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The Interplay of Soil-Transmitted Helminth Infection, Stunting, Anemia, and Wealth in Ranomafana Commune, Ifanadiana District, Madagascar

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Abstract

The Interplay of Soil-Transmitted Helminth Infection, Stunting, Anemia, and Wealth in Ranomafana Commune, Ifanadiana District, Madagascar
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Background: PIVOT, a new healthcare NGO, recently began operating in Ranomafana Commune, Madagascar, with the mission of scientifically identifying areas of intervention in order to break the cycle of poverty and disease. Due to previously described associations of soil-transmitted helminth (STH) infection and anemia, this study describes the current prevalence of STHs and anemia, and uses logistic regression to evaluate their association with wealth and developmental indicators.

Methodology: This was a cross-sectional study that randomly selected 80 households, within 4 census tracts, to participate in the study from July 1st – July 14th, 2014. For each household, in-person surveys were administered, anthropometric measurement and rapid detection anemia tests were performed, and fecal samples were obtained. A total of 76 households and 365 individuals participated in the study, with anemia data and fecal samples collected from 221 and 238 individuals, respectively. Current prevalence of STH infection in children was compared to STH prevalence twenty years ago. STH infection was evaluated for associations with anemia and wealth, as well as stunting among children.

Principle Findings: Seventy-eight percent of the population was positive for at least one STH. Forty-four percent of children less than 5 were anemic, while 12% of adults age 15-49 were. In multivariable regression, those living in the bottom 75% of the wealth distribution and who were infected with any STH had significantly lower odds [odds ratio (OR) 0.24; 95% confidence interval (CI) 0.08, 0.74] of being anemic, while those in the bottom 75% of the wealth distribution and anemic were significantly more likely to be infected with any STH (OR 3.16; 95% CI 1.61, 6.19). Those in the bottom 75% of the wealth distribution were also significantly less likely to be infected with *Trichuris trichiura* only (OR 0.35; 95% CI 0.15, 0.78).

Conclusions: Comparison to historical STH prevalence shows that current strategies to control STH infection are ineffective or have broken down over the past twenty years. Investigation into the associations between STHs, anemia, and wealth can further the understanding of how poverty and disease cycles work, and help with the design of targeted interventions to break these cycles.

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Introduction

Soil transmitted helminths (STHs) contribute greatly to the global burden of disease, causing the loss of an estimated 39 million disability-adjusted life-years (DALYs) [1]. Previous studies have linked STH infection with anemia and delayed physical and mental development in children, leading to long-term educational and economic losses [2-4]. With the assistance of foreign aid, the governments of many low and middle income countries now implement various versions of mass drug administration programs in order to mitigate the negative effects associated with high levels of STH transmission [5]. Programs are often started without a complete understanding of the transmission dynamics within the target population, which makes monitoring the effectiveness of ongoing programs difficult. A thorough understanding of the intricacies of this disease system must be understood, so interventions, such as mass drug administration, can be targeted to the specific populations most in need, or to the populations in which it will have the largest effect.

Background

Soil-Transmitted Helminths

Soil-transmitted helminths (STHs) are intestinal parasites that infect roughly two billion people worldwide [6]. Those infected usually live in extreme poverty, maintain poor hygiene practices, and have limited access to sanitation [6,7]. The most common STHs are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*) [6,8]. STHs live in the intestinal tract where they lay thousands of eggs daily, which are excreted into the feces [6]. The eggs enter the environment through contaminated feces, causing infection when people ingest vegetables or drink from water sources that harbor parasite eggs, walk barefoot through contaminated soil, or place fingers with contaminated soil into their mouths [6,9].

STHs can cause severe health effects in their hosts, with a higher intensity of infection leading to increased morbidity [10]. Symptoms of infection can manifest as general malaise, diarrhea, and abdominal pain [6,9], as well as anemia and anorexia, which have been associated with physical and developmental retardation in children [1-4]. Hookworm infections most commonly cause anemia as the adult parasites feed on blood in the intestines and cause intestinal hemorrhage [11,12]. Conversely, infection with *A. lumbricoides* and *T. trichiura* are associated with anorexia and reduced nutrient absorption, which can lead to iron deficiency and iron deficiency anemia, especially in young and school-aged children [1,2,4,13]. Individuals with clinical anemia feel fatigued, short of breath, and may experience headaches and dizziness [14] that prevent children from attending school [15] and adults from performing productive work [2].

The potential for infected individuals to experience adverse health outcomes, coupled with the high worldwide prevalence of STHs, results in a significant global disease burden. It has been estimated that there are 10.5 million disability-adjusted life-years (DALYs) lost to *A. lumbricoides* infections, 6.4 million to *T. trichiura* infections, and 22.1 million to hookworm infections [1]. In total, the three most common STH infections account for 39 million DALYs lost, compared to 35.7 million DALYs lost due to malaria [1].

Madagascar

Madagascar is home to over 23 million people and is one of the poorest countries in the world. The World Bank reports the country's GNI per capita as US$ 440, placing it in the bottom three percent of all countries [16]. Forty-eight percent of the population lives in severe poverty, while 81% live below the international poverty line of $1.25/day [17].

Similar to other low-income countries, a significant number of people living in Madagascar experience poor health and nutritional outcomes. Thirty-three percent of the population is undernourished [18], with 50% of children under the age of 5 suffering from moderate or severe stunting [17]. This may be due, in part, to heavy reliance on a single crop for subsistence agriculture – rice [19]. Rice is the main food staple in Madagascar, and as such is the most commonly grown crop, with 4,550,649 tonnes produced in 2012 [20]. Often eaten three times a day, rice is responsible for over half of the daily caloric intake for Malagasy people [20]. Meat, however, makes up less than four percent of the total food supply [20].

