INTERRELATIONSHIPS BETWEEN SOIL-TRANSMITTED HELMINTH INFECTIONS, HEMOGLOBIN LEVELS AND CHILD DEVELOPMENT: A LONGITUDINAL COHORT STUDY

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Abstract

Background: Soil-transmitted helminth (STH) infections are intestinal parasites that cause important disease burden primarily through intestinal bleeding, anemia, and competition for micronutrients. It has been proposed that STH infections affect child development, potentially due to mediation by iron levels. Limited research has been conducted on this topic in preschool children and the need to understand if STH infections affect child development has recently been emphasized. The overall objective of the current thesis, therefore, was to determine if, and how, STH infections affect child development in preschool children.

Methods: A longitudinal cohort study was conducted in Iquitos, Peru between September 2011 and July 2016. Children, originally recruited at one year of age into a randomized controlled trial, were followed-up annually to five years of age. STH infection was measured at all study visits and child development was assessed annually in 880 children. Anthropometry was measured at all study visits and hemoglobin levels were measured at the three to five years of age time points. Linear regression models were used to determine the effect of the number of detected STH infections between one and five years of age on IQ scores at five years of age. Hierarchical models were used to determine the effect of STH infections between one and two years of age on repeated measures of verbal and cognitive abilities at two to five years of age. Natural direct and indirect effects were estimated to determine if the association between *Ascaris* infection and child development was mediated by mean hemoglobin levels and malnutrition. Bayesian latent class analysis was used throughout to adjust for STH infection misclassification.

Results: A total of 781 (88.8%) children were included in the analyses. In multivariable analysis, adjusted for STH infection misclassification, increasing numbers of *Ascaris*, *Trichuris*, hookworm and any STH infections between one and five years of age were associated with lower IQ scores at five years of age (between group differences in verbal IQ scores (95% Credible Interval (CrI)) for being infected with *Ascaris* infection two, three and four or five times compared to zero or one infection, were: -8.27 (-13.85, -3.10), -6.69 (-12.05, -2.05), and, -5.06 (-10.75, 0.05), respectively). Furthermore, children found infected with *Ascaris* infection and any STH infection between one and two years of age had lower cognitive and verbal scores between two and five years of age (between group cognitive score differences (95% CrI) for

being infected with any STH once, and infected two or three times, compared to never infected, were: -4.31 (-10.64, -0.14) and -3.70 (-10.11, -0.11), respectively). The mediation analysis results suggest that the association between *Ascaris* infection between one and five years of age and total IQ score at five years of age is importantly mediated by hemoglobin levels (natural direct effects (95% CrI) and natural indirect effects (95% CrI), compared to 0 or 1 infection, were: -0.91 (-4.63, 2.82) and -4.25 (-6.92, -1.59) for the effect of 2 infections, respectively; -1.41 (-3.79, 0.98) and -1.17 (-1.95, -0.43) for the effect of 3 infections, respectively; and, -0.39 (-3.21, 2.41) and -2.65 (-4.32, -0.99) for the effect of 4 or 5 infections, respectively).

Conclusion: These results document the adverse impact of STH infection at a young age on child development. The results also suggest that this effect is importantly mediated by hemoglobin levels. Adjusting for STH infection misclassification was essential in obtaining an accurate estimation of the magnitude of this impact. Future research should take this adjustment into consideration. STH control in combination with iron-enhancing nutritional interventions targeted to preschool children as of one year of age may contribute to lowering the disease burden associated with poor child development in STH-endemic countries.

Résumé

Mise en contexte: Les géohelminthiases sont des parasites intestinaux qui posent une charge de morbidité importante, principalement par le biais de saignements intestinaux, de l'anémie, et de la malabsorption des nutriments. Il a été proposé que les géohelminthiases affectent le développement de l'enfant, par un mécanisme susceptible d'être lié aux taux de fer. Des recherches limitées ont été entreprises sur ce sujet chez les enfants d'âge préscolaire et la nécessité de développer une meilleure compréhension du lien entre les géohelminthiases et le développement de l'enfant a récemment été soulignée. L'objectif général de la présente thèse était donc de déterminer si, et comment, les géohelminthiases affectent le développement de l'enfant chez les enfants d'âge préscolaire.

Méthodes: Une étude longitudinale de cohorte a été réalisée à Iquitos, au Pérou, entre septembre 2011 et juillet 2016. Les enfants, recrutés à l'origine pour un essai contrôlé randomisé à l'âge d'un an, ont été suivis sur une base annuelle jusqu'à l'âge de cinq ans. Les géohelminthiases ont été mesurées à chacune des visites et le développement de l'enfant a été évalué annuellement chez 880 enfants. L'anthropométrie a été mesurée lors de toutes les visites et les taux d'hémoglobine ont été mesurés aux points temporels de trois et de cinq ans. Des modèles de régression linéaire ont été employés pour quantifier l'effet du nombre d'helminthiases détecté entre les âges de un et cinq ans sur les scores de QI obtenus à l'âge de cinq ans. Des effets naturels directs et indirects ont été estimés afin de déterminer si l'association entre l'infection à *Ascaris* et le développement de l'enfant avait été influencée par les taux moyens d'hémoglobine et la malnutrition. Une analyse de structure latente bayésienne a été effectuée tout au long de l'étude afin de corriger pour les erreurs de classification des géohelminthiases.

Résultats: Au total, 781 (88.8%) enfants ont été inclus dans les analyses. Une analyse multi-variables, corrigée en fonction des erreurs de classification des géohelminthiases, a démontré une association entre un nombre croissant d'infections à *Ascaris, Trichuris,* ankylostomes et toute géohelminthiase entre les âges de un et cinq ans et des scores de QI inférieurs à l'âge de cinq ans (les différences entre groupes des scores QI verbaux (Interval crédible (ICr) à 95%) pour deux, trois, et quatre ou cinq infections à *Ascaris*, par rapport à zéro ou une seule infection, étaient: -8.27 (-13.85, -3.10), -6.69 (-12.05, -2.05), et -5.06 (-10.75, 0.05), respectivement). De plus, les

enfants reconnus comme ayant été atteints d'infections à *Ascaris* et toute géohelminthiase entre les âges d'un et de deux ans ont obtenus des scores cognitifs et verbaux inférieurs entre les âges de deux et de cinq ans (les différences entre groupes des scores cognitifs (ICr à 95%) pour une et deux ou trois infections par toute géohelminthe, par rapport à aucune infection, étaient: -4.31 (-10.64, -0.14) et -3.70 (-10.11, -0.11), respectivement). Les résultats de l'analyse de médiation suggèrent que l'association entre l'infection à *Ascaris* entre les âges d'un et cinq ans et le score QI global obtenu à l'âge cinq ans est largement influencée par les taux d'hémoglobine (les effets naturels directs (ICr à 95%) et effets naturels indirects (ICR à 95%), par rapport à 0 ou 1 infection, étaient: -0.91 (-4.63, 2.82) et -4.25 (-6.92, -1.59) pour l'effet de deux infections, respectivement; -1.41 (-3.79, 0.98) et -1.17 (-1.95, -0.43) pour l'effet de trois infections, respectivement; et -0.39 (-3.21, 2.41) et -2.65 (-4.32, -0.99) pour l'effet de 4 ou 5 infections, respectivement.

Conclusion: Ces résultats documentent l'impact négatif des géohelminthiases à un âge précoce sur le développement de l'enfant. Ces résultats suggèrent également que cet impact est largement influencé par les taux d'hémoglobine. L'application d'une correction pour les erreurs de classification des géohelminthiases s'est avérée essentielle afin d'obtenir une estimation précise de l'ampleur de cet impact. Les recherches futures devraient tenir compte de cette correction. Le contrôle des géohelminthiases couplé à des interventions nutritionnelles axées sur l'augmentation des taux de fer et ciblant les enfants d'âge préscolaire dès l'âge d'un an pourraient ainsi contribuer à réduire la charge de morbidité associée au sous-développement de l'enfant dans les pays où les géohelminthiases sont endémiques.

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Dedication

Esta tesis está dedicada a Mariano, uno de mis motivadores principales y mi favorito niño preescolar peruano, quien, debido a esta tesis, aprendió a ejecutar WinBUGS antes que aprendió a leer.

Preface

Contribution of co-authors

Brittany Blouin MSc (BB) (first author on all manuscripts) was involved in all aspects of this cohort study. This included project management, human resources administration and budget oversight for the parent trial (children recruited at one year of age and followed-up to two years of age), as research coordinator. As a PhD candidate, she was responsible for all aspects of the follow-up study (children followed up between three and five years of age). She developed the thesis research questions and protocol and obtained ethics approval for the thesis protocol. She was the on-site Project Director in Iquitos, Peru from January 2014 to July 2016 (during the four and five years of age study visits). During this time, she was responsible for developing the study instruments, training research assistants, and overseeing all aspects of the data collection including field supervision and daily review of all study data. She was responsible for overseeing data entry and data cleaning. She performed all data analyses, prepared the first drafts of all three manuscripts and revised and finalized all manuscripts.

Theresa W. Gyorkos PhD (TWG) (last author on all manuscripts) was the Principal Investigator of the parent trial and a co-investigator of the extended follow-up study. She oversaw all academic, scientific, ethical and logistical aspects of both studies. She provided input on the thesis research questions, objectives and methodology. She provided input during the data analyses and interpretation of the results and she contributed to finalizing all manuscripts.

Martin Casapia MD, MSc (MC) (co-author on all manuscripts) was a co-investigator of the parent trial and Principal Investigator of the extended follow-up study. He is the Director of the research-oriented Peruvian non-governmental organization, Asociación Civil Selva Amazónica, where the data collection was based. He provided local expertise regarding ethics approvals and data collection. He reviewed all manuscripts and provided important input regarding the interpretation of the results.

Lawrence Joseph PhD (LJ) (co-author on all manuscripts) provided biostatistical expertise. He provided input into the thesis research questions and protocol. He oversaw all statistical aspects of the thesis including sample size calculations, analysis plans, data analyses and interpretation

of the results. He reviewed all manuscripts and provided important input regarding the interpretation of the results.

Jay Kaufman PhD (JK) (co-author on Manuscripts A and C) provided epidemiological expertise. He provided input in the development of the thesis research questions and protocol. He provided statistical input for the analysis plan of Manuscript C. He reviewed Manuscripts A and C and provided important input regarding the interpretation of the results.

Charles Larson MD, CM, FRCP(C) (co-author on Manuscripts A and C) provided epidemiological, pediatric and global health expertise. He provided input in the development of the thesis research questions and protocol. He reviewed Manuscripts A and C and provided important input regarding the interpretation of the results.

Originality Statement

The objectives of the current thesis were developed following three years of experience working in the area of soil-transmitted helminth (STH) research. While it has been claimed that STH infections have an important effect on child development, the research evidence to support this claim is sub-optimal. The majority of the published research conducted on STH infections and child development are cross-sectional studies and/or lack appropriate statistical analyses and adjustment for confounding. Because no gold standard diagnostic test exists for STH infection, all research conducted on this topic is limited by misclassification bias; however, no previous research on this topic has adjusted for this bias. Furthermore, the majority of the research has been conducted in school-age children. Because preschool children are in the most critical period of brain development across the lifespan, unfavorable conditions during this time period can have particularly devastating long-term effects. Preschool children are therefore, the most relevant population group for this research question. The need for research to clearly estimate the effect that STH infections may have on child development and the mechanisms driving these effects has recently been emphasized by both the STH and child development communities.

The current thesis addresses this need and contributes to the body of evidence investigating the association between STH infections and child development in preschool children. The data source is unique because it includes a large cohort of children, recruited at one year of age, who were followed-up yearly to five years of age (i.e. four years of follow-up). The longitudinal research design allowed for STH infection over time to be investigated (instead of simply investigating the effect of infection at one time point). Furthermore, it allowed for the effects of STH infections at different times throughout the preschool years to be investigated to determine if a critical time period exists when infection may have a particularly adverse effect.

The modern mediation analysis contributes to clarifying the underlying mechanisms between STH infection, hemoglobin levels, malnutrition, and child development. This is the first time that this type of rigorous mediation analysis has been done in the context of STH infection and child development and it contributes to understanding the effects and disease burden of these infections. Additionally, all analyses are adjusted for STH misclassification using Bayesian latent class analysis. This is the first time that the effect of STH infection on child development

is estimated with adjustment for this bias, leading to a more complete understanding of the complex host-parasite interactions. Finally, this research makes an important methodological contribution by performing a modern mediation analysis (investigating multiple mediators simultaneously) under the Bayesian framework allowing for adjustment of exposure misclassification.

