COMPARING THE PREVALENCE OF AND BEHAVIORS ASSOCIATED WITH HOOKWORM INFECTION IN TWO GROUPS OF RURAL INDIAN CHILDREN

By

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A THESIS

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CERTIFICATE OF APPROVAL

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CATHARINE WEBER MEMORIAL
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TRAINING CENTER.
MUTHYALPADU, KURNOOL (Dt)
DEDICATION DISCIPLINE DEVELOPMENT
BIRDS HIGH SCHOOL
MUTHYALAPADU

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Abstract

Background

More than two billion persons globally are estimated to be infected with intestinal parasites, with over 900 million of these estimated to be infected with hookworm. When measured in disability-adjusted life years, the global disease burden from hookworm alone exceeds all other major tropical infectious diseases (with the exception of malaria, leishmaniasis, and lymphatic filariasis), and includes an estimated blood loss of 7 million liters per day. 65, 000 persons are estimated to die from hookworm infection each year. A 2010 study of the global prevalence and burden of soil-transmitted helminths (STH) estimated that, of the 4.98 million years lived with disability (YLDs) attributable to STH, 65% were attributable to hookworm alone. Children and child-bearing women are particularly vulnerable to serious morbidities and outcomes associated with intestinal parasitic infections, especially hookworm. In children, chronic heavy-intensity infections are associated with growth retardation, iron-deficiency anemia, as well as intellectual and cognitive impairments. Due to the relative high prevalence among the poor—particularly persons who live on less than US $2/day—soil-transmitted helminth infections have received relatively little global attention—despite annual morbidities and mortalities in the hundreds and tens of millions, respectively.

Methods

In partnership with a successful, community-directed, local NGO (*Bharati Integrated Rural Development Society,* "BIRDS"), we performed a comparative, cross-sectional analysis of the prevalence of hookworm infection and associated health behaviors and symptoms among two groups of severely marginalized and impoverished rural southern Indian children aged 3-16 years: 72 residents of the NGO school campus and 95 non-resident students living in

surrounding villages. We collected data on several covariates to test for association with hookworm infection, including: risk behaviors (latrine use, frequency of hand washing, et al); anthropometrics (BMI-for-age percentile); and symptoms (gastro-intestinal and pulmonary). We tested the hypothesis that resident students on BIRDS campus have a lower crude prevalence of intestinal parasitosis relative to non-resident students (anticipated due to reports of more sanitary facilities available and comparatively advanced hygienic culture on BIRDS campus).

Results

We identified 14 total cases of infection with *Ancylostoma duodenale* for an **overall** prevalence of 8.4% (90%CI: 4.8, 12). Prevalence of *A. duodenale* infection among BIRDS residents was found to be 7%(5/72) (90CI: 2.1%, 12%) versus 9.5%(9/95) (90CI: 4.6%, 14%) among the group of children residing in surrounding communities. The difference in crude prevalence between the two groups was not statistically significant (p-value = 0.56) at an α-level of 0.10. Non-residents reported a higher frequency of two significant risk behaviors compared to residents: defecating outdoors more frequently (56% of non-residents claiming to do so "every time" compared to 7% of residents) and wearing footwear less frequently than the resident group (43% of non-residents reported wearing shoes "most of the time" compared to 67% of residents) (both differences significant, p-value <0.0001). Residence did not demonstrate any association with hookworm infection via unadjusted (OR: 0.73; 90% CI: 0.67, 2.3) or adjusted (OR: 0.74; 90% CI: 0.23, 2.4) logistic regression.

Associations with hookworm infection for the population **overall**: 1) Compared to those who reported hand-washing post-defecation "most of the time", subjects who reported "never" hand-washing post-defecation were more likely to be infected with hookworm (Adjusted OR=10.7; 90%CI: 1.9, 60). 2) Compared to subjects who described themselves as having equal or more appetite than their peers, subjects who described themselves as having less appetite were 6.3 times as likely to test positive for ancylostomiasis (Adjusted, 90%CI: 2.1, 19). 3) Students who were classified as defecating outdoors regularly were 3.7 times as likely to test positive for *A. duodenale* infection (Adjusted, 90%CI: 1.2, 12).

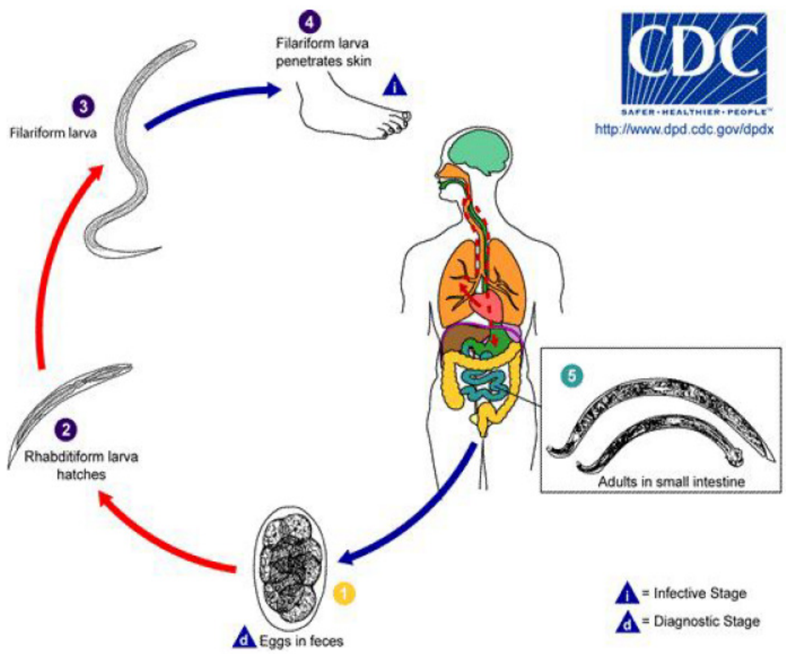
Discussion

No significant difference was observed in the crude prevalence of *A. duodenale* infection between students residing at BIRDS and those students residing off-campus. Non-residents reported a higher proportion of behaviors that put them at risk for hookworm infection, to whit: less frequent footwear use and more frequent indiscriminate defecation. Within the study population as a whole, regular hand-washing following defecation was reported significantly more frequently among those not infected with hookworm and may be protective in and of itself—or as a marker for other behaviors. *Hand-washing Frequency Post-Defecation* may actually have measured frequency of outdoor defecation. Regular outdoor defecation was also reported in greater proportion among those students with *A. duodenale* infection. Students who described themselves as having less appetite than peers were more likely to be infected with hookworm. Such a decreased appetite may indicate hookworm-induced anemia—and may also be a practical clinical indicator for presumptive treatment of ancylostomiasis.

Introduction

Soil-transmitted helminthes (STH) are parasitic worms that infect humans and other animals via soil contaminated by the feces of already-infected individuals. Of these, the species responsible for the majority of human infections are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Ancylostoma duodenale* and *Necator americanus*). The worms are widely distributed throughout the warm and humid equatorial and subtropical regions, clustering in resource-poor areas where sanitation is inadequate, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and east Asia[1–5]. Globally, estimates indicate greater than 2 billion individuals, more than 25% of the world's population,[2] are currently harboring over 2.8 billion soil-transmitted helminth infections[1,4], with about 900 million of these individuals estimated to be infected with hookworm[1–3,5–8]. Due to the focus of infections amongst the poor and least-empowered, intestinal helminth infections have received insufficient attention. Despite being one of the most common sources of infection worldwide—and the most common parasitic infection worldwide—STH infections are recognized by many, including the WHO and CDC, as Neglected Tropical Diseases (NTDs) because they cause profound disability, productive life loss, and suffering yet *can* be controlled or eliminated with existing and popularly understood methods.

Etiology and Transmission



Eggs are passed in the stool 1, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil 2, and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective 3. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed 4. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host 5. Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years.

Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

Figure 1: Life Cycle of Hookworms

When measured in disability-adjusted life years, the global disease burden from hookworm alone exceeds all other major tropical infectious diseases (with the exception of malaria, leishmaniasis, and lymphatic filariasis)[9] and includes an estimated daily blood loss of 7 million liters per day[10]. A 2010 study of the global prevalence and burden of STH infections estimated that, of the 4.98 million years lived with disability (YLDs) attributable to STH, 65% were attributable solely to hookworm[11]. The preventable annual fatalities are staggering: 65,000 persons are estimated to die each year as a result of hookworm infection.[2]

Children are a significant portion of the more than two billion persons estimated to be infected with soil-transmitted helminths [4]. The WHO estimates that over 270 million preschool-age children and over 600 million school-age children live in areas where STH are intensively transmitted, and are in need of treatment and preventive interventions.[2] Among the STH's, infection with hookworm can be particularly virulent, especially in children, for whom heavy worm burdens and resultant chronic insidious intestinal blood loss are associated with serious health problems such as iron-deficiency anemia, malnutrition, delayed cognitive development, intellectual impairment, and stunted growth[5,6,12–20]. For child-bearing mothers, HI is associated with adverse maternal-fetal outcomes[21,22]. Additionally, HI has been recognized to decrease productivity in both children and adults[5-10].

Like all the other soil-transmitted helminthiases, hookworm infection is a disease of the poor. Helminth infections are both curable and preventable, yet performing thorough and *sustainable* prevention means remediating some of the structural insults of poverty, namely providing adequate safe water and sanitation to those in need. Unanimously by region, the prevalence of hookworm infection (HI, hereafter) is strongly associated with poverty[4,7]. The following figure adapted from a 2003 meta-analysis (**Figure 2**) depicts how persons living in the worst poverty and development conditions also suffer the greatest prevalence of hookworm infection[4]. Although control/elimination measures such as improved sanitation and medication regimens are conceptually simple, their implementation is impeded by the vastness of the scale, the cost associated with practical development and deployment of the infrastructure of water and sanitation, and that STH endemicity is tied to politically and culturally entrenched conditions of poverty and underdevelopment.

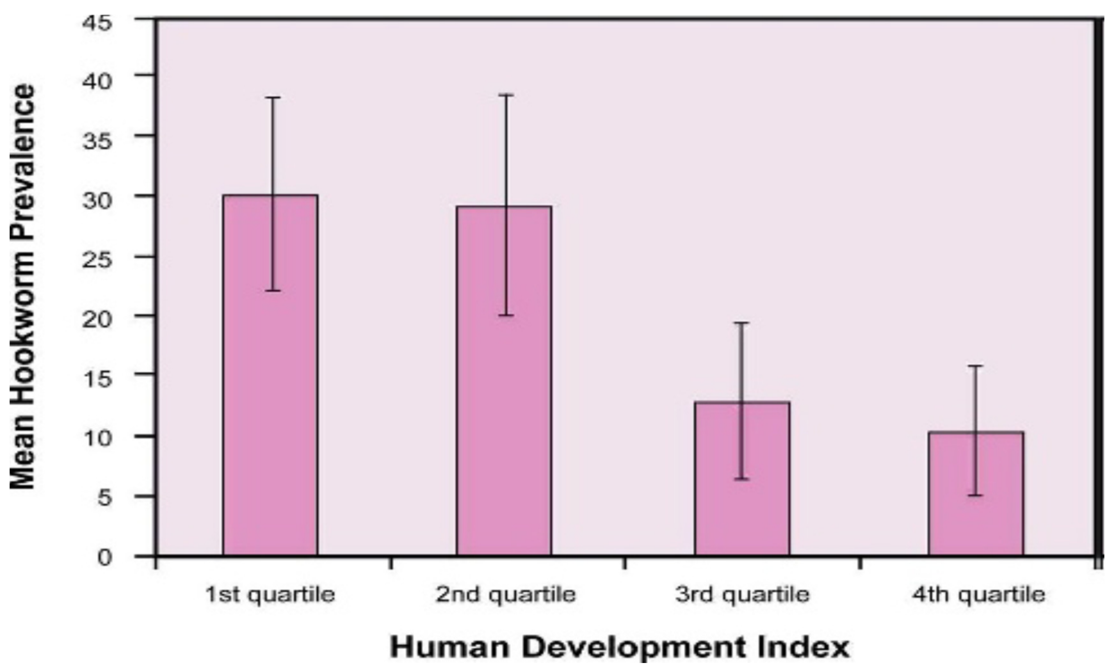


Figure 2: The Relationship between Poverty and Hookworm Prevalence

Socioeconomic status of 94 countries were assessed according to indicators as reported by the United Nation Development Program (http://hdr.undp.org/en/statistics/hdi/), including purchasing power parity-adjusted gross domestic product (GDP) per capita and human development index (HDI). Country groupings were defined by dividing the poverty measures into quartiles, so that each country is divided into most poor (1st quartile), very poor (2nd quartile), poor (3rd quartile) or least poor (4th quartile) with a mean GDP of $1467, $3043, $5880 and $15073, and a mean HDI score of 0.478, 0.636, 0.747, 0.844, respectively. Error bars represent 95% confidence intervals. Trends are significant ($p < 0.001$), as indicated by an F-test for heterogeneity, which tests for equality of variance. (Illustration: Margaret Shear, Public Library of Science, adapted from [16])

Significance

The communities surrounding (and including) our local NGO partner, BIRDS (Appendix E: Brief Profile on Bharati Integrated Rural Development Society ("BIRDS") are comprised of *dalits* (former outcastes or untouchables) and *tribals* (persons of indigenous tribes), groups which have been severely marginalized in Indian society. Chronic HI's are reasonably believed to promote long-term disability and increase the likelihood that populations with significant prevalence will remain mired in poverty.

