Risk Factors for Soil-Transmitted Helminth Infections during the First 3 Years of Life in the Tropics; Findings from a Birth Cohort

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Abstract

Background: Soil-transmitted helminths (STH) infect more than 2 billion humans worldwide, causing significant morbidity in children. There are few data on the epidemiology and risk factors for infection in pre-school children. To investigate risk factors for infection in early childhood, we analysed data prospectively collected in the ECUAVIDA birth cohort in Ecuador.

Methods and Findings: Children were recruited at birth and followed up to 3 years of age with periodic collection of stool samples that were examined microscopically for STH parasites. Data on social, demographic, and environmental risk factors were collected from the mother at time of enrolment. Associations between exposures and detection of STH infections were analysed by multivariable logistic regression. Data were analysed from 1,697 children for whom a stool sample was obtained at 3 years. 42.3% had at least one STH infection in the first 3 years of life and the most common infections were caused by A. lumbricoides (33.2% of children) and T. trichiura (21.2%). Hookworm infection was detected in 0.9% of children. Risk of STH infection was associated with factors indicative of poverty in our study population such as Afro-Ecuadorian ethnicity and low maternal educational level. Maternal STH infections during pregnancy were strong risk factors for any childhood STH infection, infections with either A. lumbricoides or T. trichiura, and early age of first STH infection. Children of mothers with moderate to high infections intensities with A. lumbricoides were most at risk.

Conclusions: Our data show high rates of infection with STH parasites during the first 3 years of life in an Ecuadorian birth cohort, an observation that was strongly associated with maternal STH infections during pregnancy. The targeted treatment of women of childbearing age, in particular before pregnancy, with anthelmintic drugs could offer a novel approach to the prevention of STH infections in pre-school children.

Introduction

Soil-transmitted helminths (STH), including A. lumbricoides lumbricoides, T. trichiura trichiura, and hookworm, are estimated to infect more than 2 billion humans worldwide [1] of which 51 million children are considered to be at risk of morbidity [2]. An estimated 35 million or more disability-adjusted life years [3] have been attributed to STH infections.

Morbidity due to STH infections has primarily been associated with anaemia, malnutrition [4], stunting [5], and cognitive impairment [6]. Effects on childhood growth have been attributed to changes in appetite, digestion, nutrient absorption and iron loss [7].

Current strategies for the control of STH infections are primarily based upon periodic treatment of schoolchildren with anthelmintic drugs, and secondarily on education and improvements in sanitation. Treatment-based control strategies aim to control morbidity through reductions in the community transmission of STH infections [8].

Previous studies have shown that the main risk factors for STH infection are rural residency, low socioeconomic status and poor sanitation [2,3]. The use of pit latrines and improved drinking water have been associated with a reduced prevalence of STH infections [4] while maternal STH infections with increased risk [9], indicating the importance of identifying risk factors amenable to targeted interventions.

There are few data on the epidemiology of STH infections and risk factors for infection in pre-school children. Such data are relevant to the control of STH infections because pre-school children constitute an important reservoir of infection and are at risk of morbidity. To investigate the epidemiology of STH infections in early childhood and to identify risk factors for infection, we analysed data collected prospectively during the first 3 years of life in the ECUAVIDA birth cohort in tropical Ecuador.
**Author Summary**

Soil-transmitted helminths (STH) are intestinal worms that cause significant morbidity in school age and pre-school children in developing countries. Infections are associated with poverty, particularly through lack of access to sanitation and clean drinking water. Current control strategies rely on periodic anthelmintic treatment of schoolchildren but new strategies are required for STH control in young children. There are few data on modifiable risk factors in pre-school children. We investigated environmental and socioeconomic risk factors for STH infection in the first 3 years of life in a birth cohort from an STH-endemic region of Latin America. Our data provide evidence that maternal STH infections documented during pregnancy are an important risk factor for infection in young children, raising the possibility of a novel intervention for the prevention of STH-associated morbidity in early childhood through the deworming of women of childbearing age, in particular before pregnancy.

**Methods**

**Study population and area**

Details of the design and methodology for the ECUAVIDA birth cohort study, an investigator-driven study, are provided elsewhere [10]. Briefly, 2,404 newborns in the district of Quinindé in Esmeraldas Province, Ecuador, were recruited at the District hospital between November 2005 and December 2009. Inclusion criteria included being a healthy baby, the collection of a maternal stool sample, and planned residence in the District for at least 3 years. The study area is tropical at an elevation of up to 200 m, average annual temperature of 30°C and relative humidity of 75%, and with a population of ~150,000 living in three towns and six rural parishes. The District is poor with limited access to clean water, sanitation, and basic services even within the towns.