Mortality in infants and in children under 5 years of age is high at 41 per 1,000 and 58 per 1,000 live births, respectively [17]. Malaria is endemic in Madagascar, with an estimated 1,300,000 malaria cases a year [21]; roughly 58 cases per 1,000 people. Anemia is also a severe health problem, with an estimated population prevalence of 68% [22]. STHs are also common, with the country-wide prevalence estimated at over 50% [23].

With a growing population and an economy largely based on subsistence agriculture, the forests of Madagascar have seen significant losses over the past centuries, with only 10% of its primary vegetation remaining [19,24]. In an effort to increase conservation of native fauna and its habitat, Ranomafana National Park (RNP) was established in 1991 [25]. A 41,000 hectare park located in the southeastern rainforest of Madagascar, RNP was originally established to protect the golden and greater bamboo lemurs [25]. While establishment of the park was important to protect the endemic biodiversity of Madagascar, it has been predicted that the people who live near the Park's border and are used to harvesting natural resources from the rainforest will suffer from a diminished standard of living [26].

There has been only one previous publication documenting the prevalence of STHs in the population living adjacent to RNP. The study was performed over a two-year period, from 1990-1992, in 1,292 children from birth to age 10 [27]. Seventy-eight percent of the children tested were positive for *A. lumbricoides*, 35% for *T. trichiura*, and 16% for hookworm [27]. Having this historical data allows for trends in STH prevalence over the past twenty years to be discussed. Of these same children, 37% were infected with two STHs and 27% were infected with three [27]. Although hemoglobin levels were not tested, many studies have found a five to eight fold increase in the odds of having anemia when children are infected with multiple parasites [28].

The high prevalence of STH infection observed in RNP is not unexpected. Only 14% of the Malagasy population has access to improved sanitation, such as a latrine [29], while only 34% of the rural population has access to safe water [29]. Due to environmental conditions and the close contact between people and livestock, there is also potential for zoonotic transmission of STHs, though this is beyond the scope of this study.

Models have been developed that couple infectious disease dynamics with economic growth models, strongly suggesting that low levels of human capital (e.g. health, education) undermines economic productivity and increases disease transmission, both of which further contribute to decreases in human capital development [30]. These poverty traps, driven by infectious diseases, such as STHs, can lead to "decreased productivity and inadequate socio-economic development" [31]. PIVOT, an NGO, was recently established in Madagascar with a goal of breaking the cycles of poverty and disease, by providing universal access to healthcare [32]. PIVOT's work is focused in the Ifanadiana District, which includes the eastern portion of the Ranomafana National Park.

The primary objective of this study was to describe the prevalence of STH infections (*T. trichiura, A. lumbricoides,* and hookworms) in the population of Ranomafana, a commune of approximately 10,000 people, located within the Ifanadiana District, and within 2 km of the RNP border. Patterns of STH infection and co-infection were evaluated for associations with age, anemia, malnutrition, and economic factors. This is based on the underlying hypothesis that STH infection causes anemia and malnutrition, and negatively impacts household economics.

Methods

Hypotheses

1. The prevalence of STH infection is expected to be high due to the low levels of access to improved sanitation in the study population.
2. No change in prevalence of STHs is expected over the past 20 years.
3. A positive association is expected between STH infection and anemia due to the lifecycle of STHs (i.e., they feed on blood and nutrients of the host).
4. A negative association is expected between STH infection and wealth since positive associations between higher incomes, education level, and access to health care are often found in developing countries.
5. A positive association is expected between STH infection and stunting in children under 5 since stunting is a measure of nutritional status.

Ethics statement

The Harvard School of Medicine Institutional Review Board initially reviewed the methods for data collection in this study and found them exempt from a human subjects review; all data collection was performed by a separate entity, the Madagascar Institute of Statistics (INSTAT), and provided to the investigators with all individual identifiers removed from the dataset. All adult participants (≥ 15 years) provided verbal and written consent for themselves and their children, and all children 6-14 years of age additionally provided assent for study participation.

Study Population

The town of Ranomafana, and several small villages immediately surrounding Ranomafana were selected for inclusion in the study. The selected area was divided into four census tracts, previously defined by the 2008-2009 Demographic and Health Survey in Madagascar; all households within the four tracts were enumerated in a baseline study performed by INSTAT for

PIVOT earlier in 2014. Twenty households were randomly selected from each census tract without replacement, for a total of 80 households.

Data

Surveys were conducted by two four-member teams from INSTAT on July 1st- July 14th, 2014. For each selected household the male and female head-of-house (HOH) were asked to participate in in-person interviews that included questions on household demographics, individual health, and household economics; the female HOH answered questions about their children. Anthropometric measurements and rapid detection anemia tests were done on all consenting household members that were less than five years of age and on adults ages 15-49; all individuals were asked to submit a fecal sample regardless of age.

A HemoCue hemoglobin test was used to determine blood hemoglobin levels in all consenting individuals. Blood was drawn using either a finger or heel stick, and was performed by a nurse working with the INSTAT team. Fresh fecal samples were collected daily in clean plastic cups provided to the selected households, and then transferred to a nearby research laboratory at Center ValBio for processing. Approximately five grams of each fecal sample was preserved in 10% formalin for light microscopy to be performed at a later date. Each day fecal floats were performed on 20% of fresh samples for comparison with results from formalin-fixed samples.

Fecal flotations were performed on three grams of feces, using a modified Wisconsin's protocol with Sheather's sugar solution (Appendix I). For each sample STH ova were identified and quantified for the three STHs of interest: *T. trichiura*, *A. lumbricoides,* and hookworms. All fecal samples were linked with individual and household level data that were collected during the in-person surveys.

Statistical Methods

Data were entered using Microsoft Excel (Redmond, WA) and analyzed using SAS 9.4 (Cary, NC). Survey data received from INSTAT were evaluated for missing or implausible values using univariate procedures for continuous variables and frequency procedures for categorical variables. Missing values were assumed to be missing at random. Survey data were linked with results of fecal parasitology using unique individual identification numbers. Household weights were calculated by INSTAT (Appendix II) and applied in all statistical procedures to account for the clustered sample survey design.

