Title

Epidemiology of Soil-Transmitted Helminth and Intestinal Protozoan Infections in Preschool-Aged Children in the Amhara Region of Ethiopia.

Journal

American Journal of Tropical Medicine and Hygiene, 96(4)

ISSN

0002-9637

Authors

Aiemjoy, Kristen
Gebresillasie, Sintayehu
Stoller, Nicole E
et al.

Publication Date

2017-04-01

DOI

10.4269/ajtmh.16-0800

Peer reviewed

Am. J. Trop. Med. Hyg., 96(4), 2017, pp. 866–872
doi:10.4269/ajtmh.16-0800

Epidemiology of Soil-Transmitted Helminth and Intestinal Protozoan Infections in Preschool-Aged Children in the Amhara Region of Ethiopia

Kristen Aiemjoy,[1]* Sintayehu Gebresillasie,[2] Nicole E. Stoller,[1] Ayalew Shiferaw,[2] Zerihun Tadesse,[2] Melsew Chanyalew,[3] Solomon Aragie,[2] Kelly Callahan,[2] and Jeremy D. Keenan[1]

[1]*Proctor Foundation, University of California San Francisco, San Francisco, California;* [2]*The Carter Center, Atlanta, Georgia;* [3]*Amhara Regional Health Bureau, Bahir-Dar, Ethiopia*

Abstract. Intestinal parasites are important contributors to global morbidity and mortality and are the second most common cause of outpatient morbidity in Ethiopia. This cross-sectional survey describes the prevalence of soil-transmitted helminths and intestinal protozoa in preschool children 0–5 years of age in seven communities in the Amhara region of Ethiopia, and investigates associations between infection, household water and sanitation characteristics, and child growth. Stool samples were collected from children 0–5 years of age, 1 g of sample was preserved in sodium acetate–acetic acid–formalin, and examined for intestinal helminth eggs and protozoa cysts ether-concentration method. A total of 212 samples were collected from 255 randomly selected children. The prevalence of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm were 10.8% (95% confidence interval [CI] 6.6–15.1), 1.4% (95% CI = 0–3.0), and 0% (95% CI = 0–1.7), respectively. The prevalence of the pathogenic intestinal protozoa *Giardia lamblia* and *Entamoeba histolytica/dispar* were 10.4% (95% CI = 6.2–14.6) and 3.3% (95% CI = 0.09–5.7), respectively. Children with *A. lumbricoides* infections had lower height-for-age *z*-scores compared with those without, but were not more likely to have stunting. Compared with those without *G. lamblia,* children with *G. lamblia* infections had lower weight-for-age and weight-for-height *z*-scores and were more than five times as likely to meet the *z*-score definition for wasting (prevalence ratio = 5.42, 95% CI = 2.97–9.89). This article adds to a growing body of research on child growth and intestinal parasitic infections and has implications for their treatment and prevention in preschool-aged children.

INTRODUCTION

Intestinal parasites (helminths and protozoa) are significant contributors to global morbidity and mortality. The soil-transmitted helminths (STHs) *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Necator americanus*, and *Ancylostoma duodenale* (hookworm) infect more than 1.5 billion people—nearly a quarter of the world population.[1] Intestinal protozoa of public health importance are *Entamoeba histolytica* and *Giardia lamblia*. Amebiasis, the disease caused by *E. histolytica*, causes 40,000–110,000 deaths annually.[2] In tropical regions, up to 45% of children are infected with *G. lamblia*.[2] *Entamoeba coli* and *Endolimax nana* are not known to be pathogenic but can be markers of environmental fecal contamination. The majority of the morbidity burden from intestinal protozoa and STHs lies in the tropics, largely in Africa.

In Ethiopia, intestinal parasite infections are the second most frequent cause of outpatient morbidity.[3] The prevalence of intestinal parasites varies across climate zones of the country, with a higher prevalence seen in the humid central highlands.[4] Previous studies have found *Ascaris* to be the most prevalent parasitic infection in Ethiopia, followed by *T. trichiura* and hookworm.[5]

Previous research on STHs and intestinal protozoa in Ethiopia has largely focused on school-aged children and used school-based surveys.[3,4,6,7] The objective of this study was to use population-based monitoring of preschool children (0–5 years of age) in rural Ethiopia to determine the prevalence of STH and intestinal protozoan infections and to investigate associations between infection, household water and sanitation characteristics, and child growth. Given that the first 5 years have been acknowledged as the critical period for determining lifelong growth faltering,[8] this is a potentially illuminating age group to study links between STH and intestinal protozoan infections with growth. In addition, data on STH and intestinal protozoan infections among preschool-aged children may support amending current treatment and prevention guidelines.

METHODS

Study design. This is a cross-sectional analysis of the baseline visit of a cluster-randomized trial. Fourteen communities in rural Ethiopia were selected for the trial, with half randomized to a water point intervention and the other half were randomized to no intervention. The present study took place in the seven intervention communities only, before water point construction began. During a single study visit in April 2014 (in the dry season), we collected stool samples and anthropometry measurements from children 0–5 years years of age. One month later, we conducted a door-to-door household survey of all households in each of the seven communities.

