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Soil-Transmitted Helminths: Determinants of Infection
in Children Aged 5 and Under in Odisha, India

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Abstract

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Helminth infection is a pervasive and persistent disease that has a long reach across the globe that has long term consequences for young children. The data was taken from a larger study evaluating the effectiveness of the Gram Vikas MANTRA intervention in the Ganjam and Gajapati districts of Odisha, India. The data used for this analysis are only from one round, which makes this analysis cross-sectional and restricted on households with children aged 5 and under in both a mixed effects model logistic regression and a conditional model logistic regression. In households with a child aged 5 and under, the proportion of household members using an improved toilet had a significant negative association with having an STH infection (OR: 0.43, 95% CI: 0.19, 0.96) in the MELR model, adjusting for age, education, caste, wealth, intervention or control village status. As the proportion of household members using an improved toilet increases, the odds a child with an STH infection reduces. After Bonferroni correction, this determinant is not statistically significant. However, an emphasis on adherence of using the improved toilet within households may be the key to a meaningful reduction in STH infection in children aged 5 and under.

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Background

Introduction

Soil-transmitted helminths (STHs), a subset of intestinal nematodes, are a neglected tropical disease that are persistent issue for the developing world. *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and hookworms (*Necator americanus* and *Ancylostoma duodenale*) are the four major species of concern (1-6). All four species share the scientific classification by the phylum of nematoda, but their common names are roundworm (*A. lumbriocoides*), whipworm (*T. trichiura*), and hookworm (*N. americanus* and *A. duodenale*) due to their unique physiology (7). Helminth infection is a pervasive and persistent disease that has a long reach across the globe. In 2010, 438.9 million persons globally were estimated to be infected with hookworms, roundworm accounted for 819 million global infections, and whipworm contributed a total of 464.6 million infections (4).

Disease Burden

The majority of STH infections worldwide occur in Asia (1, 4). India has the highest burden of STH infections within Asia, accounting for 21% of all STH infections globally(4). India, in 2009, had a reported peak prevalence of 9.5% in STH infections (1). Even with a similar large population at that time, China did not have as high a proportion of STH infections as India with a reported 18% of global infections (4). Furthermore, a combination of the most populous Sub-Saharan Africa nations (Nigeria, Ethiopia, and Democratic Republic of Congo) only account for 8% of global STH infections, combined (4).

Roundworm, whipworm and hookworm each vary in prevalence. Global prevalence of the STH infections are estimated to be 14.5% (roundworm), 8.3% (whipworm), and 7.8% (hookworm). These proportions translate into millions infected in Asia; respectively, 589, 282 and 281 million infections were estimated (4). South Asia, of which India is located, displays the highest number of infections for hookworm and roundworm infections, including 140.2, 297.8, 100.7 infections per million people of hookworm, roundworm and whipworm respectively (4). It is estimated between 2006-2012, the prevalence for hookworm, roundworm and whipworm are 17.7%, 11.2% and 4.4% in India (1). Clearly, STH infections are a pervasive public health problem in India.

While we have an idea of the pervasiveness of STH infections, it is important to understand the impact the disease has on those infected. An STH infection burden has been estimated at 5.18 million Disease Adjusted Life Years (DALYs) in 2010 due to global infection. Breaking down the STH DALYs by each helminth, there were 3.23 million DALYs due to hookworm, 1.31 million due to roundworm and .64 million due to whipworm in 2010 (4). The populations at risk at any given time is 5340.1 million globally, whereas Asia contributes 3,684.4 to global estimate (3).

Mortality & Morbidity

STH infections can have many effects on an individual's health. Mortality due to STH infections is relatively low, varying between 12,000 and 135,000 deaths annually (8). However, morbidity remains a greater concern. The symptoms of infection can include: diarrhea, abdominal pain, weakness, malnutrition, reduced physical and cognitive growth, reduced work productivity (9) and anemia (8). Anemia can be especially pronounced in vulnerable populations. Hookworm may lead to moderate to severe anemia in school-age children and have been reported to cause 30-54% anemia in pregnant women (8). Children and mothers of childbearing age share the greatest risk for morbidity (10). Moreover, developing nations are often common in chronic, multiple infections and reoccurrence (9). The intensity of infection can be due to repeated STH exposure which can lead to a heavy infection in the gut that then can cause intestinal bleeding, along with other symptoms (10). Once an individual is infected, the helminth "competes with the host for nutrients (10).

Ultimately, a child is one of the most vulnerable of all age-groups because of the long-term consequences of an STH infection. One of which may include the possibility that the child may never become a fully-functional adult capable of caring financially for him or herself, becoming a burden for the family in subsequent years. If too many children are affected with these long-term consequences, the burden could shift upon a community and even present a national crisis for work-force and a financial care burden which can then be placed upon the state.