**Study design and sample collection**

All children recruited to the ECUAVIDA birth cohort study were eligible for inclusion in this analysis. Children were actively followed from birth to 3 years of age with collection of stool samples at 3, 7, 13, 18, 24, 30 and 36 months. Sample collection took place between November 2005 and December 2012 covering the period of cohort recruitment and the period to 3 years of age of the whole cohort. Stools at 3, 18, and 30 months were collected passively (mothers were asked to provide a sample at the next time point during the previous follow-up), while mothers were actively requested for stool samples at the respective ages for the remaining time points. Household members were also asked to provide one stool sample around the time that the mother was enrolled into the study. Data on risk factors and potential confounders were collected by a questionnaire that was administered to the child’s mother by a trained member of the study team around the time of birth of the child. Categories included in the questionnaire were: maternal and paternal data (age, ethnicity, education, occupation, and number of live children [mother only]), urban versus rural location, socio-economic data (monthly income, number of material goods [household electrical appliances including refrigerator, television, Hi-Fi, and radio], a household electrical connection, household construction materials, sources of drinking water, and type of bathroom [for disposal of faeces]), number of sleeping rooms and number of people living in the household, and data on exposure to household pets, farming and farm animals.

Household overcrowding was defined as number of people living in the household per bedroom. Data on number of anthelmintic treatments during the first 3 years of life was obtained from a questionnaire administered to the mother when the child was 7, 13, 24, and 36 months.

**Stool examinations**

Single stool samples were collected and analysed for STH eggs and larvae by direct saline wet mounts (for detection of all STH including *A. lumbricoides*, *T. trichiura*, *S. stercoralis*, hookworm, and tapeworms), Kato-Katz (for quantification of *A. lumbricoides* and *T. trichiura*) and formol-ether concentration (for detection of eggs/larvae of all STH and tapeworm parasites) methods [11]. *A. lumbricoides* and *T. trichiura* infection intensities were expressed as eggs per gram (epg) of faeces using the results of Kato-Katz. The intensities of hookworm and *S. stercoralis* were not evaluated because of low prevalence and intensities in children of this age. A positive sample was defined by the presence of at least one egg or larva from any of the three detection methods.

**Statistical analysis**

A sample size of 1,697 individuals included in this analysis was estimated to provide over 80% power at P<0.05 to detect exposure effects on risk of any STH prevalence with effect sizes of or less than 0.56 (10% exposure prevalence), 0.68 (20%), 0.72 (30%), and 0.74 (40%). Any STH infection was defined by the presence of at least one STH ovum or larva in any stool sample. Potential risk factors evaluated included parental factors, child factors (gender, gestational age, and birth order), socioeconomic status and factors relating to the environment in which the child was living. A socio-economic status (SES) index was created using principal components analysis for categorical data by combining the socioeconomic variables. The first component that accounted for 30.0% of variation was divided into tertiles to represent low, middle, and high SES. The primary outcome was infection with any STH infection during the first 3 years of life. Secondary outcomes were age of first STH infection and any infection with *A. lumbricoides* or *T. trichiura* during the first 3 years of life. Estimates of effect for any STH infection and any infections with *A. lumbricoides* or *T. trichiura* during the first 3 years of life were calculated using multivariable logistic regression controlling for potential confounders using a backwards-stepwise procedure in which variables considered for inclusion were those with P<0.2 in univariate analyses. The variables, number of samples collected and number of anthelmintic treatments received and gender, were included in all analyses as potential confounding variables, the first because a greater number of samples collected increase the chances of detection of STH. Age of first STH infection was analysed by multinomial logistic regression, a technique that allows a single comparison group (uninfected) for more than one mutually exclusive outcome. This analysis allowed us to evaluate simultaneously, associations between risk factors and first STH infections acquired during the first, second and third years of life, respectively, compared to children without any documented STH infection. For multivariable multinomial logistic regression analysis, we used a backwards-stepwise method for selection of covariates – covariates considered in this analysis were those with P<0.005 in univariate analyses. The population-attributable fraction (PAFs) was calculated by: P_{nw} X (OR-1)/OR where P_{nw} is the prevalence of maternal STH infection among children with any STH infection during the first 3 years. Statistical significance was inferred by P<0.05. Analyses were done using SPSS (Version 16) and STATA version 10 (Statacorp, TX)
Ethical approval and consent

The study protocol was approved by the ethics committee of the Hospital Pedro Vicente Maldonado, Universidad San Francisco de Quito, and Pontificia Universidad Catolica del Ecuador, Ecuador. The study is registered as an observational study (ISRCTN41299086). Informed written consent was obtained from the child's mother and from household members for the collection of stool samples. Children with positive stools for STH infections were treated with a single dose of 400 mg albendazole if aged 2 years or greater and with pyrantel pamoate (11 mg/kg) if aged less than 2 years, according to Ecuadorian Ministry of Public Health recommendations [12,13].