Study population and selection. This study took place in a rural agrarian region in the Goncha Siso Enese District (woreda) of Amhara, Ethiopia, during the dry season. "Woredas" in Ethiopia are divided into administrative units known as "kebeles," and at the time of the study, "kebeles" were subdivided into government-defined units known as state teams. State teams, which consisted of approximately 275 people in our study area, are termed communities for this report.

Communities in the present study had been participating in a series of cluster-randomized trials testing different mass drug administration strategies for trachoma elimination since

*Address correspondence to Kristen Aiemjoy, Proctor Foundation, University of California San Francisco, 513 Parnassus Avenue, MedSci S309, Box 0412, San Francisco, CA 94143. E-mail: kristen.aiemjoy@ucsf.edu

2006 (clinicaltrials.gov no. NCT00322972). As part of these trials, 72 communities had received some form of mass azithromycin distribution for trachoma at least annually from 2010 to 2013. Methods for these trials are described in detail elsewhere.[9–11] From these 72 communities, we randomly selected 14 that were relatively accessible (<1-hour walk from the farthest place a four-wheel drive vehicle could reach) and had poor access to water (≤ 1 community water points). A door-to-door population census was taken in all communities before the study visit. All households in these seven communities subsequently were eligible to participate in a household survey. The 14 communities were subsequently randomized into two groups, and baseline monitoring was performed in only the seven intervention communities. From these seven communities, all households and children 0–5 years of age (i.e., up to but not including the sixth birthday) enumerated on the previous census were eligible to participate in the study.

Parasitological outcomes. Stool collection and questions were performed in the field by trained laboratory technicians according to a standardized protocol. Caregivers were asked if their child had experienced diarrhea in the past 7 days using the standard World Health Organization (WHO) definition of three or more loose or watery stools in a 24-hour period. Each caregiver was instructed to have their child defecate on a plastic sheet, transfer the sample to a collection cup, and return the cup to the stool collection station. Children that could not produce a stool within 2 hours were given supplies to collect the stool at home. Caregivers were instructed to collect stool the following morning, after which a study team member would come to their house to pick up the specimen. At the time the stool was collected in the field, 1 g of stool was transferred into 10 mL of sodium acetate–acetic acid–formalin, shaken vigorously, and kept out of the sun. All specimens were processed at the Bahir Dar Regional Laboratory (Bahir Dar, Ethiopia) using the diethyl ether-concentration method.[12] Briefly, the preserved specimen was gently shaken, then filtered and centrifuged for 1 minute at 2,000 revolutions per minute (rpm). The supernatant was decanted and the precipitate was mixed with 7 mL of 0.85% NaCl and 3 mL of diethyl ether. The whole sample was centrifuged again for 5 minutes at 2,000 rpm,[12] the supernatant was decanted, and the sediment examined with a microscope at 10× and 40× magnification for helminth eggs and intestinal protozoan cysts. For helminths, the number of eggs identified were counted and recorded, with a possible range of 1 up to > 100 eggs. Without counting all the eggs identified in the specimens, it was not possible to classify the egg density as moderate or high using the standardized eggs per gram of stool thresholds frequently used when using the Kato-Katz thick smear method.[13] For protozoans, the number of cysts were counted and reported as none (no cysts per slide), rare (1–5 cysts per slide), frequent (one cyst per field), and very frequent (greater than one cyst per field).[12] *Entamoeba* cysts were reported as either *E. histolytica* or *Entamoeba dispar*, since the two are morphologically identical and cannot be differentiated microscopically.[14] As quality control, an independent expert laboratory technician confirmed all positive slides, as well as every 10th negative specimen.

Anthropometry outcomes. We performed height and weight measurements according to standard WHO protocols and as described in a previous report.[15–17] Children were barefoot and wore only light clothing for the measurements. To measure height, we used a portable stadiometer (Shorr Productions, LLC, Olney, MD); if a child could not stand, the stadiometer was placed on the ground, and the length was measured with the same positioning. Height or length was measured to the nearest 0.1 mm three times for each child, with the child repositioned in between each measurement. To measure weight, we used a Seca 874 scale (Seca GmbH and Co. KG, Hamburg, Germany), with taring function for children who could not stand independently on the scale. Three separate weight measurements to the nearest 0.01 kg were assessed for each child. Anthropometrics were standardized during a 2-day training session and had good intra- and interrater reliability, as has been reported previously.[17]

We calculated age- and sex-adjusted nutritional *z*-scores (height-for-age *z*-score [HAZ], weight-for-age *z*-score [WAZ], and weight-for-height *z*-score [WHZ]) according to the 2006 WHO growth standards using a WHO macro for Stata.[18] We used the median value of the three height measurements and three weight measurements to calculate the *z*-scores, and accounted for whether the height measurement represented a standing height or lying length. *z*-scores less than −5 or greater than 5 were coded as missing because these extreme values were likely due to measurement or data entry errors. Stunting was defined as a HAZ < −2, underweight as a WAZ < −2, and wasting as a WHZ < −2 as per WHO definitions.