Even symptoms can deviate between STH infections. Roundworm can present with lack of appetite, abdomen pain, altered bowel habits and weight loss, which are not uncommon. Intestinal obstruction from the roundworm STH can be demonstrated by abdominal distension and tenderness. Whipworm can present with abdominal pain or tenderness as well and in severe cases, trichuris dysentery syndrome: digital clubbing and rectal prolapse. Anemia may also be acute. India exhibits that half of all biliary disease cases, one third of pancreatitis cases are caused by an STH infection (2). Despite the fact that most STH infections are usually asymptomatic, this only makes the disease more dangerous as individuals may continually pass infected stools in areas that can continually have the ability to infect others in the community without ever knowing that they need treatment.

Life Cycle

The prevention and control of STH infections begins with an understanding of each of their life cycles. Roundworm and whipworm both share fecal-oral transmission, while hookworm transmit by penetrating the skin (bare-feet are a commonplace infection area). Hookworm have larvae that are free-living in warm, moist sandy soil or feces. Once they penetrate the skin, the larvae are transported to pulmonary capillaries, where they pierce the alveolar wall, pass to the larynx and are swallowed. Larvae moult and develop into mature worms in small intestine over 1-2 months and then can survive months (*A. duodenale*) or years in the case of *N Americanus* (shown on Figure 1) (2, 11).

Roundworm eggs are swallowed and then hatch, moult, penetrate the intestine mucosa and migrate to the pulmonary circulation. Next, the larvae migrate to alveolar wall and then move to the tracheobronchial tree, then the larynx and the small intestine to again moult into larvae and adult worms. An adult female can produce thousands of eggs daily that pass in stool. Egg production occurs 2-3 months after infection and worms can live for a few years. Eggs viable in warm moist soil for years (shown on Figure 2) (2, 12).

Whipworm infections begin in a similar manner. The eggs are ingested from either food or hands, hatching as larvae that moult in small intestine. They deviate from roundworms life cycle in that they do not migrate to the lungs. Larvae will attach to the intestinal villi and develop into adult worms, reside in the caecum and ascending colon.

The female adult worm can also lay thousands of eggs a day for several years. The eggs pass in stool and embryonate in warm, moist soil and can survive for months there (shown on Figure 3) (2, 13).

Potential Zoonotic Transmission

In addition to their lifecycles, it is crucial that we understand their zoonotic transmission potential as that may be a vehicle for continued infection. Roundworm may have zoonotic to human transmission as there were cross infections under experimental conditions with pigs. Pigs can be sickened with Ascaris ovum while humans are afflicted with *Ascaris lumbricoides* (14). Hookworms of the *A. duodenale* are restricted to humans alone (15), but the *N. americanus* species has sometimes been recorded in non-human primates. For *N. americanus*, pigs may also have the ability to become transport host (16). On the other hand, whipworms have potential transmission with pigs with *T. trichiura*. The type of whipworm for pigs has been classified as *Trichuris suis*. However, *T. trichiuras* zoonotic potential pathways remains unknown (17).

Environment

Critically, the lifecycle of STHs, the climate needs to be conductive for viable eggs. As detailed before, warm, moist soil, found in tropical-like regions are a necessity for STH eggs. One factor that contributes to the prevalence of STH infection in India is its climate. Although India has such a large landmass that includes many differing climates, many parts of the country, including the State of Odisha, has tropical and savannah climates where STHs thrive (18).

Determinants & Considerations

However, climate is not the only reason for India's high prevalence; another is poverty (4). This is disease that needs populations to not use or have access to modern sanitation or hygiene. It has been directly stated that by tackling STH infections or other Neglected Tropical Diseases (NTDs) that it would be a part of a broader effort to reduce global poverty(4). The relationship with STHs and impoverished or underdeveloped populations is relatively clear. There is an absence of STH infection in the few nations that have a GDP per capita of $20,000. Historically, the elimination in the American South and other temperate areas have been characterized by poverty reduction and hygiene and sanitation improvements (3). Despite the fact that in the American South, the reduction of STH infection was credited to the efforts of the Rockefeller Hookworm Eradication Program, it has been argued that economic development was the true reason for halting hookworm transmission (16). Currently, mid-income nations of China and Brazil show a rapid decline in STH infection prevalence (3). Exemplifying this massive change, China had a 57.5% prevalence in 1990 and then a measured 18.6% prevalence in 2010 (4). Additionally, similar economic reforms in Japan and South Korea in the 1960s and 1970s, along with control programs, facilitated eradication (16). Economic development paves the way for other necessary changes in our daily lives that improve our health, including protection against STH transmission.