Impoverished children, like those of rural southern India, often rely on formal education as the principle vehicle with which to improve their social standing. Children who suffer sequelae associated with chronic worm infestation are less apt to succeed in school and, as a result, future opportunities for advancement and remediation of inherited chronic poverty become increasedly severely restricted[7,8,15,16,18–20]. For the children in BIRDS' region, such restriction is likely to mean the extinguishment of their one opportunity to escape poverty and marginalization.

Accurate assessment and estimation of the burden of hookworm disease and the associated risk behaviors and morbidities is essential to ensuring that any interventions are focused appropriately. Data on the morbidities due to intestinal helminth infection are largely unavailable in resource-poor areas like rural Andhra Pradesh, as the symptoms of infection are chronic and non-specific, and infrastructure for medical treatment and record-keeping are often absent. While hookworm and other intestinal parasites have been documented as endemic to many regions in India, prevalence data are lacking for the region occupied by

BIRDS[17,23]. The public health policy aphorism, 'No numbers=no problem,' is particularly applicable to the situation in Muthyalapadhu (county in which BIRDS is located) where hookworm infection is sure to be a persistent burden on the community—but data has never been obtained. The data from our study we hope will continue to enable and empower robust and strategic interventions to improve campus and community health infrastructure and education. Specifically, a primary hope is to contribute to the organizational goal of increasing the number, availability, and use of latrines at BIRDS. As BIRDS' operating budget is dependent on foreign donations, our report on the hookworm prevalence in local children provides a meaningful rallying point to encourage donor support. On an individual level, any student identified through this study as infected with any GI parasite received appropriate clinical treatment.

Thesis Objective and Specific Aims

The overall objectives of this thesis were to obtain an accurate estimate of the prevalence of *A. duodenale* infection among the students of BIRDS school, to compare the prevalence estimates between student residents of BIRDS campus and non-residents, and to test for associations between infection and known risk behaviors and suspected morbidities and symptoms.

Specific Aims

1. To obtain an accurate estimate of the prevalence of *A. duodenale* infection among the students of BIRDS school.
2. To test the hypothesis that students residing on BIRDS campus have a lower prevalence of hookworm infection compared to students who live off-campus.

3. To evaluate for differences in sanitary practices/risk behaviors and symptoms between student residents of BIRDS and non-residents.
4. To use multiple logistic regression to evaluate potential associations of covariates from the two groups *Sanitary Practices/Risk Behaviors* and *Symptoms* with hookworm infection among the overall study population.

Methods

Overview of Design

A cross-sectional, comparative analysis was performed of the prevalence of hookworm infection and associated risk behaviors and symptoms between resident students of BIRDS' campus and non-resident students. Diagnosis of HI was made by laboratory ova & parasite (O&P) exam using a single, recent stool sample for each subject. Anthropometric, symptomology, and hygiene behavior data were obtained during a single interview session. Multiple logistic regression modeling was used to assess the association between HI and other variables, including: BMI-for-age, symptoms, and sanitary practices. Pearson's χ^2 test was used to compare the unadjusted prevalence in the two groups.

Study Subjects

The population evaluated consists of school-aged (3-15 years), rural south-eastern Indian children originating from families of low socio-economic status (SES). BIRDS' catchment area (Muthyalapadhu, Andhra Pradesh, India) is peopled primarily by *dalits* (persons "formerly" identified under the Indian caste system as "scheduled castes", "untouchables", or "outcastes") and to a lesser extent by "tribals" or persons of indigenous tribes. Both aforementioned groups have an extended history of severe marginalization in Indian society.

The study population was dichotomized into students of BIRDS school who live in either the boys' or girls' hostel on BIRDS' campus ("residents"; total enrolled population≈200) and students who travel to BIRDS daily for school but reside in surrounding villages ("non-residents"; total enrolled population≈650). Because students from the resident group both attend school and live on BIRDS campus, it was thought that greater capacity to intervene in the HI transmission pathway might exist among residents than those students who only come to BIRDS to attend class for a limited time during the day. All villages supplying students to BIRDS lie within an approximate 16 km radius and exist in relatively homogeneous living conditions. Both resident and non-resident groups are comprised of similar gender proportions with females constituting ≈30%.

Selection Criteria

Table 1: Selection Criteria - Residents

Inclusion Criteria	Exclusion Criteria
Current student resident in BIRDS hostel	Not residing on campus in BIRDS hostel
Age 3-15 years	Subject age outside of designated range
Willing to submit stool sample	Not willing to submit stool sample or failing to provide a sample before conclusion of sampling period.
Willing to complete interview process	Student or parental objection to participation
No self-report of treatment for STHI within previous 6 months	Self-report of receipt of de-worming medication within past 6 months

Table 2: Selection Criteria – Non-Residents

Inclusion Criteria	Exclusion Criteria
Current day-school student at BIRDS	
Aged 3-15 years	Subject age outside of designated range
Willing to submit stool sample	Not willing to submit stool sample or failing to provide a sample before conclusion of sampling period.
Willing to complete interview process	Student or parental objection to participation
No self-report of treatment for STHI within previous 6 months	Self-report of receipt of de-worming medication within past 6 months

Design for Sampling

A complete roster of all students at BIRDS school was converted to electronic format (Microsoft Excel). The record for each student included: name, age, grade, gender, and residency status. Using MS Excel: students were segregated into “Resident” and “Non-Resident” groups; each student was assigned a random number; segregated lists were sorted by random number; 80 students were selected in consecutive order of random number from the Resident group; 120 students were selected in consecutive order of random number from the Non-Resident group.

Power and Sample Size

Power and sample size calculations were performed manually using tabulated information and equations in the WHO publication Hookworm Infection and Anaemia: Approaches to Prevention and Control[24] (**Table 3**). A correction formula was applied to sample size calculations to account for the finite population sizes. The sample sizes selected reflect an α-level of 0.1 and 80% power to detect an effect size (difference in proportions) of 0.1. The *a priori* prevalence estimates were obtained through evaluating the Global Atlas of Helminth Infection website (http://www.thiswormyworld.org/) and through considering previous studies among similar populations, globally and specifically within the Indian subcontinent (including Pakistan)[3–8,15,20,25–28]

Table 3: Power and Sample Size Data (α=0.1; β=0.2)

Population	Total Size	*A priori* Prevalence Estimate	Sample Size
Residents	200	0.2	80
Non-Residents	650	0.3	120

Subject Recruitment

The primary investigator (PI) made presentations to BIRDS students (separately by age group) to provide background information and explain the purpose and goals of the study. A form letter detailing the purpose, goals, and methods of the study was co-authored by the

PI and BIRDS' Executives Director, translated into the local language (Telugu), and sent to the parent(s) or guardian of each selected subject (English draft: Appendix C). Parents who did not consent to their child's participation in the study were asked to inform BIRDS administration. BIRDS' Executive Director, Paul Raja Rao, was able to provide participatory consent for students residing at BIRDS, as he is the functional legal guardian of these students. Assent was obtained from subjects during interview session, with those subjects who did not assent to participate being allowed to opt-out. As incentive to comply with stool sample submission, subjects were given a sticker with the stool sample collection kit and a small dress accessory (e.g. bangles, hair clips, hair ties; max. value≈$.25 USD/each) once their stool samples were logged as received by BIRDS' lab technician.

Measurements & Data Collection

Main Predictor Variables

Variable Choice Rationale

Sanitary Behavior Variables – The Transmission Pathway

The sanitary behavior variables were chosen based upon the known transmission pathway for hookworm (*hookworm life cycle: Appendix B*). All the STH, including hookworm—and many other pathogenic GI parasites—are capable of being transmitted via the fecal-oral route. *Hand washing* is known to decrease the oral-transmission risk of HI; thus data on hand washing frequency was collected to test for a (protective/negative) association with HI. Defecation in the open environment (i.e. "indiscriminate defecation") is logically consistent with an increased risk of exposure to the hookworm transmission pathway. *Frequency of outdoor defecation/frequency of latrine use* were thus collected as a potential predictor variable. Given that hookworm transmission is known to occur primarily via lower extremity skin penetration by filariform larvae, and the WHO recommends footwear use to decrease the risk of transmission, data on *frequency of footwear use* was collected as a potential positive risk behavior.

Symptom Variables

Symptoms that commonly occur with HI have been well documented. Unfortunately for the goal to operationalize diagnosis of HI in resource-poor settings, many of the common symptoms of HI overlap with various other related and unrelated pathologies. In spite of the potential for overlap, data collection on gastro-intestinal (GI) and respiratory symptomology was performed to probe for a possible association with HI. At the study planning stage, we projected that demonstration of a significant association between the symptom variables and HI could facilitate presumptive treatment. Since O&P examination performed by a qualified laboratory is unavailable to the overwhelming majority of the population under consideration, presumptive treatment is the option most likely to be utilized by local clinicians.

Table 4: Predictor Variables

Variables	Collection Method	Value
Sanitary Behaviors: frequency of hand washing frequency of latrine usage frequency of footwear usage	Interview	Ordinal
GI Symptoms: abdominal bloating abdominal pain/cramps	Interview	Binary (P/A)
Respiratory Symptoms: coughing difficulty breathing	Interview	Binary (P/A)

The Interview Process

The Questionnaire:

The primary regional language is *Telugu*, and staff and students have varying English language abilities; thus, BIRDS staff members fluent in *Telugu* and with advanced English language ability were selected by the executive director of BIRDS and assigned to work with the PI throughout the interview process. To enhance the likelihood that subjects would be both as comfortable and candid as possible during the interviews, staff members most familiar with/to the children were selected: one male staff to interview male subjects and one female staff to interview female subjects. Due to the personal nature of some of the questionnaire items, it was believed that matching interviewer and subject by gender would best meet the double aim of subject comfort and candidness. The primary male staff member was one of the "wardens" who lives in the BIRDS boys' hostel and coordinates the hostel residents' daily activities and needs. The female staff members were BIRDS primary school teachers who live in staff housing on BIRDS campus.

Prior to conducting interviews, the PI met with interviewers to explain study details and review the interview questionnaire (*Appendix D: Questionnaire*). Focus group meetings were conducted with BIRDS staff and CHW to solicit information on HI in the local population and any factors they believed to be associated. PI solicited feedback from the interviewers to attempt to phrase questions utilizing any local vernacular that could facilitate subject understanding of the questions asked during interview.

The daily group was assembled using a classroom on BIRDS campus. Employing one of the interviewers for language translation, PI presented a brief explanation of study goals, interview process, and instructions for stool sample collection. Subjects were then separated into two rooms by gender where they were interviewed by a staff member of the corresponding gender. Interviewers used a hard copy of the questionnaire to ask questions of participants and enter responses. As subjects completed the interview, they brought the questionnaire to PI, which PI briefly reviewed for logical consistency of responses. PI then obtained height and weight for each subject. Standing height was obtained by dorsal approximation to a flexible tape measure previously affixed to wall. Weight was obtained (without footwear) by digital scale.

Table 5: Outcome Variable

Variable	Collection Method	Value
Hookworm infection (HI)	Ova & parasite exam of single stool specimen	Binary (Presence/Absence)

Sample Collection & Processing

A daily ledger was created listing all subjects interviewed, residency status, school standard (grade) level, and age. PI provided a copy to BIRDS lab technician who received samples from subjects each day at the campus clinic. Each day, lab tech actively investigated any samples from subjects on previous day's ledger which were not received. Samples received at the clinic were logged and stored under refrigeration at the clinic. Some samples were presented by subjects to PI who would then send or take the samples to the campus clinic as soon as practical (delay to receipt at campus clinic ranged approximately between 15 min. and 2 hours, empirically estimated average of 1 hour). Samples were transported 6 days/week, on average, in portable ice-containing insulated cooler by PI or assigned BIRDS staff member to the Microbiology Department Laboratory at Santhiram Medical College (SMC) in Nandyal. Unless accompanying PI in passenger car, staff traveled with samples via public bus, a trip which requires two to three hours each direction (duration of one-way trip by car was ≈ 1.5 hr.). Samples were left with SMC lab staff along with a copy of the ledger.

The O&P Exam:

All samples received macroscopic physical examination to include: color, consistency, and presence/absence of: frank blood and adult parasites. Each sample was analyzed microscopically via direct saline wet mount (two slides) and concentration (two slides). The concentration technique employed utilized the formol-ether method, as described in the Practical Guide to Diagnostic Parasitology[29]. A digital image capture of the microscope viewing field was saved for each positive identification.