Household exposure assessment. Locally trained staff conducted a door-to-door household survey in each of the seven communities in Amharic, the local language. Heads of households or spouses were asked a series of standardized questions about water, sanitation, hygiene practices, and economic status. We selected the following risk factors for childhood parasitic infection a priori: self-reported household income, self-reported time to water source, self-reported quality of water source, direct observation of

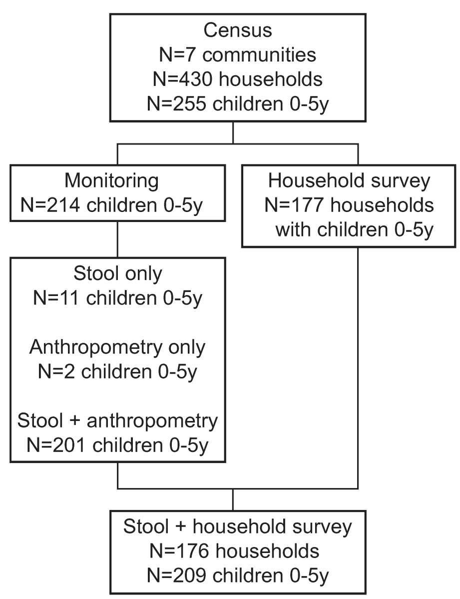

FIGURE 1. Flow diagram of survey sample.

TABLE 1
Prevalence of intestinal parasites among children 0–5 years of age in rural Amhara, Ethiopia

	Age						
	< 1 year	1 year	2 year	3 year	4 year	5 year	Total
No. children	19	31	24	41	42	55	212
Helminths							
Any helminth	0 (0.0%)	2 (6.5%)	7 (29.2%)	2 (4.9%)	7 (16.7%)	7 (12.7%)	25 (11.8%)
Ascaris lumbricoides	0 (0.0%)	2 (6.5%)	6 (25%)	2 (4.9%)	6 (14.3%)	7 (12.7%)	23 (10.8%)
Trichuris trichiura	0 (0.0%)	0 (0.0%)	1 (4.2%)	0 (0%)	1 (2.4%)	1 (1.8%)	3 (1.4%)
Hookworm	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Protozoans							
Any protozoa	2 (10.5%)	12 (38.7%)	12 (50.0%)	21 (52.5%)	30 (71.4%)	51 (92.7%)	113 (53.6%)
Giardia lamblia	0 (0.0%)	4 (12.9%)	2 (8.3%)	5 (12.2%)	7 (16.7%)	4 (7.3%)	22 (10.4%)
Entamoeba histolytica/dispar	0 (0.0%)	1 (3.2%)	0 (0.0%)	2 (4.9%)	3 (7.1%)	1 (1.8%)	7 (3.3%)
Entamoeba coli	2 (10.5%)	2 (6.5%)	9 (37.5%)	12 (29.3%)	22 (52.4%)	29 (52.7%)	76 (35.8%)
Blastocystis hominis	1 (5.3%)	7 (22.6%)	5 (20.8%)	8 (19.5%)	4 (9.5%)	4 (7.3%)	29 (13.7%)
Iodamoeba butschlii	0 (0.0%)	1 (3.2%)	1 (4.2%)	4 (9.8%)	2 (4.8%)	9 (16.4%)	17 (8%)
Endolimax nana	0 (0.0%)	3 (9.7%)	2 (8.3%)	9 (22%)	15 (35.7%)	22 (40%)	51 (24.1%)
Entamoeba hartmanni	0 (0.0%)	0 (0%)	0 (0%)	1 (2.4%)	1 (2.4%)	1 (1.8%)	3 (1.4%)

household sanitation, direct observation of a household wash station (e.g., Tippy Tap), and direct observation of soap availability for handwashing at the household. The time from the household to the water source was assessed as a round-trip, according to the following categories: water in the compound, < 30 minutes, 30 minutes to 1 hour, and > 1 hour. Water source quality was categorized as protected (covered hand-dug wells and/or springs surrounded by a fence) versus unprotected (hand-dug wells or springs that were not covered and/or not surrounded by a fence, or surface water). Household income was collected in Ethiopian birr and subsequently converted into the equivalent U.S. dollar value using the exchange rate at the time of the survey. The household survey information was linked to each child using a unique identification number.