Adequate sanitation is a positive result from improving socioeconomic conditions and is another important determinant of STHs. Since the lifecycle of STH eggs relies on infected stools to be introduced to humans as an exposure either through fecal-oral or by free-living in the environment, it is crucial to break possible exposure routes. On a community level, adequate sanitation accounts for a 40-50% reduced odds ratio for the availability of sanitation facilities and prevalence of infection (3). Poverty drives poor sanitation and hygiene which then drives STH infection.   In India, it is estimated 66% of the total rural population do not have toilet facilities. It has been estimated that only 1 in 3 wash after defecation (19). This leaves the fecal-oral route open for STH transmission.

Inextricably linked, sanitation and hygiene are essential for long-term control of STH infection (20). If there is a deficiency in sanitation, this will in turn compromise hygiene of an individual. The relationship between sanitation and hygiene can be characterized by disparities in access to safe toilet facilities of which vary by urban and rural settings and even caste. Urban access is estimated at 80.4% and rural at 30.7%. Caste also is heterogeneous as Scheduled Castes have a 62% coverage, Scheduled Tribes 75%, while the general population has 50% coverage (21). The area in which humans defecate can make all the difference in infection. For example, availability of latrines is associated with reduced hookworm intensity. The drawbacks of only a sanitation intervention is that it may not be evident until decades later and is not completely effective by itself (20). There are other determinants relating to poverty and its subsequent lack of sanitation (and thus, STH infection).

The parent’s ability to provide for their children can help determine the risk of an STH. Preschool children are at higher risk for undernutrition within a scheduled tribe in comparison with their rural counterparts (22). The undernutrition is a result of poor food intake or from a lack of access. As a potential indicator for poverty and susceptibility to a STH infection, undernutrition is an important to consider. Combining undernutrition with sociocultural taboos and limited health care access, scheduled tribe preschool children may face a dire situation. Demonstrating this large issue in India, 43%, 48% and 20% prevalence was recorded for underweight, stunting and wasting respectively. Furthermore, tribal children faced a greater risk of these challenges with a corresponding prevalence of 55%, 54% and 28% (22). Poverty has a foothold due to pervasive socio-economic conditions. Traditional divisions in caste and religion underpin India's poverty (23).

Poverty and caste are intimately linked. In 2004-2005, the India Human Development Survey was conducted to see the divisions of caste and poverty. At best, Forward Caste Hindus were 12% impoverished, while 32%, 50% and 23% were classified as impoverished for Dalits, Adivasis, and OBCs. In parallel to Forward Caste Hindus, other minority groups shared the same poverty prevalence. Recently, there have been improvements in reducing poverty. In 2012, the distribution of poverty was 14%, 20%, 27% and 42% for Forward, OBC, Dalit and Advasis groups in that order (23).

Demographically, age is a key factor in those that are infected. School age children, ages 5-14, contribute a large portion to any STH infection (1019.1 million globally). Within Asia, 646.7 million school age children are approximated are infected with an STH (3). The age trend is at its highest of 13% prevalence from ages 6 to 10 (1). The World Health Organization in 2003 gave a detailed description of age demographics: 228 million children infected from ages 0 to 4, 616 million aged 5 to 14 and 1,910 million aged 15 and up (24). Intense STH infections varies with age, but crests in childhood (20). Sex is another key factor that has been associated with STH infection; males has been identified as more likely to be positive for intestinal parasites (25). Additionally, STH infections are reported to "…highly aggregate in the host populations, with a small proportion of individuals harboring disproportionately high proportion of the total worm burden” (20). This suggests that those who are STH positive are in fact more prone to have more than one type of STH infection and or having a very heavy infection with a single STH species.

Study Aims

The aim of this study is to identify the specific water, sanitation and hygiene (WASH) factors contributing to the prevalence of STH infections in a population in rural India. The study draws son data collected by a team of Emory-led investigators in connection with an evaluation of a community intervention by Gram Vikas, a local NGO. The goal of this research is to inform program implementers in order to improve programs to reduce STH infections in endemic populations.

Methods

Data source & study design

The data for this thesis was taken from a larger study evaluating the effectiveness of the Gram Vikas MANTRA intervention in the Ganjam and Gajapati districts of Odisha, India(26). The objective of the program was to implement the intervention, "…that provides household-level piped water connections contingent on full community-level toilet coverage". The study collected data during 4 rounds. Matching of households with children under 5 years old in randomly selected intervention villages to control villages (a total of 90 villages) was followed for over 17 months. Control villages were selected through restriction to avoid spill-over effect from other intervention villages. The intervention villages were restricted on start date of the invention (2003-2006) and completion of the intervention (January 2013). The data used for this analysis is only from one round, which makes this analysis cross-sectional and restricted on households with children aged 5 and under. This secondary analysis will be performed in order to identify the factors contributing to the continued presence of STH infections within their villages.

Confounder Classification

Potential confounders were pre-selected based on biological plausibility and through careful literature review. Due to children's cognitive and physical growth, age was classified into four categories: 0 – 6 months, 6 -12 months, 12 – 24 months and 24 – 60 months. Household wealth was assigned quintiles, designated as poorest, poor, middle, rich and richest. Caste or tribe was assigned into quartiles: scheduled caste, schedule tribe, other backward caste and other caste. Education was drawn from the child care giver as this individual is the most proximal to the child and would have the highest impact. It was classified as: less than a year completed, completed primary, completed secondary, and more than secondary completed.