List of Acronyms

Bayley	The Bayley Scales of Infant and Toddler Development
Bayley-III	The Bayley Scales of Infant and Toddler Development, Third edition
BCG	Bacillus Calmette-Guérin
BIC	Bayesian information criterion
BMI	body mass index
CI	confidence interval
CrI	credible interval
DAG	directed acyclic graph
DALY	disability-adjusted life year
DDST	Denver Developmental Screening Test
epg	eggs per gram
FEC	formol-ether concentration
ICC	intraclass correlation coefficient
IQ	intelligence quotient
LMIC	low and middle-income country
MCMC	Markov Chain Monte Carlo
MD	Maryland
MDG	millennium development goals
NDE	natural direct effect
NIE	natural indirect effect
NTD	neglected tropical disease
qPCR	quantitative polymerase chain reaction
REF	reference
RCT	randomized controlled trial
SD	standard deviation
SDG	sustainable development goals
SES	socioeconomic status
STH	soil-transmitted helminth
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TDS	*Trichuris* Dysentery Syndrome

TE	total effect
Th2	T-helper-2
UN	United Nations
UNICEF	United Nation's Children Fund
USA	United States of America
WHO	World Health Organization
WPPSI	Wechsler Preschool and Primary Scale of Intelligence
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence, Third edition
WRAML	Wide Range Assessment of Memory and Learning
WRAT	Wide Range Achievement Test

List of Abbreviations

Asc	*Ascaris*
C	vector of covariates
C_{DS}	specificity of the direct smear technique
C_{KK}	specificity of the Kato-Katz technique
g/dL	grams per decilitre
g/L	grams per litre
i	study time point
kg	kilograms
KK_i	indicator variable indicating technique used to analyze stool specimen at time point i (KK_i= 1 represents use of the Kato-Katz technique; KK_i= 0 represents use of the direct smear technique)
M_{haz}	mean height-for-age z-score between one and five years of age
M_{hb}	mean hemoglobin levels between three and five years of age
mL	millilitre
$mSTH_i$	measured STH infection status at time point i
N	number
Sept.	September
S_{DS}	sensitivity of the direct smear technique
S_{KK}	sensitivity of the Kato-Katz technique
$tSTH_i$	latent, true STH infection status at time point i
vs	versus

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1. Introduction

It has recently been estimated that over 200 million (approximately 43%) children under five years of age, living in developing countries, are at risk of not fulfilling their developmental potential [1]. Ensuring healthy child development (which encompasses sensory-motor, cognitive-language and social-emotional skills) is a critical step to achieving many of the recently adopted Sustainable Development Goals (SDGs), especially SDG target 4.2, to ensure universal access to high-quality early childhood development, care, and pre-primary education [2]. It has been proposed that investing in early childhood development around the world will ensure the future health, well-being, economic productivity, prosperity, peace and security of all individuals and nations [3].

The soil-transmitted helminths (STH) are intestinal parasites that infect humans. Together, they constitute one disease of the 20 tropical diseases identified by the World Health Organization (WHO) as the "Neglected Tropical Diseases" (NTDs), a group of infections that have historically been neglected despite causing considerable disease burden [4]. STH-attributable morbidity results primarily from nutritional impairment caused by intestinal bleeding, anemia, nutrient malabsorption, protein malnutrition, competition for micronutrients, loss of appetite, reduction of food intake and diarrhea [5, 6]. These parasites infect their host through fecal-oral transmission or through skin penetration, and are therefore linked with poor hygiene and sanitation and are most commonly found in populations living in poverty [7]. STH infections are among the most common and persistent parasite infections worldwide with 103 countries considered endemic and between one and two billion people at risk of morbidity [8].

It has been proposed that STH infection can impair child development [9-11]. This is primarily theorized to be due to mediating factors like malnutrition and, more specifically, to inadequate iron levels [12]. While some previous research has linked STH infection to impaired child development [13-25], the interpretation of this evidence base is limited primarily because of poor research designs and inadequate statistical analyses. In addition, the majority of the research conducted to date used school-age children as the study population. Two previously published studies, conducted in school-age children, performed simple mediation analyses investigating iron levels and malnutrition as potential mediators of the relationship between STH infection and

child development [17, 22]. Both studies found that iron levels mediated the relationship between *Trichuris* infection and child development. One study also found that malnutrition was a mediator of the relationship between *Trichuris* infection and child development and that hemoglobin levels mediated the relationship between *Ascaris* infection and child development [22]. No mediation analysis of the relationship between STH infection and child development has yet been performed in preschool children. However, because the preschool years are considered to be the most critical period for cognitive development across the lifespan, preschool children are likely the more relevant study population for this research question.

A recent systematic review summarized the available published evidence on STH infections and child development and concluded that there was a need for improved methodological approaches to assess the effect of STH infection on cognitive function, especially in preschool children [26]. Furthermore, the need for research to clarify the effects that STH infections may have on child development, and the mechanisms driving these effects, has recently been emphasized by both the STH and child development communities [9, 27-31]. The present research investigates the long-term effect of cumulative or recurring STH infections over time on child development in preschool children who are in the most critical period of development. Furthermore, a modern mediation analysis is used to clarify the underlying mechanisms between STH infection, iron levels, malnutrition, and child development. Finally, the present research takes into account STH misclassification, an important bias. Because no gold standard diagnostic technique exists for diagnosing STH infection, conclusions from previous research which failed to take this misclassification bias into account may be questionable. No previous research has yet taken this important bias into consideration.

The current thesis, therefore, addresses important gaps in knowledge and contributes to the body of evidence regarding the relationship between STH infections and child development in preschool children.

2. Literature Review

2.1. Child development

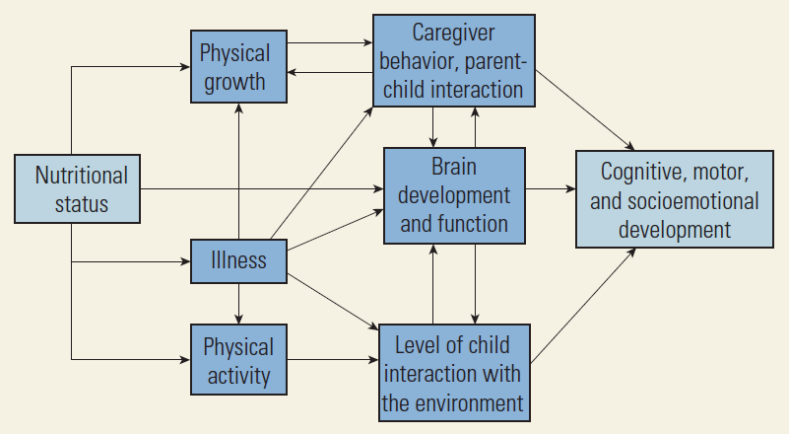
Child development refers to the emergence and development of sensory-motor, cognitive-language and social-emotional skills from birth through to adolescence [32]. It is an on-going developmental process encompassing physical, mental and social development underpinning the ability to think, learn, remember and articulate ideas [28]. The foundations of brain architecture are laid down in the early years of life through dynamic interactions between genetic, biological and psychosocial factors [29]. Therefore, poor development during childhood has been linked to poor academic performance in school, lower incomes later in life and general loss of human potential [33]. Individuals and societies are not only healthier but more productive if they have mature mental skills [28]. Therefore, healthy brain development is critical to allow children to grow and thrive into healthy adults who can make productive contributions to society [34].

The early childhood period is considered to be the most important development period across the lifespan [34]. During the first few years of life the brain develops rapidly and small perturbations in this process can have long-term effects on its structural and functional capacity [33]. Because brain plasticity is greatest during the very early years, protective interventions targeting this time period are expected to have the greatest impact [35]. Conversely, this suggests that unfavorable conditions during this time period can have particularly devastating long-term effects. During the early childhood period, if brain synapses are not used (i.e. due to lack of stimulation or due to a lack of available energy for brain activity) they are gradually deleted which can result in severe impairment [28]. These impairments can not only be life-long but are increasingly irreversible as time goes on. The early childhood period, therefore, is the most cost-effective period in a child's life for disease prevention investment because events during this time period can affect learning and productivity into adulthood. Investing in early child development has the potential to increase the effectiveness of educational expenditures and reduce school drop-out rates. Furthermore, improved development and education for girls has been shown to have long-term effects on their own children's survival, growth and development such that the positive effects are passed down to future generations [32].

The two most recognized risk factors for impaired development during the first five years of life include lack of psychosocial stimulation and malnutrition [9, 28]. Psychosocial stimulation is thought to affect child development in many ways. Objects and events in the environment elicit physiological and psychological responses and they provide opportunities to play and converse in ways that stretch thought processes and understanding of speech [28]. Conversation between an adult and a child, for example, stimulates the child's speech perception sites in the brain which maintain the neural connections throughout the brain's language sites [28]. Challenging play materials that children can manipulate and combine are also necessary to help them learn about mass, weight and problem-solving [28]. A lack of appropriate stimulation can result in dysregulation of the hypothalamic-pituitary-adrenocortical system [36] and can change the electrical activity of the brain related to efficiency of cognitive processing [37]. Age-appropriate stimulation, including conversation and play, is therefore an essential component to ensure healthy brain development.

Adequate nutrition during the early years is also crucial to ensure healthy brain development. The framework outlining the link between nutrition and child development is shown in Figure 1.

Figure 1: Framework illustrating pathways from nutritional status to child development [38]



There are several pathways by which child development can be affected by nutritional status. The direct pathway involves nutrients that support the structure and activity of the brain sites responsible for child development [28]. The other indirect pathways involve an enhanced engagement with the environment associated with good nutrition, which, in turn, promotes

healthy child development [28]. Short child length/height is strongly correlated with child development and this is likely because it is a strong indicator of chronic malnutrition [28]. While healthy child development requires a sufficient intake and absorption of all nutrients, iron and iodine have been found to be particularly important and a congruent body of evidence exists documenting their effects on brain development during the early years of life [9]. It has repeatedly been found that anemic children have lower levels of child development compared to non-anemic children [39]. Iron deficiency can cause brain abnormalities because iron is essential for proper neurogenesis and for the differentiation of certain brain cells and regions [40-42]. Based on animal models, it has been proposed that iron deficiency impairs myelination, dendritogenesis, synaptogensis and neurotransmission, as these processes are highly dependent on iron-containing enzymes and hemoproteins [43, 44]. It has also been shown that anemic children are at a higher risk for weariness and lethargy which reduces their exposure to psychosocial stimulation [28]. It has been estimated that, in children, a 10 g/L decrease in hemoglobin concentration lowers intelligence quotient (IQ) by 1.73 points [45]. Iodine is a constituent of thyroid hormones which affect the central nervous system development and regulate many physiological processes [46]. Iodine deficiency can lead to congenital hypothyroidism and is the most common preventable cause of mental retardation [46-48]. Previous research has shown that iodine deficiency can reduce IQ scores by approximately 12.5-13.5 points, on average [49, 50].

The other risk factors that have been found to affect child development include infectious diseases (e.g. malaria and HIV), maternal mental health (e.g. maternal depression), violence (e.g. exposure to armed conflict), low-birth weight with intrauterine growth restriction and environmental exposure to metals (e.g. lead and arsenic) [9, 28, 29, 51]. It has also been found that breastfeeding and maternal education act as protective factors for child development [29]. The effect of breastfeeding is likely due to improved nutrition while the effect of maternal education is likely due to improved nutrition, less maternal depression, improved quality of the child-rearing environment and improved ability to access and benefit from interventions [29, 52]. When evaluating a child's overall risk of impaired development it is important to consider that many risk factors are likely to co-occur and that the effects of cumulative risks are complex [29]. This also highlights the importance of an integrated approach to childhood health intervention

packages, and their delivery, so that co-occurring risk factors can be addressed simultaneously [29].

The most common and the most researched interventions to promote child development focus on preventing or minimizing the effects of inadequate stimulation, stunting, iodine deficiency and iron-deficiency anemia [32]. Different types of early child development programs have been designed and implemented in different parts of the world with the overall goal of promoting child development, preventing the risks of impaired development and mitigating the negative effects of risks [32]. While specific programs vary greatly in different countries and few systematic evaluations of early child development programs exist in developing countries, they all include some combination of specific evidence-based interventions, including: improving the diets of pregnant women, infants and toddlers [9, 53, 54], universal salt iodization [55], preventive iron supplementation [9], parenting programs [56] and early stimulation [57, 58]. Recently, an emphasis has been placed on the importance of nurturing care during the early years of child development. Nurturing care is a multi-dimensional integrative concept that is characterized as a stable environment that is sensitive to children's health and nutrition needs, with protection from threats, opportunities for early learning, and interactions that are responsive, emotionally supportive, and developmentally stimulating [56]. Nurturing care has been found to be positively associated with children's health, growth and development and is an essential foundation for human capital development [59-61]. Currently, no international set of standard indicators exists to monitor child development programs. Development of these types of indicators is needed to improve countries' abilities to set targets, allocate resources, monitor progress, ensure accountability, and contribute to an overall improvement in child development around the world [32].

Measuring child development cannot be done with a single tool and requires measuring the specific constructs that it represents. These constructs include cognition (the ability to solve small problems), receptive language (the ability to understand the meaning of words, sentences and abstract categories), expressive language (the ability to use sounds, gestures and spoken word to communicate), fine motor (hand-eye coordination), gross motor (i.e. sitting and walking), and social-emotional skills (emotional security, secure attachment, personal-social

skills and the purposeful and social expression of emotions) [28]. Many developmental measures have been designed to measure these constructs, some of which are based on parental assessment of the child's abilities and others directly test the child [62]. Standardized behavioural tests are the most common measures of child development after the newborn period. Examples of standardized tests that have been developed to measure specific constructs of child development include: Ages and Stages Questionnaire [63], Denver Developmental Screening Test (DDST) [64], WHO Motor Milestones [65], the Bayley Scales of Infant and Toddler Development (Bayley) [66], Movement Assessment Battery for Children [67], Griffiths Developmental Scales [68], Wechsler Preschool and Primary Scale of Intelligence (WPPSI) [69, 70], Means-end one, two, and three Step Problem-solving Test [71-73], Kaufman Assessment Battery for Children [74], MacArthur-Bates Communicative Development Inventories [75, 76] and Wolke's Behavior Rating [77]. Each of these scales has been designed to measure some, or all, of the child development constructs at specific ages. Most provide raw scores on a continuous scale that can be converted into scaled or composite scores and compared to a reference norm [62].