Clinical Treatment of Subjects

The medical directors of BIRDS, Dr. Virginia Feldman and Dr. George Feldman, evaluated each subject who tested positive for any parasite and composed treatment recommendations which were forwarded to BIRDS' executive director via email. The O&P exams identified multiple GI parasites apart from *A. duodenale* (list included in Appendix F: Comprehensive List of Parasites Discovered through O&P Assay); while treatment recommendations were provided for all of these parasites, they were not included in statistical analysis.

Data Entry/Processing:

PI entered all data from questionnaires and lab results into MS Excel (2007). Given the small size of the sample (N≈200), each record was cross-checked by PI with the hard copy once all entries were completed. Data used in statistical analysis was de-identified by replacing each name with a unique subject ID number upon completion of data entry. MS Excel files were password-protected and stored on PI's laptop, which is also password protected.

Statistical Analysis

All statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC) save for the calculation of percentiles (BMI-for-age [BAP], weight for age [WAP], and height-for-age [HAP]) which were performed with WHO AnthroPlus software (WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009 [http://www.who.int/growthref/tools/en/]).

Approach to Statistical Analyses

Exploratory bivariate analysis was conducted by determining the Pearson's correlation coefficient between each predictor variable and the outcome variable (data not shown). Proportion of HI was calculated for each group as: $\frac{\boldsymbol{n}\ positive\ subjects}{\boldsymbol{n}\ subjects\ with\ O\&P\ results}$. An independent two-sample Pearson's χ^2 test of proportions was used to compare the crude (unadjusted) prevalence of HI in residents to non-residents. Alpha level was set at 0.1 for calculation of 90% confidence intervals (CI) around the prevalence estimates. An α-level of

0.1 was chosen to account for the relatively small sample size—and the resultant reduction in power to detect differences of interest.

Multivariable logistic regression was used to assess the association between HI and potential risk and protective factors (explanatory variables). The best subsets method utilizing Mallow's C_p values was employed to consider potential models. Explanatory variables were evaluated for significance using a forward-stepwise procedure with significance level of 0.2 used as a cutoff for entry into the model, as recommended by Hosmer and Lemeshow[30]. The model resulting from forward-stepwise selection was compared to that obtained through best subsets/Mallow's C_p.

The model was tested for goodness-of-fit using the Hosmer-Lemeshow test. Model diagnostics were based on analysis of individual residuals and graphs of the following statistics: Change in Pearson's χ^2; Standardized Pearson's Residual; Change in Deviance; Leverage; Cook's Distance; and DfBetas (data not shown).

Associations were calculated for (significant) model variables by using SAS estimates of the variable coefficients and were equivalent to the statement (the probability of HI | status of predictor variable). Odds ratios were estimated to compare HI between categories of predictor variables.

Confounding was assessed by comparing the crude and adjusted Pearson's χ^2 tests of HI proportions between residents and non-residents and odds ratios (for HI by status of explanatory variable). An explanatory covariate was considered a potential confounder if it changed an estimate (OR, χ^2) by ≥10%.

Results

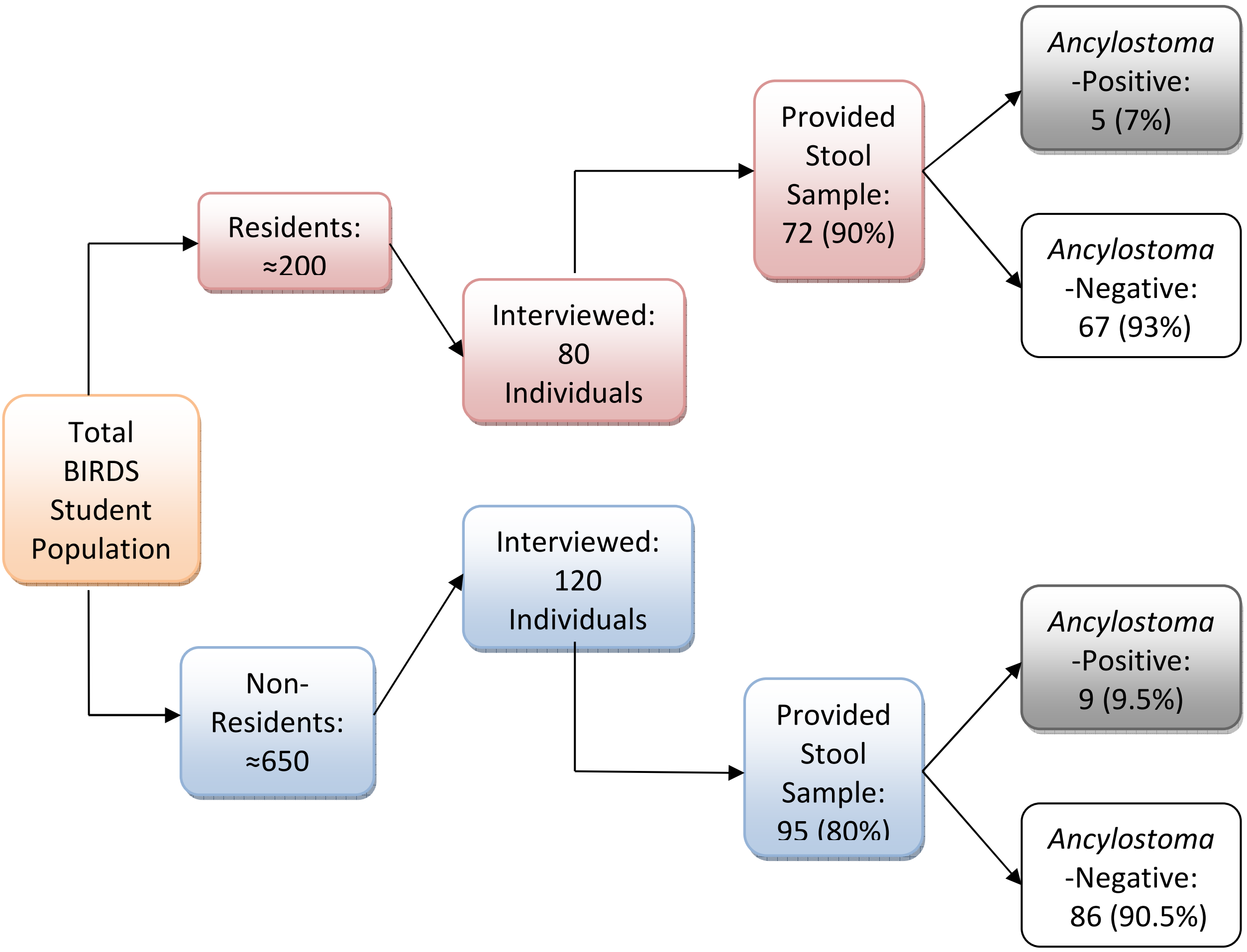


Figure 3: Study Population Processing and Results

Study Sample

The flowchart in **Figure 3** depicts the number of subjects and process results at each stage of the study. While 200 total subjects were interviewed (80 Residents; 120 Non-Residents), 33 failed to provide a stool sample and thus had to be excluded from the test of hypothesis (comparison of *A. duodenale* infection prevalence) and evaluation of covariate association with hookworm infection. Of the BIRDS campus Residents interviewed, 90%

(72/80) provided a stool sample, whereas among the Non-Residents, 80% (95/120) submitted a sample. The total number of subjects interviewed and whose samples were examined (167) represent roughly 20% of the total BIRDS student population (167/850).

Descriptive Statistics

<u>Combined</u> Subject Population

Table 6 (a): Total Study Population Characteristics (N=167) - *HI, Anthropometrics**

	Variable	
(n=167)	**Hookworm Infection**	14 (8.4%)
(n=167)	**Age**	
	Mean	10 years
	Median	10 years
	Range	3-16
(n=167)	**Sex:**	
	Male	104 (63%)
	Female	63 (37%)
(n=167)	**BMI**	
	Mean	15.4
	Median	14.9

*Sample sizes ("n's") for each variable may be less than 167, as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

Table 6 (b): Total Study Population Characteristics (N=167) - *Risk Behaviors**

	Variable	
(n=164)	**Hand Washing Frequency Post-Defecation**	
	Most of the Time	136 (83%)
	Sometimes	20 (12%)
	Never	8 (5%)
(n=164)	**Hand Washing Frequency Before-Eating**	
	Most of the Time	135 (83%)
	Sometimes	25 (15%)
	Never	4 (2%)
(n=161)	**Outdoor Defecation Frequency-*Home***	
	Every Time	110 (68%)
	Sometimes	23 (14%)
	Never	28 (17%)
(n=157)	**Outdoor Defecation Frequency-*School***	
	Every Time	55 (35%)
	Sometimes	43 (27%)
	Never	59 (38%)
(n=164)	**Footwear Use Frequency**	
	Most of the Time	87 (53%)
	Sometimes	46 (28%)
	Never	31 (19%)

*Sample sizes ("n's") for each variable may be less than 167, as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

Table 6 (c): Total Study Population Characteristics (N=167) - *Symptomologies**

	Variable	
(n=162)	**Current GI Pain**	
	Y	69 (43%)
	N	93 (57%)
(n=162)	**Current Cough**	
	Y	46 (28%)
	N	116 (72%)
(n=164)	**Appetite vs. Peers**	
	More	18 (11%)
	Same	124 (76%)
	Less	22 (13%)
(n=164)	**Energy vs. Peers**	
	More	49 (30%)
	Same	80 (49%)
	Less	35 (21%)

*Sample sizes ("n's") for each variable may be less than 167, as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

The effective study population consisted of 167 rural Southern Indian children aged 3-16 years with a median age of 10 years. 63 (37%) of all subjects were female, 104 (63%) male. Participant gender proportions reflected those of the student body at BIRDS (65% male, 35% female). The median BMI for all subjects was calculated as 14.9. With regard to the known risk behaviors for HI: 67% of students report defecating outdoors every time while at home, whereas 35% report defecating outdoors every time at school; only about half (53%) report wearing footwear most of the time, and 19% indicate never using footwear.

Overall Prevalence of A. duodenale

In the total student body overall, 14 subjects (8.4% [90%CI: 4.8, 12]) tested positive for hookworm infection.

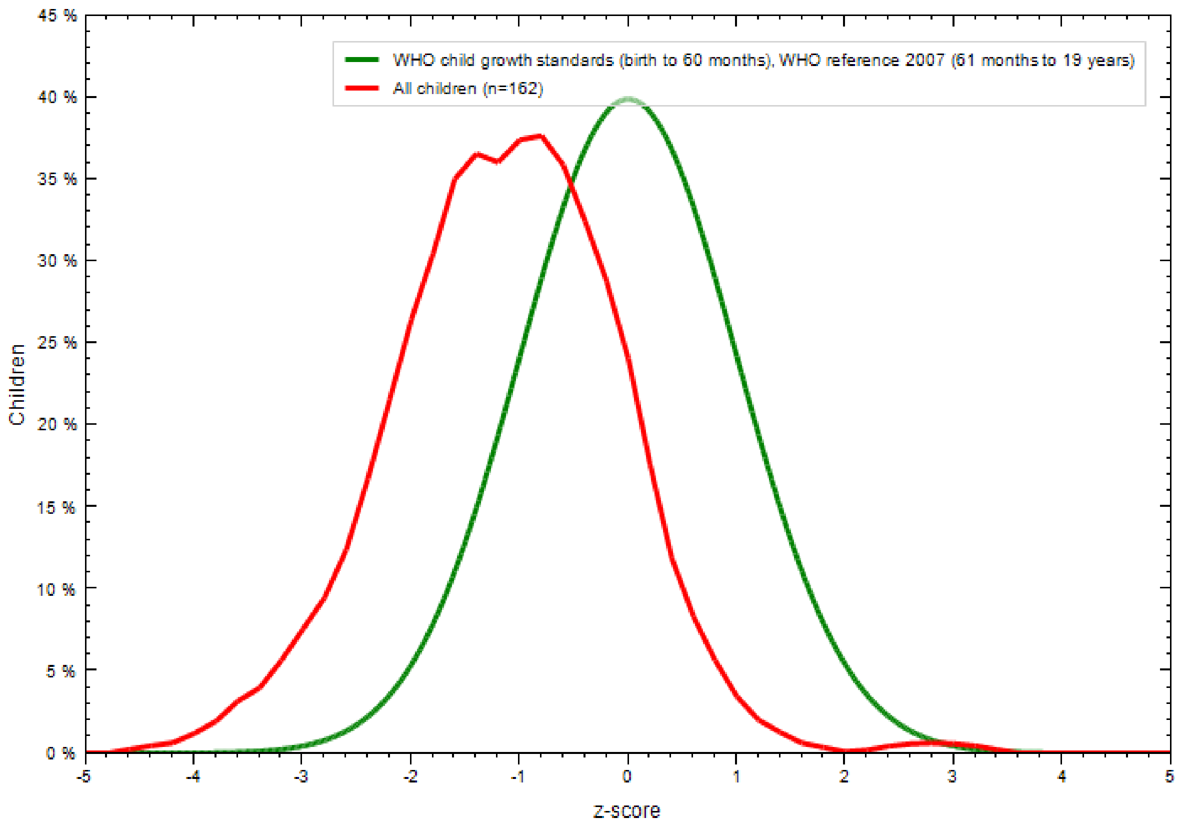


Figure 4: BIRDS All Children – BMI for Age Percentiles – (2011)

The school children at BIRDS (both residency groups combined) were found to have significantly lower average weight and height compared to the WHO international standards for children of the same ages. **Figure 4** displays that schoolchildren at BIRDS have a significantly lower average BMI-for-age (z-score) than the WHO standard.