Potential determinants for soil-transmitted helminth infection were selected based on their biologic plausibility and through a literature review. Any feces observed in home exposure included any observation of oxen, cattle, goats, sheep, chickens, ducks, turkeys, pigeons, human, pig, dog and monkey feces as an indicator of poor hygiene for the household. The handwashing station compared those who had a station with water and soap to: no station, a station without water, a station without soap or by any combination of the previous. In households with children 5 and under, the proportion of the children using an improved toilet was continuous. The location of last defecation was classified by using a toilet and other (latrine, potty, diaper, in clothes, on a cloth and open defecation). The location of defecation is important as the care giver will be exposed to the feces unless the child uses a toilet. Child feces disposal was categorized as safe (rinsed or put into an improved toilet) and unsafe (not put into an improved toilet). The disposal is also crucial in determining whether the environment is potentially contaminated with STH nematodes. Hand appearance was determined through visual inspection of the palms and fingerpads. An unclean appearance or visible dirt on any one of these parts was assigned as dirty and, if not, clean. Consumption of soil was assessed if the child had been observed eating soil in the last week. Sanitation was classified as having improved sanitation and open defecation or unimproved sanitation. Improved sanitation included those who had access and used bathrooms or toilets. Water source was classified by on-premise piped water or anything other water source.

Data Analysis

The analysis used to identify meaningful determinants for soil transmitted helminths in children aged 5 and under was a mixed effects logistic regression model for each of the 9 exposures of interest. This decision was made because there were too few events (106 total) of infection cases in the subpopulation of interest which made a fuller model with all 9 exposures and 6 covariates, without any observations to make any estimates.

Mixed effects logistic regression was conducted for each exposure to obtain prevalence odds ratios. The nature of the model testing was impeded by the dearth of events (106). The limited events were further spread too thin due to the numerous levels of each confounder which created a convergence issue. To overcome non-convergence, all confounders identified in each exposure model were grouped together to determine their probability of association with exposure which combined their magnitude as a whole. This probability was used in the mixed effects modeling in place of the confounders. A random effect term for village was used to account for clustering of child observations within each village. Additionally, the Fisher's exact test was used to obtain point estimates.

Due to the separate analysis of each exposure in their own models, the Bonferroni correction to the alpha level of .05 was used for significance testing of p-values. As there are nine exposures, the new alpha used was .006.

Ethics

The study protocol was reviewed and approved by the ethics boards of the London School of Hygiene and Tropical Medicine, UK and KIIT University, Bhubaneswar, India. Trial registration number: NCT02441699. Un-identifiable data was provided to Emory University for this analysis under a data transfer agreement between LSHTM and Emory University.

Results

The population analyzed had total sample size of 2,457 children aged 5 and under. The age distribution for cases of STH infections were 5, 9, 20 and 70 for ages 0 – 6 months, 6 - 12 months, 12 – 24 months and 24 – 60 months, respectively (Table 1). Wealth was distributed into quintiles: poorest - 32 cases, poor – 17 cases, middle – 11 cases, rich – 17 cases and richest – 27 cases. Child care give education level had 29, 18, 55 and 2 cases for under 1 year completed of school, completed primary, completed secondary and more than secondary completed respectively (N= 2,451). All demographic results are displayed in Table 1.

Interaction was assessed between each exposure and village intervention status. All models found that the interaction terms were not statistically significant (Table not shown). Thus, all final adjusted models for the exposure did not include an interaction term. All model results are presented in Table 2, adjusted for age, child care giver education, caste and wealth.

Among children aged 5 and younger, having a handwashing station with soap and water compared to not having a handwashing station with soap and water, had a 58% reduction for STH infection (95% CI: 0.31, 1.09). The handwashing station with soap and water is associated with a protective effect on soil transmitted helminth infection for children aged 5 and under; however, this finding is not statistically significant.

In households with a child aged 5 and under, the proportion of household members using an improved toilet had a significant negative association with having an STH infection (OR: 0.43, 95% CI: 0.19, 0.96). As the proportion of household members using an improved toilet increases, the odds a child with an STH infection reduces.

Among children aged 5 and younger, observed feces in the home, estimated a prevalence odds ratio of 1.13 (95% CI: .53, 2.45) for STH infection. This suggests that there is a 13% increase in STH infection when the child care giver observes the child eating dirt. However, the finding is not statistically significant as the CI includes the null of 1.