It has recently been estimated that over 200 million children (approximately 43%) under five years of age, living in developing countries, are at risk of not fulfilling their developmental potential [1]. Children living in low and middle-income countries (LMICs) have an increased risk of impaired development compared to children living in high income countries [34] due to poverty, poor health and nutrition, and inadequate care [33]. Poverty increases children's exposure to both the biological and psychosocial risks of impaired development, affecting brain structure and function and behavior [9]. Poverty is associated with increased risk of inadequate food, poor sanitation and hygiene, infections, stunting, poor maternal education, higher maternal stress and depression, and inadequate stimulation in the home [33, 78-81]. All of these factors have a negative impact on child development [78, 81] and lead to fewer years of schooling and less learning per year in school [33]. It has been estimated that, on average, each year of schooling increases wages by approximately 9% [82, 83]. For children at risk of poor development, it has been estimated that, as adults, their average percent loss of income per year will be approximately 26%, trapping families in poverty [3]. Without appropriate interventions

to address the poverty-development cycle, therefore, the cycle of poverty becomes intergenerational [32].

The United Nations (UN) Millennium Development Goals (MDGs) were implemented in 2000 with a global commitment to achieve a set of eight goals aimed at improving the lives of the poorest populations. Although the MDGs sparked unprecedented advances in global health and development, the 2015 deadline passed with many targets unmet [84]. Building on the successes of the MDGs and, in an attempt to address the important gaps and challenges that still exist globally, the UN officially launched the 17 Sustainable Development Goals (SDGs) on January 1, 2016 [2]. These new goals aim to integrate economic, social and environmental development over the next 15 years (target 2030). Ensuring healthy development for all children around the world is a crucial step to achieving many of the SDGs, especially SDG 1 (to end poverty in all its forms everywhere), SDG 2 (to end hunger and improve nutrition), SDG 3 (to ensure healthy lives and promote well-being for all at all ages), SDG 4 (to ensure inclusive and equitable quality education and promote lifelong learning opportunities for all), SDG 5 (to achieve gender equality), SDG 10 (to reduce inequality in and among countries), SDG 16 (to promote peaceful societies), and SDG 17 (to strengthen the means of implementation) [2, 3]. Child development is most directly mentioned in SDG target 4.2 (to ensure universal access to high-quality early childhood development, care, and pre-primary education) [2]. The SDGs represent a globally united platform under which investments must now be made in early childhood development to ensure the future health, wellbeing, economic productivity, prosperity, peace and security of all individuals and nations [3].

2.2. Soil-transmitted helminth infections and deworming

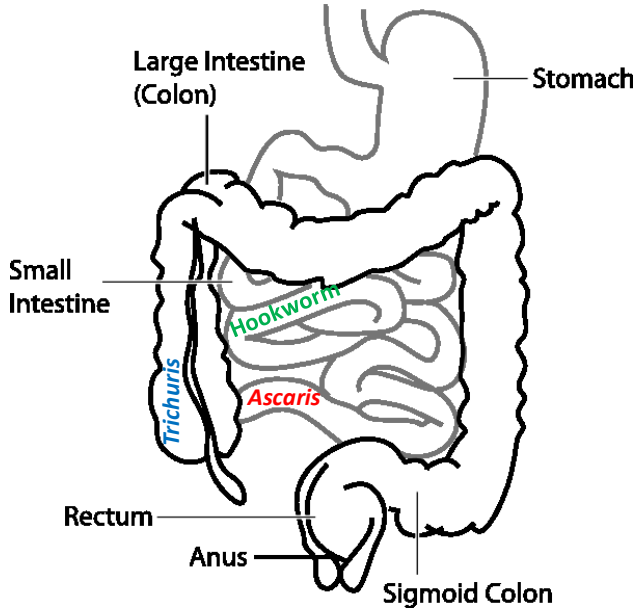
The soil-transmitted helminths (STHs) (i.e. the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura,* and the hookworms *Necator americanus* and *Ancylostoma duodenale*) are parasites that live in the human gut. Because the hookworm species are rarely differentiated, the term "hookworm infection" can refer to infection by either, or both, species. The three STH infections are considered as one disease cluster among the 20 tropical diseases identified by the World Health Organization (WHO) as the "Neglected Tropical Diseases" (NTD), a group of infections that have historically been neglected despite causing considerable disease burden [4].

Among the NTDs, STH infections are ranked first in terms of both prevalence and disease burden (measured by disability-adjusted life years (DALYs)) in Latin America [6, 7, 85]. STH infections are among the most common and persistent parasite infections worldwide. A total of 103 countries around the world are considered endemic for STH infections [8] and approximately two billion people are affected by these parasites [86].

The STHs thrive in tropical areas of the world where poor hygiene and sanitation prevail, and they are most prevalent in LMICs in Latin America, Asia and Africa [7]. Adult worms parasitize the human gut and, following mating, female worms produce thousands of eggs per day which are subsequently excreted in the host's feces [6]. An adult female *Ascaris* worm produces approximately 200,000 eggs per day; female *Trichuris* worms produce between 3,000-5,000 eggs per day; and female hookworms produce between 9,000-30,000 eggs per day (depending on the hookworm species) [6]. To become infective, excreted eggs undergo a critical developmental phase in the environment that takes approximately three weeks (depending on temperature and humidity conditions). Individuals become infected with *Ascaris* and *Trichuris* by ingesting these infective eggs. Larvae are released following egg ingestion and *Trichuris* larvae travel to the large intestine where they develop into adult worms within 12 weeks (Figure 2) [87]. *Ascaris* larvae penetrate intestinal mucosa following ingestion and then migrate to the liver, lungs, epiglottis, re-enter the gastrointestinal tract and finally parasitize the small intestine where they develop into adult worms (Figure 2) [6]. It takes approximately 9-11 weeks for *Ascaris* larvae to develop into adult worms following ingestion of infective eggs [88]. Hookworm eggs, however, hatch in the soil and their larvae enter the human body by penetrating the skin. Following penetration, hookworm larvae enter subcutaneous venules and lymphatic vessels and access the afferent circulation. They enter the lungs, epiglottis and migrate to the upper part of the small intestine where they develop into adult worms (Figure 2) [6]. Development into adult hookworms takes approximately 5-9 weeks following skin penetration [6]. In rare cases, *Ancylostoma duodenale* can also be acquired by oral ingestion of infective larvae [89]. The adult worms can live in the human gastrointestinal tract for several years (the lifespans of *Ascaris*, *Trichuris* and hookworms have been estimated at one year, one and a half to two years, and, five to seven years, respectively); however, STHs do not reproduce in the human body [6]. The developmental phase of the eggs (which are excreted in human feces) in the

environment is crucial for transmission and infection to occur [6]. Therefore, STH infection is closely linked with contamination of the environment with the feces of infected individuals [90]. Climate is also an important factor for transmission to occur because STH eggs require an adequately moist and warm temperature to be become infective [91, 92]. Adult STH worms vary in size and females are generally larger than males. Adult *Ascaris*, *Trichuris* and hookworms measure between 150-400 mm, 30-50 mm and 7-13 mm, respectively [87].

Figure 2: Diagram showing where the three STH species reside in the human intestine.



STHs are thought to survive in humans by altering the host's immune response [93]. STH infections induce the T-helper-2 (Th2) immune response which includes the production of cytokines, parasite-specific immunoglobulins and non-specific immunoglobulin E, and the expansion and mobilization of mast cells, eosinophils and basophils [94]. This Th2 immune response leads to larval killing, goblet-cell hyperplasia and increased mucus secretion [95]. To combat the host's immune response and to facilitate their survival, STHs secrete molecules inside their host which interact with the host tissues and induce regulatory responses that dampen immune response activity [6, 96]. Hookworms, for example, secrete molecules that inhibit host coagulation. This ensures that blood flow is continuous and that bleeding occurs where the hookworm attaches to the intestinal wall [97]. These molecules also initiate a multi-enzyme cascade involved in red-blood cell lysis and hemoglobin digestion [98]. *Ascaris* worms secrete

pepsin inhibitors that are thought to protect it from digestive enzymes in the stomach [6]. Finally, *Trichuris* worms secrete large amounts of TT47, proteins that create ion-conducting pores in lipid bilayers, allowing the parasite to protrude into the host gut, maintaining its anterior end in the syncytial environment in the cecal epithelium [99].

Migrating STH larvae can cause reactions in some of the host tissues through which they pass. When *Ascaris* larvae pass through the lungs, larval antigens can cause a major inflammatory response resulting in an acute transient pneumonitis known as Loeffler's syndrome (fever, cough, wheezing and marked eosinophilia) [6, 100]. Repeated skin exposure to hookworm larvae can cause ground itch [89]. Hookworm larvae in the lungs can also cause pneumonia and orally ingested hookworm larvae can lead to Wakana syndrome (nausea, vomiting, pharyngeal irritation, cough, dyspnea and hoarseness) [89, 101]. *Trichuris* larvae, however, do not appear to cause these types of reactions, likely due to their limited migration in the host.

Clinical manifestation of STH infection is insidious as symptoms usually go unnoticed, especially when infection is of low intensity. STH-attributable morbidity results primarily from nutritional impairment caused by intestinal bleeding, anemia, nutrient malabsorption, protein malnutrition, competition for micronutrients, loss of appetite, reduction of food intake and diarrhea [5, 6]. The presence of worms in the intestines can induce damage to the intestinal mucosa which reduces the host's ability to extract and absorb nutrients from food [102]. In extreme cases, large numbers of worms in the gut can cause intestinal or biliary obstruction (from *Ascaris* infections) or rectal prolapse (from *Trichuris* infections) [5]. Large quantities of *Ascaris* worms in the small intestine can cause abdominal distension and pain, lactose intolerance and malabsorption of vitamin A, protein, lactose and fat-soluble vitamins [103, 104]. Malabsorption of nutrients associated with *Ascaris* infection is hypothesized to be due to the erosion of the intestinal villi responsible for nutrient absorption (i.e. villous atrophy) [102]. Intestinal obstruction (especially in children) can arise and lead to bowel infarction and intestinal perforation, which can be fatal [6]. Adult *Ascaris* worms can also enter the appendix and the bile duct causing tissue-specific morbidity [6]. Adult *Trichuris* worms that burrow into the cecum cause inflammation that may result in colitis. If colitis is present long-term, it can produce a clinical disorder similar to inflammatory bowel disease which is associated with impaired growth

and anemia [105]. Direct blood loss also occurs at the site of parasite attachment which can lead to anemia and iron deficiency [106-109]. Heavy *Trichuris* infection can cause *Trichuris* Dysentery Syndrome (TDS), resulting in chronic dysentery and rectal prolapse [105]. The primary clinical manifestation of hookworm infection results from intestinal blood loss due to the hookworms invading and burrowing deeply into the mucosa and submucosa of the distal duodenum and proximal jejunum [89]. Following attachment, hookworms release active peptides that reduce host inflammation, prevent blood-clotting and degrade host connective tissue components [110-116]. This maximizes blood loss and can lead to iron-deficiency anemia and chronic protein loss leading to hypoproteinemia and anasarca [89]. It has been estimated that with heavy infections, each adult hookworm can cause up to 0.2 mL of blood loss per day and that *Ancylostoma duodenale* causes greater blood loss than *Necator americanus* [89, 102].

STH infections are most commonly diagnosed in humans by identifying STH eggs in stool specimens under microscopic examination. The most commonly used microscopic techniques to identify STH eggs include direct smear microscopy, the Kato-Katz technique, formol-ether concentration (FEC), the McMaster technique, FLOTAC and Mini-FLOTAC. All of these techniques rely on visual examination of a small quantity of stool to determine the presence and/or number of STH eggs [117]. The intensity of infection is quantified by counting the number of species-specific STH eggs per gram of stool (epg). This method for quantifying the intensity of infection relies on the assumption that STH eggs are uniformly distributed in the stool specimen which is achieved through homogenization prior to microscopy. All of the above mentioned microscopic techniques are capable of detecting both the presence and intensity of STH infection, with the exception of direct smear microscopy which measures the presence, and not the intensity, of infection. Stool specimens should generally be analysed on the same day of deposition to avoid clearing and the hatching of hookworm eggs [118]. The WHO cut-offs for the different levels of infection intensities for each of the STH species are presented in Table 1 [119].

Table 1: Classes of intensity for soil-transmitted helminth infections [119]

Parasite	Light intensity infections	Moderate intensity infections	Heavy intensity infections
Ascaris	1-4,999 epg	5,000 – 49,999 epg	≥ 50,000 epg
Trichuris	1-999 epg	1,000 – 9,999 epg	≥ 10,000 epg
Hookworm	1-1,999 epg	2,000 – 3,999 epg	≥ 4,000 epg

*epg: number of eggs per gram of stool

The direct smear technique involves examing a smear of stool under a microscope with no previous preparation. The Kato-Katz technique involves using a standard quantity of filtered stool and staining it with either glycerol-malachite green or glycerol-methylene blue solution, making STH eggs more visible, prior to microscopic examination [117]. The FEC method is a concentration technique that involves centrifuging a solution made of stool combined with formalin and ether and examining a drop of the suspension under a microscope [117]. The McMaster, FLOTAC and Mini-FLOTAC methods are flotation techniques that allow STH eggs to separate from stool by 'floating' to the top of a flotation suspension, making microscopic egg identification easier. The McMaster technique involves centrifuging a filtered suspension of stool, mixing in flotation solution and pipetting 1 mL of the solution onto McMaster slides which are examined under a microscope [120]. The FLOTAC technique uses a specific FLOTAC apparatus with flotation chambers and flotation solution and involves centrifuging a filtered suspension of stool two times and subsequently examining the apical portion of the floating suspension under a microscope [121]. Finally, the Mini-FLOTAC is a simplified adaptation of the FLOTAC technique that can be performed without the use of a centrifuge. The stool is manually homogenized with flotation solution and the same FLOTAC apparatus is used and examined under a microscope [122].