Subject Population Stratified by Residency Group

Table 7 (a): Subject Population Characteristics by Residency Group - *Anthropometrics**

	Variable	Residents (n=72)	Non-Residents (n=95)	Statistical Test (α=0.1) [*p-value*]
(n=167)	**Age**			<0.0001
	Mean	12 years	9 years	
	Median	12 years	9 years	
	Range	4-15 years	3-16 years	
(n=167)	**Sex:**			0.36
	Male	42 (58%)	62 (65%)	
	Female	30 (42%)	33 (35%)	
(n=167)	**BMI**			0.18
	Median	15.8	14.4	
	Mean	16.3	14.7	

*Sample sizes ("n's") for each variable may be less than 72 Residents and 95 Non-Residents, as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

Table 7 (b): Subject Population Characteristics by Residency Group - *Risk Behaviors**

	Variable	Residents (n=72)	Non-Residents (n=95)	Statistical Test[θ] (α=0.1) [*p-value*]
(n=164)	**Hand Washing Frequency Post-Defecation**			0.61
	Most of the Time	60 (86%)	76 (81%)	
	Sometimes	8 (11%)	12 (13%)	
	Never	2 (3%)	6 (6%)	
	missing	2	1	
(n=164)	**Hand Washing Frequency Before Eating**			0.41
	Most of the Time	61 (87%)	74 (78%)	
	Sometimes	8 (11%)	17 (18%)	
	Never	1 (1.4%)	3 (3%)	
	missing	2	1	
(n=161)	**Outdoor Defecation Frequency-*Home***			0.67
	Every Time	47 (69%)	63 (68%)	
	Sometimes	11 (16%)	12 (13%)	
	Never	10 (15%)	18 (19%)	
	missing	4	2	
(n=157)	**Outdoor Defecation Frequency-*School***			**<0.0001**
	Every Time	5 (7%)	50 (56%)	
	Sometimes	23 (34%)	20 (22%)	
	Never	39 (58%)	20 (22%)	
	missing	5	5	
(n=167)	**Regular Outdoor Defecation††**			**<0.0001**
	Yes	3 (4%)	42 (44%)	
	No	69 (96%)	53 (56%)	
(n=164)	**Footwear Use Frequency**			**0.002**
	Most of the Time	47 (67%)	40 (43%)	
	Sometimes	17 (24%)	29 (31%)	
	Never	6 (9%)	25 (27%)	
	missing	2	1	

*Sample sizes ("n's") for each variable may be less than 72 Residents and 95 Non-Residents, as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

[θ]Fisher's exact test was used for variables containing ≥25% expected cell counts less than 5.

††The variable "Regular Outdoor Defecation" was generated by consolidating the 2 Outdoor Defecation Frequency Variables (OD Frequency-Home; OD Frequency – School); A response of "Every Time" to both of these translates to a "Yes" entry while all other responses translated to a "No" entry. A subject who indicated a practice of defecating outdoors "Every Time" both at home and at school is believed to perform OD as a regular behavior.

Table 7 (c): Subject Population Characteristics by Residency Group - *Symptomologies**

	Variable	Residents (n=72)	Non-Residents (n=95)	Statistical Test (α=0.1) [*p-value*]
(n=164)	**Appetite vs. Peers**			0.97
	More	8 (11%)	10 (11%)	
	Same	53 (76%)	71 (76%)	
	Less	9 (13%)	13 (14%)	
	missing	2	1	
(n=164)	**Energy vs. Peers**			**0.078**
	More	25 (36%)	24 (26%)	
	Same	27 (39%)	53 (56%)	
	Less	18 (26%)	17 (18%)	
	missing	2	1	
(n=162)	**Current GI Pain**			0.60
	Y	31 (43%)	38 (41%)	
	N	38 (55%)	55 (59%)	
	missing	3	2	
(n=162)	**Current Cough**			**0.004**
	Y	28 (40%)	18 (20%)	
	N	42 (60%)	74 (80%)	
	missing	2	3	

*Sample sizes ("n's") for each variable may be less than 72 Residents and 95 Non-Residents, as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

[θ]Fisher's exact test was used for variables containing ≥25% expected cell counts less than 5.

Several significant differences were observed between the two residency groups (**Table 7 [a-c]**). While Residents more frequently reported a current cough (p = 0.0043), a current cough was not found to be significantly associated with hookworm infection (p≈1; **Table 9 [c],** page 31).

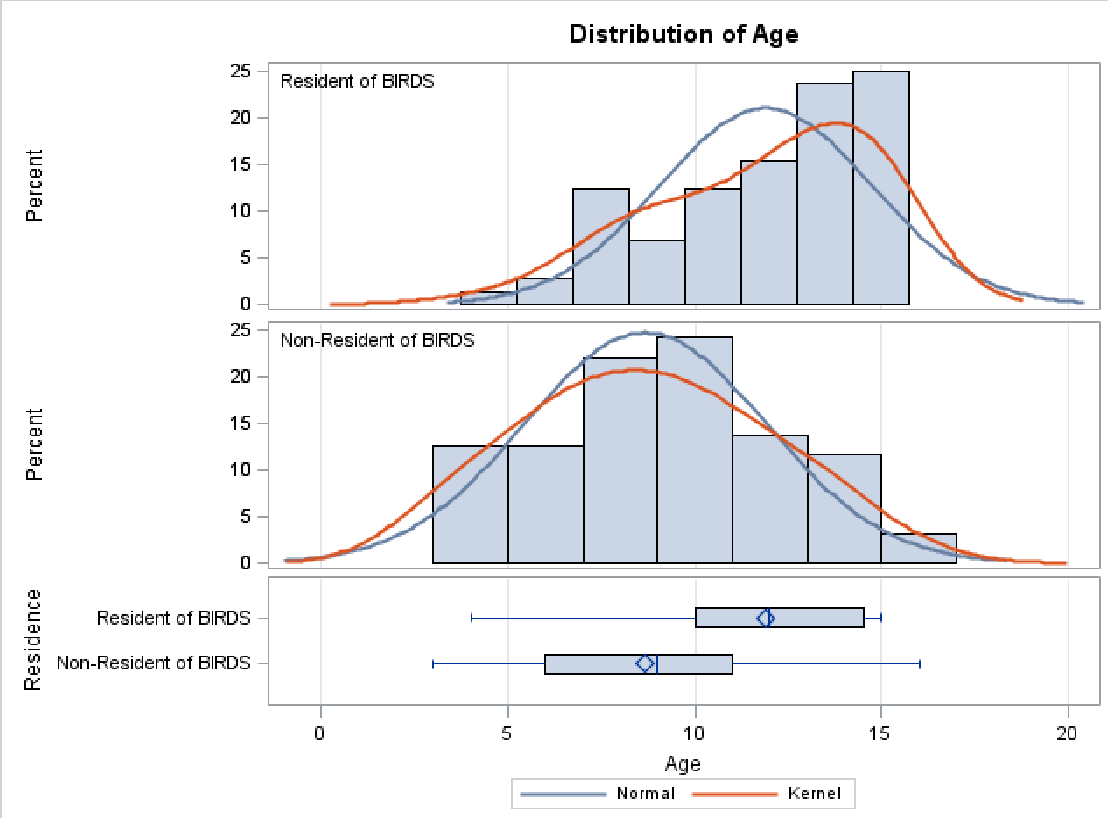


Figure 5: Differences in Age Distribution between Residency Groups

The difference in the distribution of age among residency groups is depicted in **Figure 6**. The median ages for each residency group (9 yrs. for Residents vs. 12 yrs. for Non-Residents) differs significantly (p <0.0001). Age did not, however, demonstrate any significant association with HI (p=0.26), regardless of whether it was used as a continuous or categorical variable. Neither residency group reported more frequent *Hand-Washing* than the other, neither *Post-Defecation* nor prior to eating. While at their homes, both Residents and Non-Residents reported similar relative frequencies of *Outdoor Defecation*, with the great majority (approx. 70% both groups) indicating they defecate outside every time. Since the majority of homes in the area do not have latrines or plumbing, outdoor defecation as a usual practice was expected. When questioned about defecation habits at

BIRDS school (where latrines are available), however, only 7% of Residents claimed to go out-of-doors every time compared to 56% of Non-Residents. Students living at BIRDS school reported significantly more frequent use of footwear than students living off-campus; with 67% of residents claiming to use footwear most of the time and 43% of non-residents indicating such frequency. Substantially different responses were received between residency groups (p=0.078) when subjects were asked to rate their own energy level compared to peers. Specifically, 26% of Residents estimated having less energy than their peers compared to 18% of Non-Residents.

Despite the significant differences between residency groups in the two aforementioned established risk behaviors for HI (*Outdoor Defecation; Footwear Use Frequency*), we fail to reject the null hypothesis that no significant difference in the crude prevalence of HI exists between Residents and Non-Residents (**Table 8**).

Table 8: Hookworm Infection Crude Prevalence by Residency Group

	Residents (n=72)	Non-Residents (n=95)	Statistical Test (α=0.1) [*p-value*]
Hookworm Infection:	5 (7.0%) (90%CI: 2.1, 12)	9 (9.5%) (90%:CI: 4.6, 14)	0.56

Several differences were observed in the self-reported characteristics and behaviors between students who tested positive for *A. duodenale* and those who tested negative, though not all yielded significant measures of association (OR) (**Table 9 (a-c)**, page 28). Hookworm-positive individuals more frequently reported *Current GI Pain* (p=0.09), washed their hands less frequently after defecating (p=0.034), and considered themselves to have both less appetite (p=0.0056) and less energy (p=0.0027) than their peers. Bivariate analysis indicated that reporting never hand-washing after defecating, and having less appetite than peers were most strongly associated with ancylostomiasis. The unadjusted odds of reporting current GI pain among those who tested positive for hookworm are 2.4 times those who did not yield a positive test (90% CI: 0.85, 6.9). The unadjusted odds of reporting never hand-washing after defecating among hookworm-infected individuals are 7.5 times the odds of making such a report among individuals not infected (90% CI: 1.5, 36). Individuals who tested positive for *A. duodenale* had unadjusted odds of reporting less appetite and less energy 5.4 and 2.6 times the odds of these reports from individuals who tested negative, respectively (90% CI's: 1.6, 17; 0.84, 8.1).

Table 9 (a): Subject Population Characteristics by Hookworm Infection Status - *Anthropometrics**

	Variable	Positive Test (n=14)	Negative Test (n=153)	OR (90% CI)	Statistical Test (α=0.1) [*p-value*]
(n=167)	**Age**			1.08 (0.95, 1.3)	0.26
	Mean	11 years	11 years		(*Satterthwaite*)
	Median	12 years	10 years		
	Range	6-15 years	3-16 years		
(n=167)	**Sex:**				0.87
	Female	5 (36%)	58 (38%)	0.92 (0.38, 2.2)	
	Male	9 (64%)	95 (62%)	*Referent*	
(n=167)	**BMI**			0.71 (0.44, 1.1)	0.78
	Mean	15.1	15.7		
	Median	14.8	14.9		

*Sample sizes ("n's") for each variable may be less than 14 HI(+) and 153 HI(-), as subjects without a response for a particular variable were excluded from tabulation of the respective variable.
*Percentages calculated based on sample sizes excluding missing respondents.
*All OR's and statistical tests values are bivariate, unadjusted.

Table 9 (b): Subject Population Characteristics by Hookworm Infection Status - *Risk Behaviors**

	Variable	Positive Test (n=14)	Negative Test (n=153)	OR (90% CI)	Statistical Test[θ] (α=0.1) [*p-value*]
(n=164)	**Hand Washing Frequency Post-Defecation†**				**0.022**
	Most of the Time + Sometimes	11 (79%)	145 (97%)	*Referent*	
	Never	3 (21%)	5 (3%)	**7.9 (2.1, 29)**	
	missing		*3*		
(n=164)	**Hand Washing Frequency Before Eating[φ]**				0.30
	Most of the Time + Sometimes	12 (92%)	147 (98%)	*Referent*	
	Never	1 (8%)	3 (2%)	3.8 (0.53, 26)	
	missing	*1*	*2*		
(n=161)	**Outdoor Defecation Frequency-*Home***				0.48
	Every Time	12 (86%)	98 (67%)	3.3 (0.41, 26)	
	Sometimes	1 (7%)	22 (15%)	1.2 (0.11, 13)	
	Never	1 (7%)	27 (18%)	*Referent*	
	missing		*6*		
(n=157)	**Outdoor Defecation Frequency-*School***				0.73
	Every Time	6 (43%)	49 (34%)	1.6 (0.55, 5.1)	
	Sometimes	4 (28%)	39 (27%)	1.4 (0.41, 4.7)	
	Never	4 (29%)	55 (38%)	*Referent*	
	missing		*10*		
(n=167)	**Regular Outdoor Defecation††**				0.20
	Yes	6 (43%)	39 (25%)	2.19 (0.86, 5.6)	
	No	8 (57%)	114 (75%)	*Referent*	
(n=164)	**Footwear Use Frequency**				0.53
	Most of the Time	8 (57%)	79 (53%)	*Referent*	
	Sometimes	5 (36%)	41 (27%)	1.2 (0.37, 3.9)	
	Never	1 (7%)	30 (20%)	0.33 (0.04, 2.7)	
	missing		*3*		

*Sample sizes ("n's") for each variable may be less than 14 HI(+) and 153 HI(-), as subjects without a response for a particular variable were excluded from tabulation of the respective variable.
*Percentages calculated based on sample sizes excluding missing respondents.
*All OR's and statistical tests values are bivariate, unadjusted.
[θ]Fisher's exact test was used for variables containing ≥25% expected cell counts less than 5.