Results

Selection of study participants for analysis

Of 2,404 newborns recruited, 1,697 (70.6%) of children had a stool sample examined at 3 years of age and these were the children included in the present analysis. Follow-up to 3 years of age for stool sampling for the whole cohort is illustrated in Figure 1. There were no significant differences between excluded and included children except for non-maternal STH infections among household members that were more frequent among excluded children (data provided in Table S1).

Epidemiology of STH infections in pre-school children

Of the 1,697 children analysed here, 718 (42.3%) were infected with at least one STH infection during the first 3 years of life. The most frequent STH infection was A. lumbricoides (33.2% of children had at least one documented infection during the first 3 years), followed by T. trichiura (21.2%), Strongyloides stercoralis (1.4%) and hookworm (0.9%). Other enteric parasites observed during the first 3 years of life included Hymenolepis spp. (2.2% of children), Giardia lamblia (44.8%) and Entamoeba histolytica/dispar (26.5%). The prevalence of STH infections by age is shown in Figure 2. STH infections appeared after 3 months of age and prevalence increased with age. The diagnostic methods used were not optimal for the detection of S. stercoralis: the infection was first detected at 13 months and prevalence did not vary substantially between 13 months and 3 years (0.4% at 13 months, 0.1% at 18 months, 0.6% at 24 months, 0.8% at 30 months, and 0.5% at 36 months. Mean age at first infection with an STH parasite among the study children was 23 months (SD 9 months, range 7–36 months). For comparisons with other studies, we stratified the children's infection intensities according to WHO recommendations [14]. The majority of infections were of low intensity (Figure 3). Geometric mean infection intensities among children with A. lumbricoides or T. trichiura infections, respectively, were: 13 months - 931 epg (range 35-182,910) or 244 epg (range 35-6,860); 24 months - 1,859 epg (range 35-176,750) or 248 epg (range 35-21,875); 36 months - 1,973 epg (range 35-283,930) or 243 epg (range 35-47,425). Anthelmintic treatments were widely available and were provided by the study team with a positive stool examination for STH infections and were also obtained directly from pharmacies by the mothers: 75.6% of children were treated.
at least once during the first 3 years of life and the proportions receiving anthelmintic treatments at 0–7, 8–13, 14–24, and 25–36 months were 0.8%, 16.5%, 44.5%, and 47.0%, respectively. No differences were observed in the number of anthelmintic treatments received between infected and uninfected children (Table 1). At enrolment, STH infections were present in 45.7% of mothers, 31.0% of fathers and 53.3% of other household members. The estimated prevalence of *S. stercoralis* among mothers was 4.0%.

**Risk factors associated with any STH infection**

The distributions of covariates between children with any documented STH infection and those without a documented infection are shown in Table 1. Factors that were significantly more frequent among infected children were: being lower in the birth order, having a younger mother or Afro-Ecuadorian mother or illiterate mother, being of lower socioeconomic status, urban residence, living in a more crowded household, having a mother infected with an STH parasite during pregnancy particularly having a mother with moderate to high parasite burdens with *A. lumbricoides* or *T. trichiura*, and having a father or other household member infected with an STH parasite after the child’s birth. The risk of infection was greater among children who provided 5 or more stool samples during the 3 years of observation. The number of anthelmintic treatments received by the child did not affect the risk of having any STH infection. There was some evidence that the prevalence of *S. stercoralis* was higher among mothers of children with STH infections compared to those without (6.0% vs. 2.3%, P<0.001). The results of univariate and multivariable analyses are shown in Table 2. In multivariable analyses, being lower in the birth order, maternal Afro-Ecuadorian ethnicity and younger age, being of low socioeconomic status, urban residence, and household overcrowding were significant independent predictors of STH infection during the first 3 years of life. Intensity of maternal *A. lumbricoides* infection produced the highest odds ratios; children were 11.6 times more likely to have STH if their mothers had moderate to heavy infection intensities with *A. lumbricoides*. This estimate was imprecise with high confidence intervals but is consistent with an analysis in which maternal *A. lumbricoides* infection intensities were stratified as tertiles and the highest tertile was compared with negatives (adj. OR 4.46 (95% CI 2.68–7.41), O<0.001) (data not shown). Having household members with STH infections also increased the child’s chance of having an STH infection as did the number of stool samples collected for each child. In a separate analysis, we estimated the association between any maternal STH infection and any STH infection in children, excluding the other maternal STH variables shown in Table 2, giving an adjusted OR of 1.88 (95% CI 1.46–2.41, P<0.001). This estimate corresponds to a fraction of child STH infections attributable to maternal STH infections (PAF%) of 27.9%. To distinguish the effects of increased risk of child infection associated with STH during pregnancy from that of having a mother with STH infection during the first year of life, we stratified the data according to whether the mother was the child’s primary carer during the first year of life or not: data were available for 1,639 children of whom mothers were the primary carer for 95.1%. The association between maternal STH infection and any child infections was greater among children with primary mother carers.
Risk factors associated with *A. lumbricoides* or *T. trichiura* infections