Statistical methods. We used population means, standard deviations (SDs), and proportions with 95% confidence intervals (CIs) to describe the study population and the prevalence of intestinal parasites. We conducted two main types of analysis: first, we defined *Ascaris* and *Giardia* infections as outcomes and assessed whether several prespecified exposure variables from the household survey were associated with these intestinal infections, and second, we defined the anthropometric indices as both continuous and dichotomous outcomes and assessed whether *Ascaris* or *Giardia* infections were associated with smaller anthropometric measurements. The other pathogenic protozoa were not used in this analysis due to an insufficient number of cases. We used multivariate mixed-effects logistic regression models with random effects for community to investigate associations between 1) household characteristics and 2) wasting, stunting, and underweight with *Ascaris* and *Giardia* infections. We used marginal risks from our logistic regression models to calculate prevalence ratios.[19] We used linear mixed-effects models with random effects for community to investigate associations between *Ascaris* and *Giardia* infections and HAZ, WAZ, and WHZ. The models were adjusted for household income. The analyses in this report were designated a priori as hypothesis generating, and therefore we used a significance level of 0.05 for all analyses. The sample size was based on the ocular *Chlamydia* outcome of the underlying clinical trial. The seven communities provided 80% power to detect a 14% difference in STHs between comparison groups, assuming enrollment of approximately 30 children from each community, an intraclass correlation coefficient for helminthic infections of 0.028,[20] an STH prevalence of 24%,[7] and a two-sided alpha of 0.05. All analyses were run in Stata 14 (StataCorp, College Station, TX).

Ethics statement. Ethical committees at the University of California San Francisco, Emory University, the Amhara Regional Health Bureau, the Food, Medicine and Health Care Administration and Control Authority of Ethiopia, and the Ethiopian Ministry of Science and Technology granted approval for this study. We obtained verbal informed consent in Amharic from all caregivers and household participants. All data were deidentified prior to analysis.

RESULTS

Participation and final study sample. Of the 255 children 0 to 5 years of age from the preceding census, 214 children participated in the study, 212 children provided stool samples for parasitological analysis, and 203 children had

TABLE 2
Household survey results among households with children 0–5 years of age, rural Amhara, Ethiopia

	N	%
Total households	177	
Household income		
More than 1 USD/day	67	37.9
Less than 1 USD/day	106	59.9
Main water source		
Protected	129	72.9
Unprotected	45	25.4
Time to collect water		
< 30-minute walk	149	84.2
> 30-minute walk	25	14.1
Tippy Tap (observation)		
Yes	38	21.5
No	136	76.8
Soap in household (observation)		
Yes	123	69.5
No	50	28.2
Latrine use (observation)		
Covered latrine	48	27.1
Uncovered latrine	76	42.9
No latrine/open defecation (85)	49	27.7

TABLE 3
Household WASH and *Ascaris* and *Giardia* infections in rural Amhara, Ethiopia

	Ascaris lumbricoides			*Giardia lamblia*		
	N (%)	PR* (95% CI)	*P* value	*N* (%)	PR* (95% CI)	*P* value
Household income						
More than 1 USD/day (*N* = 76)	2 (2.6)	1.0		11 (14.5)	1.0	
Less than 1 USD/day (*N* = 134)	21 (15.7)	6.68 (1.01, 44.34)	0.042	11 (8.2)	0.85 (0.27, 2.72)	0.785
Main water source						
Protected (*N* = 157)	15 (9.6)	1.0		13 (8.3)	1.0	
Unprotected (*N* = 54)	8 (14.8)	1.85 (0.57, 6.02)	0.290	9 (16.7)	1.95 (0.96, 3.99)	0.049
Time to collect water						
< 30-minute walk (*N* = 184)	21 (11.4)	1.0		18 (9.8)	1.0	
> 30-minute walk (*N* = 27)	2 (7.4)	1.00 (0.14, 6.95)	0.999	4 (14.8)	0.89 (0.10, 7.76)	0.919
Tippy Tap (observation)						
Yes (*N* = 44)	3 (6.8)	1.0		5 (11.4)	1.0	
No (*N* = 167)	19 (12.2)	1.03 (0.31, 3.37)	0.963	17 (10.2)	1.45 (0.82, 2.57)	0.185
Soap in household (observation)						
Yes (*N* = 152)	19 (12.5)	1.0		15 (9.9)	1.0	
No (*N* = 57)	4 (7.0)	0.60 (0.19, 1.80)	0.344	7 (12.3)	1.09 (0.51, 2.35)	0.815
Latrine use (observation)						
Covered latrine (*N* = 56)	4 (7.1)	1.0		6 (10.7)	1.0	
Uncovered latrine (*N* = 92)	9 (9.8)	1.31 (0.66, 2.62)	0.423	8 (8.7)	0.94 (0.21, 4.18)	0.937
No latrine/open defecation (*N* = 62)	10 (16.1)	1.84 (0.59, 5.82)	0.294	8 (12.9)	0.94 (0.41, 2.14)	0.969

CI = confidence interval; PR = prevalence ratio.
*Mixed-effects logistic regression model with random effect for community, adjusted for all variables in model.

anthropometry measurements taken. A total of 201 children had complete anthropometry and parasitological data. In all, 177 households had children under 5 years of age and 209 children in 176 households had complete parasitological and household data. See Figure 1 for flow diagram.