Descriptive statistics for outcome variables, exposure variables and covariates were calculated using the entire study population where data were available. Prevalence was determined for the STHs of interest, stratified by age group and presented as seven binary (positive/negative) variables: (1) "No STH" was defined as negative for all three STHs of interest, (2) "Any STH" was defined as positive for at least one STH, (3) "*T. trichiura,*" was defined as positive for *T. trichiura* with or without other STH infections, (4) "*A. lumbricoides*," was defined as positive for *A. lumbricoides* with or without other STH infections, (5) "Hookworms" was defined as positive for either *N. americanus* or *A. duodenale* with or without other STH infections, (6) "*T. trichiura* only" was defined as positive for *T. trichiura* only with no other STHs present, and (7) "All" was defined as all three STHs are present in the samples. With the exception of "No STH" the categories are neither exhaustive nor mutually exclusive.

Prevalence of anemia was determined for children less than five and adults 15-49 years old, and nutritional indicators (i.e. stunting, underweight, and wasting) were determined for children less than five. Anemia was defined using the WHO guidelines of hemoglobin levels of less than 110 g/l for children less than five, less than 120 g/l for adult women, and less than 130 g/l for adult men [33]. Nutritional indicators were defined following UNICEF guidelines of less than two standard deviations from the median measure [34].

The wealth index used as a measure of socio-economic status was calculated by INSTAT using Principal Component Analysis and traditional household wealth indicators used in the Malagasy Demographic Health Survey (Appendix II). A binary wealth variable was created for the analyses based on this wealth index, which compares the bottom 75% of the population to the top 25%.

Prevalence of *A. lumbricoides*, *T. trichiura*, and hookworms was determined for children ages 0 to 14, allowing for a comparison of the STH prevalence in 2014 to the STH prevalence 20 years ago as reported by Kigthlinger et al [27] for children 0 – 11 years old. Chi-square tests were used to make the comparisons.

Bivariate associations were examined between four selected categories of STH infection (any STH, Hookworms, *T. trichiura* only, and All) and age, anemia, wealth, stunting, and sex; associations were assessed using the Wald Chi-squared test with $\alpha = 0.05$. All associations were restricted to those less than five years of age and 15-49 year olds, except for stunting, which was restricted to those less than five years of age. Results of the bivariate analyses and literature review informed the initial multivariate logistic regression models.

The first two models evaluated the association between anemia and infection with any STH infection and anemia with hookworm infection. Models three and four examined the association between any STH infection and the binary wealth variable, and *T. trichiura* only and the binary wealth variable. Model five examined the association between stunting and infection with STH in children under 5 years of age.

Models were specified to include possible confounders and interaction terms. Interaction was assessed using likelihood ratio tests, while confounding was assessed through comparisons of unadjusted and adjusted ORs, as well as using information from the literature.

Results

Participant Characteristics

Seventy-six households elected to participate in the study, providing information on a total of 365 individuals (Table 1). The age distribution of participants was 14% children under the age of five years (n = 50), 29% children age 5-14 years (n = 105), 47% adults age 15-49 years (n = 173) and 10% adults 50 years and older (n = 37). Gender distribution was evenly split between males (49.9%; n = 183) and females (50.1%; n = 182). Seventy-three percent of the adults surveyed reported being employed (n = 150) with 41% (n = 72) reporting employment in the agriculture and livestock sector; approximately 20% reported working as day laborers (n = 34). The median household income of participating households was MGA 1,614,975 (US$573) per year. Nearly 60% of the households surveyed (n = 45) reported having an unimproved latrine, while 18% had an improved latrine or flushing toilet (n = 14), and the remaining 22% had no latrine at all (n = 17). Households tend to be large with nearly 33% of the households holding six to nine people.

STH infection, anemia, and nutritional indicators

The prevalence of STH infection, anemia, and nutritional indicators for the population of Ranomafana and surrounding villages was determined (Table 2). Seventy-eight percent (95% CI: 72.7, 83.2) of the population was positive for at least one STH (*T. trichiura*, *A. lumbricoides*, and/or hookworm). When evaluated by type of STH, 67.6% (95% CI: 61.4, 73.8) were positive with *T. trichiura*, 48.2% (95% CI: 39.0, 57.3) with *A. lumbricoides*, and 22.7% (95% CI: 16.5, 29.0) with hookworms. Twenty-five percent (95% CI: 19.1, 31.6) were positive for only *T. trichiura*. Only 15.9% (95% CI: 9.9, 22.0) had co-infection of all three STHs. By age group, those that were 5-14 years of age had the highest prevalence of STH, with 93% (95% CI: 86.7, 100.0) positive for any STH and 21% (95% CI: 10.9, 31.2) positive with all three STHs. Children less than 5 years of age had the lowest prevalence of STH, with only 60.6% (95% CI: 41.3, 79.9) positive for any STH and 5.3% (95% CI: 0.0, 12.5) for all three STHs.

Forty-four percent (95% CI: 28.6, 58.9) of children less than five years of age and 11.5% (95% CI: 6.1, 16.8) of adults age 15-49 were anemic. Nutritional indicators evaluated for children less than five years of age revealed that while only 9.1% (95% CI: 0, 19.2) were wasted, 54.6% (95% CI: 34.3, 74.9) were stunted, and 47.3% (95% CI: 28.6, 66.1) were underweight.

Chi-square Comparison of Prevalence

Chi-square tests determined that the prevalence of *A. lumbricoides* has significantly decreased (p-value < 0.0001) in children over the past 20 years from 78% in 1994 [27] to 53.4% (95% CI: 41.1, 65.8) in 2014 (Table 3). *Trichuris trichiura* prevalence significantly increased (p-value < 0.0001) during the past 20 years, from 38% in 1994 [27] to 70.2% (95% CI: 60.9, 79.6) in 2014 (Table 3). The prevalence of hookworms has also significantly increased (p-value = 0.004) since 1994 [27], from 16% to 22.8% (95% CI: 16.7, 28.8) (Table 3).