Most STH infections during the first 3 years of life were caused by *A. lumbricoides*. Risk factors for any *A. lumbricoides* or any *T. trichiura* infection during the first 3 years of life in multivariable analyses were similar to those documented for any STH infection and showed strong associations with maternal STH infections: 1) Any *A. lumbricoides* infections was associated with greater maternal infection intensities with *A. lumbricoides* (Moderate/heavy vs. uninfected, adj. OR 3.88, 95% CI 2.12–7.08, P<0.001) and maternal *T. trichiura* infections (adj. OR 1.38, 95% CI 1.05–1.82, P = 0.021); 2) Any *T. trichiura* infection was associated with having a mother with greater maternal infection intensities with *A. lumbricoides* (Moderate/high vs. uninfected, adj. OR 5.85, 95% CI 3.29–10.40, P<0.001) or infection with *T. trichiura* (adj. OR 1.71, 95% CI 1.36–2.56, P<0.001) during pregnancy. (Complete results are provided in the Table S2).

Risk factors associated for age at first infection with any STH parasite

Multivariable analyses showed that maternal infections with either *A. lumbricoides* or *T. trichiura* were consistently strong predictors of age at first infection with STH parasites across the first 3 years of life (Table 3). Maternal infection with *A. lumbricoides* during pregnancy doubled the odds of a child being infected during the first year of life (adj. OR 2.34, 95% CI 1.61–3.40) - a significant effect was seen also for first infections acquired in the second year of life. Similar effects were observed for age of first infection among children born to mothers with *T. trichiura* infections. Maternal Afro-Ecuadorian ethnicity and low educational level were also associated with an increased risk across the first 3 years of life, although the association was not significant for low maternal educational level in the second year of life. Being lower in the birth order and having a household member with an STH infection were associated only with early infections with STH in children (i.e. first year of life). Household overcrowding was associated with first infections after the 1st year of life.

Discussion

In the present analysis, we investigated the epidemiology of and risk factors for STH infection during the first 3 years of life in a birth cohort from a largely rural District in tropical Ecuador. Over 40% of children had at least one STH infection documented during the first 3 years of life. Almost all infections (96.9%) were caused by *A. lumbricoides* and *T. trichiura*, although few of these pre-school children harboured heavy parasite burdens. Markers of poverty were independent risk factors for any STH infections or infections with individual parasites. STH infection risk in the cohort children was strongly associated with maternal STH infections during pregnancy, particularly children with mothers with moderate to high infection intensities with *A. lumbricoides* who had an approximately 12-fold increased risk of infection.

Potential limitations to the present study include losses to follow-up – we collected a stool sample from 70% of the original cohort at 3 years of age. Such losses could lead to selection bias. However, baseline variables were generally similar between those included and excluded from the analysis indicating that selection bias is
Table 1. Characteristics of 1,697 study participants stratified by the presence or absence of any soil-transmitted helminth (STH) infection during the first 3 years of life.