While it is generally accepted that the specificities of these diagnostic techniques are high, they all have limited sensitivities and, unfortunately, a perfect gold standard diagnostic technique for STH infection does not exist [123]. Recent estimates of the sensitivities of the most common techniques are presented in Table 2 [123]. While the FLOTAC method has the highest sensitivity values, it is limited by higher costs and the need for a centrifuge, making this technique unfeasible in resource-limited settings where STH infections are endemic [123]. The Mini-FLOTAC is becoming increasingly popular; however, to date, it does not appear to

outperform the less expensive Kato-Katz technique [123]. The Kato-Katz technique, therefore, continues to be the technique recommended by WHO because it can be used to quantify both prevalence and intensity of STH infections and because it is simple and can be performed at a low cost [119, 124]. Molecular diagnostic techniques (e.g. quantitative polymerase chain reaction (qPCR)) have recently been developed to diagnose STH infections with markedly increased sensitivities compared to the standard microscopic techniques [125]. The high cost associated with these techniques, however, makes them currently unfeasible for use in resource-poor settings.

Table 2: Sensitivities of the most common microscopic techniques to identify STH eggs in human stool [123]

	Ascaris **[Sensitivity (95% CrI*)]**	***Trichuris*** **[Sensitivity (95% CrI*)]**	**Hookworm [Sensitivity (95% CrI*)]**
Direct Smear	52.1% (46.6, 57.7)	62.8% (56.9, 68.9)	42.8% (38.3, 48.4)
Kato-Katz	63.8% (59.1, 68.6)	82.2% (80.1, 84.5)	59.5% (56.9, 62.2)
FEC	56.9% (51.1, 63.5)	81.2% (73.0, 89.2)	53.0% (48.6, 57.5)
McMaster	61.1% (56.3, 65.9)	81.8% (79.6, 84.2)	58.9% (55.7, 62.2)
FLOTAC	79.7% (72.8, 86.0)	91.0% (88.8, 93.5)	92.4% (87.6, 96.2)
Mini-FLOTAC	75.5% (54.0, 95.9)	76.2% (33.9, 99.4)	79.2% (72.7, 85.9)

* 95% CrI: 95% Bayesian Credible Interval

Three population groups at particularly high risk for STH infections have been identified by WHO. These include preschool children (1-4 years of age), school-age children (5-14 years of age), and women of reproductive age (15-49 years of age) [126]. These population groups have the highest STH-attributable prevalence and disease burden. While there have been rare reports of STH infection present at birth, most STH infections begin to be acquired once the child starts crawling [127]. Once children begin crawling and walking, they are more likely to be exposed to eggs in the environment. The risk of infection is further exacerbated if they constantly put their fingers in their mouths, for example, to suck on fingers or bite nails [127]. Infection rates tend to increase with age, peak at 5-14 years, and then decline somewhat in late adolescence and adulthood [90]. School-age children carry the highest morbidity burden of STHs since they typically have the highest prevalence and intensity of infection [90]. Preschool children and women of reproductive age are considered high-risk groups because the adverse effects of

infection are more pronounced during early childhood and pregnancy, crucial times for growth and development. Together, these three population groups are usually considered the primary targets for public health interventions aimed at reducing STH infections.

The deworming drugs albendazole and mebendazole are the most common drugs used to treat STH infections [126] and it is generally accepted that they are both safe and effective [128]. These benzimidazole drugs act by binding to the β-tubulin protein of the worms and inhibiting parasite microtubule polymerization [129]. This causes the worm to die within a few days and it is subsequently excreted in the host's feces [6]. While both albendazole and mebendazole are effective at combating *Ascaris* infection with a single dose, albendazole tends to be more efficacious in treating hookworm infection and mebendazole tends to be more efficacious in treating *Trichuris* infection [130-132]. To combat STH infections at the population level, WHO, the United Nation's Children Fund (UNICEF), and the World Bank, among others, recommend that school-based deworming programs be carried out in areas of the world where STH prevalence exceeds 20% (Table 3) [126, 133, 134]. In these programs, single-dose deworming drugs are administered to all children enrolled (and, in some cases, non-enrolled) in schools one to two times per year (depending on the baseline prevalence), regardless of infection status [126]. School-based deworming campaigns are considered to be one of the most cost-effective global public health control measures [135-137]. Pharmaceutical companies are now donating mebendazole and albendazole free of cost to WHO for Ministries of Health of STH-endemic countries to be used in national deworming campaigns. These donations are further increasing the cost-effectiveness of this public health intervention [138]. WHO also recommends that preschool children be targeted for deworming treatment in endemic areas as of one year of age (Table 3)[134]. Reaching preschool children for deworming is usually achieved through piggy-backing onto vaccination programs or micronutrient supplementation campaigns [138]. To date, the disease burden associated with infection in this age group has not been rigorously quantified and the optimal delivery strategy to target this age group remains unclear. Therefore, political motivation to reach this age group for intervention continues to be a major challenge [139].

Table 3: WHO recommendations for large-scale deworming programs [134]

Recommen-dation type	Condition	Action
Strong	Prevalence of any STH in population group > 50%	1. Treat all young children (12-23 months of age), preschool children (24-59 months of age) and school-age children *two times/year* 2. Treat non-pregnant adolescent girls (10-19 years of age) and non-pregnant women of reproductive age (15-49 years of age) *two times/year*
Strong	Prevalence of any STH in population group ≥ 20% and ≤ 50%	1. Treat all young children (12-23 months of age), preschool children (24-59 months of age) and school-age children *one time/year* 2. Treat non-pregnant adolescent girls (10-19 years of age) and non-pregnant women of reproductive age (15-49 years of age) *one time/year*
Conditional	i) Prevalence of hookworm and/or *Trichuris* ≥ 20% in pregnant women AND ii) Prevalence of anemia is ≥ 40% in pregnant women	1. Treat pregnant women, after the first trimester

The primary objective of deworming campaigns is to reduce STH morbidity caused by moderate and heavy intensity infections. Without permanent improvements in environmental conditions, hygiene and sanitation, access to clean and potable water, better housing, and sustained socioeconomic development to reduce exposure to STH eggs in the environment, however, re-infection following treatment is almost inevitable [27, 140]. It has been estimated that, following community-wide mass deworming, hookworm infection can reach 80% of the pre-treatment prevalence within 30-36 months [141], *Ascaris* infection can reach 55% of the pre-treatment prevalence within 11 months [142] and *Trichuris* infection can reach 44% of the pre-treatment prevalence within 17 months [143]. Health educational campaigns that promote hygiene and sanitation (e.g. hand washing, clean water sources, appropriate defecation behaviours, etc.) have been found to effectively reduce re-infection rates following mass treatment [144] and WHO recommends that a health education component always be incorporated into school-based deworming programs [119].

In the World Health Assembly of 2001, all Member States unanimously ratified Resolution 54.19 which advocated for periodic administration of deworming to at least 75% of school-age children at risk of STH infection (i.e. who live in endemic areas) by 2010 [145]. This target was not met [146]. By 2010, only approximately one third of children living in STH-endemic countries received deworming [147]. Moving forward, in 2012, WHO published a Strategic Plan for the control of STH infections with the following objectives for the 2011-2020 period: 1) Reduce the prevalence of moderate and heavy intensity STH infections in both preschool and school-age children to less than 1% by 2020; 2) By 2015, all STH-endemic countries should have started national STH control programs; and 3) By 2020, in all STH-endemic countries, STH control programs should reach 75% national coverage and 100% geographical coverage [138]. The most recent coverage rates reported to date show that, in 2016, global coverage of deworming in endemic countries was 50.8% and 69.5% in preschool and school-age children, respectively [8]. These are some of the highest coverage rates ever reported and show that, although progress is still needed in order to reach the objectives outlined in the WHO strategic plan [138], global efforts to deliver deworming in endemic countries are rising. One of the major barriers that needs to be overcome to improve the success of national deworming programs is the lack of strong long-term political commitment in endemic countries. This is largely due to neglect of the magnitude of the disease burden caused by STH infections and lack of funding for their control programs [138].

2.3. The effect of soil-transmitted helminth infections on child development

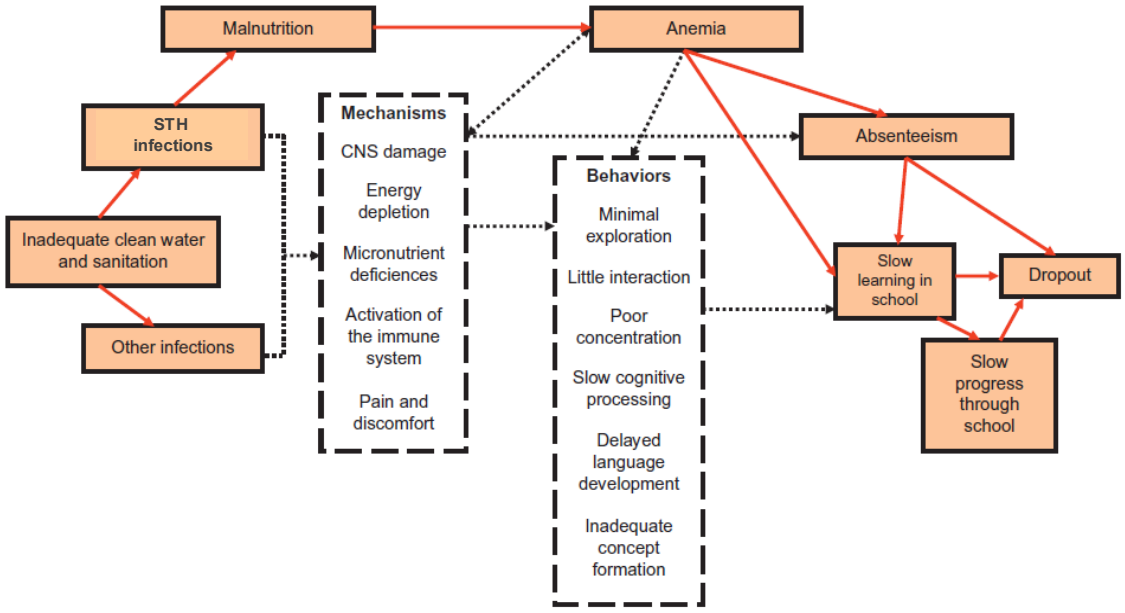
2.3.1. Theory

It has been proposed that STH infections adversely affect child development [9-11]. This is primarily theorized to be due to mediating factors including malnutrition and, most specifically, iron levels. Malnutrition caused by STH infections results from worms feeding on host tissues and gut contents, maldigestion and malabsorption of nutrients, inflammatory responses leading to reduced appetite, modification of the host's metabolism and storage of nutrients, and diversion of nutrients and energy to fight responses to infection [148]. These effects of STH infection on the nutritional status of the host are likely to affect cognitive development. Furthermore, it is known that *Trichuris* [149] and hookworm [149, 150] infections are important risk factors for anemia, one of the primary risk factors for impaired child development, especially in developing

countries [9]. It has also been suggested that the effect of STH infection on child development may be modified by nutritional status, such that malnourished children will suffer greater consequences of infection compared to healthy children who become infected [148, 151].

While the primary pathway linking STH infection to possible effects on child development involves malnutrition, other pathways have also been hypothesized. One such pathway is that inflammation caused by STH infection leads to specific cytokine actions (i.e. chemical signals released by immune cells) that could affect brain function and behaviour [12]. Furthermore, while the exact mechanisms have not yet been determined, based on research involving the effect of neurocysticercosis on epilepsy, it has also been proposed that STH infections may produce substances that interfere with neuronal transmission and that the immunological mechanism involved in combating STH infections may affect brain function [12, 152]. Finally, it has recently been proposed that possible effects of helminths on cognitive development may be due to helminth-induced changes to gut microbiota composition and diversity, especially in young children [153]. A framework detailing the different pathways through which STH infections may affect poor educational outcomes associated with poor child development is presented in Figure 3 [12].

Figure 3: Pathways linking STH infections to poor educational outcomes (modified from [12]).



The timing of infection in terms of the stage of development of the host is also likely to play a role in determining the magnitude of the effect that STH infections may have on child development. During the first few years of life, brain plasticity is greatest as the brain grows rapidly; and, at this particular stage of development, perturbations caused by STH infections, for example, could have important and long-term effects on brain development [33-35]. It is therefore likely that the effect of STH infection on child development is more pronounced in preschool children, who are at the greatest risk of developmental impairment, compared to school-age children and adults [12].

2.3.2. Previous research

The interpretation of the current body of evidence for the effect of STH infections on child development is limited. A total of 14 observational studies were identified that document the effect of one or more STH infections on standardized test scores of child development (Table 4) [13-25, 154]. All of these studies, with only one exception [154], document some kind of association between one or more STH infections and lower scores on different standardized tests of child development. The quality of the evidence, however, is weak. Of the 14 identified studies, 10 are cross-sectional [13-15, 17-19, 21, 22, 24, 25] and therefore, they are unable to establish temporality with respect to when the infections were acquired and if these infections affected child development over time. Additional major methodological flaws of the identified studies include: failure to adjust for crucial confounding variables (e.g. socioeconomic status (SES)), very small sample sizes, unconventional study designs that are not appropriate for the research question, grouping STH infections with other parasites in the analyses, and inappropriate statistical analyses. Only four of these studies investigated the effect of STH infection in preschool children [16, 19, 20, 23], the population group that is most likely to be affected. The majority of research done to date has been done in school-age children.

