generalized estimating equation algorithms. In addition to controlling for the school effect, schools were grouped into four geographic clusters of four schools each that were used in the analysis. Final models included control variables and variables of statistical significance (P < 0.05). Nonparametric analysis (Mann-Whitney U test, Kruskal Wallis test) was used when comparing non-normally distributed variables, including baseline epg, mean cell volume, and red cell distribution width.

RESULTS

Intestinal parasite assessment of study population. Fecal samples were obtained from 279 persons, representing a range of 7–36 students/school. The overall prevalence of hookworm in the study population was 39.1% (109/279), and of those that were infected, 82.6% (90/109) were classified as having light infections (<1,000 epg). Other intestinal parasites were present in 15% of the population, including *Hymenolepis nana* (10.7%), *Taenia* spp., and other unidentified tapeworm species. *Ascaris lumbricoides* and *Trichuris trichiura* were not detected. The prevalence of hookworm infection varied significantly across the schools, ranging from 15% to 70%.

Socioeconomic status and risk of hookworm infection. Fewer than half of the households used an improved water source (well, rainwater, borehole, spring) or latrine (Table 1). Ownership of mosquito nets was high (78%). Most of the male and female household heads (80%) and female caregivers (63%) were farmers. More than two-thirds of the female caregivers (69.7%) and heads of household (67.3%) reported no schooling. Among the socioeconomic variables considered, parental occupation (farmer), larger household size, and ownership of a pig were associated with significantly higher prevalence of hookworm infection in bivariate analyses (Table 1). Children who had not recalled seeing a health care provider within the past year had significantly higher prevalence of hookworm infection (Table 1), and this effect remained significant in multivariate analysis (Table 2).

Anthropometric and nutritional indicators. Only 3.5% (10) of the participants had BAZ scores < –2.0. Almost all (≥ 95%) of the children reported consuming grain, vegetables, roots/tubers, and fish on at least a weekly basis, and ≥ 85% reported having consumed those food groups the previous day. Seventy-nine percent of the households reported some food insecurity, and > 30% reported moderate-to-severe food insecurity (Table 3). Animal source foods (meat, organ meats, eggs, dairy, and fish) showed significant variation with household food security and wealth (P < 0.05). The average blood hemoglobin level among study participants was 10.5 g/dL, and 71.8% of the children were below the WHO designation for anemia (11.5 g/dL). Compared with children with blood hemoglobin levels ≥ 11.5 g/dL, those with levels < 11.5 g/dL had significantly lower mean cell volumes (80.5 versus 83.3; P < 0.01) and significantly higher mean RDWs (14.0 versus 13.5; P < 0.05), which is consistent with microcytic or iron deficiency anemia.

Nutritional status, dietary intake, and risk of hookworm infection. No anthropometric measures were associated with hookworm infection at baseline, including BAZ (Table 3). However, lower food insecurity and above average consumption of ASF were each associated with a reduced odds of hookworm infection (P < 0.01 for both). The relationship between ASF consumption and hookworm infection remained significant in multivariate analysis, with children in the below average ASF group having a three-fold higher risk for hookworm infection at baseline (P < 0.001) (Table 2).

Malaria infection. Using the malaria rapid diagnostic test kit, we found that 84.7% (210/249) of participants were positive...
likely to have hookworm infection. Based on univariate analysis, children with malaria were more specifically identified as P. falciparum and all were asymptomatic. The malaria species was definitively associated with hookworm infection status. In multivariate analysis, malaria infection remained significantly associated with hookworm infection, with the odds ratio of 0.001 times more likely to be infected at baseline compared with those in the lowest quartile of antibody responses. This result is consistent with our prior investigation of the correlation between antibodies to A. ceylanicum ES and hookworm infection status.

**Response to treatment with albendazole.** One hundred nine children who were positive for hookworm infection at baseline were treated with a single 400-mg oral dose of albendazole and 107 (96.4%) provided follow-up fecal samples. As shown in Table 4, the albendazole cure rate was 43% (46 of 107), and all children had < 1,000 epg at follow-up. The FECR rate was 87.3%, based on the arithmetic mean of group egg counts before and after treatment, and 70.3% using individual arithmetic means. There was a slightly higher baseline epg in persons who remained positive post-treatment (P = 0.045) based on a nonparametric rank test. However, analysis based on a theoretical framework of predictors of response to albendazole did not identify any significant predictors of response to treatment among the variables collected. The BMI for age score was not associated with response to treatment, nor were household food security, hunger, dietary diversity, or pre-treatment anti-hookworm antibody levels.