† (Hand Washing Frequency Post-Defecation) The categorical responses of "Sometimes" (1[7%]-HI(+); 19 [13%]-HI(-)) were collapsed into "Most of the Time" due to inadequate cell sizes for statistical analysis.
Φ(Hand Washing Frequency Before-Eating) The categorical responses of "Sometimes" (1[7%]-HI(+); 24 [16%]-HI(-)) were collapsed into "Most of the Time" due to inadequate cell sizes for statistical analysis.
††The variable "Regular Outdoor Defecation" was generated by consolidating the 2 Outdoor Defecation Frequency Variables (OD Frequency-Home; OD Frequency – School); A response of "Every Time" to both of these translates to a "Yes" entry while all other responses translated to a "No" entry. A subject who indicated a practice of defecating outdoors "Every Time" both at home and at school is believed to perform OD as a regular behavior.

Table 9-c: Subject Population Characteristics by Hookworm Infection Status - *Symptomologies**

	Variable	*Positive* Test (n=14)	*Negative* Test (n=153)	OR (90% CI)	Statistical Test[θ] (α=0.1) [*p-value*]
(n=162)	**Current GI Pain**				**0.09**
	Y	9 (65%)	60 (41%)	2.4 (0.85, 6.9)	
	N	5 (36%)	88 (59%)	*Referent*	
	missing		*5*		
(n=162)	**Current Cough**				1
	Y	4 (29%)	42 (28%)	1 (0.37 – 2.8)	
	N	10 (71%)	106 (72%)	*Referent*	
	missing		*5*		
(n=164)	**Appetite vs. Peers[¥]**				**0.0044**
	More + Same	8 (57%)	134 (90%)	*Referent*	
	Less	6 (43%)	16 (10%)	**6.3 (2.3, 17)**	
	missing		*3*		
(n=164)	**Energy vs. Peers[φ]**				**0.012**
	More + Same	7 (50%)	122 (82%)	*Referent*	
	Less	7 (50%)	28 (18%)	**4.4 (1.7, 11)**	
	missing				

*Sample sizes ("n's") for each variable may be less than 14 HI(+) and 153 HI(-), as subjects without a response for a particular variable were excluded from tabulation of the respective variable.

*Percentages calculated based on sample sizes excluding missing respondents.

*All OR's and statistical tests values are bivariate, unadjusted.

[θ]Fisher's exact test was used for variables containing ≥25% expected cell counts less than 5.

[¥](Appetite vs. Peers) The categorical responses of "Same" (8[57%]-HI(+); 116 [77%]-HI(-))were collapsed into "More" (0-HI(+); 49 [33%]-HI(-))due to inadequate cell sizes for statistical analysis.

[φ](Energy vs. Peers) The categorical responses of "Same" (7 [50%]-HI(+); 73 [49%]-HI(-))were collapsed into "More" (0-HI(+); 49 [33%]-HI(-))due to inadequate cell sizes for statistical analysis.

Table 10: Testing for Factors Associated with Hookworm Infection**

	Variable	OR (90% CI)	Wald Chi-Square (α=0.1) [*p-value*]
N=164	**Appetite vs. Peers¥**		**0.0063**
	More + Same	*Referent*	
	Less	**6.3 (2.1, 19)**	
	Hand Washing Frequency Post-Defecation†		**0.022**
	Most of the Time	*Referent*	
	Never	**10.7 (1.9, 60)**	
	Regular Outdoor Defecation††		**0.0543**
	Yes	**3.7 (1.2, 12)**	
	No	*Referent*	
	Footwear Use Frequency		
	Most of the Time	*Referent*	
	Sometimes	0.60 (0.18, 1.9)	0.47
	Never	**0.08 (0.008, 0.82)**	**0.074**

**OR's adjusted for all variables included in this table.

¥(Appetite vs. Peers) The categorical responses of "Same" (8[57%]-HI(+); 116 [77%]-HI(-))were collapsed into "More" (0-HI(+); 49 [33%]-HI(-))due to inadequate cell sizes for statistical analysis.

† (Hand Washing Frequency Post-Defecation) The categorical responses of "Sometimes" (1[7%]-HI(+); 19 [13%]-HI(-)) were collapsed into "Most of the Time" due to inadequate cell sizes for statistical analysis.

††The variable "Regular Outdoor Defecation" was generated by consolidating the 2 Outdoor Defecation Frequency Variables (OD Frequency-Home; OD Frequency – School); A response of "Every Time" to both of these translates to a "Yes" entry while all other responses translated to a "No" entry. A subject who indicated a practice of defecating outdoors "Every Time" both at home and at school is believed to perform OD as a regular behavior.

As reported in **Table 9 (c)**, bivariate analyses performed through simple logistic regression revealed that while *Current GI Pain* yielded a significant statistical test, the unadjusted Odds Ratio did not show a significant association with hookworm infection. Among the results of bivariate analyses, *Hand-Washing Frequency-Post Defecation*, *Energy vs. Peers*, and *Appetite vs. Peers* demonstrated significant unadjusted OR's that did not include the null value (1).

Because both *Appetite vs. Peers* and *Energy vs. Peers* included a count of "0" in the cell for subjects who both tested positive for *Ancylostomiasis* and reported having "More" appetite/energy than peers, the categorical responses "Same" and "More" were collapsed (refer to Table 9 notes for specifics) to allow for evaluation of association through logistic regression. From an epidemiologic and clinical perspective, it is worth noting that no student found to be infected with hookworm reported having either more appetite or more energy than fellow classmates.

After adjusting for the other factors included in the final MLR model, *Hand-Washing Frequency-Post Defecation* and *Appetite Vs. Peers* remained significantly associated with HI, while *Regular Outdoor Defecation* and *Footwear Frequency (never* vs. *most of the time)* achieved significant association (**Table 10**). *Footwear frequency,* however, barely crossed the (alpha=0.1) level of significance. Students who reported never performing hand-washing following defecation were 10.7 times as likely to be infected with hookworm than students who reported hand-washing most of the time (Adjusted, 90%CI: 1.9, 59). Compared to subjects who described themselves as having equal or more appetite than their peers, subjects who described themselves as having less appetite were 6.3 times as likely to test positive for ancylostomiasis (Adjusted, 90%CI: 2.1, 19). Students who defecate outdoors regularly were 3.7 times more likely to be infected with A. duodenale than students who do not (Adjusted, 90%CI: 1.2, 12). Finally, individuals who claimed to never wear shoes had 0.08 times the odds of infection with hookworm as individuals who reported wearing shoes most of the time (90%CI: 0.008, 0.82).

Discussion

The Prevalence Estimates

As mentioned previously, it is difficult to make comparisons between the overall prevalence of *Ancylostoma duodenale* infection obtained in this study (8.4%; Ages 3-16 yrs.) and prior estimates, since no research which estimated hookworm prevalence in children of like ages and gender distribution and living in the Indian province represented in our study in have been identified. When considering all ages, gender distributions, and all other Indian provinces, estimates of HI prevalence cover a wide range from 1.3-84% but with the overwhelming majority trending much higher than the 8.4% obtained in our study.[17,20,23,25–27,31] Having evaluated those studies judged to represent a population and environment closest to that studied here, we arrived at the *a priori* estimate of 20-30% prevalence. A couple factors likely contributed to the point prevalence estimate from our study emerging as lower than we expect the actual *A. duodenale* prevalence to be, notably the low sensitivity of the O&P exam and the absence of serial testing.

Hookworm eggs can be susceptible to desiccation and require some ambiental moisture to successfully pass through the three larval stages and achieve entry into a human host. Previous experimental and field research has demonstrated the connections between the amount of hookworm transmission and environmental conditions including heat and moisture[3,6,7,32]. One previous study found that the prevalence of hookworm (in an area south of Bangladesh) increased by about 14%

from pre-monsoon season (March-June) to post-monsoon season (November-February).[33] Since the majority of the present study was conducted June-August, and the monsoon season was just beginning in August, it appears quite possible that the prevalence of hookworm infection would have increased over the coming months in relation to the increased amount of precipitation and resultant ambiental moisture.

Limitations:

Low Sensitivity of the O&P Exam

The greatest limiting factor in our study is undoubtedly the low sensitivity of the ova and parasite examinations performed on the stool samples from our subjects. The absence of any degree of serial testing assures that the estimate of prevalence generated from our single sample per individual will significantly underestimate the true proportion of students infected. In large diagnostic laboratories in the U.S., such as Kaiser Permanente's Northwest Laboratory, the standard is to test no less than 3 samples collected over a 72-hour period.[24,34] Even under optimal laboratory testing conditions, such as those employed by Kaiser and large university medical facilities, single O&P examinations have demonstrated a sensitivity of ≤ 79% when using a single sample.[34] The data depicted in **Figure 7** from a 1998 study that took place in an Indian province (Tamil Nadu) neighboring that in which ours was conducted (Andhra Pradesh) indicated a sensitivity of slightly less than 40% for the use of 1 sample[27]. Additionally, it was not possible to keep all samples under refrigeration from the time of collection to the time of analysis. With usual daytime temperatures ranging from the low 90's to over 100° F, it could be expected that some ancylostoma ova present in samples degraded past the point of detectability. Even if we were to presume that additional factors apart from the lack of serial testing did not diminish the sensitivity in our study, such a metric (**Figure 7**) would put our prevalence estimate at 21%. Due to cost, burden of stool sample collection on subjects and partner NGO staff, fluctuating subject generosity with stool sample remittance, and the onerous task of transporting samples daily to the laboratory some three hours away by bus, we accepted the limitation of a single O&P examination. In light of the absence of serial testing, the lack of consistent sample refrigeration, and various less-than-ideal collection measures, we are confident that our estimate of hookworm prevalence is significantly lower than the actual prevalence.

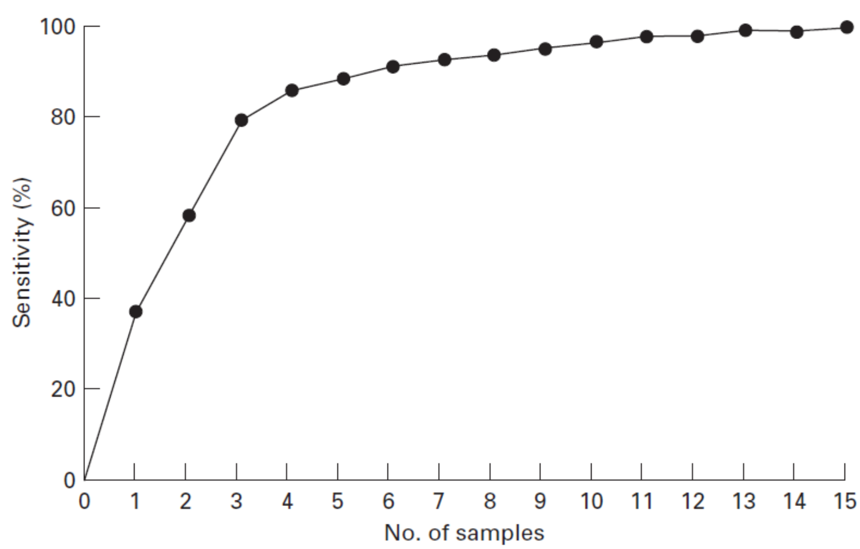


Figure 6: The effect of serial testing on the sensitivity of O&P assay[27]

At the study design stage, we created a seemingly obvious exclusion criterion stating that a subject's receipt of de-worming medication within six months prior to participating in the study would be grounds to exclude that subject. We logically expected that an individual treated with an anti-helminthic drug would be less likely to test positive for hookworm infection. While conducting the active study in Muthyalapadhu, however, it was not obvious whether subjects had received de-worming treatment or not. Initial reports from BIRDS administration indicated that no campus resident had received de-worming medication in the six months prior to initiation of the study. The boys hostel warden (who served as the primary interpreter and interviewer) confirmed to the researcher on–site that no distribution of de-worming medication had been conducted at BIRDS school in recent memory. While attempting to review student medical records at BIRDS campus clinic and confirm that no individual or group of students had been given anti-helminthic treatment, a tub containing a few hundred doses of the common anti-helminthic drug albendazole was observed in the clinic pharmacy. It was also learned that medical records are not usually kept for patients seen in the BIRDS clinic and thus could not be used to determine receipt of deworming treatment. Although clinic staff were not in total agreement, some thought that the boys warden had widely distributed albendazole to residents of the hostels some months prior (though the time was also unclear). Thus, while our best guess was that student residents of BIRDS had not received recent de-worming treatment, some residual doubt persists. To assist with confirmation, each subject was asked during interview whether or not he/she had received any de-worming medication recently. No participant responded "yes" to this question.