| Characteristic                          | Uninfected (n = 979) (N, %) | Infected (n = 718) (N, %) | P value |
|----------------------------------------|-----------------------------|--------------------------|---------|
| **Child Factors**                      |                             |                          |         |
| Sex                                     |                             |                          |         |
| Male                                    | 497 (50.8%)                 | 357 (49.7%)              | 0.671   |
| Gestational age (weeks) [Mean/SD]      | 39 (2)                      | 39 (2)                   | 0.791   |
| Birth order                            |                             |                          |         |
| 1–2                                     | 517 (52.8%)                 | 306 (42.6%)              |         |
| 3–4                                     | 311 (31.8%)                 | 235 (32.7%)              |         |
| >5                                      | 151 (15.4%)                 | 177 (24.7%)              | <0.001  |
| **Maternal Factors**                   |                             |                          |         |
| Age [Mean/SD]                          | 26 (6)                      | 25 (6)                   | 0.048   |
| Ethnicity                              |                             |                          |         |
| Afro-Ecuadorian                         | 187 (19.1%)                 | 259 (36.1%)              | <0.001  |
| Other                                   | 792 (80.9%)                 | 459 (63.9%)              |         |
| Educational level                      |                             |                          |         |
| Illiterate                              | 103 (10.5%)                 | 155 (21.6%)              |         |
| Complete primary                       | 562 (57.4%)                 | 448 (62.4%)              |         |
| Complete secondary                     | 314 (32.1%)                 | 115 (16.0%)              | <0.001  |
| **Paternal Factors**                   |                             |                          |         |
| Age [Mean/SD]                          | 30 (8)                      | 30 (9)                   | 0.516   |
| Ethnicity                              |                             |                          |         |
| Afro-Ecuadorian                         | 175 (18.3%)                 | 204 (29.4%)              | <0.001  |
| Other                                   | 779 (81.6%)                 | 491 (70.6%)              |         |
| Educational level                      |                             |                          |         |
| Illiterate                              | 114 (12.4%)                 | 133 (20.9%)              |         |
| Complete primary                       | 486 (52.8%)                 | 354 (55.7%)              |         |
| Complete secondary                     | 321 (34.9%)                 | 149 (23.4%)              | <0.001  |
| **Socioeconomic status**               |                             |                          |         |
| Low                                     | 328 (33.5%)                 | 312 (43.5%)              |         |
| Medium                                  | 303 (30.9%)                 | 230 (32%)                |         |
| High                                    | 348 (35.5%)                 | 176 (24.5%)              | <0.001  |
| **Environmental Factors**              |                             |                          |         |
| Area of residence                       |                             |                          |         |
| Urban                                   | 652 (66.6%)                 | 528 (73.5%)              | 0.002   |
| Household overcrowding                 |                             |                          |         |
| ≥3 people                               | 511 (65.0%)                 | 490 (80.7%)              | <0.001  |
| **Maternal STH Infections**            |                             |                          |         |
| Any STH infection                      |                             |                          |         |
| Yes                                     | 346 (35.5%)                 | 425 (59.5%)              | <0.001  |
| *A. lumbricoides* infection             |                             |                          |         |
| Yes                                     | 184 (18.9%)                 | 273 (38.2%)              | <0.001  |
| *A. lumbricoides* intensity (epg)      |                             |                          |         |
| Negative                                | 790 (81.1%)                 | 441 (61.7%)              |         |
| Light                                   | 174 (17.9%)                 | 200 (28.0%)              |         |
| Moderate                                | 10 (1.0%)                   | 67 (9.4)                 |         |
| Heavy                                   | 0 (0%)                      | 6 (0.8%)                 | <0.001  |
| **T. trichiura infection**             |                             |                          |         |
| Yes                                     | 197 (20.2%)                 | 279 (39.1%)              | <0.001  |
| T. trichiura intensity (epg)           |                             |                          |         |
Table 1. Cont.

| Characteristic                          | Uninfected (n = 979) (N, %) | Infected (n = 718) (N, %) | P value |
|----------------------------------------|-----------------------------|---------------------------|---------|
| Negative                               | 777                         | 438                       | 61.2%   |
| Light                                  | 180                         | 224                       | 31.4%   |
| Moderate                               | 17                          | 47                        | 6.6%    |
| Heavy                                  | 0                           | 6                         | 0.8%    | <0.001 |
| Hookworm                               |                             |                           |         |
| Yes                                    | 41                          | 63                        | 8.8%    | <0.001 |
| Paternal STH infection                 |                             |                           |         |
| Yes                                    | 82                          | 104                       | 40.9%   | <0.001 |
| Other household member with STH        |                             |                           |         |
| Yes                                    | 408                         | 428                       | 59.6%   | <0.001 |
| Number of stool samples from child     |                             |                           |         |
| 1–4                                    | 442                         | 288                       | 40.1%   |         |
| ≥5                                     | 537                         | 430                       | 59.9%   | 0.022  |
| Number of anthelmintic treatments*     |                             |                           |         |
| Received by child                      |                             |                           |         |
| 0                                      | 250                         | 164                       | 22.8%   |         |
| 1                                      | 451                         | 323                       | 45%     |         |
| ≥2                                     | 278                         | 231                       | 32.2%   | 0.191  |

P values were calculated using Chi-squared or Student’s t tests, as appropriate. Ethnicity ‘other’ represents: mothers: 1,245 Mestizo/6 Indigenous; fathers 1264 Mestizo/6 Indigenous. Socioeconomic status represents tertiles of z scores obtained using a factor analysis. Overcrowding is defined as the number of people living in the household per sleeping room. STH infections were detected using direct saline, Kato-Katz and formal-ether concentration methods. SD – standard deviation. Infection intensities were estimated using the Kato-Katz method. STH infection intensity categories were: A. lumbricoides (light- <5,000 eggs per gramme of stool [epg]; moderate 5,000–49,999; heavy – ≥50,000); T. trichiura (light - <1,000 epg; moderate – 1,000–9,999; heavy – ≥10,000).

* Treatments with any of: albendazole, mebendazole, oxantel/pyrantel, piperazine, nitazoxanide, and flubendazole. Numbers of missing values (brackets) were: gestational age (312), maternal ethnicity (6), paternal ethnicity (54), household overcrowding (304), maternal STH infection (9), and paternal STH infection (1100).