**In vitro susceptibility of hookworm isolates.** An in vitro EHA was used to characterize the susceptibility of hookworm

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**Table 1** Socioeconomic and health indicators of study population for hookworm infection, Ghana*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Hookworm negative (n = 170)</th>
<th>Hookworm positive (n = 109)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 8 years</td>
<td>32.4 (91)</td>
<td>37.6 (64)</td>
<td>24.3 (27)</td>
<td>0.056</td>
</tr>
<tr>
<td>Age 8–9 years</td>
<td>34.2 (96)</td>
<td>32.9 (56)</td>
<td>36.9 (41)</td>
<td>0.469</td>
</tr>
<tr>
<td>Age ≥ 10 years</td>
<td>33.1 (93)</td>
<td>29.4 (50)</td>
<td>38.7 (43)</td>
<td>0.589</td>
</tr>
<tr>
<td>Female</td>
<td>51.6 (145)</td>
<td>55.3 (94)</td>
<td>45.9 (51)</td>
<td>0.432</td>
</tr>
<tr>
<td>Improved water sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td>45.9 (129)</td>
<td>47.6 (81)</td>
<td>43.2 (48)</td>
<td>0.661</td>
</tr>
<tr>
<td>Cooking</td>
<td>43.4 (122)</td>
<td>44.7 (76)</td>
<td>41.4 (46)</td>
<td>0.219</td>
</tr>
<tr>
<td>Bathing</td>
<td>43.4 (122)</td>
<td>45.3 (77)</td>
<td>40.5 (45)</td>
<td>0.340</td>
</tr>
<tr>
<td>Use of a latrine</td>
<td>34.9 (98)</td>
<td>35.9 (61)</td>
<td>33.3 (37)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mosquito net ownership</td>
<td>77.2 (217)</td>
<td>75.3 (128)</td>
<td>80.2 (89)</td>
<td>0.219</td>
</tr>
<tr>
<td>Bicycle</td>
<td>85.1 (239)</td>
<td>82.9 (141)</td>
<td>88.3 (98)</td>
<td>0.314</td>
</tr>
<tr>
<td>Car/motorcycle</td>
<td>19.6 (55)</td>
<td>18.8 (32)</td>
<td>20.7 (23)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pig</td>
<td>12.1 (34)</td>
<td>6.5 (11)</td>
<td>20.7 (23)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bank account</td>
<td>44.5 (125)</td>
<td>42.9 (73)</td>
<td>46.8 (52)</td>
<td>0.520</td>
</tr>
<tr>
<td>Household head occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>80.4 (226)</td>
<td>75.7 (128)</td>
<td>88.3 (98)</td>
<td>0.023</td>
</tr>
<tr>
<td>Small trader/other</td>
<td>14.2 (40)</td>
<td>17.2 (29)</td>
<td>9.9 (11)</td>
<td>0.219</td>
</tr>
<tr>
<td>None</td>
<td>5.0 (14)</td>
<td>7.1 (12)</td>
<td>1.8 (2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Household head schooling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No schooling</td>
<td>67.3 (189)</td>
<td>63.0 (104)</td>
<td>76.6 (85)</td>
<td>0.076</td>
</tr>
<tr>
<td>Primary school</td>
<td>11.4 (32)</td>
<td>13.3 (22)</td>
<td>9.0 (10)</td>
<td>0.219</td>
</tr>
<tr>
<td>More than primary school</td>
<td>29.5 (55)</td>
<td>23.6 (39)</td>
<td>14.4 (16)</td>
<td>0.002</td>
</tr>
<tr>
<td>Female caregiver occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>63.2 (175)</td>
<td>53.9 (89)</td>
<td>80.4 (86)</td>
<td>0.001</td>
</tr>
<tr>
<td>Small trader/other</td>
<td>26.7 (74)</td>
<td>35.8 (59)</td>
<td>14.0 (15)</td>
<td>0.001</td>
</tr>
<tr>
<td>None</td>
<td>8.3 (23)</td>
<td>10.3 (16)</td>
<td>5.6 (6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Female caregiver schooling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No schooling</td>
<td>79.4 (193)</td>
<td>77.1 (111)</td>
<td>82.4 (84)</td>
<td>0.105</td>
</tr>
<tr>
<td>Primary school</td>
<td>14.0 (34)</td>
<td>13.2 (19)</td>
<td>15.7 (16)</td>
<td>0.001</td>
</tr>
<tr>
<td>More than primary school</td>
<td>7.6 (16)</td>
<td>9.7 (14)</td>
<td>2.0 (2)</td>
<td>0.001</td>
</tr>
<tr>
<td>No. children ≤ 5 years of age</td>
<td>25.6 (72)</td>
<td>32.4 (55)</td>
<td>15.3 (17)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1 child ≤ 5 years of age</td>
<td>32.4 (91)</td>
<td>34.1 (58)</td>
<td>29.7 (33)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 2 children ≤ 5 years of age</td>
<td>42.0 (118)</td>
<td>33.5 (57)</td>
<td>55.0 (61)</td>
<td>0.001</td>
</tr>
<tr>
<td>Above average wealth</td>
<td>49.5 (139)</td>
<td>48.2 (82)</td>
<td>51.7 (57)</td>
<td>0.558</td>
</tr>
<tr>
<td>Household size</td>
<td>8.1 ± 4.1</td>
<td>7.51 ± 3.8</td>
<td>8.95 ± 4.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Mosquito nets/person</td>
<td>0.39 ± 0.20</td>
<td>0.41 ± 0.2</td>
<td>0.37 ± 0.2</td>
<td>0.174</td>
</tr>
<tr>
<td>Deworming in the last year</td>
<td>15.4 (42)</td>
<td>19.3 (32)</td>
<td>9.3 (10)</td>
<td>0.002</td>
</tr>
<tr>
<td>Health care access within last year</td>
<td>50.0 (132)</td>
<td>70.5 (93)</td>
<td>29.5 (39)</td>
<td>0.002</td>
</tr>
<tr>
<td>More than one year</td>
<td>33.5 (88)</td>
<td>54.5 (48)</td>
<td>45.5 (40)</td>
<td>0.001</td>
</tr>
<tr>
<td>Never</td>
<td>16.7 (44)</td>
<td>43.2 (19)</td>
<td>56.8 (25)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Cells indicate the proportion of children that are hookworm negative (or positive) for each characteristic. Values are % (no.) for categorical variables and mean ± SD for continuous variables.