Consumption of soil and dirty hand appearance displayed a protective association for STH infection among children aged 5 and under with prevalence odds ratios (PORs) of 0.56 and 0.82. This indicates that there is reduction in the odds of an STH infection with consuming soil and dirty hand appearance. However, both findings are not significant. Additionally, the use of an improved toilet for the last defection and child feces disposal into an improved toilet was estimated to have PORs of 1.43 and 1.14, respectively. Thus, the odds of an STH infection in both had a harmful association with an STH infection. However, both findings were not significant.

The on-premise piped water source was estimated a 0.95 prevalence odds ratio for STH infection in children aged 5 and under compared to other water sources. Thus, there was a reduction in association between on-premise piped water source compared to other water sources. Sanitation had a 57% reduction in the odds of an STH compared to open defecation and unimproved sanitation (95% CI: .27, 1.21). Both of these findings were not significant.

After Bonferroni correction, no risk factors were statistically significant. The Bonferroni's Correction was used to account for the 9 separate models. This correction set the alpha level at .006. Thus, the proportion of household members using an improved toilet in households with children aged 5 and under were not significant.

Discussion

In summary, it was estimated that proportion of household members using an improved toilet had significant and protective associations with the STH infection in children aged 5 and under. However, after applying the Bonferroni's Correction (BC), the estimate became not statistically significant at the new alpha .006 level. The other models held wide CIs that included the null value, so there could be no determination about the exposures association on STH infection.

The mixed effects logistic regression was used to account for village level clustering of cases due to unknown confounding. Additionally, conditional logisitic regression was conducted to see if there were notable differences due to this village level clustering (refer to Tables 3 and 4 and Appendices). While there was a notable difference between the estimated point estimate for the POR association on STH infection for children under 5 (feces observed in the home) between the two models, the CIs precision was essentially unchanged for only sanitation and child feces disposal into an improved toilet with an estimated .04 to 0 difference in the models, respectively. This suggests that random effect of village level clustering of cases was important to calculate, as most of the models precision deviated somewhat substantially. Therefore, the main conclusion of these results should only be drawn from the MELR models.

When we focused on the any feces observed in the home model, it was selected as an exposure because it stood in as a proxy for poor hygiene and the possibility for zoonotic transmission. In one systematic and meta-analysis, hygiene was identified as an important factor to measure for STH infection (19). Feces observed in the home only extrapolates on whether at that point in time if the home had a fecal mess, not how long the mess had been there and who had been in contact with it. Additionally, potential zoonotic transmission was considered. Each STH was analyzed through literature review. Under experimental conditions, there may be cross-infection between pigs and humans for roundworm and whipworm species (14). With hookworm (*N americanus*) species, there is also a potential for non-human primates in beings a transport host (15, 16). However, there is not any strong evidence that suggests that this is a significant predictor in exposure. Due to these reasons, the observed estimate for any feces observed in the home, may not be the best measure to predict STH infection.

Handwashing station with soap and water compared to other was another proxy of hygiene used to estimate the association with STH infection. In the same meta-analysis study, many studies focused on handwashing as a determinant and found that handwashing before eating and defecating had a significant effect on STH infection (19). While the estimated association was protective in association against STH infection as in line with previous findings, the CI overlapped the null in this analysis. This may be due to lack of event power.

Observed consumption of soil was estimated due to it direct biologic plausibility with children aged 5 and under. During this developmental period, children put many things in their mouths of which soil may be one such substance. Soil is the environment that the offspring or larvae of the STH remain, where they either hatch in the case of hookworms or are consumed through fecal-oral transmission in case of roundworm and whipworm (2, 17). Thus, estimating the potential predictor became of interest. However, the observance was recorded by the child care giver, which may or may not be accurate as it is hard to always observe a child. Additionally, only 2 were recorded to have consumed soil and have an infection. The lack of power may be the reason that the prevalence odds ratio was protective for STH infection.

The last location of defecation of a child aged 5 and under was classified binary due to potential exposure routes to the child care giver and others. If the child defecated into a toilet, then the potential for transmission between child and others is reduced. However, if a child defecated into a latrine, potty, diaper, in clothes, on a cloth or in the open, then the exposure to others in the household was left open. The observed results suggested that instead of having a protective association, the toilet location of last defecation was harmful. Once again, these results held a wide CI overlapping the null. The last defecation area of a child may not be the best indicator for usual sanitation practices of a child. Additionally, child feces disposal into an improved toilet was a binary classification that compared the toilet to every other disposal placement that had an estimated harmful association with STH infection. The CI was also too wide to draw a conclusion. It is possible that we saw these results because there was a lack of powers of events (11 cases for both the exposures).

Limitations & Strengths

The lack of statistical significance could have been a byproduct of the lack of event power. While the study itself had plenty of participants, the number of children with an STH infection was estimated at 106 total cases. It was not possible to run an entire model with all of the exposures and covariates in entirety, as there were no observations that fit all the criteria. Furthermore, when divided into many classes by sex, age, education, caste, wealth and intervention or control status, many categories either had too few cell counts or none. This created an issue with convergence in the MELR models, as it was still necessary to adjust by these covariates.