Helminth and intestinal protozoan infection. The parasitological results of the 212 children who provided stool samples are shown in Table 1. At least one type of helminth was identified in the stool of 25 children (prevalence = 11.8%, 95% CI = 7.4–16.2). Most of these infections were *A. lumbricoides* (prevalence = 10.8%, 95% CI = 6.6–15.1), although a few cases of *T. trichiura* were also detected (prevalence = 1.4%, 95% CI = 0–3.0). No hookworm, *Schistosoma mansoni*, *Hymenolepis nana, or Taenia* sp. eggs were detected in the stool samples. At the community level, the prevalence of any STH ranged between 3.9% and 25.9%. Helminthic infections were less common among the youngest children. A total of 113 children (53.6%) showed evidence of a protozoan in their stool, among which 22 (prevalence = 10.4%, 95% CI = 6.2–14.6) had evidence of *G. lamblia* and seven (prevalence = 3.3%, 95% CI = 0.1–5.7) had evidence of *E. histolytica/dispar*. The prevalence of *Giardia* varied across the seven communities, ranging from 0% to 31.6%. Infection was more common in older children; no children under 1 year of age were infected with *Giardia* or *E. histolytica*.

Household-level water, sanitation, and hygiene indicators. Household survey responses and observations are shown in Table 2. Almost two-thirds of households earned the equivalent of less than 1 U.S. dollar a day. The majority of households spent less than 30 minutes to collect water, although nearly a third relied on an unprotected water source as their main source of water, with 24 (13.6%) using a river, 20 (11.3%) using an unprotected spring, and one (0.6%) using an unprotected hand-dug well. A jerry can handwashing station (Tippy Tap) was observed in approximately one in five households, and soap was observed in over three-quarters of households. A latrine was observed in over two-thirds of households, with 48 (27.1%) having a covered latrine, 76 (42.9%) having an open latrine, and the remaining 49 (27.7%) having no latrine and practicing open defecation. Two (1.3%) households used a shared latrine.

We performed multivariate mixed-effects regression analyses to determine whether any household-level exposures

TABLE 4
Anthropometry *z*-scores, malnutrition indicators, and *Ascaris* infection in children 0–5 years of age in rural Amhara, Ethiopia

	Ascaris lumbricoides (+) *N* = 23	*A. lumbricoides* (–) *N* = 178		
	Mean (95% CI)	Mean (95% CI)	*Coefficient (95% CI)	*P* value
HAZ	–0.70 (–1.25, –0.14)	–0.32 (–0.55, –0.09)	–0.60 (–1.03, –0.16)	0.007
WAZ	–0.77 (–1.16, –0.38)	–0.79 (–0.94, –0.63)	–0.16 (–0.33, 0.02)	0.079
WHZ	–0.58 (–0.99, –0.17)	–0.85 (–0.99, –0.70)	0.28 (0.06, 0.50)	0.013
	N (%)	*N* (%)	†Adjusted PR (95% CI)	*P* value
Stunting (HAZ < –2)	3 (13.0)	22 (12.4)	1.34 (0.42, 4.25)	0.639
Underweight (WAZ < –2)	0	16 (8.8)	–	–
Wasting (WHZ < –2)	3 (13.6)	17 (9.1)	1.94 (1.09, 3.44)	0.027
Diarrhea (past 7 days)	2 (8.7)	47 (25.3)	0.31 (0.08, 1.28)	0.089

CI = confidence interval; HAZ = height-for-age *z*-score; PR = prevalence ratio; WAZ = weight-for-age *z*-score; WHZ = weight-for-height *z*-score.
*Linear mixed-effects model with random effect for community and adjusted for household income.
†Mixed-effects logistic regression model with random effect for community, adjusted for household income.

Table 5
Anthropometry z-scores, malnutrition indicators, and *Giardia* infection in children 0–5 years of age in rural Amhara, Ethiopia

	Giardia lamblia (+) *N* = 22	*G. lamblia* (–) *N* = 189		
	Mean (95% CI)	Mean (95% CI)	*Coefficient (95% CI)	*P* value
HAZ	–0.78 (–1.40, –0.15)	–0.32 (–0.54, –0.09)	–0.38 (–1.05, 0.28)	0.257
WAZ	–1.40 (–1.91, –0.88)	–0.71 (–0.86, –0.56)	–0.69 (–1.13, –0.24)	0.002
WHZ	–1.20 (–1.56, –0.83)	–0.77 (–0.92, –0.62)	–0.41 (–0.69, –0.14)	0.003
	N (%)	*N* (%)	†Adjusted PR (95% CI)	*P* value
Stunting (HAZ < –2)	4 (19.0)	21 (11.7)	1.72 (0.93, 3.19)	0.088
Underweight (WAZ < –2)	2 (9.1)	14 (7.7)	1.06 (0.29, 3.90)	0.926
Wasting (WHZ < –2)	5 (22.7)	15 (8.0)	5.42 (2.97, 9.89)	< 0.001
Diarrhea (past 7 days)	6 (28.6)	43 (22.9)	1.10 (0.50, 2.42)	0.814

CI = confidence interval; HAZ = height-for-age *z*-score; WAZ = weight-for-age *z*-score; WHZ = weight-for-height *z*-score.
*Linear mixed-effects model with random effect for community and adjusted for household income.
†Mixed-effects logistic regression model with random effect for community, adjusted for household income.

were associated with *Ascaris* and *Giardia* infection (Table 3). These analyses demonstrated that *Ascaris* infections were more common in children living in households with lower incomes (prevalence ratio = 6.68, 95% CI = 1.01–44.34) and that *Giardia* infections were more common in children living in households that used an unprotected water source (prevalence ratio = 1.95, 95% CI = 0.96–3.99).