We acknowledge the possibility that deworming treatment, if given, may have artificially lowered the prevalence (in either or both groups) estimated through our study. Apart from BIRDS clinic, no formal clinical services in the study locale were identified. Some local persons make the three-hour bus trip to the hospital to obtain clinical care, but most do not. As wards of BIRDS, campus residents may be more likely to receive clinical care—including anti-helminthic treatment. The slightly lower HI prevalence observed in student residents at BIRDS relative to non-residents (7% vs. 9.5%) could possibly be an artifact of such treatment, but confirmation is not possible.

As a population, the student body at BIRDS school may be self-selected for lower hookworm prevalence, burden—or both. It may be that children in this region who are severely ill do not go to school. The fact that a child attends BIRDS school may mean that he or she does not suffer from any such severe illness. Thus the population of children in the surrounding communities who do not attend school could have a greater prevalence and burden of illness—including ancylostomiasis—than the students at BIRDS. Chronic, heavy hookworm infestations in children can cause chronic diarrhea and poor intellectual and physical performance. It is not difficult to imagine how such burdensome sequelae could keep a child from attending school, or at least decrease frequency of attendance. Also, relatively higher levels of transmission and prevalence of hookworm infection among agricultural workers have been demonstrated in the literature.[2,3,6–8,26,27,33] We know that before they were students at BIRDS, many children worked in agriculture—the largest regional industry. It is reasonable to assume that the population of children in the surrounding communities who do not attend school have a larger proportion of regular agricultural workers relative to the student body at BIRDS. For the reasons discussed above, a higher prevalence of hookworm may exist in the local population of non-students.

Hand-Washing Frequency-Post-Defecation

Hand-Washing Frequency-Post-Defecation demonstrated a strong association with ancylostomiasis (OR 10.7 [90%CI: 1.9, 59]), though the practical infectious pathway (and association) is not as clear as it might at first seem. Although the predominant mode of transmission is believed to occur via direct contact of filariform larvae with unprotected skin (e.g. bare feet contacting feces, soil, or low-lying ground foliage harboring larvae), infection by *A. duodenale* can also occur orally with direct maturation in the intestine to adult stage.[1,35] In the study locality (rural Andhra Pradesh, India), the usual method of cleaning oneself following defecation if performed, consists of using a shared water pitcher ("dipper") to apply water to the anal region and performing any physical cleaning/removal of residual fecal material with an uncovered hand. Since about two weeks are required for eggs to transform into the infective form of *A. duodenale* (the filariform [third-stage] larvae), immediate auto-infection or xenoinfection by *A. duodenale* is not biologically plausible. It is unclear whether in the common outdoor defecation practice, a person's hands may come into contact with material harboring previously matured filariform larvae that then penetrate the skin or are transferred to the oral cavity—if not removed via cleansing of the hands. An important alternative explanation, however, is that *hand-washing frequency-post-defecation* may also be a marker either for outdoor defecation or another variable actually responsible for transmission of HI that we did not measure in this study (i.e. a confounder). Although BIRDS staff suggested to us that no cultural stigma existed regarding outdoor defecation, student participants may have been disinclined to self-report regular outdoor defecation. The on-site researcher conducted educational seminars with all students at BIRDS (by grade-level group) to discuss germ theory and geohelminth transmission (of which a core component is outdoor defecation). Such communications may have lead the student participants to believe that regular outdoor defecation (or other behaviors in the transmission pathway) was (were) disapproved of and thus avoided accurately describing their behavior(s). If this occurred, it would have diminished the observed association between outdoor defecation and ancylostomiasis (Adjusted OR: 3.7; 90%CI: 1.2, 12).

It is reasonable to wonder why never-washing hands post-defecation demonstrated an association with hookworm infection nearly three times that of regular outdoor defecation (Adjusted OR: 10.7 vs. 3.7). We expect that when these individuals are defecating in the fields, they are probably not washing their hands. At the least, regular outdoor defecators probably wash their hands less frequently than persons who regularly use latrines, where hand-washing sinks (at least at BIRDS) are nearby. If students felt disinclined to self-report regular outdoor defecation, perhaps they did not feel such an inhibition regarding hand-washing. It is possible that the effect (association with hookworm infection) observed with *Hand-Washing Frequency Post-Defecation* is actually due to *Regular Outdoor Defecation.*

Regular Outdoor Defecation

As expected based on the well-documented transmission pathway in similar populations, *Regular Outdoor Defecation* demonstrated a significant association with hookworm infection, with those students reporting indiscriminate defecation "every time" having 3.7 times the odds of being infected as students who reported a frequency of "sometimes" or "never" (Adjusted, 90% CI: 1.2, 12). We would expect this observed association only to increase with greater O&P assay sensitivity and a larger sample. Linking the practice of defecating outdoors to HI may serve as a rallying point for BIRDS to both provide more latrines and encourage more frequent usage.

Presumptive Clinical Treatment

The WHO recommends universal treatment with an anti-helminthic drug (e.g. albendazole, mebendazole) every 6 months for at-risk pediatric populations.[2] Although the commonly-used anti-helminthic drugs have demonstrated relatively low risk of adverse secondary effects, when deployed in large populations, even a small amount of risk will be amplified to some degree—with some individuals incurring adverse effects. Part of our rationale in seeking to identify symptoms associated with HI, was that if the administration of anti-helminthic treatment could be selectively applied only to those individuals deemed likely to harbor infections, then some of the aforementioned negative side effects of treatment might be avoided. Furthermore, since accurate O&P examination performed by a qualified laboratory is unlikely to be available for the majority of individuals from the BIRDS student population presenting with possible helminth infections, presumptive treatment is the option most likely to be utilized by local clinicians. The data failed to show any significant association between either the gastro-intestinal or respiratory symptoms usually attributed to hookworm infection.

As discussed previously in this paper, no subject who tested positive for ancylostomiasis described him/herself as having either more appetite or more energy than peers. Self-reports of less energy and less appetite vs. peers both showed significant association with HI when compared to subjects who reported having the same or more appetite or energy (unadjusted OR [90%CI]: *Energy*: 4.4 [1.7, 11]; *Appetite:* 6.3 [2.3, 17]). A significant amount of overlap in symptoms measured with the *Appetite* and *Energy vs. Peers* variables seems to have occurred. When both variables were placed in the penultimate multivariate logistic regression model, overall attenuation of effects was observed, particularly for the *Energy* and *Appetite* variables (data not shown). *Appetite vs. Peers* demonstrated the stronger association with HI and was used in the model exclusively (adjusted OR: 6.3 [90%CI: 2.1, 19]). As expected given the chronic insidious blood loss, various types of anemia have been demonstrated to occur with chronic hookworm infections in children.[3,6–8,12,15,17,19,24,25,28] Given 1) the well-established association between anemia and HI in children; 2) that decreased appetite is one of most common symptoms of anemia in children; and 3) reporting decreased appetite showed a strong association with HI in our study, it is highly possible that the decreased appetite we observed was a manifestation of hookworm-induced (or influenced) anemia. Thus, the symptom which appears as a potential indicator for presumptive treatment for hookworm infection in this population is a decreased appetite.

Footwear Use Frequency

A self-report of never wearing shoes curiously emerged as demonstrating a marginal “protective” effect with HI (Adjusted OR: 0.08 [90%CI: 0.008, 0.82]). As reported, 1/14 (7%) HI(+) individual reported never wearing shoes vs. 30/153 (20%) of HI(-) individuals. The small cell size (1) representing a [HI(+)/never shoe-user] and the relatively small number of persons testing positive for HI (14/167), made it difficult to generate meaningful comparisons in this regard. No biologically plausible explanation has been identified to suggest why not wearing shoes would be protective against a burrowing parasite that enters the host most often through exposed skin on the feet and lower extremities.

Bias

At least one result of low sensitivity would be a non-differential misclassification bias; that is, subjects who actually were positive for HI were incorrectly classified as negative. Such an effect would be non-differential with respect to the effect of residency, as no apparent reason suggests that the low sensitivity would disproportionately apply to one comparison group vs. another (Residents/Non-Residents). Failing to detect a larger proportion of the true positives in the study population means that our ability to show potential associations with HI and differences between residency groups was reduced. Because the effective sample size was less than planned, the statistical power to show any actual differences between comparison groups was further reduced below that originally projected (80%). The data from the present study did show a difference in the prevalence of HI between the residency groups, albeit non-significant: 7% of Residents vs. 9.5% of Non-Residents (p-value=0.56). The data failed to show, however, any significant association between subjects campus/off-campus residence and ancylostoma infection: Residents/Non-Residents-(Unadjusted OR: 0.73; 90% CI: 0.68, 2. 3); (Adjusted OR: 0.74 90%CI: 0.23, 2.4).

Prior to initiation of the study, we posited that those students who resided on BIRDS campus might be more likely to perform sanitary behaviors known to be protective against HI (more frequent use of latrines, more frequent use of footwear, et al). Recalling that the majority of domestic dwellings in the area do not possess latrines encouraged the supposition that students living in the hostels at BIRDS, where latrines are available, might be less exposed to the risk behavior of indiscriminate defecation. It was also posited that a comparatively advanced hygienic culture on BIRDS campus might induce less frequent exposure to risk behaviors for HI for resident students than for students living in the surrounding communities. We did observe meaningful differences in the frequency of *risk behaviors* between campus resident subjects and non-residents. Residents in BIRDS campus hostels reported less frequent outdoor defecation while at school (7% claiming to defecate outdoors every time vs. 56% of non-residents) and more frequent use of footwear (67% reporting wearing shoes most of the time vs. 47% of non-residents). Based on the significant differences observed in *risk behaviors* between Residents and Non-Residents—and the clarity of association between these *risk behaviors* and STH infections demonstrated redundantly in the literature—we suspicion that a larger sample population would likely reveal stronger associations between *risk behaviors* and ancylostomiasis.

During the course of the active study, it was learned that some resident students at BIRDS periodically returned for a few days per visit to their homes of origin in the surrounding communities, and that many students spent several weeks at home during an annual break that ended just weeks before the initiation of the present study. The fact that *A. duodenale* can persist in the human intestine for several years (avg. 1-2 yrs.) means that any subjects testing positive for infection could have acquired the infection either while spending time at BIRDS campus or at home. The previous statement also applies to Non-Resident students who spend 8-10 hours per day, six days per week at BIRDS. Thus, if a genuine significant difference in the transmission frequency of hookworm exists between the BIRDS campus and the surrounding communities, a cross-sectional comparison of ancylostoma prevalence between residence groups is a design not well-suited to demonstrate such. A more impactful approach for future studies would be to test the differences in participation in known risk behaviors between population, again attempt to evaluate for association with HI—and perhaps evaluate whether these behaviors change by age and over time.

As the local language of the study site area is Telugu, and the on-site researcher (PI) was a native English speaker with very limited Telugu capacity, misunderstandings in communication may have introduced errors in data collection. Informational and training sessions were conducted for interviewers by the PI, however, with the aim to increase the likelihood that interviewers attained adequate understanding of study goals and questionnaire. Contrary to plan, however, it was neither possible to conduct interviews of all subjects employing only the two planned, trained, interviewers nor to have both of these interviewers present for the entire study. It was also not possible to make a record of which interviewer conducted each interview, and thus was not possible to evaluate any differences that may or may not have arisen between interviewers. Understandably, inter-interviewer differences in both submission of the questionnaire and recording of responses may have occurred. Although we do not have evidence that any interviewer differences manifested disproportionately in one comparison group vs. the other, we recognize the possibility. The fact that the primary interviewer was also responsible for managing the group of students living on campus could have influenced him to elicit or inaccurately record responses from the residents to make their behaviors appear more desirable than the non-resident students. Many conversations were had between the on-site researcher and the primary interviewer regarding sanitary behaviors and helminth transmission. As discussed previously, the data demonstrate a lesser proportion of risk behaviors among residents. Ultimately, no evidence is available to qualitatively or quantitatively evaluate whether interviewer bias occurred.

The use in this study of self-reported data is accompanied by at least two potential sources of information error: recall bias and the false reporting of behaviors perceived by the subject as desirable when the actual behavior was not concurrent with that reported. The latter occurrence could in theory lead to either differential misclassification bias (if one of the comparison groups gave false self-reports more than the other) or non-differential misclassification bias (if the frequency of false reports did not differ significantly by comparison group). During each pre-interview subject orientation, participants were assured that: 1) all responses would be kept confidential; 2) only anonymous responses would be used for population-level evaluation; 3) that no particular responses were sought; and 4) students were encouraged to answer truthfully to assist in the goal of improving the health of all students at BIRDS school. While several of the behavioral variables are intended to assess actions that have occurred over time, the habitual, daily aspect and current practice of these behaviors should have enabled accurate reporting by subjects and reduced the potential for recall bias.