Table 4: Previous research documenting the effect of STH infections on child development

First author, date [reference]	Study location	Study popula-tion	Study design	N	Measure of developmental outcome	Main results	Major limitations
Gall, 2017 [25]	South Africa	Children 8-12 years of age	Cross-sectional	835	-Selective attention (d2-test) -Academic achievement (end of year grades)	-STH infection associated with lower selective attention and lower academic achievement	-Cross-sectional study design -Linear regression likely inappropriate for the outcome (score between 0-7) -No species-specific analyses
Kuong, 2016 [13]	Cambodia	Children 6-16 years of age	Cross-sectional	1,760	-Raven's Colored Progressive Matrices -Block Design -Picture Completion	-Hookworm infection was associated with lower scores on all three scales	-Cross-sectional study design -Residual confounding was likely an issue (i.e. no adjustment for SES)
Liu, 2015 [14]	China	Children 9-11 years of age	Cross-sectional	2,179	-4 subscales of the Wechsler Intelligence Scale for Children	-Any STH infection was associated with lower Working Memory Indexes and Processing Speed Indexes -Any STH infection was associated with lower standardized math test scores -*Trichuris* infection seemed to be responsible for effects	-Cross-sectional study design -Residual confounding may have been an issue (the adjustment for SES was not optimal)
Lobato, 2012 [154]	Brazil	Children 6-10 years of age	Longitu-dinal (random-ized controlled trial (RCT) of an educational interven-tion)	87	-Raven's Coloured Standard Progressive Matrices -Draw-a-Person Test 3rd ed - Wechsler Intelligence Scale for Children	-There was no effect of hookworm infection on any of the measures of development	-Statistical analyses were not appropriate and no adjustment for important confounders was made -Small sample size (study was likely underpowered) -The timing of measurement of STH infection was unclear, potentially affecting the relevance of the results

Shang, 2010 [15]	China	Children 9-12 years of age	Cross-sectional	116	-Wechsler Intelligence Scale for Children	-STH infection was associated with lower scores on the Full Scale IQ and on the 3 subscales	-Cross-sectional study design -No adjustment for important confounders -No species-specific analyses -Small sample size
Santos, 2008 [16]	Brazil	Preschool children	Cohort	346	-Wechsler Preschool and Primary Scale of Intelligence	-STH infections were associated with lower Full Scale IQ scores	-No adjustment for important confounders (e.g. SES) -STHs grouped with *Giardia* -STHs only measured at one time point and definition of 'infection' was arbitrary
Ezeamama, 2005 [17]	Philip-pines	Children 7-18 years of age	Cross-sectional	319	-Wide Range Assessment of Memory and Learning (WRAML) -Verbal Fluency -Philippines Non-verbal Intelligence Test	-*Ascaris* infection was associated with lower scores on the memory subscale of WRAML (this effect did not appear to be mediated by hemoglobin level or malnutrition) -*Trichuris* infection was associated with lower scores on Verbal Fluency (this effect appeared to be mediated by hemoglobin levels) - All other comparisons did not reach statistical significance	-Cross-sectional study design -Mediation analysis was not optimal -Study was likely underpowered
Sakti, 1999 [18]	Indonesia	Children 8-9 & 11-13 years of age	Cross-sectional	432	-Picture Search -Number Choice -Stroop -Categorical Fluency -Digit-span Forwards -Digit-span Backwards -Corsi Block -Free Recall	-Hookworm infection was associated with lower scores on 6 subscales -Significant interaction of hookworm with age on 4 subscales (as age increased, effect of hookworm increased) -*Trichuris* infection was associated with lower scores on 2 subscales -*Ascaris* infection was	-Cross-sectional study design -Analyses adjusted for potential mediators (hemoglobin, body mass index (BMI) and stunting), therefore effects may be underestimated

					-Verbal Analogies -Pegboard -Bead threading -Mazes	associated with lower scores on 1 subscale -No significant effect was found for any helminth on 6 subscales	
Oberhelman, 1998 [19]	Nicaragua	Children less than 6 years of age	Cross-sectional	573	-DDST II	-Any intestinal parasite was associated with 'suspect' (i.e. poorer) results on the language scale of the DDST II	-Cross-sectional study design -Analyses grouped all intestinal parasites together and the most common parasite was *Giardia* -Authors didn't specify which variables were controlled for in multivariable analyses
Callender, 1998 [20]	Jamaica	Children 3-10 years of age	-Cohort -4 years of follow-up -Those infected with *Trichuris* received treatment every 3-6 months	35	-Stanford-Binet Intelligence Scale -Verbal Fluency -Digit-span Forwards -French Vocabulary -Corsi Blocks -Ravens Progressive Matrices -Verbal Analogies -Peabody Picture Vocabulary Test -Grooved Pegboard Test -Wide Range Achievement Test (WRAT)	-Children with *Trichuris* Dysentery Syndrome (TDS) had lower scores on all tests of development after four years of follow-up with treatment	-Unconventional study design that was not ideal to investigate the effect of *Trichuris* on child development -Very small sample size -Multivariable analyses were unclear and species-specific results were not presented
Hutchinson, 1997 [21]	Jamaica	Grade 5 school-children	Cross-sectional	800	-WRAT	-*Trichuris* infection was associated with lower scores on three subscales of WRAT -*Ascaris* infection was associated with lower scores on	-Cross-sectional study design -Stool specimens appear to have often been read more than 24 hours after being deposited -Analyses were adjusted for

						two subscales of WRAT	height-for-age (a likely mediator); therefore, effects are likely underestimated
Simeon, 1994 [22]	Jamaica	Children in grades 2-5	Cross-sectional	616	-WRAT	-*Trichuris* infection was associated with lower scores on two subscales of the WRAT (these effects appear to have been mediated by BMI, height-for-age, hemoglobin levels and ferritin levels) -*Ascaris* infection was associated with lower scores on three subscales of the WRAT (this effect appears to have been mediated by hemoglobin and ferritin levels but not by BMI or height-for-age)	-Cross-sectional study design -Mediation analysis was not optimal and results were not fully presented -Light intensity *Trichuris* infections and severely malnourished children were not included -No adjustment for clustering by schools
Callender, 1992 [23]	Jamaica	Children 3-6 years of age	-Cohort -One year follow-up -Those with *Trichuris* were treated every three months	38	-4 subscales of Griffiths Mental Development Scales for Young Children	-At baseline, children with TDS had lower scores on all four subscales -At follow-up (with treatment every three months), the TDS children improved significantly more than controls on one subscale only	-Unconventional study design that was not ideal to investigate the effect of *Trichuris* on child development -Very small sample size -No multivariable analyses (however, exposure groups were matched on age, sex, neighbourhood and SES)
Waite, 1919 [24]	Australia	Children 6-14 years of age	Cross-sectional	340	-Goodard's 1911 Revision of Binet-Simon Scale -Porteus Mazes	-Hookworm infection was associated with lower scores on both scales of development	-Cross-sectional study design -No multivariable analyses or formal statistical testing

A recent meta-analysis attempted to calculate pooled estimates for the effect of STH infection on cognitive development in school-age children [155]. The researchers found that STH-infected children had deficits in learning, memory, reaction time and innate intelligence compared to uninfected children; however, they also noted that the results should be interpreted with caution due to high heterogeneity and high risk of bias of the included studies [155]. Additionally, a recent systematic review reviewed the available evidence linking STH infection to cognitive development and concluded that there is a need for improved methodological approaches to assess the effect of STH infection on cognitive function of children [26]. This review also noted the lack of evidence in preschool children, the population group in the most critical stage of human cognitive development, and, recommended that future research focus on the effects of chronic STH infections on cognitive function using appropriate study designs and measurement tools [26].

Two studies were identified that performed simple mediation analyses (comparing multivariable regression models with and without possible mediators included), investigating malnutrition (i.e. body mass index (BMI) or height-for-age) and iron status (i.e. hemoglobin and/or ferritin levels) as potential mediators of the relationship between STH infection and child development [17, 22]. Both studies were performed in school-age children and concluded that the effects of *Trichuris* infection on development are likely mediated by hemoglobin and that effects of *Ascaris* infection are not mediated by malnutrition. One of the two mediation analyses also identified malnutrition as a mediator of the relationship between *Trichuris* and child development and identified hemoglobin as a mediator of the relationship between *Ascaris* and child development [22]. Unfortunately, the assumptions required for this simple type of mediation analysis are likely not met (i.e. that the mediating variables investigated are independent) as malnutrition and iron levels are known to be associated. To date, no study has performed a modern mediation analysis of the effect of STH infection on child development to decompose total effects and determine the magnitude of the effect mediated by specific variables. Furthermore, no type of mediation analysis regarding the effect of STH infection on child development has ever been performed in preschool children.

2.4. The effect of deworming on child development

Some previous research has been dedicated to investigating the effect of deworming on child development. Deworming drugs are currently the best intervention that exists to combat STH infections and, if STH infections impair child development, it is logical to assume that deworming would mitigate these effects. Research investigating the effect of deworming, as opposed to STH infection, has the advantage of using an intervention that can be randomized. Therefore, randomized controlled trials (RCTs) can be performed to document the unconfounded effect of deworming on child development.

A recent Cochrane Review (updated in 2015) reviewed RCTs and quasi-RCTs investigating the effect of deworming on cognitive development in children [156]. Ten trials were included; however, comparison of results was difficult because of the different deworming strategies used (i.e. mass treatment, screening and treating, single dose and multiple doses) and because of the different tests of cognitive development used. The authors of the review concluded that "For infected children, deworming drugs may increase average weight gain over one to six months, but we do not know if there is an effect on haemoglobin or cognitive function" [156]. The authors specified that a single dose of deworming may have little or no effect on cognition; that multiple doses over six months to three years probably have little or no effect on cognition; and, that the mechanisms for any potential effects are unknown [156].

Of the ten trials included in this review, only one documented a positive effect of deworming on three out of ten tests of cognitive development used [157]. The remaining nine trials did not find any effect of deworming on cognitive development [158-166]. The majority of the trials were done in school-age children. Four trials included preschool children, none of which documented an effect of deworming on cognitive test scores [162-165]. Follow-up time of the majority of the trials was relatively short with eight of the ten trials having a follow-up time of one year or less [157-161, 163, 165, 166], one trial having two years of follow-up [162] and one trial having a long follow-up time of five years [164]. While it is possible that deworming has no effect on cognitive development, several other explanations may account for the results observed to date. First, four trials [157, 158, 160, 161] only provided a single dose of deworming and, considering the high probability of rapid re-infection following treatment, this type of intervention is likely

not sufficient to cause an important effect on a potentially chronic condition [167]. Second, seven of the trials included both infected and non-infected children (to mimic a deworming program) [160-166]. This type of deworming strategy, where deworming drugs are administered to both infected and non-infected children, is done for feasibility purposes in public health programs because the drugs have negligible adverse risks and costs are kept to a minimum. Diagnosing infection prior to treatment is considerably more costly. Therefore, since it is not expected that deworming would have any effect in non-infected children, depending on the baseline prevalence of infection in the population, and the species-specific prevalences, effect dilution alone could explain the null results observed to date [167, 168]. Third, one year of follow-up is probably insufficient to detect an effect considering the clinical pathway including infection, treatment, mediators, brain development and measurable differences on tests of cognitive development [167]. Unfortunately, the longest follow-up study (i.e. five years) conducted to date [164] was critically flawed by providing deworming to participants in the placebo group if they were found positive for STH infection at the quarterly study visits. While this is understandable from an ethical perspective, this aspect of the study design removed any possibility of detecting differences between the intervention arms. Proper interpretation of the results of the Cochrane Review must take into account these limitations of the individual trials included. Further research that addresses these limitations is needed to accurately assess the effects that deworming may have.

2.5. The knowledge gap

The need for research to clearly estimate the health effects caused by helminths and specifically, to clarify the effects that STH infections and deworming may have on child development, and the mechanisms driving these effects, has recently been emphasized by both the STH and child development communities [9, 27-31]. High quality observational studies with a longitudinal design, sufficiently long follow-up and appropriate adjustment for confounding variables is urgently needed. The current thesis addresses this need and contributes to the body of evidence on STH infections, deworming and child development in preschool children, for which very little rigorous evidence exists. The present research investigates the long-term effect of cumulative or recurring STH infections over time during the most critical time of development. This research is unique in that a large cohort of children, recruited at one year of age, was followed-up yearly

to five years of age (i.e. four years of follow-up). While it is unlikely that a single STH infection at any one time will have an important effect on child development, the long-term cohort design of the present research allows for the effect of cumulative infections over time to be investigated. Furthermore, the modern mediation analysis contributes to clarifying the underlying mechanisms between STH infection, iron levels, malnutrition, and child development. This is the first time that this type of rigorous analysis has been done in the context of STH infections and child development and it contributes to understanding the effects and disease burden of these infections.

3. Objectives

The overall research question of this thesis is: do STH infections affect child development in young children living in the Amazon region of Peru?

The specific objectives include:

1. To determine the effect of the number of times found STH-infected between one and five years of age on child development at five years of age.
   Hypothesis: Children with more STH infections detected between one and five years of age will have lower development scores compared to children who were never found infected.
   - *Aim 1.1:* To determine the effect of the number of times found infected with at least one STH infection and with species-specific infections (i.e. *Ascaris*, *Trichuris* and hookworm) between one and five years of age on full scale IQ scores at five years of age.
   - *Aim 1.2:* To determine the effect of the number of times found infected with at least one STH infection and with species-specific infections (i.e. *Ascaris*, *Trichuris* and hookworm) between one and five years of age on performance IQ score at five years of age.
   - *Aim 1.3:* To determine the effect of the number of times found infected with at least one STH infection and with species-specific infections (i.e. *Ascaris*, *Trichuris* and hookworm) between one and five years of age on verbal IQ score at five years of age.