†By chi-square test or t test.

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and all were asymptomatic. The malaria species was definitively identified as P. falciparum on 97.5% of the smears. Based on univariate analysis, children with malaria were more likely to have hookworm infection (P = 0.026), and the prevalence of co-infection in the study population was 35.1%. In multivariate analysis, malaria infection remained significantly associated with hookworm infection, with the odds ratio for hookworm infection increasing with higher malaria parasite density (Table 2).

**Hookworm antibody levels.** The level of serum IgG against A. ceylanicum excretory secretory antigens was also positively associated with hookworm infection status (P < 0.001, by univariate analysis). As shown in Figure 2, in each of the four regions surveyed, the level of antigen-specific IgG, as measured by serum ELISA, was lower among those who were negative for hookworm at baseline compared with those who were infected. Based on multivariate analysis (Table 2), those with antibody levels in the second, third, or fourth highest quartile were 2.22 (P = 0.25), 6.47 (P = 0.001), or 11.76 (P < 0.001) times more likely to be infected at baseline compared with those in the lowest quartile of antibody responses. This result is consistent with our prior investigation of the correlation between antibodies to A. ceylanicum ES and hookworm infection status.21
isolates to albendazole.\textsuperscript{38} As shown in Figure 3, pre-treatment samples (n = 71) exhibited reduced overall hatching rates in the presence of increasing concentrations of albendazole, ranging from a mean ± SD hatch rate of 92.1 ± 8.0% at 0.1 \( \mu \)g/mL to 23.2 ± 17.2% at 5 \( \mu \)g/mL. At the highest concentration of drug (5 \( \mu \)g/mL), the median hatch rate for post-treatment samples was 2.3 fold higher in post-treatment samples than in those collected before treatment (34.0% versus 14.8% (Figure 3). The differences in hatch rate were not statistically significant (\( P = 0.06 \)), presumably because of the small number of post-treatment samples (n = 10), we observed an increase (\( P < 0.05 \)) in hatch rate at 5 \( \mu \)g/mL of albendazole in three samples, a decrease in three samples, and no substantial change in four samples. However, a similar disparity in egg hatch rates was also noted at 2 \( \mu \)g/mL of albendazole (73.6% in post-treatment samples versus 50.6% in pre-treatment samples), suggesting that exposure to albendazole may select for parasites with reduced susceptibility to albendazole.