Strengths:

The cross-sectional design of this study would seem to preclude assessment of causality between the explanatory variables (i.e. *Risk Behaviors*) and the outcome variable (*A. duodenale* infection). However, because both the biology and etiology of soil-transmitted helminth infection have been well-understood and repeatedly examined over many studies and years, we are confident that documented participation in at least one aspect of the known transmission cycle (*Appendix: A*) can be causally linked to HI status at the population level. Furthermore, as a primary purpose of this study was not to re-confirm the established transmission cycle but to provide population-level data on factors associated with HI in order to inform community health interventions, the study design was reasonably well-suited to this goal.

The WHO recommends that any program aimed at controlling morbidity due to soil-transmitted helminthiasis should begin with a baseline survey [36]. We have conducted a study that not only meets the WHO's criterion by yielding important individual and community-level data on the prevalence of intestinal parasitosis but also provides motivating information to guide the development and implementation of critical public health interventions. Furthermore, community health improvements galvanized by this study to prevent transmission of parasites (e.g. improving sanitation and hygienic behaviors) will also serve to reduce the transmission of bacterial and viral pathogens—due to common transmission pathway aspects.

While developing community partnerships and support is often one of the greatest challenges to trans-cultural health projects, our study was anchored and supported by the established strong partnership with the local NGO, BIRDS. BIRDS has been integrally linked to the local population since its inception, and the majority of staff are members of and originate from local communities. Through more than twenty-five years of community development and health programs, BIRDS has developed great credibility and working relationships with local people. Partnership with BIRDS thus encouraged high levels of acceptance and cooperation from the study subject population than would have been possible otherwise.

In partnership with BIRDS and the student participants, we have produced actionable health behavior data for a population extremely vulnerable due to social marginalization, poverty, and lack of public health infrastructure—and for whom this data has never been collected and reported. These sub-populations of children are particularly vulnerable to serious consequences of unmitigated soil-transmitted helminth infection because of their nutritional needs for normal growth and cognitive development—and the lifetime cascade of restricting consequences that can result from growth retardation and reduced learning ability[5-10]. It is our hope that the educational sessions on community disease transmission and preventive health behaviors conducted with all BIRDS students will lead them to diffuse the knowledge and behaviors throughout their communities. For the reasons stated above, BIRDS is well-positioned to advance the development and deployment of improved sanitation and health-guarding hygiene behaviors both on campus and in the surrounding communities. We hope the associations between hookworm infection and the sanitary risk behaviors described through our study will provide motivating information to guide the development and implementation of critical public health improvements at BIRDS in the surrounding communities.

Future Studies

By making our baseline prevalence data available, future studies will be able to demonstrate whether the actual population prevalence is actually higher than our estimate—as we suspect. Subsequent research efforts in this population or region would do well to consider focusing on the frequency of modifiable risk behaviors and reevaluating their association with hookworm (and other helminth) infections. Finally, future research can serve the communities of Muthyalapadhu by studying temporal trends in prevalence and risk behaviors as well as by evaluating the impact of public health interventions implemented subsequent to this study.

References

1. CDC - Soil-Transmitted Helminths. Available at: http://www.cdc.gov/parasites/sth/index.html. Accessed February 19, 2014.

2. WHO | Soil-transmitted helminth infections. 2013. Available at: http://www.who.int/mediacentre/factsheets/fs366/en/. Accessed February 18, 2014.

3. Brooker S, Clements ACA, Bundy DAP. Global epidemiology , ecology and control of soil-transmitted helminth infections. *Adv Parasitol*. 2006;62:221–261.

4. De Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol*. 2003;19(12):547–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14642761.

5. Brooker S. Estimating the global distribution and disease burden of intestinal nematode infections : Adding up the numbers – A review. *Int J Parasitol*. 2010;40(10):1137–1144. doi:10.1016/j.ijpara.2010.04.004.Estimating.

6. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm infection. *N Engl J Med*. 2004;351(8):799–807. doi:10.1056/NEJMra032492.

7. Hotez PJ, Bethony J, Bottazzi ME, Brooker S, Buss P. Hookworm: "the great infection of mankind". *PLoS Med*. 2005;2(3):e67. doi:10.1371/journal.pmed.0020067.

8. Brooker S, Bethony J, Hotez PJ. Human Hookworm Infection in the 21st Century. *Adv Parasitol*. 2004;58:197–288.

9. Guilbert JJ. The world health report 2002 - reducing risks, promoting healthy life. *Educ Heal Abingdon Engl*. 2003;16(2):230. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14741909.

10. Ghaffar A. Parasitology Chapter 4: Nematodes (Roundworms). In: *Medical Microbiology 6th edition*. 6th ed. Mosby; 2009. Available at: http://www.us.elsevierhealth.com/microbiology/medical-microbiology-paperback/9780323054706/. Accessed March 16, 2014.

11. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors*. 2014;7(1):37. doi:10.1186/1756-3305-7-37.

12. Stoltzfus, Rebecca J;Chwaya, Hababu M.; Tielsch, James M.; Schulze, Kerry J; Albonico, Marco; Savioli S. Epidemiology of Iron-deficiency Anemia in Zanzibari schoolchildren: the importance of of hookworms. *Am J Clin Nutr*. 1997;65:153–9.

13. Stephenson LS. Helminth parasites, a major factor in malnutrition. *World Health Forum*. 1994;15(2):169–172. Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=med3&AN=8018283.

14. Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M. Hookworm control as a strategy to prevent iron deficiency. *Nutr Rev*. 1997;55(6):223–232. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9279058.

15. Brooker S, Peshu N, Warn PA, et al. The epidemiology of hookworm infection pre-school children on the Kenyan Coast and its contribution to anaemia among. *Trans R Soc Trop Med Hyg*. 1999;30(3):240–246. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10492749.

16. Sakti H, Nokes C, Hertanto WS, Hendratno S, Hall A, Bundy DA. Evidence for an association between hookworm infection and cognitive function in Indonesian school children. *Trop Med Int Heal TM IH*. 1999;4(5):322–334. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10402967.

17. Rao, VG ;Aggrawal, MC; Yadav, R; Das, SK; Sahare, LK; Bondley, MK; Minocha R. Intestinal parasitic infections, anaemia and undernutrition among tribal adolescents of madhya pradesh. *Indian J Community Med*. 2003;XXVIII(1):26–29.

18. Ezeamama AE, Friedman JF, Acosta LP, et al. Helminth infection and cognitive impairment among Filipino children. *Am J Trop Med Hyg*. 2005;72(5):540–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1382476&tool=pmcentrez&rendertype=abstract.

19. Oberhelman R a, Guerrero ES, Fernandez ML, et al. Correlations between intestinal parasitosis, physical growth, and psychomotor development among infants and children from rural Nicaragua. *Am J Trop Med Hyg*. 1998;58(4):470–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9574794.

20. S., Ananthakrishnan; P., Nalini; S.P. P. Intestinal Geohelminthiasis in the Developing World -.pdf. *Natl Med J India*. 1997;10(2):67–71.

21. Christian P, Khatry SK, West KP. Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lance*. 2004;364(9438):981–983. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15364190.

22. Bundy DA, Chan MS, Savioli L. Hookworm infection in pregnancy. *Trans R Soc Trop Med Hyg*. 89(5):521–522.

23. Nagoba BS, Basutkar SH, Bhat SD. Status of intestinal parasitic infections in Loni--a rural area of Ahmednagar District of Maharashtra. *J Commun Dis*. 1992;24(1):58–59.

24. Pawłowski, Z. S.; Schad, G. A.; Stott GJ. *Hookworm Infection and Anaemia: Approaches to Prevention and Control*. Geneva, Switzerland: World Health Organization; 1991.

25. Chakma T, Rao P V, Tiwary RS. Prevalence of anaemia and worm infestation in tribal areas of Madhya Pradesh. *J Indian Med Assoc*. 2000;98(9):567, 570–571.

26. Kaliappan SP, George S, Francis MR, et al. Prevalence and clustering of soil-transmitted helminth infections in a tribal area in southern India. *Trop Med Int Health*. 2013;18(12):1452–62. doi:10.1111/tmi.12205.

27. Kang G, Mathew MS, Rajan DP, et al. Prevalence of intestinal parasites in rural Southern Indians. *Trop Med Int Health*. 1998;3(1):70–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9484973.

28. Mehraj V, Hatcher J, Akhtar S, Rafique G, Beg MA. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PLoS One*. 2008;3(11):e3680. doi:10.1371/journal.pone.0003680.

29. Garcia LS. *Practical guide to diagnostic parasitology*. Washington, DC: ASM Press; 2009.

30. Hosmer DW, Lemeshow S. *Applied Logistic Regression*. 2nd ed. Hoboken: John Wiley & Sons; 2004.

31. Mani GG, Rao ST, Madhavi R. Estimation of hookworm intensity by anthhelmintic expulsion in primary schoolchildren in South India. *Trans R Soc Trop Med Hyg*. 1993;87:634–635.

32. Saathoff E, Olsen A, Sharp B, Kvalsvig JD, Appleton CC, Kleinschmidt I. Ecologic covariates of hookworm infection and reinfection in rural Kwazulu-natal/south Africa: a geographic information system-based study. *Am J Trop Med Hyg*. 2005;72(4):384–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15827274.

33. Lyndem L, Tandon V, Yadav A. Hookworm infection among the rural tribal populations of Meghalaya (Northeast India). *J Parasit Dis*. 2002;26:60–68.

34. Staat MA, Rice M, Donauer S, et al. Intestinal Parasite Screening in Internationally Adopted Children: Importance of Multiple Stool Specimens. *Pediatrics*. 2011. doi:10.1542/peds.2010-3032.

35. Government of Canada PHA of C. Ancylostoma duodenale - Pathogen Safety Data Sheets - Public Health Agency of Canada. 2012. Available at: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/ancylostoma-duodenale-eng.php. Accessed March 14, 2014.

36. Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L. *Guidelines for the Evaluation of Soil-transmitted Helminthiasis and Schistosomiasis at the Community Level*. Geneva, Switzerland; 1998. Available at: http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB0QFjA

A&url=http://whqlibdoc.who.int/hq/1998/WHO_CTD_SIP_98.1.pdf&ei=wavRToumNMPe
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Appendix A: Life Cycle of Hookworms

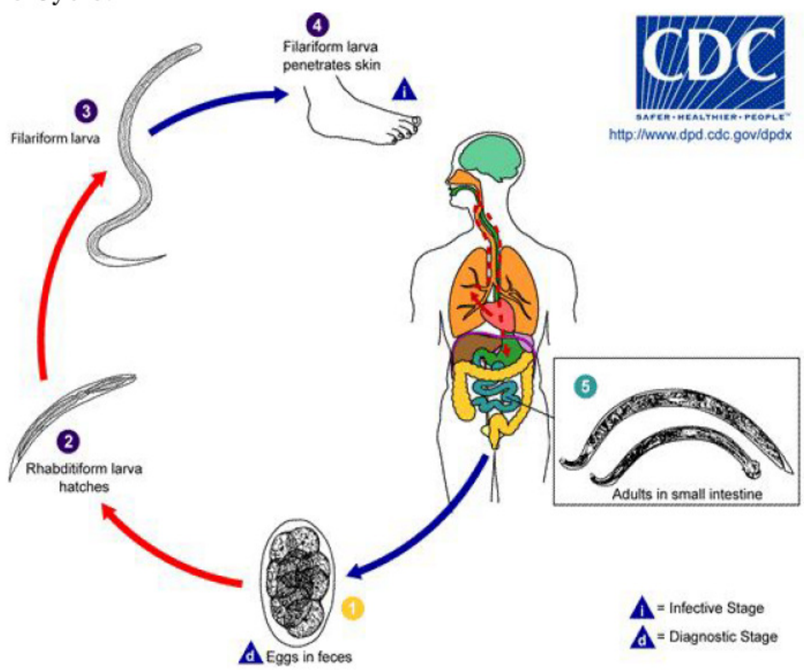
Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

Biology

Causal Agent:

The human hookworms include the nematode species, *Ancylostoma duodenale* and *Necator americanus*.

Life Cycle:



Eggs are passed in the stool 1, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil 2, and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective 3. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed 4. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host 5. Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years.