Bivariate Associations

Results of bivariate associations are presented in Table 4. There was a significant positive association between wealth category (referent = top 25%) and infection with any STH and infection with hookworms (OR 1.95; 95% CI 1.13, 3.36 and OR 12.08; 95% CI 1.80, 81.33, respectively). A significant negative association was found between wealth category and infection with *T. trichiura* only (OR 0.35; 95% CI 0.16, 0.76). There was also a significant positive association between sex (referent = women) and infection with hookworms and all STH (OR 3.48; 95% CI

1.53, 7.93, and OR 3.39; 95% CI 1.24, 9.26, respectively). No other bivariate associations were found to be significant.

Logistic Regression

Five models were constructed using logistic regression to evaluate the associations between anemia and STH infection, STH infection and wealth, and STH infection and stunting in children less than 5 (Table 5).

The first model examined the association between infection with any STH and anemia (Model 1 in Table 5). A significant interaction was found between infection with any STH and wealth (p=0.009), therefore results stratified on wealth category are presented. Among those in the bottom 75% of the wealth distribution, infection with any STH lead to a significant decrease in the odds of anemia, controlling for age (OR 0.24; 95% CI 0.08, 0.74). There was no significant association between any STH and anemia among those in the top 25% of the wealth distribution, controlling for age (OR 4.56; 95% CI 0.70, 29.90).

The second model (Model 2 in Table 5) examined the association between infection with hookworms and anemia. Due to overstratification, interaction between infection with hookworms and wealth could not be assessed, since no one in the top 25% of the wealth distribution was both anemic and positive for hookworms. No other significant interactions were identified. In the final model, there was no significant association between hookworm infection and anemia, when controlling for age and wealth (OR 0.67; 95% CI 0.17, 2.59).

The third model was then created to examine the associations between STH infection and wealth (Model 3 in Table 5). A significant interaction was found between wealth and anemia (p = 0.018). Among those who were anemic, individuals in the bottom 75% of the wealth distribution had a significant increase in their odds of infection with any STH, controlling for age (OR 3.16;

95% CI 1.61, 6.19). Among those without anemia, there was no association between wealth distribution and infection with any STH, controlling for age (OR 0.21; 95% CI 0.03, 1.63).

The fourth model was specified to identify any association between infection with only *T. trichiura* and wealth (Model 4 in Table 5). The odds of being infected with only *T. trichiura* was significantly decreased among those in the bottom 75% of the wealth distribution compared to those in the top 25%, controlling for age (OR 0.35; 95% CI 0.15, 0.78).

The fifth and final model examined the association between stunting and infection with any STH (Model 5 in Table 5). Due to overstratification of the model it was not possible to look at interaction between wealth and any STH infection or between sex and any STH infection. There was no significant association between infection with any STH and stunting, controlling for wealth and sex (OR 0.20; 95% CI 0.02, 2.63).

Discussion

Prevalence of STHs

This was the first study to examine prevalence of STHs in this region since 1994. The 1994 study by Kightlinger et al. [27] found a prevalence of 78% for *A. lumbricoides*, 38% for *T. trichiura*, and 16% for hookworms in children from birth to 11 years old. This can be compared to the prevalence found in this study for children from birth to 14 years old: 53% for *A. lumbricoides*, 70% for *T. trichiura*, and 23% for hookworms. While the prevalence of *A. lumbricoides* has significantly decreased over the past 20 years, the prevalence of both *T. trichiura* and hookworms has significantly increased (Table 3). These results suggest that current deworming programs in the area have not been effective at controlling STHs. The fact that the highest prevalence of STHs is in school aged children (Table 2), shows that either, (1) the area surrounding RNP does not practice school based mass drug administration (MDA) of anthelmintic medications, or (2) the WHO guidelines [35] that promote school based MDA to control the prevalence of STHs are not effective in this region.

Multivariate Models

A significant association between wealth and infection with any STH, among those with anemia, and controlling for age was found in this study (Table 6). Among those with anemia, those in the bottom 75% of the wealth distribution were more likely to be infected with any STH, controlling for age. The wealth distribution variable was based on a wealth index calculated to describe a households' standard of living, and is based in part on a households' toilet facilities and type of water source. This association is therefore expected, since those in the bottom 75% of the wealth distribution more commonly lack access to improved sanitation, such as a latrine or flush toilet, and have less ability to maintain good hygiene practices, both of which are risk factors for

STH infection [6,7]. This model helps to identify the populations for which PIVOT should target interventions that aim to control and reduce STH infection in and around Ranomafana.

Those in the bottom 75% of the wealth distribution are significantly less likely to be infected with *T. trichiura* only compared to those in the top 25% of the wealth distribution, controlling for age (Table 6). This finding suggests that an unequal administration of anthelmintic medication among the study population, based on wealth, may occur. One possible explanation is that those in the top 25% of the wealth distribution have enough disposable income to purchase anthelmintic medication and have received enough education to know the importance of deworming. However, most pharmacies sell a single dose of 400mg albendazole, which will eliminate *A. lumbricoides* and hookworms [36,37], but will not eliminate *T. trichiura,* which requires 400 mg of albendazole given three days in a row [38]. This may explain the observed association of infection with *T. trichiura* being more common among the top 25% of the wealth distribution because a larger proportion of individuals self-administer anthelmintic medication, compared to those in the lower 75% of the wealth distribution who do not deworm and therefore have a lower proportion of individuals who are positive with only *T. trichiura* infection.

Among the bottom 75% of the wealth distribution, those infected with any STH were less likely to have anemia (Table 6). Based on our understanding of the feeding behaviors of intestinal macroparasites and the mechanisms through which they cause anemia, the direction of this association is unexpected and does not support the original hypothesis. One possible reason for this result is the potential for residual confounding within the lowest wealth category. This category contains 75% of the population and there may be significant variation in wealth, standard of living, nutrition, or behavior among those in the bottom 75% of the distribution. If residual confounding is strong, it may bias the OR across the null, resulting in a protective effect, rather than the expected harmful effect. This possibility should be examined in future studies with a larger study population, where the wealth index can be further stratified to better represent differences in wealth distribution.