**Speciation of hookworm isolates using PCR.** To define the predominant hookworm species causing infection in KNM, genomic DNA was extracted from hookworm eggs isolated from persons who were positive by fecal microscopy. Coding sequences corresponding to the internal transcribed spacer 2 region of ribosomal DNA were amplified by using a nested PCR protocol.\textsuperscript{27} Definitive speciation results were obtained from 94 (91.2%) of 103 samples that were tested by PCR. All 94 PCR results identified *N. americanus*, and two persons (1.9%) were negative.

<table>
<thead>
<tr>
<th>Table 2 Risk factors for hookworm infection, Ghana*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>Age ≤ 7 years (reference)</td>
</tr>
<tr>
<td>Age 8–9 years</td>
</tr>
<tr>
<td>Age ≥ 10 years</td>
</tr>
<tr>
<td>Wealth (reference = lowest tertile)</td>
</tr>
<tr>
<td>Highest tertile</td>
</tr>
<tr>
<td>Middle tertile</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Health care (reference = within 1 yr)</td>
</tr>
<tr>
<td>&gt; 1 year</td>
</tr>
<tr>
<td>Never</td>
</tr>
<tr>
<td>School Region South (reference)</td>
</tr>
<tr>
<td>Mid South</td>
</tr>
<tr>
<td>Mid North</td>
</tr>
<tr>
<td>North</td>
</tr>
<tr>
<td>Malaria (reference = 0 parasites/( \mu )L)</td>
</tr>
<tr>
<td>1–499 parasites/( \mu )L</td>
</tr>
<tr>
<td>500–1,999 parasites/( \mu )L</td>
</tr>
<tr>
<td>≥ 2,000 parasites/( \mu )L</td>
</tr>
<tr>
<td>Anti-ES IgG (reference = lowest quartile)</td>
</tr>
<tr>
<td>Second lowest quartile</td>
</tr>
<tr>
<td>Third quartile</td>
</tr>
<tr>
<td>Highest quartile</td>
</tr>
<tr>
<td>Below average animal source foods</td>
</tr>
</tbody>
</table>

*For adjusted model, n = 212. ES = excretory–secretory.
†Adjusted for all other variables in this model and for clustering within schools.

**Figure 2.** IgG concentration by hookworm status and school region, Ghana. Median IgG levels (horizontal bar) are presented as a function of infection status (positive versus negative) across the four school regions. Bottom of the box represents the 25th percentile, and top represents the 75th percentile. Error bar represents the 5–95% percentile, and solid circles are outlier data points. OD = optical density.

<table>
<thead>
<tr>
<th>Table 3 Anthropometry and nutritional indicators of study population for hookworm infection, Ghana*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>BAZ mean (SD)</td>
</tr>
<tr>
<td>BAZ &gt; 0</td>
</tr>
<tr>
<td>BAZ –0.99–0</td>
</tr>
<tr>
<td>BAZ –1.99–1.0</td>
</tr>
<tr>
<td>BAZ ≤ -2.0</td>
</tr>
<tr>
<td>Hemoglobin, mean (SD)</td>
</tr>
<tr>
<td>RDW, mean (SD)</td>
</tr>
<tr>
<td>MCV, mean (SD)</td>
</tr>
<tr>
<td>Household food secure</td>
</tr>
<tr>
<td>Some food insecurity</td>
</tr>
<tr>
<td>Moderate food insecurity</td>
</tr>
<tr>
<td>Severe food insecurity</td>
</tr>
<tr>
<td>No household hunger</td>
</tr>
<tr>
<td>Some hunger</td>
</tr>
<tr>
<td>Severe hunger</td>
</tr>
<tr>
<td>Above average dietary diversity</td>
</tr>
<tr>
<td>Above average ASF groups</td>
</tr>
</tbody>
</table>

*BAZ = body mass index for age; RDW = red cell distribution width; MCV= mean cell volume; ASF = animal source foods.