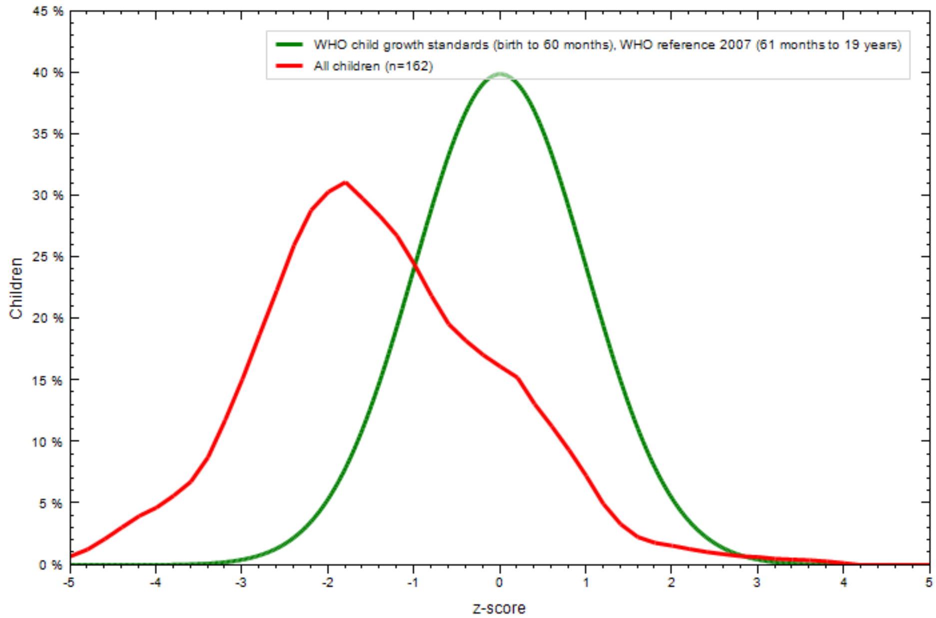
Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

Life cycle image and information courtesy of DPDx (http://dpd.cdc.gov/) .

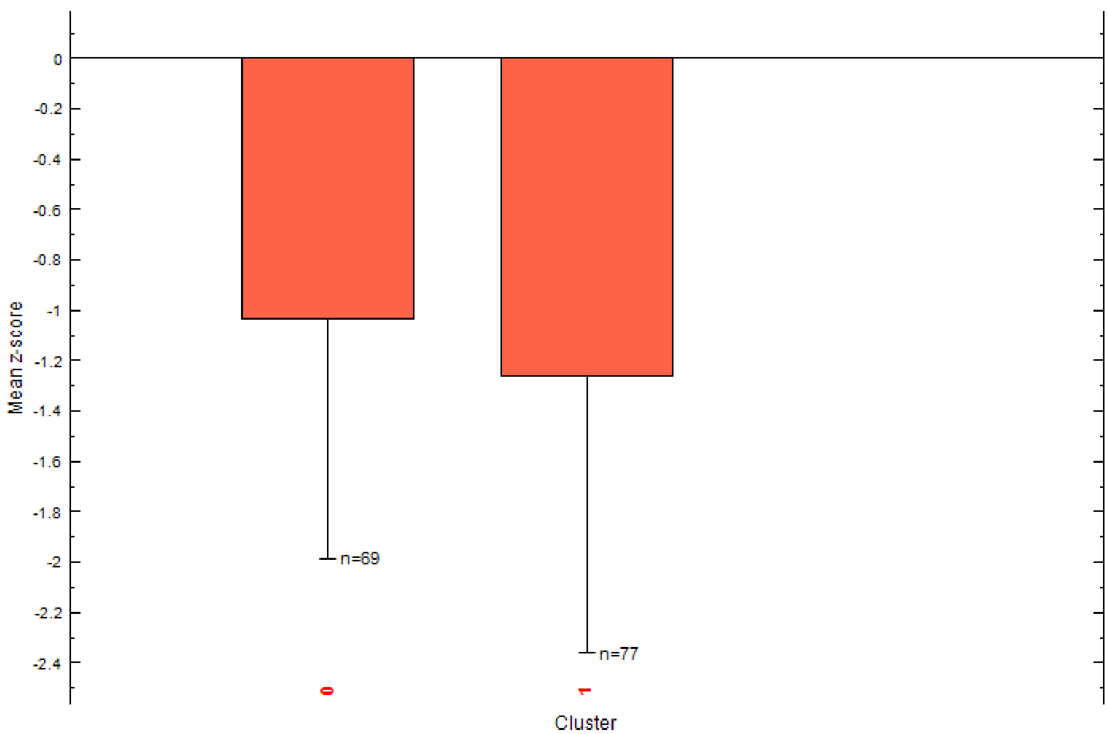
Page last reviewed: November 2, 2010

Appendix B: Additional Anthropometric Data

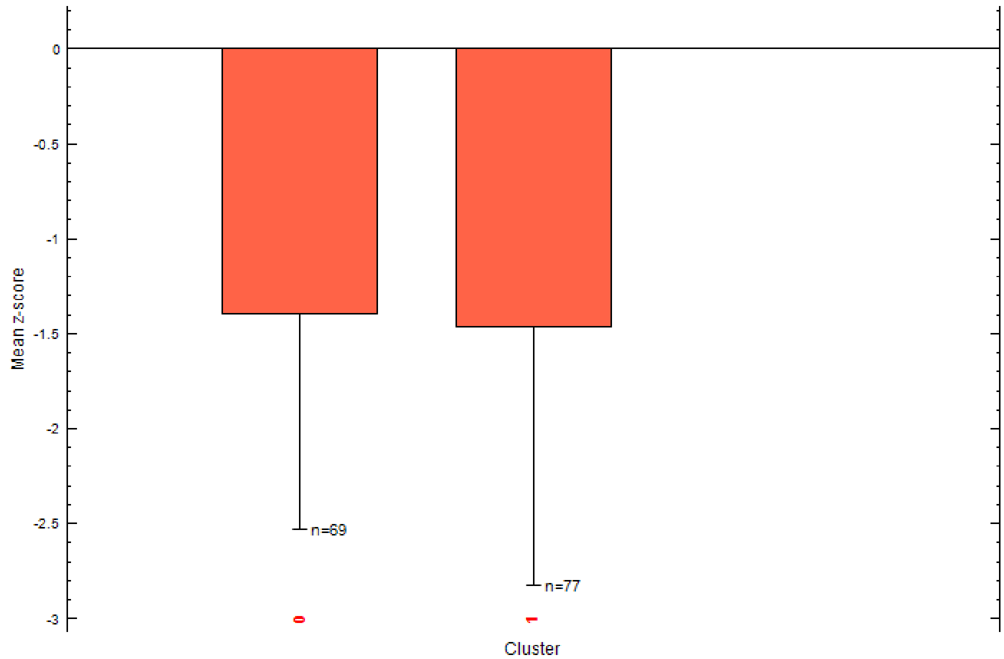
BIRDS ALL CHILDREN – HEIGHT FOR AGE PERCENTILES– (2011)



BIRDS RESIDENTS (0) VS. NON-RESIDENTS (1) – BMI FOR AGE PERCENTILES– (2011)



BIRDS RESIDENTS (0) VS. NON-RESIDENTS (1) – HEIGHT FOR AGE PERCENTILES– (2011)



Appendix C: Parental Information/Consent Letter

English

****Note: The below letter was translated into Telugu before distribution.****

Dear Parents,

We wish to inform you about the project at BIRDS school in which your child has been selected to participate. Our purpose is to ensure that all children at BIRDS are in the best health and succeeding in their studies to their full potential. We will be testing a random group of students at BIRDS for intestinal parasites. To perform the test, we will collect one stool sample from each student selected to participate. We will also record each participant's height and weight to see if they are growing properly. The results will tell us how much of a parasite problem children at BIRDS have and how we can best assure that children at BIRDS remain parasite-free.

The three main benefits of this project are:

1. **Educational**: children who are worm-free become better students in school than children who continue to have worms. These children go farther in school and are more successful as adults.
2. **Physical:** children who are worm-free grow better. For girl children, when they become mothers, their babies will be healthier.
3. **Hygiene:** the results of the project will tell us about the children's hygiene and if more attention is needed in personal and community hygiene.

We inform you that the actions that prevent intestinal worms are the same to prevent many causes of diarrhea and yellow jaundice. We thank you for your support and honor your child for helping us in this important project to increase the health of all BIRDS school children.

If you should desire that your child NOT participate in the study, please inform BIRDS administration at the earliest possible opportunity. No penalties or any other negative consequences will be imposed on your child for selecting not to participate.

Appendix D: Questionnaire

Interview Questions for Project (English)

1. Is your stool usually **(CIRCLE ONE)**:
   A. Watery B. Soft (like a ripe banana) C. Firm (like mango) D. Hard like rocks

2. What is the usual color of your stool? **(CIRCLE ONE)**:
   A. Brown B. Yellow C. Green D. White E. Black

3. Do you usually see **blood** in your stool? **(CIRCLE ONE)**
   A. Yes B. No

If answer "Yes": **When was the last time? (Write date/time as answer)***

4. Have you ever seen worms in your stool? **(CIRCLE ONE)**
   A. Yes B. No

If answer "Yes": **When was the last time? (Write date/time as answer)***

5. Have you been given any de-worming medicine during the past year? (albendazole, mebendazole)
   A. Yes B. No

If answer "Yes": **When was the last time? (Write date/time as answer)***

6. Is your stomach swollen or bloated now? **(CIRCLE ONE)**
   A. Yes B. No

7. Have you felt any **pain or cramps** in your belly or intestines area? **(CIRCLE ONE)**
   A. Yes B. No
   a. **If answer "Yes"**: How long have you felt it? **(Write date/time as answer)***
   b. **If answer "Yes"**: How **often** do you feel it? **(CIRCLE ONE)**
      i. Some days ii. Every day
   c. **If answer "Yes"**: How long does it last? **(CIRCLE ONE)**
      i. A few seconds ii. Most of the day

8. How is your breathing? **(Write answer, <u>if</u> student gives description)**

   a. Do you have any **coughing**? **(CIRCLE ONE)**
      i. Yes **ii. No**

   If answer "Yes": How long have you had it? **(Write date/time as answer)***

   b. Is it hard for you to breathe, or do you experience any **shortness of breath**? **(CIRCLE ONE)**
      i. Yes **ii. No**

   If answer "Yes": How long have you had it? **(Write date/time as answer)***

9. How often do you wash your hands before eating? **(CIRCLE ONE)**
   A. Never **B. Sometimes** **C. Most of the time**

10. How often do you wash your hands after defecating? **(CIRCLE ONE)**
   A. Never **B. Sometimes** **C. Most of the time**

11. How often each day do you use the field/go outside to defecate? **(CIRCLE ANSWERS)**

   a. At **School**: **(CIRCLE ONE)**
      i. Every time **ii. Sometimes** **iii. Rarely** **iv. Never**

   b. At **Home**: **(CIRCLE ONE)**
      i. Every time **ii. Sometimes** **iii. Rarely** **iv. Never**

12. How often each day do you use the latrine to defecate? **(CIRCLE ONE)**
   A. Never **B. Sometimes** **C. Most of the time**

13. How often do you wear shoes or slippers? **(CIRCLE ONE)**
   A. Never **B. Sometimes** **C. Most of the time**

14. How is your **appetite** compared to the other children in your class? **(CIRCLE ONE)**
   A. Less than theirs **B. About the same as theirs** **C. Greater than theirs**

15. How is your **energy level** <u>compared to the other children in your class</u>? **(CIRCLE ONE)**

    A. Less than theirs B. About the same as theirs C. Greater than theirs

16. What is your age? "**Mi vayasu entha?"**

Appendix E: Brief Profile on *Bharati Integrated Rural Development Society ("BIRDS")*

Born into rural poverty but managing to obtain formal higher education (including a law degree and M.S. in sociology), V. Paul Raja Rao founded the non-profit BIRDS with the aim to aid, include, and empower the many marginalized persons in his community of origin. Rural populations in the Indian state of Andhra Pradesh (AP), where BIRDS is located, consist primarily of *dalits* (formerly untouchables or outcastes) and tribals (persons of indigenous tribes). These populations, having been systematically deprived of good education, capital, and health care, have existed for centuries as a permanent underclass of Indian society. An integral organizational principle of BIRDS is a holistic approach to social justice based on awareness that health, education, economics, and ecological sustainability are intertwined. Guided by this holistic principle and the goal to bring the marginalized into the mainstream, BIRDS staffing and projects are based on inclusion of and collaboration with communities targeted for aid.

BIRDS began with a women's empowerment and micro-finance program, providing small loans and business and artisan skills training to participants. Recognizing an historic inability to own land and the resources needed for agricultural production, Paul worked to change legislation to allow *dalit* farmers to own land and developed a community-based water management program. The water management program was designed to remedy frequent drought conditions in AP, and the practice of wealthy land-owners monopolizing water and only using *dalits* and tribals as cheap, expendable labor. BIRDS documented significant increases in the living standard for farmers participating in the program. In 2004 Drs. George and Virginia Feldman founded BIRDS Health Center and a community health worker (CHW) training program. The clinic treats common diseases at no cost to patients; and CHW, working in their villages of residence, treat minor disease, provide health information and immunizations, and help connect villagers with social services. In 2005 BIRDS started a boarding school for orphans and former child laborers. With the subsequent closure of a nearby government school, BIRDS school expanded to enroll ≈650 non-resident students from surrounding villages.

Contact us:

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Appendix F: Comprehensive List of Parasites Discovered through O&P Assay

Parasite	Type	# of Subjects
A. duodenale	STH	14
H. nana	STH	5
G. lamblia	Protozoa	3
T. solium	STH	1
E. hystolytica	Protozoa	2
Entamoeba coli	Amoeba	2
6	**4**	**18**