A second reason we may observe this result is the potential for confounding due to an unmeasured variable, possibly diet. Information on diet was not measured during the surveys and therefore not controlled for in the analysis. Diet may create confounding since it is likely associated both with STH infection (if food being consumed is not properly washed and is contaminated with STHs) and with anemia (diets low in iron can cause iron-deficiency anemia) [4,9]. If this confounding is large enough, it could be responsible for biasing the OR across the null leading to the unexpected directionality seen.

Limitations

The bivariate associations (Table 4) made evident the limitations of the small sample size and the possibility of over-stratifying the data to create unstable ORs, leading to a bivariate categorization of weight and limiting how STH infections could be categorized. Due to how the age variable was categorized, the comparison between STH prevalence today to twenty years ago does not use identical age groupings (birth to 14 years old vs birth to 11 years old). Missing hemoglobin data for the age group with the highest prevalence of STHs (5-14 year olds) further decreased the sample size and limits how representative the models are of the study population, especially since age can act as a confounder. The high prevalence of STHs in the study population was a problem since ORs cannot be interpreted as risk ratios since the rare disease assumption is not met. Diet could also contribute to both anemia and STH infection and was not measured.

Conclusions and Recommendations

Although this was a cross-sectional study, interesting and useful conclusions can be drawn. Normally, due to the constraints of a cross-sectional study design, no statements can be made about trends over time, since only point prevalence is calculated. However, a study 20 years previously allows for prevalence trends over time to be described through a comparison with results presented by Kightlinger et al. [27] and the findings of the current study. That comparison showed that either any efforts to reduce STH prevalence in the past 20 years have not been successful, or that any successful interventions have not been sustained. The increasing prevalence of STHs may be due, in part, to population growth in and around Ranomafana. If the population has been growing, with no improvement to sanitation, parasite transmission would increase. The recommendation is PIVOT should evaluate what programs, if any, currently exist to reduce STH infection and to then design and implement new interventions that will successfully reduce the burden of STHs in and around Ranomafana.

Based on the hypothesis that those in the top 25% of the wealth distribution are incorrectly self-administering anthelmintic medication, PIVOT should work with community leaders and pharmacists to properly educate them on the proper administration of albendazole. This would lead to a greater proportion of the population living without infection with STH, lowering the ova density in the environment, potentially leading to lower rates of transmission and infection.

References

1. Stephenson LS, Latham MC, Ottesen EA (2000) Malnutrition and parasitic helminth infections. Parasitology 121 Suppl: S23-38.
2. Crompton DW, Nesheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr 22: 35-59.
3. Engels D, Savioli L (2006) Reconsidering the underestimated burden caused by neglected tropical diseases. Trends Parasitol 22: 363-366.
4. Golden CD, Fernald LC, Brashares JS, Rasolofoniaina BJ, Kremen C (2011) Benefits of wildlife consumption to child nutrition in a biodiversity hotspot. Proc Natl Acad Sci U S A 108: 19653-19656.
5. USAID (2014) Countries Supported by USAID's NTD Program.
6. WHO (2013) Soil transmitted helminth infections.
7. Albonico M, Montresor A, Crompton DW, Savioli L (2006) Intervention for the control of soil-transmitted helminthiasis in the community. Adv Parasitol 61: 311-348.
8. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, et al. (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367: 1521-1532.
9. CDC (2013) Parasites - Soil Transmitted Heminths (STHs).
10. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, et al. (2008) Helminth infections: the great neglected tropical diseases. J Clin Invest 118: 1311-1321.
11. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, et al. (2004) Hookworm infection. N Engl J Med 351: 799-807.
12. Hotez PJ, Bethony J, Bottazzi ME, Brooker S, Buss P (2005) Hookworm: "the great infection of mankind". PLoS Med 2: e67.
13. Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, et al. (1997) Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. Am J Clin Nutr 65: 153-159.
14. NHLBI (2012) What are the signs and symptoms of anemia?
15. Miguel E, Kremer M (2004) Worms: Identifying Impacts on Education and Health in the Presence of Treatment Externalities. Econometrica 72: 159-217.
16. World Bank (2015) Gross national income per capita 2013, Atlas method and PPP.
17. Malik K (2014) 2014 Human Development Report. New York.
18. von Grebmer K, Headey D, Bene C, Haddad L, Olofinbiyi T, et al. (2013) 2013 Global Hunger Index: The Challegne of Hunger: Building Resilience to Achieve Food and Nutrition Security. Bonn
19. Gorenflo L, Corson C, Chomitz K, Harper G, Honzak M, et al. (2011) Exploring the association between people and deforestation in Madagascar. In: Cincotta R, Gorenflo L, editors. Human Population: Its Influences on Biological Diversity. Berlin: Springer-Verlag. pp. 197-221.
20. FAOSTAT (2011) Madagascar Top Ten commodities: Availability for consumption 2011. FAO of the UN.
21. WHO (2012) Malaria, Estimated cases, Data by country.
22. WHO (1993-2005) Worldwide prevalence of anaemia 1993-2005. In: de Benoist B, McLean E, Egli I, Cogswell M, editors: WHO Global Database on Anaemia.
23. WHO (2010) Working to Overcome the Global Impact of Neglected Tropical Diseases. First WHO Report on Neglected Tropical Diseases. Geneva.
24. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853-858.
25. Wright P, Erhart E, Tecot S, Baden A, Arrigo-Nelson S, et al. (2012) Long-Term Lemur Research at Centre Valbio, Ranomafana National Park, Madagascar. In: Kappeler P,