\( ^* \) For adjusted model, n = 212. ES = excretory–secretory.
† Adjusted for all other variables in this model and for clustering within schools.
were found to have a mixed infection with *N. americanus* and *A. duodenale*. No isolated *A. duodenale* infections were detected.

**DISCUSSION**

Mass drug administration is recommended by WHO for control of four geohelminth infections. The goal of preventive chemotherapy using MDA is to reduce the intensity of infection (i.e., worm burden) among persons, which also has the potential to reduce transmission of infection within disease-endemic communities. Concerns about reduced effectiveness of commonly used anthelmintics have focused on the potential emergence of hookworm resistance, especially against the benzimidazoles mebendazole and albendazole.

Ultimately, control of hookworm infection will likely require a multi-faceted approach that includes efforts to identify intrinsic or modifiable host and parasite factors that mediate susceptibility to infection (and reinfection), as well as the response to therapy. Nutritional factors, such as dietary content, may influence risk of infection, and supplementary feeding has been proposed as a potential method to control gastrointestinal nematodes in livestock. This study confirms that low-level consumption of animal source foods groups is significantly associated with human hookworm infection status, along with older age, higher malaria parasite density, lack of access to health care, school region, and higher hookworm antibody levels, even after controlling for wealth and sex. To our knowledge, increased prevalence of hookworm infection in school children who consume below average amounts of animal source food groups has not been reported.

Animal source foods are an important source of dietary protein, as well as micronutrients such as iron, vitamin A, vitamin B-12, riboflavin, calcium, and zinc. Low consumption of animal source foods has been linked to deficiencies in multiple micronutrients, particularly in children from low-income countries. Numerous cross-sectional studies have confirmed an association between poor nutritional status and intestinal helminth infections, and recent studies suggest that malnutrition increases susceptibility to helminth infection and re-infection. Our data are also supported by animal studies showing that protein supplementation confers protection against nematode infections. Thus, the finding in KNM of lower rates of hookworm among children who consume higher amounts of animal source foods provides further evidence that nutritional status may affect susceptibility to hookworm infection in this population. Importantly, the data analyses do not enable assignment of causality, but nonetheless point to an interesting association between dietary intake and hookworm infection status.

As in a prior study, children in KNM who were positive for malaria had a higher prevalence of hookworm infection at baseline, and the risk of hookworm increased with the level of malaria parasitemia. The high prevalence of malaria and helminth co-infections among school children is consistent with prior analyses of geographic distribution and age-related patterns of the two infections. Recent reviews suggest that helminth infections have specific effects on the epidemiology and pathogenesis of malaria. Overall, however, reports of the effects of helminth co-infection on malaria prevalence and clinical phenotype vary. Cross-sectional studies of school children found a higher prevalence of helminth infections among children with *P. falciparum*; although a study of young children (6–23 months of age) found a lower prevalence of helminth infections among children with malaria. A deworming trial in Nigeria found a significantly lower rate of increase in malaria prevalence and intensity among children who were treated with albendazole. In Thailand, *Ascaris* co-infection decreased the risk of cerebral malaria, although a prospective community-based observational study by the same authors found a higher risk of symptomatic malaria among helminth infected individuals. Other studies have found no association between hookworm and malaria, demonstrating that interpretation of results is confounded by marked differences in study populations, presence of multiple helminths with varying intensities, and in the study definition of malaria used. In general, current opinion seems to hold that hookworm, in contrast
studies conducted in northern Ghana. Given that there are > 400 million cases of *P. falciparum* malaria each year, and that > 500 million persons are infected with hookworm, a better understanding of the dynamics of coinfection could lead to the development of more effective control measures for both diseases.