The strength of this analysis is that it collected exhaustive information from the participants, including data gathered by direct observation of the researchers. Potential confounders have been accounted for in each model. This analysis is relevant in that it continues to update the efforts of the MANTRA intervention to further prevent and control STH infections.

Conclusion

While soil-transmitted helminths are a neglected tropical disease, on-going studies and NGO's that address water, sanitation and hygiene interventions can provide avenues to look into soil-transmitted helminth infection determinants. The analysis above sought to find meaningful determinants to inform the Gram Vikas MANTRA intervention, in order to best identify what parts of their work they could work on. While the finding for proportion of household members using an improved toilet was not statistically significant, an emphasis on adherence of using the improved toilet within households may be the key to a meaningful reduction in STH infection in children aged 5 and under.

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Tables

Table 1. Descriptive characteristics of study population			
	N	**Any STH Infection**	
		No (%)	**Yes (%)**
Sex	2436		
Male		1171 (96%)	49 (4.02%)
Age	2457		
0 - 6 Months		125 (85.8%)	5 (14.2%)
>6 - 12 Months		217 (96%)	9 (4%)
>12 - 24 Months		642 (97%)	20 (3%)
>24 - 60 Months		1369 (95.1%)	70 (4.9%)
Wealth Quintile	2457		
Poorest		482 (93.8%)	32 (6.2%)
Poor		396 (95.9%)	17 (4.1%)
Middle		429 (97.5%)	11 (2.5%)
Rich		456 (96.4%)	17 (3.6%)
Richest		590 (95.6%)	27 (4.4%)
Caste/Tribe	2412		
Scheduled Caste		457 (95.6%)	21 (4.4%)
Scheduled Tribe		366 (92.9%)	28 (7.1%)
Other Backward Caste		772 (96.6%)	27 (3.4%)
Other Caste		713 (96.2%)	28 (3.8%)
Child Care Giver Education	2451		
<1 Year Completed		836 (96.7%)	29 (3.3%)
Completed Primary		223 (92.5%)	18 (7.5%)
Completed Secondary		1193 (95.6%)	55 (4.4%)
More Than Secondary Completed		95 (97.9%)	2 (2.1%)
Religion	2236		
Other/Christian		36 (100%)	0
Hindu		2104 (95.6%)	96 (4.4%)
MANTRA Intervention Village	2457		
Intervention		1105 (96.8%)	37 (3.2%)

Table 2. Mixed Effects Model - Logisitic Regression*

	Adjusted OR	95% C.I.		P-Value
Observed Feces in the Home				
No	1.00			
Yes	1.13	0.53	2.45	0.75
Handwashing Station				
No Station/No Water/No Soap	1.00			
Station with Water and Soap	0.58	0.31	1.09	0.09
Observed Consumption of Soil[1]				
No	1.00			
Yes	0.56	0.12	2.62	0.46
Location of Last Defecation				
Other	1.00			
Toilet	1.43	0.63	3.27	0.39
Location of Child Feces Disposal				
Other	1.00			
Improved Toilet	1.14	0.51	2.52	0.75
Hand Appearance				
Clean	1.00			
Dirty	0.82	0.31	2.14	0.69
Water Source				
Other	1.00			
On-Premise Piped	0.95	0.61	1.49	0.82
Sanitation				
Open Defecation/ Umimproved Sanitation	1.00			
Improved Sanitation	0.57	0.27	1.21	0.14
Proportion of Household Members Using an Improved Toilet				
Continuous	0.43	0.19	0.96	0.04

*Each model was controlled for age, wealth, child care giver education and intervention/control status with a random effect term included for village clustering of

[1] Observed by the child care giver in the last week

Table 3. Conditional Logisitic Regression Interaction Assessment for Any STH Infection*

	P-Value
Observed Feces in the Home	0.05
Handwashing Station	0.61
Observed Consumption of Soil‡ [1]	0.9
Location of Last Defecation	0.57
Location of Child Feces Disposal	0.96
Hand Appearance‡	0.16
Water Source	0.23
Sanitation	0.49
Proportion of Household Members Using an Improved	0.7

*Each model was controlled for age, wealth, child care giver education and intervention/control status