Child growth indicators. Of the 201 children who had both anthropometric and stool measurements, the mean HAZ was –0.37 (SD = 1.52) and 25 (12.4%, 95% CI = 8.2–17.8) were classified as having stunting (HAZ < –2). The mean WAZ among these children was –0.78 (SD 1.06), and 16 (7.9%, 95% CI = 4.6–12.5) were classified as being underweight (WHZ < –2). The corresponding values for WHZ among these 201 children were a mean of –0.82 (SD = 1.00), with 20 (9.6%, 95% CI = 5.9–14.4) classified as having wasting (WAZ < –2).

We performed mixed-effects regression models to determine whether intestinal infections as exposure variables were associated with lower anthropometric indices as outcomes. *Ascaris* infections were significantly associated with lower HAZ measurements, although not with the dichotomous outcome of stunting (Table 4). *Giardia* infections were significantly associated with both lower WAZ and WHZ measurements and the dichotomous wasting outcome (Table 5). Kernal density plots of HAZ, WAZ, and WHZ across *Ascaris* and *Giardia* infection status are depicted in Figure 2.

DISCUSSION

In this cross-sectional population-based survey of preschool children from seven communities in rural Ethiopia, we found stool samples containing STHs *A. lumbricoides* in 10.8% of children and *T. trichiura* in 1.4%. Stool samples were positive for the pathogenic intestinal protozoan *G. lamblia* in 10.4% of children and *E. histolytica/dispar* in 3.3%.

We found that children from households with a lower daily income (< 1 USD/day) were more likely to be infected with *Ascaris* and that children were more likely to have a *Giardia* infection when their household used an unprotected water source, compared with children from households using a protected water source. Given the same caveat regarding the inability to determine causality from a cross-sectional study, this finding is logical since unprotected water sources can be easily contaminated by *Giardia* cysts from infected humans and other domestic animals (mainly cats, dogs, and cattle).[21]

We found that children with *Ascaris* infections had lower HAZs compared with children without *Ascaris* infections, but were not more likely to be classified as stunted (HAZ < –2). Children with *Giardia* had both lower WHZs and were five times more likely to have wasting (WHZ < –2) compared with children without *Giardia* infections. Children with *Giardia* also had lower average WAZ scores but were not more likely to be classified as underweight. Our findings are consistent with previous studies in Brazil and Iran that have linked pediatric *Giardia* infections to wasting,[22,23] and supported by mouse models that have demonstrated that persistent *Giardia* infections impair growth.[24] Our findings are also in line with previous studies that have shown that *Ascaris* and other STHs are associated with impaired child growth.[25]

There was no significant difference in 7-day diarrhea prevalence in children with *Giardia* or *Ascaris* compared with children without these parasites. A recent comprehensive case-control study of pediatric diarrhea in 14 sites (Global Enteric Multicenter Study) found that 72% of

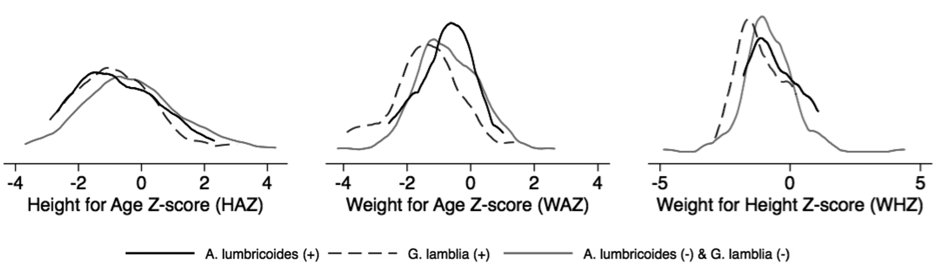

Figure 2. Kernal density plots of anthropometry *z*-scores and *Ascaris* and *Giardia* infections.

children under 5 years of age with no clinical signs of diarrhea were infected with at least one pathogenic virus, bacteria, protozoan, or helminth.[26] There is a growing body of evidence that asymptomatic enteric infections are common and may still negatively impact on child growth.[27,28] Caregiver-reported symptoms also may not accurately measure genuine diarrheal disease.