In this study, we confirmed previous observations that antibodies directed at adult ES proteins from a laboratory strain of *A. ceylanicum* correlate with infection status. Further, this finding validates *A. ceylanicum* as a useful tool for characterizing human hookworm responses because those with the highest antibody responses were > 11 times more likely to be infected at the time of sampling, a difference that was highly statistically significant (*P* < 0.001). Although the hookworm ELISA lacks specificity to predict infection status on an individual basis (Figure 2), this assay could potentially serve as an effective screening tool to monitor control programs at the community level. If deworming or other intervention programs resulted in a substantial reduction in prevalence, we would anticipate that seroreactivity would also decrease over time.

To define the molecular epidemiology of hookworm in KNM, we undertook studies to identify the species of hookworm causing infection in this region. Using a PCR-based assay, we determined that *N. americanus* and *A. duodenale* are endemic to KNM, although infection with *N. americanus* is significantly more prevalent (100% of infected persons versus 2%). The predominance of *N. americanus* in Ghana is consistent with prior studies conducted in northern Ghana. To our knowledge, however, this is the first report of the species distribution of hookworm in the more centrally located KNM. These data can now serve as a baseline for future studies of the molecular epidemiology of hookworm in central Ghana.

Current recommendations of WHO call for single-dose therapy with one of four anthelmintics, including albendazole, as part of preventive chemotherapy for soil-transmitted nematodes. Reported cure rates for hookworm infection from single dose (400 mg) therapy with albendazole range from 40% to 100%, and an average cure rate of 78.4%.

In light of the prevailing opinion that intensity, i.e., worm burden, is the most significant factor mediating the clinical sequela of hookworm infection, it has been proposed that cure rate is not as important a measure of deworming efficacy as the FECR rate, which more closely correlates with the impact of treatment on worm burden. In the present study, we observed an albendazole cure rate of 43%, which is less than the cure rate of 61% we reported in a study of children and adults in Kintampo. The FECR rate ranged from 70.3%, when calculated by using individual arithmetic means, to 87.3% by using group arithmetic means of epg (Table 4). These FECR rates are similar to what has been reported from other disease-endemic populations. A recent collaborative report, which included representation from WHO, suggested that hookworm FECR rates > 90% (measured by using group arithmetic means) after albendazole treatment should increase concern about the possibility of resistance, a position with which we concur.

By validating our initial observations from 2007, we have now established that the cure rate and FECR rate in Kintampo have reached a level requiring careful monitoring of deworming effectiveness.

Having been alerted to the potential emergence of benzimidazole resistance by results from the prior study, we integrated into the 2010 field study application of a *in vitro* analysis of albendazole susceptibility by using hookworm eggs harvested from individual persons before and after treatment. The data are notable for two findings. First, and consistent with the diversity in cure rates and FECR rates, we noted a wide range of susceptibility among isolates between study participants, which suggests that the populations of hookworm within KNM are distinct in terms of benzimidazole susceptibility. To date, few studies have applied *in vitro* susceptibility testing to human hookworm isolates by using the EHA. In 1997, de Clercq and others reported a lower than expected response to mebendazole in Mali, as well as EHA data suggesting that the human isolates were less susceptible than a laboratory strain of *N. americanus*. Subsequently, Kotze and others successfully defined 50% lethal dose values for thiabendazole and albendazole by using a small number of (pre-treatment) *N. americanus* isolates from Papua New Guinea. Albonico and others used an *in vitro* assay similar to the one reported here to characterize susceptibility of human isolates from Pemba Island to mebendazole and thiabendazole. Important differences with our study include the fact that they pooled 10 participant samples before testing, and that susceptibility to albendazole was not tested.

Despite the limited number of matched samples in our study, the trend toward reduced albendazole susceptibility observed in samples collected post-treatment (Figure 3) suggests that treatment selects for isolates that are more resistant to the drug. The data also demonstrate the utility of field-based *in vitro* susceptibility testing, and show that samples from individual persons can be broadly distinguished based on drug susceptibility. Work is currently underway aimed at defining molecular markers that correlate with albendazole susceptibility with treatment response and molecular markers.

In summary, hookworm infection is prevalent among school age children in KNM, and dietary intake of animal source foods inversely correlates with infection status. Although no specific host factors were associated with treatment failure, we observed that post-treatment isolates of *N. americanus* exhibited reduced *in vitro* susceptibility to albendazole. Because of the modest cure rate and fecal egg count reduction rate observed, further investigation of potential benzimidazole resistance in Ghana is warranted.

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