Watts D, editors. Long-Term Field Studies of Primates. Berlin: Springer-Verlag. pp. 67-100.
26. Ferraro P (2002) The local costs of establishing portected areas in low-income nations: Ranomafana National Park, Madagascar. Ecological Economics 43: 261-275.
27. Kightlinger LK, Seed JR, Kightlinger MB (1995) The epidemiology of Ascaris lumbricoides, Trichuris trichiura, and hookworm in children in the Ranomafana rainforest, Madagascar. J Parasitol 81: 159-169.
28. Ezeamama AE, Friedman JF, Olveda RM, Acosta LP, Kurtis JD, et al. (2005) Functional significance of low-intensity polyparasite helminth infections in anemia. J Infect Dis 192: 2160-2170.
29. World Bank (2010) Madagascar.
30. Ngonghala CN, Plucinski MM, Murray MB, Farmer PE, Barrett CB, et al. (2014) Poverty, disease, and the ecology of complex systems. PLoS Biol 12: e1001827.
31. Lustigman S, Prichard RK, Gazzinelli A, Grant WN, Boatin BA, et al. (2012) A research agenda for helminth diseases of humans: the problem of helminthiases. PLoS Negl Trop Dis 6: e1582.
32. PIVOT (2014) Madagascar.
33. WHO (2011) Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: Word Health Organization.
34. UNICEF (n.d.) Nutrition.
35. WHO (2011) Helminth control in school-age children. A guide for managers of control promrammes. Geneva: WHO.
36. CDC (2013) Parasites - Hookworm. Resources for Health Professionals.
37. Merck (2013) Ascariasis.
38. CDC (2013) Parasites - Trichuriasis. Resources for Health Professionals.

Tables

Table 1. Household and Individual Characteristics of Study Population of Ranomafana Commune, Ifanadiana District, Madagascar.

	No.	%/Median
Sex		
Female	183	50.14
Male	182	49.86
Age, years		
<5	50	13.7
5-14	105	28.77
15-49	173	47.4
>49	37	10.14
Household Latrine		
None	17	22.37
Unimproved	45	59.21
Improved or Flushing Toilet	14	18.42
Employed		
Yes	150	72.82
No	56	27.18
Principle Employment		
Agriculture and Livestock	72	41.38
Permanent Employment	35	20.11
Trade	24	13.79
Day Labor	34	19.54
Other	9	5.17
People per Household		
1-2	9	11.84
3-5	42	55.26
6-9	25	32.89
Median Income[1]	-	1614075

[1] Income reported in Malagasy Ariary (1 USD = 2,860 MGA)

Table 2. Weighted Prevalence of Soil-Transmitted Helminths, Anemia, and Nutritional Indicators, Stratified by Age of Study Population of Ranomafana Commune, Ifanadiana District, Madagascar.

	Total Participants		<5 years		5-14 years		15-49 years		>49 years	
	W % (n)	W 95% CI	W % (n)	W 95% CI	W % (n)	W 95% CI	W % (n)	W 95% CI	W % (n)	W 95% CI
STH (n=238)										
No STH	22.0 (49)	16.7, 27.3	39.4 (10)	20.1, 58.7	6.5 (4)	0.0, 13.1	23.7 (28)	17.2, 30.3	29.3 (7)	10.6, 48.1
Any STH	78.0 (189)	72.7, 83.2	60.6 (17)	41.3, 79.9	93.4 (63)	86.7, 100.0	76.2 (87)	69.7, 82.8	70.7 (22)	51.9, 89.4
Trichuris trichiura	67.6 (166)	61.4, 73.8	42.2 (12)	24.9, 59.5	84.3 (58)	72.0, 96.4	68.9 (79)	61.8, 75.9	53.1 (17)	33.6, 72.7
Ascaris lumbricoides	48.2 (124)	39.0, 57.3	26.3 (8)	10.5, 42.1	67.0 (47)	51.7, 82.2	43.1 (53)	33.1, 53.1	52.8 (16)	30.1, 75.6
Hookworms	22.7 (62)	16.5, 29.0	13.1 (5)	2.3, 23.9	27.6 (21)	17.1, 38.1	22.5 (28)	14.2, 30.8	23.6 (8)	5.1, 42.0
Trichuris trichiura only	25.3 (53)	19.1, 31.6	26.4 (6)	9.8, 42.9	22.5 (13)	10.9, 34.1	28.3 (28)	19.5, 37.0	17.9 (6)	3.1, 32.6
All	15.9 (45)	9.9, 22.0	5.3 (2)	0.0, 12.5	21.0 (16)	10.9, 31.2	15.0 (20)	7.8, 22.1	20.6 (7)	1.9, 39.3
Anemia (n=221)	19.7 (46)	14.4, 25.1	43.8 (22)	28.6, 58.9	-	-	11.5 (20)	6.1, 16.8	-	-
Nutritional Indicators (n=47)										
Stunting	54 .0 (25)	35.3, 72.7	54.6 (21)	34.3, 74.9	-	-	-	-	-	-
Underweight	46.0 (22)	28.9, 63.2	47.3 (19)	28.6, 66.1	-	-	-	-	-	-
Wasting	7.9 (4)	0, 16.9	9.1 (4)	0.0, 19.2	-	-	-	-	-	-

Table 3. Chi-Square Comparison of the Prevalence of Soil-Transmitted Helminths in 1994[1] and 2014 of Ranomafana Commune, Ifanadiana District, Madagascar

	1994	2014		Comparison	
	0-11 year olds	0 - 14 year olds			
	% (n)	W % (n)	W 95% CI	Chi-Square	P-Value
Ascaris lumbricoides	78 (951)	53.4 (55)	41.1, 65.8	18.53	<0.0001*
Trichuris trichiura	38 (463)	70.2 (70)	60.9, 79.6	48.18	<0.0001*
Hookworms	16 (195)	22.8 (26)	16.7, 28.8	8.48	0.004*

[1]1994 prevalence as reported in Kightlinger et al. 1995 [27]

*Significant at α = 0.05

Table 4. Bivariate Associations of Soil-Transmitted Helminths and Age, Anemia, Wealth, Stunting, and Sex of Study Population of Ranomafana Commune, Ifanadiana District, Madagascar.