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probably not an important issue. Although we attempted to control for potential confounders, we cannot exclude confounding by uncontrolled factors or by highly correlated exposures as an alternative explanation for our findings. Approximately 76% of children were reported by mothers to have received at least one anthelmintic treatment during the first 3 years of life. This proportion did not vary significantly between infected and uninfected children indicating that mothers of children who did not receive anthelmintic treatment for their child for a positive stool examination were extremely likely to obtain anthelmintic drugs through other sources irrespective of a negative stool examination and concurs with our own experience that a lot of illness in children is attributed by mothers to the presence of ‘parasites’ and that self-medication is extremely common. Although 30% of all children had received 2 or more doses of anthelmintic drugs, number of treatments was not associated with risk of STH infections, an observation that might be explained by misclassification of this variable (number of anthelmintic treatments) or by high rates of reinfection over the year following treatment. The use of questionnaires to collect data on exposures is exposure-dependent. Unsurprisingly STH infection prevalence increased with age in the cohort with the highest prevalence of 24.9% observed at 36 months. This is within the 19.6–35.5% estimate by PAHO-WHO of STH prevalence in Ecuadorian preschool children [14]. Age-specific estimates of prevalence and intensity may have been lower than expected in the present study because of the ethical requirement to provide treatment whenever a positive sample was detected. Schoolchildren would be expected to have a higher prevalence of STH infections: a previous study of schoolchildren living in two other rural Districts in Esmeraldas Province estimated a prevalence of 74.9% using the same diagnostic methods and a single stool sample [15]. The use of several diagnostic methods as done in the present study is useful to maximise the sensitivity for STH detection [16]. However, the methods we used have limited sensitivity for the detection of S. stercoralis and could be improved by using more sensitive molecular diagnostics such as PCR.

The strongest and most consistent risk factor for infection with STH in the first 3 years of life was maternal STH infections, particularly among children whose mothers harboured moderate to high parasite burdens with A. lumbricoides during pregnancy. A previous case-control analysis of a single stool sample collected from
1,004 children aged 7 months to 3 years, nested within the same cohort, showed that children of mothers infected with STH parasites during pregnancy had a higher risk of infection compared to children of uninfected mothers [9]. We now have extended these analyses to look prospectively at the acquisition of infection during the first 3 years of life in the whole cohort. Our data suggest that

### Table 2. Univariate and multivariable associations between risk factors and having any STH infection during the first 3 years of life.

| Variable | Univariate | Multivariable |
|----------|------------|---------------|
|          | OR (95% CI) | P value | OR (95% CI) | P value |
| Child Factors | | | | |
| Sex: Male vs. Female | 0.96 (0.79–1.16) | 0.671 | | |
| Gestational age: <39 vs. ≥39 weeks | 0.95 (0.76–1.19) | 0.639 | | |
| Birth Order: ≥5th vs. <5th | 1.79 (1.41–2.29) | <0.001 | 1.85 (1.31–2.60) | 0.001 |
| Maternal Factors | | | | |
| Age: <26 vs. ≥26 years | 1.19 (0.98–1.44) | 0.082 | 1.52 (1.15–2.01) | 0.003 |
| Ethnicity: Afro vs. Other | 2.38 (1.91–2.97) | <0.001 | 2.11 (1.61–2.75) | <0.001 |
| Educational level | | | | |
| Primary vs. Illiterate | 0.53 (0.40–0.70) | <0.001 | | |
| Secondary vs. Illiterate | 0.24 (0.18–0.34) | <0.001 | | |
| Paternal Factors | | | | |
| Age: <30 vs. ≥30 years | 0.88 (0.73–1.07) | 0.197 | | |
| Ethnicity: Afro vs. Other | 1.84 (1.46–2.31) | <0.002 | | |
| Educational level | | | | |
| Primary vs. Illiterate | 0.62 (0.47–0.83) | 0.001 | | |
| Secondary vs. Illiterate | 0.40 (0.29–0.55) | <0.001 | | |
| Socioeconomic status | | | | |
| Medium vs. Low | 0.80 (0.63–1.01) | 0.056 | 0.79 (0.60–1.06) | 0.118 |
| High vs. Low | 0.53 (0.42–0.68) | <0.001 | 0.54 (0.40–0.74) | <0.001 |
| Environmental Factors | | | | |
| Area of residence: Urban vs. Rural | 1.39 (1.13–1.72) | 0.002 | 1.72 (1.31–2.27) | <0.001 |
| Household overcrowding: ≥3 vs. <3 | 2.25 (1.76–2.89) | <0.001 | 1.81 (1.38–2.39) | <0.001 |
| Maternal STH Infections | | | | |
| Any geohelminth: Yes vs. No | 2.67 (2.19–3.26) | <0.001 | | |
| A. lumbricoides: Yes vs. No | 2.66 (2.13–3.31) | <0.001 | | |
| A. lumbricoides intensity (epg) | | | | |
| Light vs. Negative | 2.07 (1.63–2.62) | <0.001 | 1.50 (1.13–1.99) | 0.005 |
| Moderate/heavy vs. Negative | 13.1 (6.7–25.6) | <0.001 | 11.6 (4.83–27.8) | <0.001 |
| T. trichiura: Yes vs. No | 2.53 (2.04–3.14) | <0.001 | | |
| T. trichiura intensity (epg) | | | | |
| Light vs. Negative | 2.21 (1.76–2.77) | <0.001 | | |
| Moderate/heavy vs. Negative | 5.53 (3.16–9.67) | <0.001 | | |
| Hookworm: Yes vs. No | 2.20 (1.47–3.30) | <0.001 | | |
| Paternal STH infection: Yes vs. No | 1.83 (1.35–2.49) | <0.001 | | |
| Household member with STH infection: Yes vs. No | 2.07 (1.70–2.51) | <0.001 | 1.44 (1.13–1.83) | 0.003 |
| Number of stool samples from child | | | | |
| ≥5 vs. 1–4 | 1.23 (1.01–1.49) | 0.039 | 1.28 (1.01–1.62) | 0.041 |
| Number of anthelmintic treatments | | | | |
| 1 vs. 0 | 1.09 (0.86–1.39) | 0.48 | | |
| ≥2 vs. 0 | 1.27 (0.97–1.65) | 0.078 | | |