2. To determine the effect of the number of detected STH infections during a critical window of development (i.e. between one and two years of age) on child development at two, three, four and five years of age.
   Hypothesis: Children who acquire STH infection during the second year of life will have lower development scores at two, three, four and five years of age compared to children who have not acquired infection during this time.

- *Aim 2.1:* To determine the effect of the number of times found infected with at least one STH infection and with species-specific infections (i.e. *Ascaris* and *Trichuris*) between one and two years of age on cognitive scores at two, three, four and five years of age.
- *Aim 2.2:* To determine the effect of the number of times found infected with at least one STH infection and with species-specific infections (i.e. *Ascaris* and *Trichuris*) between one and two years of age on verbal scores at two, three, four and five years of age.

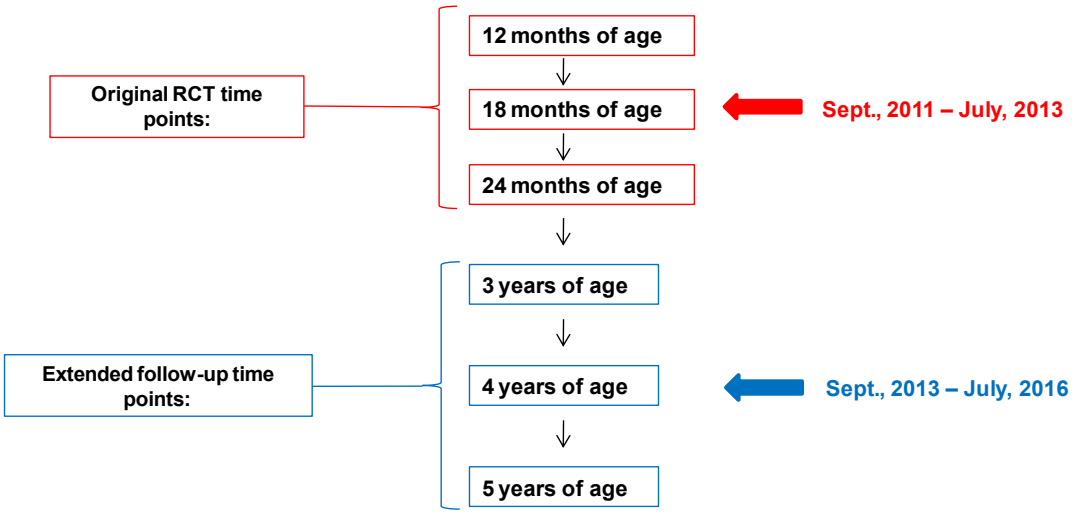
3. To investigate the role of hemoglobin levels and malnutrition as potential mediators of the relationship between *Ascaris* infection between one and five years of age and child development at five years of age.
   Hypothesis: A considerable amount of the total effect of *Ascaris* infection on child development is mediated by hemoglobin levels and/or malnutrition.
   - *Aim 3.1:* To determine the amount of the total effect of *Ascaris* infection on child development that is due to mediation by mean hemoglobin levels between three and five years of age.
   - *Aim 3.*2: To determine the additional mediating contribution of malnutrition between one and five years of age (apart from, and independent of, hemoglobin levels) of the relationship between *Ascaris* infection and child development.

4. Methods

4.1. Parent study

The objectives of the current thesis were derived from research questions identified following the completion of a parent randomized controlled trial (RCT) conducted in Iquitos, Peru between 2011 and 2013 [169]. Details of this RCT's methods are provided here to clarify recruitment and assembly of the cohort. In the RCT, 1,760 children between 12 and 14 months of age had been randomized to one of four intervention arms, receiving the assigned interventions as follows: 1) Deworming with mebendazole at 12 months of age and placebo at 18 months of age; 2) Deworming with mebendazole at 12 and 18 months of age; 3) Placebo at 12 months of age and deworming with mebendazole at 18 months of age; and, 4) Placebo at 12 and 18 months of age. Children were included in the trial regardless of their STH infection status such that the intervention resembled a mass deworming program where treatment is given to all individuals without previously screening for infection. The primary objective was to determine the effect of the timing and frequency of deworming during the second year of life on infant weight gain between one and two years of age [169]. A secondary outcome was child development at two years of age [170]. The RCT did not find a consistent effect of deworming compared to placebo on either weight gain or cognition at two years of age, and this was hypothesized to be due to the short follow-up period (i.e. only one year) and the relatively lower than expected prevalence of STH infections [169, 170]. Follow-up of these children continued on an annual basis to five years of age (Figure 4). The present thesis uses both the original RCT data and the extended follow-up data of the trial child cohort to investigate the long term effects of STH infection on child development. The study referred to subsequently, therefore, is a cohort study of children who initially participated in the original RCT and who were followed up to five years of age.

Figure 4: Data collection time points of the original RCT and the extended follow-up study

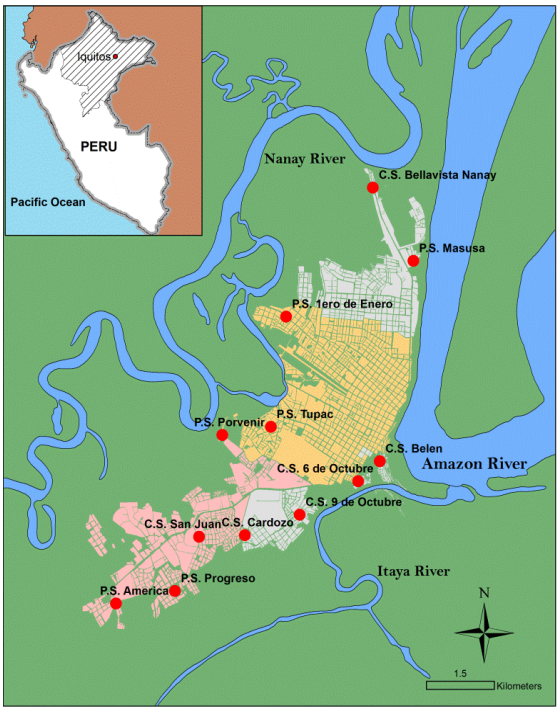


4.2. Study location and population

The study took place in the rural and peri-urban communities surrounding Iquitos, the capital of the Loreto region of the Peruvian Amazon, an STH-endemic region of Peru. Iquitos cannot be accessed by road (only by plane or boat) and as a result, it is a very isolated and under-developed area of Peru. It borders the Amazon, Itaya and Nanay rivers and during the rainy season (i.e. November – April) flooding is extremely common. Houses in the communities close to the river are built on stilts to avoid being flooded. STH infection is highly endemic in this region [127, 144], largely due to persistent environmental fecal contamination resulting from a lack of adequate sanitation and waste management, exacerbated by the tropical climate and seasonal flooding.

The target population for this study included preschool children between one and five years of age living in the rural or peri-urban areas surrounding Iquitos. The study population consists of eligible children living in the catchment areas of the twelve major health centres serving the three rural/peri-urban communities surrounding Iquitos (i.e. Nanay, Belén and San Juan), who, during study recruitment, were between 12 and 14 months of age. Figure 5 shows a map of the study area and the location of the participating health centres.

Figure 5: Map of the study area in and around the city of Iquitos in the Loreto region of the Peruvian Amazon.

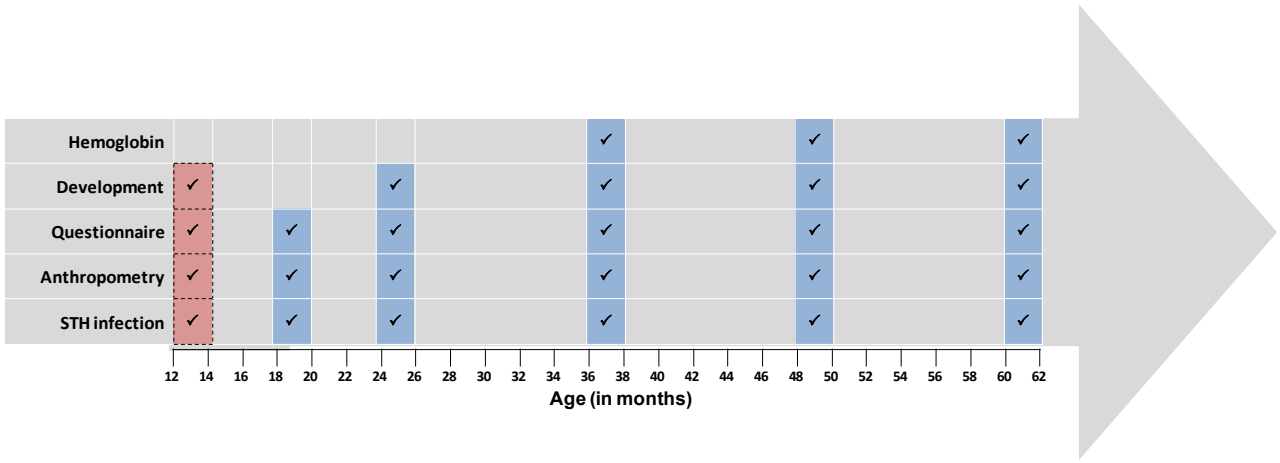


*The red dots show the location of the participating health centres.

4.3. Overall study design

The overall study design is a longitudinal cohort study. A schematic diagram of the study design can be found in Figure 6. Children recruited between 12 and 14 months of age completed follow-up assessments at 18 months and at two, three, four and five years of age. At each time point, parents of participating children were visited in their home by a research assistant one or two days before their scheduled study visit. Study visits were scheduled to coincide with children's routine healthy growth and development check-ups, which take place in the health centres. However, depending on the availability and willingness of the child's parent/guardian, study visits either took place in a participating health centre or in the child's home. Parents were reimbursed for travel to the health centre.

Figure 6: Schematic diagram of the longitudinal cohort study indicating measures taken at each study time point.



* Red cells with dotted borders indicate measures taken at the recruitment visit and blue cells with solid borders indicate measures taken at follow-up visits.

4.4. Recruitment

A sampling frame for the study was obtained from participating health centre records and from a door-to-door census conducted in the study area by the research team before initiation of the study. All eligible children were approached for recruitment into the study until the desired sample size was reached. The recruitment phase took place over two visits. The first 'screening' visit took place in potential participants' homes. At this visit, the potential participant was assessed for eligibility, the child's parents/guardians were informed of the study and if interested, the informed consent form was read to them. If the parents agreed to participate, both the participating child's mother and father signed the informed consent form. The baseline questionnaire was administered to a parent/guardian of the participating child and she/he was given the materials and instructions necessary to collect a stool specimen from the participating child. The second 'enrollment' visit took place in the health centre. This visit usually took place within one to two days following the screening visit. At this visit, the parent/guardian gave the research assistant the child's stool specimen (which had been collected within the past 24 hours), the child's height and weight were measured, and the child's development was assessed.

4.5. Inclusion and exclusion criteria

The inclusion and exclusion criteria were specified based on the scientific and feasibility considerations of the original parent study. These criteria included:

4.5.1. Inclusion criteria

1) Children between 12 and 14 months of age at recruitment; 2) Children attending one of the participating health centres for their 12-month routine healthy growth and development visit (Note that the parent/guardian of any child who was identified as a potential participant from the sampling frame but who did not attend his/her routine visit at 12 months of age was contacted at home by a research assistant and encouraged to attend); 3) Children who were not consulting medical advice for a suspected STH infection; 4) Children who had not been dewormed in the six months prior to their recruitment into the study; 5) Children who did not have any serious congenital or chronic medical condition.

4.5.2. Exclusion criteria

1) Children who lived outside of the identified study area; 2) Children whose family planned to move outside of the study area in the year following recruitment; 3) Children whose parents did not consent to participate in the study.

4.6. Exposure ascertainment

4.6.1. STH infection

STH infection was measured at all study time points (i.e. 12 months, 18 months, two years, three years, four years and five years). Detailed instructions and all materials necessary to collect a stool specimen were given to the parent/guardian of the participating child at the home visit one or two days before their official study visit. Parents collected stool specimens from their child in the 24 hours preceding their scheduled study visits and gave this specimen to the research assistant at their study visit. When the parents gave the research assistants the stool specimen, the research assistants confirmed that the specimen had been collected from the participating child within the preceding 24 hours. All stool specimens were transported to the study laboratory where they were analysed by trained microscopists. At the two, three, four and five-year visits, all stool specimens were analyzed with the Kato-Katz technique within 24 hours of

collection of the specimen. This technique is recommended for the assessment of STH prevalence and also to quantify the intensity of infection [171]. All participants were treated with mebendazole at the two-year visit by research personnel and participants found infected at the three, four and five-year visits were referred to the health centre for treatment.

Stool specimens collected at the 12 and 18-month time points (when treatment allocation took place) required special processing. This was because ethics guidelines required any individual found positive for STH infection to be treated. Therefore, if found to be STH-infected, those randomized to the placebo group would have needed to be treated (which would have obviously compromised the trial). Due to clinical equipoise of the trial research question, however, it was deemed acceptable to collect stool specimens from all children participating in the RCT and only immediately analyze those specimens from children who were randomized to receive deworming (this ensured that those found positive would be treated). Stool specimens from children randomized to receive deworming were therefore analyzed immediately with the Kato-Katz technique. Stool specimens from children randomized to receive placebo, at both 12 and 18-months, were stored in 10% formalin until they were analyzed using the direct smear technique, following their two-year visit (i.e. when all children were treated, ensuring that those found positive would receive treatment). (Note that it is not possible to analyze stored stool specimens with the Kato-Katz technique.)