The results of this study must be considered in the context of several limitations. First, the prevalence estimates are based on cyst and egg detection in 1 g of stool from a single specimen. Helminth egg output varies within and between stool samples from the same individual.[29,30] It is possible that we were unable to detect some infections thus underestimating the true prevalence of infection and reducing our statistical power to detect associations between infection, household water, sanitation, and hygiene status, and child growth. In addition, because we used the ether-concentration method rather than Kato-Katz, we were unable to determine the intensity of infection. However, for determining STH prevalence, the ether-concentration method is as sensitive as Kato-Katz[31] and is more sensitive for detecting protozoan cysts.[32] We elicited self-reported household hygiene and sanitation behaviors, which may be susceptible to social desirability bias, a form of measurement error that may have resulted in overreporting hygiene behaviors. However, wash station, latrine use, and soap were measured by direct observation and should have been less subject to this form of measurement error. Finally, given that this study is cross-sectional, we are unable to determine the causal pathway of the associations. For example, chronic *Giardia* infection may lead to impaired growth, but it is also plausible that impaired growth could cause dysregulation of the immune system and hence increase the likelihood of *Giardia* infection.

Despite these limitations, this article adds to the growing body of research looking at the interplay between intestinal parasitic infections, child growth, and household water and sanitation characteristics. It is one of the few studies to measure STH and intestinal protozoan prevalence in preschool children in rural Ethiopia. The findings suggest that both protozoan and STH infections are present at a high enough level to warrant interventions among preschool children. Moreover, the associations between *Giardia* and *Ascaris* with stunting and wasting in this population suggest that interventions for these pathogens could be considered for preschool children. Early intervention could be more effective than interventions with school-aged children since stunting is not thought to be reversible after the preschool years.[8] Further interventional studies would be useful to determine the benefit of deworming for preschool children.

Received October 11, 2016. Accepted for publication January 6, 2017.

Published online February 6, 2017.

Acknowledgments: We thank the participation of communities, households, and individuals in this study. We are thankful for collaboration with the Carter Center of Ethiopia and the Amhara Regional Health Bureau. We are very appreciative of the time, effort, and commitment of all field teams, drivers, and laboratory technicians.

Financial support: This study was supported by the National Institutes of Health—National Eye Institute (U10 EY016214), That Man May See, and the Sara and Evan Williams Foundation (San Francisco, CA).

Authors' addresses: Kristen Aiemjoy, Nicole E. Stoller, and Jeremy D. Keenan, Proctor Foundation, University of California San Francisco, San Francisco, CA, E-mails: kristen.aiemjoy@ucsf.edu, nicole.stoller@ucsf.edu, and jeremy.keenan@ucsf.edu. Sintayehu Gebresillasie, Ayalew Shiferaw, Zerihun Tadesse, Solomon Aragie, and Kelly Callahan, The Carter Center, Atlanta, GA, E-mails: sintayehugs@gmail.com, ayalewsisu2003@gmail.com, zerihun.tadesse@cartercenter.org, solomon.aragie@cartercenter.org, and kelly.callahan@cartercenter.org. Melsew Chanyalew, Amhara Regional Health Bureau, Bahir-Dar, Ethiopia, E-mail: yeshiwork97@yahoo.com.

REFERENCES

1. World Health Organization, 2016. *Soil-Transmitted Helminth Infections Fact Sheet*. Geneva, Switzerland: World Health Organization.
2. Kelly P, 2014. Intestinal protozoa. Farrar J, ed. *Manson's Tropical Diseases*. Philadelphia, PA: Saunders.
3. Fikresilasie S, 2015. Status of soil-transmitted helminths infection in Ethiopia. *Am Jf Health Res 3:* 170–176.
4. Abera B, Alem G, Yimer M, Herrador Z, 2013. Epidemiology of soil-transmitted helminths, *Schistosoma mansoni*, and haematocrit values among schoolchildren in Ethiopia. *J Infect Dev Ctries 7:* 253–260.
5. Tadesse Z, Hailemariam A, Kolaczinski JH, 2008. Potential for integrated control of neglected tropical diseases in Ethiopia. *Trans R Soc Trop Med Hyg 102:* 213–214.
6. Zerdo Z, Yohanes T, Tariku B, 2016. Soil-transmitted helminth reinfection and associated risk factors among school-age children in Chencha District, southern Ethiopia: a cross-sectional study. *J Parasitol Res 2016:* 4737891.
7. King JD, Endeshaw T, Escher E, Alemtaye G, Melaku S, Gelaye W, Worku A, Adugna M, Melak B, Teferi T, 2013. Intestinal parasite prevalence in an area of Ethiopia after implementing the SAFE strategy, enhanced outreach services, and health extension program. *PLoS Negl Trop Dis 7:* e2223.
8. Victora CG, de Onis M, Hallal PC, Blössner M, Shrimpton R, 2010. Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics 125:* e473–80.
9. Gebre T, Ayele B, Zerihun M, Genet A, Stoller NE, Zhou Z, House JI, Yu SN, Ray KJ, Emerson PM, Keenan JD, Porco TC, Lietman TM, Gaynor BD, 2012. Comparison of annual versus twice-yearly mass azithromycin treatment for hyperendemic trachoma in Ethiopia: a cluster-randomised trial. *Lancet 379:* 143–151.
10. Aiemjoy K, Stoller NE, Gebresillasie S, Shiferaw A, Tadesse Z, Sewnet T, Ayele B, Chanyalew M, Callahan K, Stewart A, Emerson PM, Lietman TM, Keenan JD, Oldenburg CE, 2016. 'If an eye is washed properly, it means it would see clearly': a mixed methods study of face washing knowledge, attitudes, and behaviors in rural Ethiopia. *PLoS Negl Trop Dis 10:* e0005099.
11. Aiemjoy K, Stoller NE, Gebresillasie S, Shiferaw A, Tadesse Z, Sewent T, Ayele B, Chanyalew M, Aragie S, Callahan K, Stewart A, Emerson PM, Lietman TM, Keenan JD, Oldenburg CE, 2016. Is using a latrine "a strange thing to do"? A mixed-methods study of sanitation preference and behaviors in rural Ethiopia. *Am J Trop Med Hyg 96:* 65–73.
12. Utzinger J, Botero-Kleiven S, Castelli F, Chiodini P, Edwards H, Köhler N, Gulletta M, Lebbad M, Manser M, Matthys B, N'Goran EK, Tannich E, Vounatsou P, Marti H, 2010. Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories. *Clin Microbiol Infect 16:* 267–273.
13. Committee WE, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *World Health Organ Tech Rep Ser 912:* i–vi, 1–57.
14. Gonin P, Trudel L, 2003. Detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* isolates in clinical samples by PCR and enzyme-linked immunosorbent assay. *J Clin Microbiol 41:* 237–241.
15. de Onis M, Onyango AW, Van den Broeck J, Chumlea CW, Martorell R, 2004. Measurement and standardization protocols