Multivariable analyses included data from 1,381 children for whom complete data were available. Paternal STH infection was excluded from the multivariate model because of missing data. Odds ratios (ORs), 95% confidence intervals (95% CI) were estimated using logistic regression. STH were detected using all 3 microscopic detection methods. Overcrowding was defined as number of household members per sleeping room. SES (socioeconomic) index shows tertiles of Z scores calculated using principal components analysis. Paternal and maternal age, overcrowding, and gestational age used the mean as cut-off. STH infection intensity categories were: A. lumbricoides (light- <5,000 eggs per gramme of stool [epg]; moderate = 5,000–49,999; heavy – ≥50,000); T. trichiura (light - <1,000 epg; moderate – 1,000–9,999; heavy – ≥10,000).

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Risk Factors for STH Infections in the Tropics

Table 3. Univariate and multivariable polytomous logistic regressions for factors associated with age of first infection with any STH parasite.

| Variable                              | Univariate | Multivariable |
|---------------------------------------|------------|--------------|
|                                       | First year | Second year  | Third year |
|                                       | OR (95% CI)| OR (95% CI) | OR (95% CI) |
|                                       | P value    | P value      | P value    |
| Birth Order                           |            |             |            |
| ≤5 vs. <5                             | 3.0 (2.13–4.20) | <0.001      |            |
| Maternal Ethnicity                    |            |             |            |
| Afro-Ecuadorian vs. Other             | 2.31 (1.66–3.21) | <0.001      |            |
| Maternal Educational Level            |            |             |            |
| ≤Primary vs. Illiterate               | 0.31 (0.214–0.449) | <0.001      |            |
| Paternal Ethnicity                    |            |             |            |
| Afro-Ecuadorian vs. Other             | 1.71 (1.20–2.44) | 0.003       |            |
| Paternal Educational level            |            |             |            |
| ≤Primary vs. Illiterate               | 0.43 (0.29–0.63) | <0.001      |            |
| SES Index                             |            |             |            |
| Medium/High vs. Low                   | -0.54 (0.40–0.74) | >0.001      |            |
| Area of residence                     |            |             |            |
| Urban vs. Rural                       | 1.16 (0.83–1.62) | 0.385       | 1.43 (1.02–2.02) |
| Household overcrowding                |            |             |            |
| ≤3 vs. <3                             | 2.02 (1.46–2.80) | <0.001      |            |
| Maternal STH Infection                |            |             |            |
| Yes vs. No                            | 4.58 (3.26–6.43) | 0.001       | 1.93 (1.43–2.61) |
| A. lumbricoides                       |            |             |            |
| Yes vs. No                            | 4.10 (2.97–5.66) | <0.001      | 1.90 (1.36–2.66) |
| T. trichiura                          |            |             |            |
| Yes vs. No                            | 3.73 (2.71–5.14) | <0.001      | 1.98 (1.43–2.74) |
| Hookworm                              |            |             |            |
| Yes vs. No                            | 2.67 (1.55–4.62) | <0.001      | 2.02 (1.13–3.61) |
| Householder with STH infection         |            |             |            |
| Yes vs. No                            | 2.71 (1.97–3.74) | <0.001      | 1.76 (1.30–2.38) |

Multivariable analyses included data from 1,381 children for whom we had complete data. Associations between risk factors and age at first infection were compared to children without any infection in the first 3 years of life using univariate and multivariable multinomial logistic regression. STH were detected using all 3 microscopic detection methods. Variables with more than 2 groups in Tables 1 and 2 were redefined as binary. Overcrowding was defined as number of household members per sleeping room using the mean as cut-off. SES (socioeconomic) index shows tertiles of Z scores calculated using principal components analysis. The analysis controlled also for gender of the child, number of stool samples collected and number of anthelmintic treatments received. Maternal infection intensities with A. lumbricoides and T. trichiura were not included. Paternal STH infection was excluded from the multivariate model because of missing data. doi:10.1371/journal.pntd.0002718.t003

maternal STH infections, particularly moderate to heavy parasite burdens with *A. lumbricoides*, are an important independent determinant of risk of STH infections during early childhood.