4.7. Outcome ascertainment

Because no single high-quality scale of child development exists for both one and five-year-old children, two separate scales were used to measure child development throughout this study: The Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) and the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III).

4.7.1. Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III)

The cognitive, receptive language, expressive language and fine motor subtests of the Bayley-III were used to measure child development at the one, two and three years of age time points (Table 5). The Bayley-III is one of the most widely used and preferred scales to measure child development in children between one and 42 months of age [172]. Research assistants were

trained health care personnel (i.e. nurses or nurse mid-wives) with a minimum bachelor's degree education and received extensive training in the scale administration from an expert psychologist. Research assistants administered the scale to participating children in a secluded area to avoid distractions. According to the guidelines for administering this scale, in the event that a child did not seem to be performing at his/her optimal ability, either a long break was taken or the visit was postponed to a future date. The Bayley-III subtests were administered, at the one and two-year visits, to all 1,760 study participants; and at the three-year visit, for feasibility reasons, to a random sample of 880 children of the original study population who had completed all previous study visits. In the event that a child who was randomly sampled to be administered the Bayley-III was lost to follow-up at the three-year visit, the next child scheduled to pass their study visit from the same health centre catchment area, who had not originally been randomly selected, was selected to be administered the Bayley-III.

4.7.2. Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III)

The WPPSI-III was used to measure child development in children at the four and five years of age time points. This scale is designed for use in children between 2.5 and 7.25 years of age and has been adapted and translated for use in Spanish (note that the more recent version, the WPPSI-IV, had not been translated for use in Spanish when the four years of age follow-up visit started) [173]. The WPPSI-III contains 14 subscales and generates the following intelligence scores: 1- full IQ score; 2- verbal IQ score; 3- performance IQ score; 4- processing speed quotient; and, 5- general language composite; however, only seven of the 14 subscales are required to generate the full IQ score, the verbal IQ score and the performance IQ score (Table 5). As described for the Bayley-III, the WPPSI-III was administered by highly trained research assistants in a secluded area. In the event that a child did not seem to be performing at his/her optimal ability, either a long break was taken or the visit was postponed to a future date. At the four-year visit, the entire WPPSI-III (i.e. all 14 subtests) was administered to the same random sample of 880 participants who had completed the Bayley-III at the three years of age visit. At the five-year visit, because the full IQ score, the verbal IQ score and the performance IQ scores were considered most relevant for the research question and, for feasibility purposes (to reduce time burden), only the seven subscales needed to generate these scores were administered to the same random sample of 880 participants.

Table 5: Specific constructs measured by the subscales of the Bayley-III and WPPSI-III administered in this study to measure verbal and cognitive abilities of preschool children between one and five years of age.

	Verbal		Cognitive	
	Subscale	**Constructs measured**	**Subscale**	**Constructs measured**
Bayley-III (ages 1-3 years)	Expressive language	Expressive preverbal communication, vocabulary development, morpho-syntactic development (babbling, gesturing, etc.).	Fine Motor	Visual tracking, reaching, object manipulation, grasping, movement quality, functional hand skills, sensory integration.
	Receptive language	Receptive preverbal communication and vocabulary development (identifying objects, pronouns, plurals, etc.).	Cogni-tion	Counting, visual/tactile exploration, object assembly, puzzle board completion, matching colours, discrimination patterns, etc.
WPPSI-III (ages 4-5 years)	Vocab-ulary	Word knowledge, verbal concept formation, fund of knowledge.	Matrices	Visual information processing and abstract reasoning, fluid intelligence, general intellectual ability.
	Informa-tion	Crystallized intelligence, ability to acquire, retain and recuperate general factual knowledge, long-term memory.	Picture concepts	Abstract and categorical reasoning.
	Word reasoning	Verbal reasoning, verbal comprehension, general and analog reasoning, verbal abstraction, knowledge of domain and ability to generate alternate concepts.	Block design	Ability to analyze/ synthesize abstract visual stimuli, nonverbal concept formation, visual perception/organization, simultaneous processing, visual-motor coordination, learning, ability to separate figure/ground in visual stimuli.

4.8. Other relevant covariates

4.8.1. Anthropometry

Child weight and length/height were measured at all time points (i.e. 12 months, 18 months, two years, three years, four years and five years). Children were measured without clothes or shoes.

Weight was measured using a portable electronic scale accurate to the nearest 0.01 kg. At the 12 and 18 months of age visits, children were weighed in the sitting position and at the two to five years of age visits, children were weighed in the standing position. Length/height was measured using a portable stadiometer, accurate to the nearest millimetre. Child length (i.e. measured in the recumbent position) was measured at the one to two years of age visits and child height (i.e. measured in the standing position) was measured at the three to five years of age visits.

4.8.2. Hemoglobin levels

Child hemoglobin levels were measured at the three, four and five years of age visits. Hemoglobin levels were measured by research assistants using a portable HemoCue® machine, accurate to within 1.5% of the gold standard reference [174]. Blood for this test was drawn from finger-prick using disposable lancets. According to the manufacturer's instructions, the first two drops of blood were wiped away and the third drop of blood was used for the analysis. According to the WHO definition of anemia, three and four-year-old children were considered anemic if their hemoglobin levels were lower than 110 g/L and five-year-old children were considered anemic if their hemoglobin levels were lower than 115 g/L [175]. Anemic children were referred to the health centre for consultation and treatment.

4.8.3. Questionnaire-based covariates

A questionnaire was administered by research assistants to the mother or primary guardian of the participating child at all study time points to obtain information on socio-demographic characteristics and other relevant variables. These variables include: maternal education, maternal occupation, number of people living in the home, material of house, household assets (electricity, radio, television, potable water, toilet/latrine), child's sex, characteristics of the child's birth, child's breastfeeding behaviours and diet, history of child's healthy growth visits, history of child's vaccinations, history of micronutrient supplementation, history of deworming, history of child hospitalizations, and child morbidity in the last two weeks (i.e. Integrated Management of Childhood Illness indicators).

At the one to four years of age study visits, all questionnaire data were collected on paper questionnaires. At the five years of age study visit, questionnaire data were collected on portable

Samsung Galaxy tablets using a custom designed mobile application. The mobile application was specifically designed for global health research data collection and was designed to minimize missing data and transcription errors and increase standardization of study processes among research assistants. All data were collected in the field 'offline' and were later uploaded daily to a central database located on a local server in the research office. During data collection, all collected study data (including consent forms, paper questionnaires, mobile questionnaires and child development tests) were reviewed daily by research coordinators. At the end of every day research coordinators met with each research assistant to review all data collected that day to ensure that the information collected was consistent, accurate and complete.

4.9. Statistical analyses

4.9.1. Data management, descriptive statistics and missing data

Anonymized data were stored on password-protected computers in locked offices. Double data entry was performed for all entered data and data cleaning followed a comparison of the two databases. Summary statistics and graphs were generated for each variable to ensure that all data were within a plausible range. When possible, variables were cross-referenced (e.g. age and date of birth) to ensure consistency. If implausible or inconsistent data arose, the original questionnaires were reviewed.

For all thesis objectives, summary statistics were generated to describe the study population at baseline. Means and standard deviations are presented for continuous variables and counts and proportions are presented for dichotomous and categorical variables. Histograms and scatterplots were generated to observe the distribution of variables included in the analyses.

For all objectives, missing data due to item non-response was minimal because questionnaires were administered by highly trained research assistants and reviewed daily by research coordinators. Missing data are, however, present due to loss to follow-up over time. Both types of missing data were imputed using multiple imputation. Separate imputation models were specified for each analysis model. The imputation models included all covariates in the analysis models and variables that were found to highly predict the missing data.

All analyses were performed in Stata version 13.1, R version 3.0.2 and WinBUGS version 1.4.3.

4.9.2. Manuscript A analyses

Linear regression models were used to determine the effect of the number of times a child was found STH-infected between one and five years of age on child development scores at five years of age. To give equal weight to infection at all years throughout the study period (between one and five years), the number of times a child was found STH-infected was counted using data at five time points: at one, two, three, four and five years of age. Several different models were used to investigate the effect of the number of times a child was found infected with at least one of the three STH species (any STH infection) and species-specific infections (i.e. number of times found infected with *Ascaris* infection, number of times found infected with *Trichuris* infection and number of times found infected with hookworm infection) on the three measures of child development at five years of age (i.e. full scale IQ score, verbal IQ score and performance IQ score). For any STH infection, a child was considered infected at each study timepoint if, at that timepoint, s/he was infected with at least one of the three STH species. Number of times found infected was categorized into six categories: never infected, infected once, infected twice, infected three times, infected four times and infected five times. Due to sparse data in some of the categories (e.g. infected four and five times), these categories were combined. Each category was compared to the reference category of never infected. The categorization of this variable allows for the relationship between the number of times found infected and development scores to be non-linear.

Analyses were adjusted for relevant variables that were found to be common causes of both STH infection and child development. The covariates included in the multivariable models were chosen based on theoretical knowledge (variables that are thought to be associated with both the exposure and outcome of interest without being mediators of this relationship) and statistical criteria. Variables that were considered during model selection included: socioeconomic status (i.e. residential district, urban/rural status, mother's marital status (i.e. married/common-law vs single), maternal education (i.e. completed secondary school), mother employed, father or mother's partner employed, number of people living in the home, house material, cooks using gas, presence of electricity in the home, working radio ownership, working television ownership,

water source, toilet with water and connection to public sewage in the home, and household income); sex; healthcare seeking behavior (i.e. number of healthy growth visits attended from birth to one year of age and vaccines up to date at baseline); hygiene (i.e. number of baths per day and use of soap for bathing); hospitalizations since birth; baseline anthropometry/ malnutrition (i.e. stunted, underweight, wasted, birth weight); baseline development scores (i.e. Bayley-III cognition raw score, Bayley-III receptive language raw score, Bayley-III expressive language raw score and, Bayley-III fine motor raw score); breastfeeding (i.e. exclusively breastfed to six months and continued breastfeeding at one year); and, number of years in preschool by five years of age. Univariable linear regression models were used to identify variables that were associated with the outcome and univariable multinomial regression models were used to identify variables that were associated with the exposure. Correlations and 2 x 2 tables were also used to observe relationships between the confounding variables. The final multivariable models included: socioeconomic status (i.e. maternal education (completed secondary school), cooks using gas and, has a toilet with water and connection to public sewage in the home), baseline nutritional status (i.e. stunted), use of health care (i.e. number of healthy growth visits attended from birth to one year of age), baseline development scores (i.e. Bayley-III cognition score) and number of years in preschool by five years of age.

Bayesian latent class regression models were used to adjust for misclassification of the exposure due to imperfect sensitivity and specificity of the diagnostic techniques used. This analysis allowed for adjustment with individual variation in sensitivity and specificity values (here, due to the fact that, while the majority of stool specimens were analyzed with the Kato-Katz technique, some stool specimens were analyzed with the direct smear technique). This type of analysis is particularly useful in situations where a gold standard test does not exist (as is the case for diagnosing STH infection) and therefore the sensitivity and specificity values are not exactly known. It has been described in detail elsewhere [176, 177] and used previously to correct for misclassification of STH infections [178]. Briefly, in this analysis, three types of models were specified: 1) *The outcome model* is a linear regression model that models child development scores conditional on latent STH infection (i.e. the true, unmeasured exposure) and other relevant covariates; 2) *The exposure models* are logistic regressions that model true latent STH infection status at each study time point, conditional on covariates that predict species-specific STH

infection. These variables were chosen based on a Bayesian information criterion (BIC). The exposure models allow for differences in the probabilities of being STH-infected between various groups of children to be accounted for; and 3) *The misclassification models* model the measured exposure status at each of the five time points according to the latent STH infection status at each time point and the sensitivity and specificity values of the diagnostic technique used (i.e. Kato-Katz technique or direct smear technique) at each time point, according to:

$$P(mSTH_i = 1) = \begin{matrix} S_{KK}(tSTH_i)(KK_i) + (1 - C_{KK})(1 - tSTH_i)(KK_i) + \\ S_{DS}\,(tSTH_i)(1 - KK_i) + \,(1 - C_{DS})(1 - tSTH_i)(1 - KK_i) \end{matrix}$$

Where:

i = study time points

$mSTH_i$ = Measured STH infection status at time point *i* (dichotomous variable)

$tSTH_i$ = Latent, true STH infection status at time point *i* (dichotomous variable)

KK_i= Indicator variable indicating technique used to analyze stool specimen at time point *i* (KK_i= 1 represents use of the Kato-Katz technique; KK_i= 0 represents use of the direct smear technique)

S_{KK}= Sensitivity of the Kato-Katz technique

C_{KK}= Specificity of the Kato-Katz technique

S_{DS}= Sensitivity of the direct smear technique

C_{DS}= Specificity of the direct smear technique

The different sensitivities of the two separate diagnostic techniques are incorporated into the measurement model to account for the lower sensitivity of the direct smear technique compared to the Kato-Katz technique. Diffuse, non-informative priors were used for all regression coefficients in the outcome and exposure models. Informative prior distributions were developed for the sensitivity and specificity values of the two diagnostic techniques, which summarize published estimates of these parameters [123] (Table 6). Clinical priors representing the best summary of the information from past literature and expert opinion, [123] as well as optimistic and pessimistic priors, were specified for sensitivity analyses. Optimistic priors assume higher accuracy for the techniques than the "best estimate" clinical priors would suggest, and similarly, pessimistic priors assume lower accuracy than the clinical priors would suggest. All prior densities on the sensitivity and specificity parameters were assumed to follow a beta

distribution. Although unrealistic, for comparison purposes, analyses were also run assuming that the sensitivity and specificity values were 100%. The three models were jointly estimated using the Gibbs sampler, such that the latent exposure was essentially imputed within the misclassification and exposure models (analogous to missing data techniques) and subsequently modeled in the outcome model.