for anthropometry used in the construction of a new international growth reference. *Food Nutr Bull 25:* S27–S36.
16. World Health Organization, 1995. *Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee. Technical Report Series No. 854.* Geneva, Switzerland: World Health Organization.
17. Ayele B, Aemere A, Gebre T, Tadesse Z, Stoller NE, See CW, Sun NY, Gaynor BD, McCulloch CE, Porco TC, Emerson PM, Lietman TM, Keenan JD, 2012. Reliability of measurements performed by community-drawn anthropometrists from rural Ethiopia. *PLoS One 7:* e30345.
18. Leroy J, 2011. *ZSCORE06: Stata Module to Calculate Anthropometric Z-Scores Using the 2006 WHO Child Growth Standards*. Chestnut Hill, MA: Boston College.
19. Cummings P, 2011. Estimating adjusted risk ratios for matched and unmatched data: an update. *Stata J 11:* 290–298.
20. Casapía MW, Joseph SA, Núnez C, Rahme E, Gyorkos TW, 2007. Parasite and maternal risk factors for malnutrition in preschool-age children in Belen, Peru using the new WHO Child Growth Standards. *Br J Nutr 98:* 1259–1266.
21. Heymann DL, 2008. *Control of Communicable Diseases Manual*. Washington, DC: American Public Health Association.
22. Prado M, Cairncross S, Strina A, Barreto ML, Oliveira-Assis A, Rego S, 2005. Asymptomatic giardiasis and growth in young children; a longitudinal study in Salvador, Brazil. *Parasitology 131:* 51–56.
23. Nematian J, Gholamrezanezhad A, Nematian E, 2008. Giardiasis and other intestinal parasitic infections in relation to anthropometric indicators of malnutrition: a large, population-based survey of schoolchildren in Tehran. *Ann Trop Med Parasitol 102:* 209–214.
24. Bartelt LA, Roche J, Kolling G, Bolick D, Noronha F, Naylor C, Hoffman P, Warren C, Singer S, Guerrant R, 2013. Persistent *G. lamblia* impairs growth in a murine malnutrition model. *J Clin Invest 123:* 2672–2684.
25. Hall A, Hewitt G, Tuffrey V, De Silva N, 2008. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Matern Child Nutr 4:* 118–236.
26. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque ASG, Zaidi AKM, Debasish Saha, Alonso PL, Boubou Tamboura, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM, 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet 382:* 209–222.
27. McCormick B, 2014. *Frequent Symptomatic or Asymptomatic Infections May Have Long-Term Consequences on Growth and Cognitive Development*. Old Herborn University Seminar Monographs. Herborn, Germany: Institute for Microbiology und Biochemistry, 23–39.
28. Petri WA, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL, 2008. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest 118:* 1277–1290.
29. De Vlas S, Gryseels B, 1992. Underestimation of *Schistosoma mansoni* prevalences. *Parasitol Today 8:* 274–277.
30. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, Marti H, Utzinger J, 2008. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis 2:* e331.
31. Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, Rinaldi L, Cringoli G, N'Goran EK, Utzinger J, 2010. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *PLoS Negl Trop Dis 4:* e754.
32. Marti H, Escher E, 1990. SAF: an alternative fixation solution for parasitological stool specimens [in German]. *Schweiz Med Wochenschr 120:* 1473–1476.