	Odds Ratio	95% CI	P value
STH = age[1]			
Any STH	1.45	0.92, 2.28	0.113
Hookworms	1.39	0.86, 2.25	0.184
Trichuris trichiura only	1.05	0.64, 1.73	0.852
All	1.78	0.89, 3.54	0.101
STH = Anemia			
Any STH	0.53	0.21, 1.35	0.186
Hookworms	0.62	0.20, 1.98	0.420
Trichuris trichiura only	0.74	0.25, 2.19	0.583
All	0.38	0.08, 1.90	0.239
STH = Wealth[2]			
Any STH*	1.95	1.13, 3.36	0.017
Hookworms*	12.08	1.80, 81.33	0.010
Trichuris trichiura only*	0.35	0.16, 0.76	0.008
All	-	-	-
STH = Stunted			
Any STH	0.65	0.107, 3.958	0.640
Hookworms	0.29	0.02, 4.07	0.356
Trichuris trichiura only	1.49	0.25, 8.95	0.661
All	-	-	-
STH = Sex[3]			
Any STH	0.97	0.47, 2.00	0.928
Hookworm*	3.48	1.53, 7.93	0.003
Trichuris trichiura only	0.75	0.32, 1.80	0.523
All*	3.39	1.24, 9.26	0.017

*Significant bivariate association
[1]Referent is less than 5 year olds
[2]Referent is top 25%
[3]Referent is female

Table 5. Three Sets of Multivariate Models Examining Associations between (1) Soil-Transmitted Helminth Infection and Anemia, (2) Soil-Transmitted Helminth Infection and Wealth, (3) Soil-Transmitted Helminth Infection and Stunting of Study Population of Ranomafana Commune, Ifanadiana District, Madagascar.

	Coefficient	95% CI	p-value
Outcome = anemia			
Model 1			
Intercept	-1.36	-3.44, 0.73	0.203
Any STH	1.52	-0.36, 3.40	0.113
Age	-1.90	-2.99, -0.81	0.001
Wealth	2.23	-.022, 4.48	0.052
Any STH * Wealth	-2.93	-5.13, -0.72	0.009
Model 2			
Intercept	-0.34	-1.32, 0.63	0.489
Hookworms	-0.40	-1.75, 0.95	0.562
Age	-1.80	-2.85, -0.76	0.001
Wealth	0.29	-0.72, 1.30	0.571
Outcome = Infection with any STH			
Model 3			
Intercept	-0.14	-1.10, 0.81	0.767
Wealth	1.15	0.48, 1.82	0.001
Anemia	1.44	-0.49, 3.37	1.144
Age	0.66	-0.30, 1.62	0.179
Wealth * Anemia	-2.73	-5.00, -0.46	0.018
Outcome = Infection with only Trichuris trichiura			
Model 4			
Intercept	-0.45	-1.32, 0.42	0.315
Wealth	-1.06	-1.87, -0.25	0.010
Age	0.2	-0.86, 1.26	0.714
Outcome = Stunting			
Model 5			
Intercept	-1.17	-3.71, 1.38	0.369
Any STH	-1.61	-4.18, 0.97	0.222
Wealth	4.31	1.29, 7.34	0.005
Sex	-1.71	-4,39, 0.97	0.211

Table 6. Odds Ratios, 95% CI, and P-Values for the 5 Multivariate Models of Study Population of Ranomafana Commune, Ifanadiana District, Madagascar.

	Outcome	Predictor	Strata	Odds Ratio	95% CI	P Value
Model 1[1]	Anemia	Any STH	Bottom 75% of wealth distribution	0.24	0.08, 0.74	0.013*
			Top 25% of wealth distribution	4.56	0.70, 29.90	0.113
Model 2[2]	Anemia	Hookworms	-	0.67	0.17, 2.59	0.562
Model 3[1]	Any STH	Wealth	Not Anemic	0.21	0.03, 1.63	0.135
			Anemic	3.16	1.61, 6.19	0.001*
Model 4[1]	*T. trichiura only*	Wealth	-	0.35	0.15, 0.78	0.010*
Model 5[3]	Stunting	Any STH	-	0.20	0.02, 2.63	0.222*

*significant at α= 0.05
[1]controlling for age
[2]controlling for age and wealth
[3]controlling for wealth and sex

Appendix I

Modified Wisconsin's Protocol for fecal floatations.

1) Measure out 3 grams of feces into a paper cup
2) Mix with 10ml of Sheather's sugar solution*
3) Place a doubled over piece of cheesecloth over the paper cup
4) Pour the contents of the cup into the Falcon tube (expect to get ~6ml back, depending on the sample)
5) Fill the Falcon tube to the top with Sheather's – there should be a slight meniscus over the top of the tube
6) Place a coverslip onto the meniscus
7) Centrifuge for 5 minutes at 1,500 rpm
8) Let sit for 5 min
9) Place the coverslip onto a slide and look at the entire slide under the microscope at 10x objective
10) Identify and quantify STH ova

*Sheather's sugar solution: In an Erlenmeyer flask 355 ml of filtered water was combined with 454 grams of granulated sugar. This mixture was slowly heated on a hot plate, until sugar was fully dissolved. The mixture was then allowed to cool and 6 mL of 30% formaldehyde was added. The mixture was kept at room temperature.

Fecal Directs

1) Using a toothpick smear a small amount of fresh feces onto a slide

2) Add a drop of saline on top of the feces

3) Mix the feces and saline together with the toothpick

4) Place a coverslip over the mixture

5) Look at the entire slide at 20x and 40x objective

Appendix II – Weighting Procedures and Wealth Index from the Malagasy Institute of Statistics

Context

These procedures were used with **PIVOT baseline survey for the whole district of Ifanadiana.** At this level stratum was each commune of the district, 80 cluster were sampled.