‡ Each model was controlled sex

[1] Observed by the child care giver in the last week

Table 4. Conditional Logisitic Regression for Any STH Infection*

	Any STH Infection		Adjusted OR	95% C.I.		P-Value
	No (%)	Yes (%)				
Observed Feces in the Home						
No	808 (96.4%)	30 (3.6%)	1.00			
Yes	255 (94.8%)	14 (5.2%)	0.91	0.42	1.96	0.8
Handwashing Station						
No Station/No Water/No Soap	1458 (95.1%)	76 (4.9%)	1.00			
Station with Water and Soap	812 (97.1%)	24 (2.9%)	0.51	0.29	0.90	0.02
Observed Consumption of Soil‡ [1]						
No	440 (95.6%)	20 (4.4%)	1.00			
Yes	79 (97.5%)	2 (2.5%)	0.57	0.12	2.75	0.48
Location of Last Defecation						
Other	1431 (95.78%)	63 (4.22%)	1.00			
Toilet	264 (96%)	11 (4%)	1.39	0.63	3.07	0.42
Location of Child Feces Disposal						
Other	1283 (95.06%)	56 (4.18%)	1.00			
Improved Toilet	318 (96.66%)	11 (3.34)	1.14	0.51	2.52	0.75
Hand Appearance‡						
Clean	374 (92.1%)	32 (7.9%)	1.00			
Dirty	543 (86.5%)	85 (13.5%)	0.46	0.14	1.53	0.21
Water Source						
Other	1496 (95.04%)	78 (4.96%)	1.00			
On-Premise Piped	856 (97.05%)	26 (2.95%)	0.83	0.41	1.67	0.6
Sanitation						
Open Defecation/Umimproved Sanitati	810 (94.7%)	45 (5.3%)	1.00			
Improved Sanitation	877 (96.8%)	29 (3.2%)	0.54	0.25	1.15	0.11
Proportion of Household Members Using an Improved Toilet						
Continuous	3330 (90.6%)	347 (9.4%)	0.50	0.22	1.14	0.1

*Each model was controlled for age, wealth, child care giver education and intervention/control status

‡ Each model was controlled sex

[1] Observed by the child care giver in the last week

Figures

Figure 1. Life Cycle of Hookworm

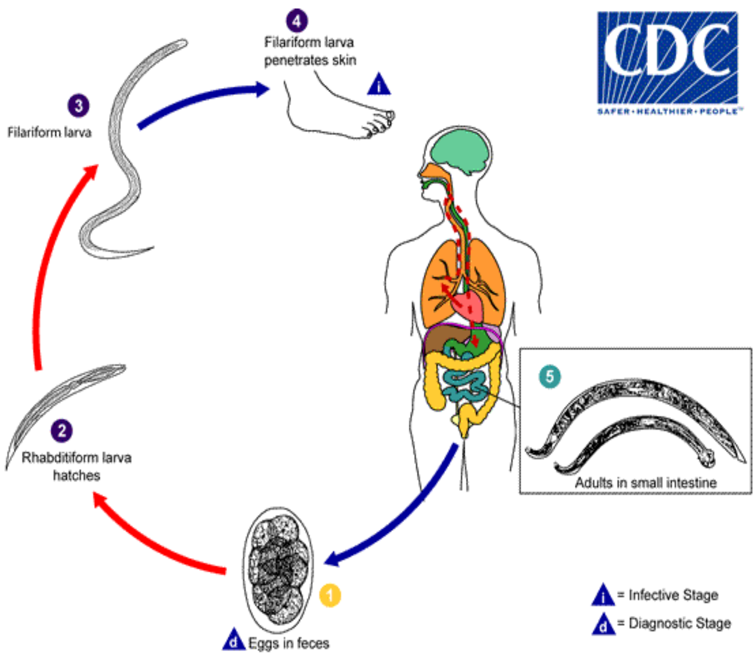
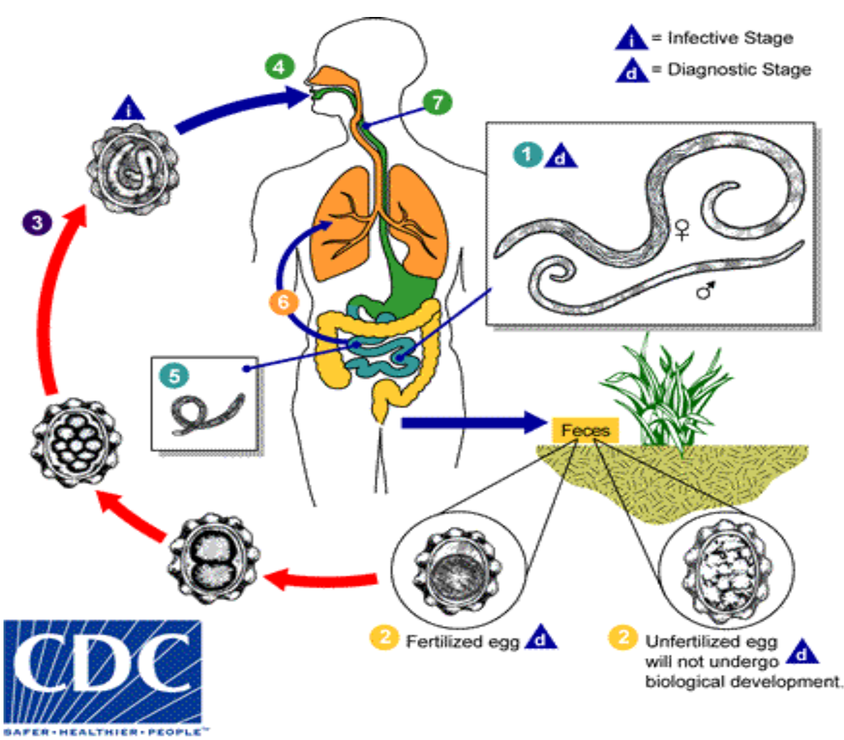
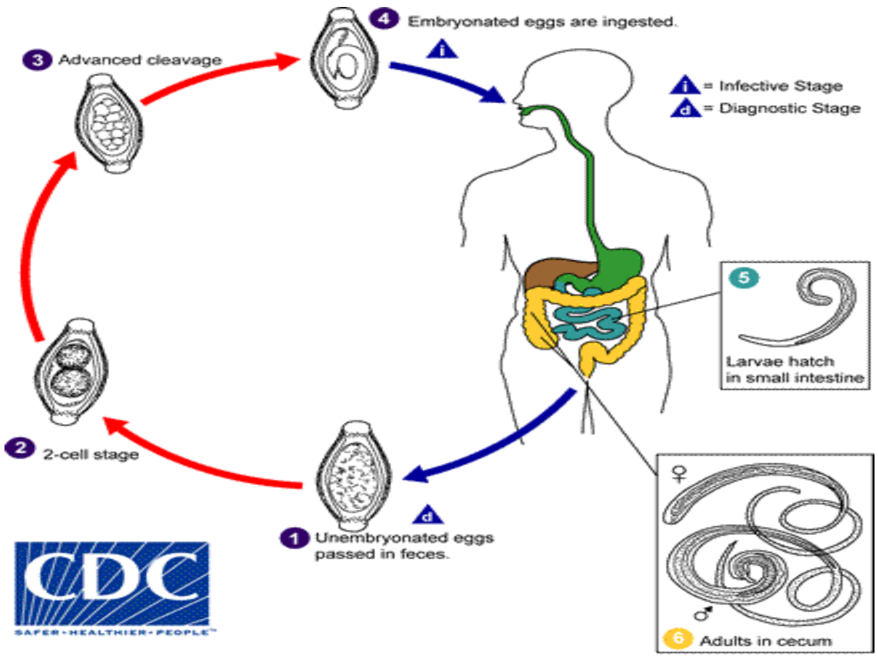