The association between STH infections in mothers and infections of children has two possible explanations. 1) STH infections of the mother during pregnancy may increase susceptibility to infection in offspring through tolerization to parasite antigens - *A. lumbricoides* antigens can be detected in the circulation of infected individuals [17] that may cross the placenta and engage with the foetus’s developing immune system. There is evidence from animal models of helminth infection [18] and studies in humans [19,20,21] that maternal infections may induce foetal tolerance to parasites and increase susceptibility to helminth infection in offspring. We have shown previously in the same study population that maternal STH infections induce immunologic sensitization to *A. lumbricoides* antigens in utero [22] and that the cord blood of newborns born to infected mothers has elevated levels of the immune regulatory cytokine IL-10 compared to those born of uninfected mothers [9]. Such tolerization could have a genetic basis, and there is ample evidence that susceptibility to STH infections is associated with immune genes [23,24]. A recent study of children in urban Brazil provided evidence that IL-10 polymorphisms were associated with susceptibility to STH infections [25]. 2) The higher risk of infection in children of infected mothers may reflect a shared environment particularly during the first year of life when the child is completely dependent on the mother. This explanation is supported partly by the observation that among children whose primary carer during the first year of life was the mother, the association between maternal STH infection in pregnancy and any STH infection in offspring was stronger than for children whose primary carer was not the mother, although small numbers in the latter group yielded a very imprecise estimate.

Maternal STH infections were a common risk factor - approximately 46% of mothers were infected with STH in their third trimester of pregnancy, with an estimated attributable fraction of 27.9%. Our observations, therefore, have identified a potentially modifiable exposure - maternal STH infections - that could be evaluated in an intervention programme using currently available and highly efficacious anthelmintic treatments. An intervention in which anthelmintic drugs are given before
pregnancy for women planning to have a family, during pregnancy or soon after birth or even periodically to women of child-bearing age could substantially reduce the risk of infection and potential morbidity during early childhood. Development of immune tolerance begins after 14 weeks gestation [26] so it might be beneficial to deworm women before pregnancy or if pregnant, in the second trimester before the foetal immune system is capable of developing tolerance to parasite antigens. Clearly, deworming of mothers before pregnancy would be preferable because giving anthelmintic drugs to pregnant or lactating women carries a risk of potential adverse effects on the foetus or neonate, respectively. Albendazole has been indicated by WHO for use in pregnant women after the first trimester in areas that are highly endemic for hookworm because of the risks to mother and child of maternal hookworm anaemia [27]. We now suggest another potential benefit of deworming mothers with other STH infections – to reduce the risk of STH infection and associated morbidity in early childhood. There are still limited data on the safety of anthelmintic drugs in pregnant and lactating women although benzimidazole drugs are believed to be safe when used after the first trimester or during breast-feeding [27]. Such a strategy (to reduce the risk of STH infection and associated morbidity in early childhood) will only be useful in areas where *A. lumbricoides* and *T. trichiura* are endemic because hookworm is less a problem in pre-school children among whom prevalence is generally low even in highly endemic areas [28]. The use of anthelmintic treatment in mothers, therefore, to prevent child infections will require careful consideration of the balance of potential adverse effects in the mother and child versus the clinical consequences of infections in the pre-school child for which there is growing evidence of important nutritional effects [29]. Such decisions will almost certainly have to be made locally depending on the epidemiology of STH parasites in any specific area or region. An alternative strategy would be the periodic treatment of women of childbearing age.

Figure 4. Model of the potential effects of risk factors on the risk of STH infections. The model shows potential effects of environmental and socioeconomic risk factors on risk of STH infections in early childhood and morbidity. Potential interventions to reduce risk of infection are illustrated with red crosses.

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with immediate benefits and without major municipal investment in local infrastructure and services. The targeting of anthelminthic treatment to pregnant women attending antenatal clinics, and mothers and their young children attending public vaccination clinics that serve the most vulnerable sections of the population in STH endemic areas, provides a rapid and easily implemented strategy for the control of STH infections in pre-school children.

In conclusion, our study identified risk factors for STH infection during the first 3 years of life in a birth cohort conducted in a rural District in coastal Ecuador. Over 40% of children were infected at least once with STH parasites during the first 3 years of life and risk factors for infections were those associated with poverty. We identified maternal STH infections as an important and potentially modifiable risk factor that could be evaluated in future intervention studies for the control of STH infections in pre-school children.

Supporting Information

Checklist S1  STROBE Checklist. (DOC)

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