Another survey were conducted at 4 clusters in the Commune of Ranomafana. Sample design weight for household in these clusters are the same as used in PIVOT baseline survey. Final and normalized weight are equal to sample design weight updated by nonresponse during fieldwork.

Considering this sampling procedure, we could not say if these 4 clusters are statistically representative of Commune of Ranomafana.

Weighting Procedures

In order for the sample estimates from the basic survey to be representative of the population, it is necessary to multiply the data by a sampling weight. The basic weight for each sample household would be equal to the inverse of its overall probability of selection, calculated by multiplying the probabilities at each sampling stage. A household weight will be attached to each sample household record in the data files; in addition, woman weights and child weights will be attached to the corresponding data files. The sampling probabilities at each stage of selection will be maintained in an Excel spreadsheet with information from the sampling frame for all the sample cluster so that the corresponding weights can be calculated. Following the fieldwork it will be necessary to enter in this spreadsheet the total number of households listed and the final number of completed household interviews in each sample cluster.

Based on the proposed sample design, the overall probability of selection for the sample households can be expressed as follows:

$$p_{hij} = \frac{z_h \times N_{hi}}{N_h} \times p_{Shij} \times \frac{m_{hij}}{M'_{hij}},$$

Where:

- p_{hij} = probability of selection for the sample households in the j-th sample segment in the i-th sample cluster in stratum (commune) h
- z_h = number of sample cluster (or segments) selected in stratum h

- N_{hi} = total population in the frame for the i-th sample cluster in stratum h

- N_h = total population in the sampling frame for stratum h (cumulated measures of size)

- p_{Shij} = probability of selecting the j-th sample segment in the i-th sample cluster in stratum h

- m_{hij} = 20 = number of sample households selected in the j-th sample segment in the i-th sample cluster in stratum h

- M'_{hij} = total number of households listed in the j-th sample segment in the i-th sample cluster in stratum h

In the case of the sample cluster that are not divided into segments, p_{Shij} = 1. For the remaining (large) sample cluster, the formula for p_{Shij} will depend on whether the sample segment within the cluster is selected with PPS or equal probability.

The basic household weight is calculated as the inverse of this probability of selection. Based on the previous expression for the probability, the weight can be simplified as follows:

$$W_{hij} = \frac{N_h \times M'_{hij}}{z_h \times N_{hi} \times p_{2hij} \times m_{hij}},$$

Where:

- W_{hij} = basic weight for the sample households in the j-th sample segment in the i-th sample cluster in stratum h

If m_{hij} is constant for each segment (for example, 20 households), the sample will be approximately self-weighting within each stratum. The variability in the weights within each stratum depends on the correlation between the population in the frame for the cluster and the number of households listed in the sample segment (multiplied by the number of segments in the cluster).

It is also important to adjust the basic weights for the sample households to take into account the nonresponse in each sample cluster. Since the weights will be calculated at the level of the sample cluster, it would be advantageous to adjust the weights at this level. The final weight (W'_{hij}) for the sample households in the i-th sample cluster in stratum h can be expressed as follows:

$$W'_{hij} = W_{hij} \times \frac{m'_{hij}}{m''_{hij}},$$

Where:

- m'_{hij} = total number of valid (occupied) sample households selected in the j-th sample segment in the i-th sample cluster in stratum h

- m''_{hij} = number of sample households with completed interviews in the j-th sample segment in the i-th sample cluster in stratum h

Following the adjustment of the household weights for nonresponse, these weights are generally normalized (standardized) in data files so that relative weights can be used for the analysis of the survey data. In this way the sum of the relative weights will be equal to the number of sample households. The household weights are normalized by dividing each weight by the average weight at the whole-district level (that is, the sum of the weights for all sample households divided by the number of sample households). Therefore the relative weights will have a mean value of 1.

Given that sometimes it is not possible to complete a woman questionnaire for each woman identified in the household roster, it is also necessary to have a separate woman weight with an additional nonresponse adjustment factor applied to the household weight. The woman weight can be expressed as follows:

$$W_{whij} = W'_{hij} \times \frac{w_{hij}}{w'_{hij}},$$

Where:

- W_{whij} = adjusted weight for data in woman questionnaires for the j-th sample segment the i-th sample cluster in stratum h

- w_{hij} = total number of women age 15 to 49 years identified in the questionnaire roster for all sample households in the j-th sample segment in the i-th sample cluster in stratum h

- w'_{hij} = number of women with completed interviews for all sample households in the j-th sample segment i-th sample cluster in stratum h

There will also be cases where men questionnaire is not completed for all eligible men in some sample households. Therefore it is necessary to have a separate men weight with an additional nonresponse adjustment factor applied to the household weight. The men weight can be expressed as follows:

$$W_{chij} = W'_{hij} \times \frac{c_{hij}}{c'_{hij}},$$

Where:

- W_{chij} = adjusted weight for data in child questionnaires for the j-th sample segment in the i-th sample cluster in stratum h

- c_{hij} = total number of children under 5 years identified in the questionnaire roster for all sample households in the j-th sample segment in the i-th sample cluster in stratum h

- c'_{hij} = number of women with completed interviews for all sample households in the j-th sample segment in the i-th sample cluster in stratum h

Wealth index

Wealth index calculations are exactly the same as in DHS and MICS surveys. The wealth index is a background characteristic that is used throughout the report as a proxy for long-term standard of living of the household. It is based on the data on the household's ownership of consumer goods; dwelling characteristics; type of drinking water source; toilet facilities; and other characteristics that are related to a household's socio-economic status. To construct the index, each of these assets was assigned a weight (factor score) generated through principal component analysis, and the resulting asset scores were standardized in relation to a standard normal distribution with a mean of zero and standard deviation of one (Gwatkin et al., 2000).

Each household was then assigned a score for each asset, and the scores were summed for each household. Individuals were ranked according to the total score of the household in which they resided.

The sample was then divided into quintiles from one (poorest) to five (richest). A single asset index was developed on the basis of data from the entire.