Figure 2. Life Cycle of *Ascaris lumbriodes*



<u>Figure 3. Life Cycle of *Trichuris trichuria*</u>



Appendices

Conditional logistic regression modeling was also conducted. Each exposure of interest was put into a model in order to obtain prevalence odds ratio estimates. Village intervention status was adjusted for in all of the models. Potential effect modification of each risk factor by village intervention status was assessed.. Inclusion of the interaction term in the final model for each factor was assessed through its p-value. Confounders, child age, caste, wealth and caregiver's education were included in all of the adjusted models. Child sex was included as a confounder in the observed consumption of soil and hand appearance models, due to previous studies observing that males have a higher infection rate[11]. Additionally, both of these models directly measure the individual WASH behavior of a child as opposed to the other exposures that are not related to the sex of a child. The Fisher's exact test was used to obtain point estimates.

Interaction was considered in Table 3. Ultimately, all interaction terms with intervention or control status was not statistically significant. Thus, all final models did not contain an interaction term. All results for the conditional logistic regression are reported in Table 4, adjusted for age, sex, wealth, caste and child care giver education.

Among children aged 5 and younger, having a handwashing station with soap and water compared to not having a handwashing station with soap and water has a protective association with a prevalence odds ratio of 0.51 (95% CI: .29, .90) for STH infection. This finding is statistically signification.

In the CLR adjusted model, the proportion of household members using an improved toilet had an estimated .50 prevalence odds ratio for STH infection (95% CI:

0.22, 1.14). This suggests that the odds of an STH infection reduces was the proportion of the household using an improved toilet increases.

When comparing the two models, feces observed in the home had a significant shift in prevalence odds ratio from a harmful association in the MELR model to a protective association in the CLR model. Among children aged 5 and younger, observed feces in the home, had a prevalence odds ratio of 0.91 for STH infections compared to no observed feces in the home. This association is not significant (95% CI: .42, 1.96).

The on-premise piped water source compared to other water source was estimated a slightly stronger protective association of 0.83 POR. Similarly, improved sanitation compared to open defecation and unimproved sanitation in STH infection, had an estimated protective association of 0.54 prevalence odds ratio (95% CI: .25, 1.15).

After Bonferroni correction, no risk factors were statistically significant. The Bonferroni's Correction was used to account for the 9 separate models. This correction set the alpha level at .006. Thus, the previously significant handwashing station with soap and water was no longer significant.