Table 6: Probability ranges and coefficients of the beta prior densities for the sensitivities and specificities of the Kato-Katz and direct smear techniques used in the Bayesian latent class analyses to adjust for misclassification of STH infection

		Clinical priors			Optimistic priors			Pessimistic priors		
		Range*	Beta distribution coefficients		Range*	Beta distribution coefficients		Range*	Beta distribution coefficients	
			α	β		α	β		α	β
Kato-Katz	*Ascaris*:									
	Sensitivity	0.55-0.75	55.86	29.63	0.70-0.80	214.34	70.81	0.50-0.60	208.45	170.38
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Trichuris:									
	Sensitivity	0.75-0.90	77.85	15.75	0.85-0.95	116.06	12.05	0.70-0.80	214.34	70.81
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Hookworm:									
	Sensitivity	0.52-0.68	85.65	56.78	0.63-0.73	226.28	105.98	0.47-0.57	198.73	183.37
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
Direct smear	*Ascaris*:									
	Sensitivity	0.40-0.60	47.30	47.30	0.55-0.65	220.49	146.68	0.35-0.45	146.68	220.49
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Trichuris:									
	Sensitivity	0.55-0.75	55.86	29.63	0.70-0.80	214.34	70.81	0.50-0.60	208.45	170.38
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Hookworm:									
	Sensitivity	0.35-0.55	42.02	51.57	0.50-0.60	208.45	170.38	0.30-0.40	121.41	226.29
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26

* Prior probability ranges were developed based on previous research [123] and expert opinion.

Sample size. The methodology for performing sample size calculations using Bayesian latent class models to correct for exposure misclassification has not yet been developed. Therefore, sample size calculations were performed for a simplified scenario. A sample size of 880 children was available for analysis. For Aim 1.1, the outcome, full scale IQ score at five years of age, was compared between children who were never infected to children who were infected one, two, three, and four or five times. For Aims 1.2 and 1.3, the same analysis is performed comparing verbal IQ scores and performance IQ scores. Based on expert opinion in child development, a difference in the mean IQ score of 5 points (i.e. 1/3 of a standard deviation) was considered the minimum clinically significant effect size. Assuming that the standard deviation of the full scale, verbal and performance IQ scores in both exposure groups is 15 [172], and assuming that approximately 20% of the population is unexposed (were never STH-infected) and that 20%, 25%, 25% and 10% of the population would have been found STH-infected 1, 2, 3, and 4-5 times, respectively (based on preliminary data, Appendix 1), a sample size of 880 is able to detect a difference of 5 points between each infection category and the never infected category, with a total 95% confidence interval width of 6.28, 5.95, 5.95 and 7.68 for 1, 2, 3 and 4-5 times found infected, respectively (details shown in Appendix 2). Calculations for STH species-specific analyses are also presented in Appendix 2. Note that for an effect size of 5, a total confidence interval width of less than 10 would ensure that the CI would not cross the null value. The sample size, therefore, allowed for a high level of precision for all comparisons (the widest total confidence interval was 9.16). It should be noted that some additional loss of precision was expected in the analysis that adjusted for misclassification of the exposure.

4.9.3. Manuscript B analyses

Hierarchical linear regression models were used to estimate the effect of STH infection during the second year of life on cognitive and verbal composite scores over time (i.e. at two, three, four and five years of age). Measures of each child's cognitive abilities were obtained using the age-scaled cognitive composite score from the Bayley-III at two and three years of age and using the age-scaled performance IQ score (a summary of the block design, matrices and picture concept subscales) from the WPPSI-III at four and five years of age. Measures of each child's verbal abilities were obtained using the age-scaled language composite score from the Bayley-III (a summary of the expressive language and receptive language scores) at two and three years of age

and using the age-scaled verbal IQ score (a summary of the vocabulary, information and word reasoning subscales) from the WPPSI-III at four and five years of age. The primary exposure was the number of times detected infected with any STH infection (i.e. found infected with at least one of the three STH species) and secondary exposures included number of times detected infected with *Ascaris* and number of times detected infected with *Trichuris*. The specific effect of hookworm infection was not assessed due to the very low prevalence of hookworm in this age group. The exposures were categorized into: never infected (reference category), infected once, and, infected two or three times (with the maximum number of times found infected corresponding to the number of study visits between one and two years of age, inclusive). Confounding variables were identified using the same methodology as described for Manuscript A. Analyses were adjusted for relevant confounding variables, including socioeconomic status (i.e. maternal education (completed secondary school), cooks using gas, has a toilet with water and connection to public sewage in the home), baseline nutritional status (i.e. stunted), use of health care (i.e. number of healthy growth visits attended from birth to one year), baseline development scores (i.e. Bayley-III cognition raw score) and age. The hierarchical model accounts for the repeated measures of the outcome on each child (i.e. at two, three, four and five years of age). An individualized intercept term was used to allow each child's baseline development score to be different and an individualized slope for age was used to allow children to develop at different rates. The age variable was centered to reduce posterior correlation between the individualized intercept and slope, in order to aid convergence of the Markov Chain Monte Carlo (MCMC) process.

As described for Manuscript A, Bayesian latent class models were used to adjust for exposure misclassification using the informative priors for the sensitivity and specificity values of the Kato-Katz and direct smear techniques presented in Table 6. For comparison purposes, analyses were also conducted assuming that the sensitivities and specificities of the diagnostic techniques were perfect.

Sample size. A sample size of 880 children with four measures of the outcome per child (i.e. at two, three, four and five years of age) was available for analysis. For Aim 2.1, the outcome, cognitive scores, were compared between children who were never infected, to children who

were found infected once, and to children who were found infected two or three times. For Aim 2.2, the same analysis was performed comparing verbal scores. No estimate of the intraclass correlation coefficient (ICC) for repeated measures of cognitive and verbal abilities of children between two and five years of age was found in the literature. Therefore, based on preliminary data that were available when this study was being conceptualized (i.e. from the one and two years of age visits), an ICC for cognitive ability between one and two years of age was estimated to be 0.2 and that for verbal ability was estimated to be 0.3. This corresponds to a design effect of 1.6 and 1.9 (design effect = 1 + (# observations per child – 1) × ICC) for cognitive and verbal scores, respectively. Assuming that the repeated observations were independent, a total sample size of 3,520 would be available (880 × 4). Taking the correlation between repeated measures into account, the effective sample size is 2,200 (3,520 / 1.6) for the analysis of cognitive scores and 1,852 (3,520 / 1.9) for the analysis of verbal scores. Based on expert opinion in child development, a difference in the mean scores of 5 points (i.e. 1/3 of a standard deviation) is considered the minimum clinically significant effect size. Assuming that the standard deviation of the cognitive and verbal scores is 15, and having known from preliminary data that 43% of the population was unexposed (never STH-infected) and that 36% and 22% of the population were found STH-infected once, and two or three times, respectively, an effective sample size of 2,200 would be able to detect a difference of 5 points in cognitive scores between never infected and infected once, and between never infected and infected two or three times, with total 95% confidence interval widths of 2.84 and 3.34, respectively (Appendix 3). Furthermore, an effective sample size of 1,852 would be able to detect a difference of 5 points in verbal scores between children who were never infected and children who were infected once, and between children who were never infected and children who were found infected two or three times, with a total 95% confidence interval width of 3.09 and 3.64, respectively (Appendix 4). Species-specific calculations are also presented in Appendices 3 and 4. Because the ICC values used here are based on data between one and two years of age only, and therefore, may underestimate the ICC for repeated observations between two and five years of age, sample size calculations were also performed under a worst-case scenario, using an ICC of 0.9 (Appendix 5). Note that, for an effect size of 5, a total confidence interval width of less than 10 would ensure that the CI would not cross the null value. The sample size available, therefore, allows for a high level of precision for all comparisons, including those under the worst-case scenario. Again, it should be

noted that some loss of precision was expected in the analysis adjusted for misclassification of the exposure.

4.9.4. Manuscript C analyses

Iron levels (i.e. mean hemoglobin levels between three and five years of age) and malnutrition (i.e. mean height-for-age z-scores between one and five years of age) were investigated as mediators of the association between the number of detected *Ascaris* infections between one and five years of age and total IQ scores at five years of age using the methodology for assessing multiple mediators when the sequential order of the mediators can by hypothesized [179]. The two directed acyclic graphs (DAGs) used to conceptualize the mediation analysis are presented in Figure 7. *Ascaris* infection was theorized to affect hemoglobin levels, which contribute to malnutrition, which, in turn contributes to child IQ scores. In order to assess the mediators sequentially, the contribution of hemoglobin as a mediator was first assessed according to the DAG in Figure 7a. This allowed for the portion of the effect mediated through hemoglobin levels to be determined [179]. Two regression models were fit: 1.1) a linear regression model, modeling total IQ scores at five years of age (IQ), conditional on the number of times found infected with *Ascaris* between one and five years of age (Asc), mean hemoglobin levels between three and five years of age (M_{hb}), and other covariates (C); and 1.2) a linear regression model, modeling the mean hemoglobin levels between three and five years of age (M_{hb}) conditional on number of times found *Ascaris*-infected (Asc) and other covariates (C):

1.1 $E(IQ|Asc, M_{hb}, C) = \theta_0 + \theta_1 Asc + \theta_2 M_{hb} + \theta_4 C$

1.2 $E(M_{hb}|Asc, C) = \beta_0 + \beta_1 Asc + \beta_2 C$

From these regression models the following decomposed effects were calculated:

1) The natural direct effect (NDE): This is the effect of *Ascaris* infection on IQ scores when hemoglobin levels are fixed to the value they would take when the number of times found *Ascaris* infected is 0 or 1 (i.e. the dashed path in Figure 7a).

$$E(NDE|c) = \theta_1$$

2) The natural indirect effect (NIE): This is the effect on IQ scores of changes in *Ascaris* infection that operate through hemoglobin levels (i.e. the dotted path in Figure 7a). This represents the effect on IQ scores of changing the mediator from what it would

be in the absence of the exposure to what it would be in the presence of the exposure, assuming that the exposure is set to some level.

$$E(NIE|c) = \theta_2\beta_1$$

3) The total effect (TE): This is the overall effect of *Ascaris* infection on IQ scores and is simply the sum of the NDE and NIE (i.e. the dashed and dotted paths in Figure 7a).

$$E(TE|c) = \theta_1 + \theta_2\beta_1$$

To avoid imposing a linear relationship between the number of *Ascaris* infections between one and five years of age and IQ scores, the number of infections was treated as a categorical variable and included in the regression models as dummy variables according to the methodology for including multicategorical independent variables in mediation analyses [180].

Figure 7: Directed acyclic graphs (DAGs) used to conceptualize the mediation analysis

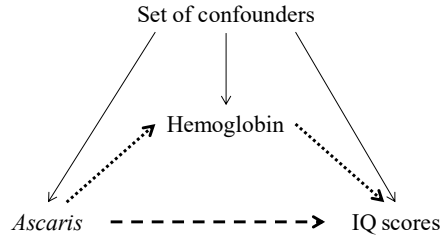


Figure 7a: DAG for analysis only considering hemoglobin as a mediator of the relationship between *Ascaris* infection and IQ scores

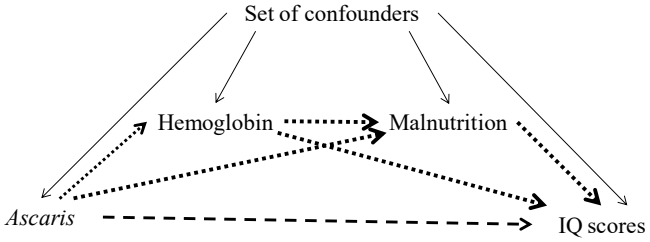


Figure 7b: DAG for analysis considering both hemoglobin levels and malnutrition jointly as mediators of the relationship between *Ascaris* infection and IQ scores

Direct effects Indirect effects

Second, the additional mediating contribution of malnutrition (mean height-for-age z-scores) was assessed according to the DAG in Figure 7b. Three regression models were fit: 2.1) the same linear regression model as 1.1 but also including mean height-for-age z-score as a covariate; 2.2) the same linear regression model as 1.2; and, 2.3) a linear regression model, modeling the mean height-for-age z-scores between one and five years of age (M_{haz}) conditional on number of times found *Ascaris*-infected (Asc) and other covariates (C):

2.1 $E(IQ|Asc, M_{hb}, C) = \theta_0 + \theta_1 Asc + \theta_2 M_{hb} + \theta_3 M_{haz} + \theta_4 C$

2.2 $E(M_{hb}|Asc, C) = \beta_0^i + \beta_1^i Asc + \beta_2^i C$

2.3 $E(M_{haz}|Asc, C) = \beta_0^{ii} + \beta_1^{ii} Asc + \beta_2^{ii} C$

From these regression models, the following decomposed effects were calculated:

1) NDE: This is the effect of *Ascaris* infection on IQ scores when hemoglobin levels and height-for-age z-scores are fixed to the value they would take when the number of times found *Ascaris* infected is 0 or 1 (i.e. the dashed path in Figure 7b).

$$E(NDE|c) = \theta_1$$

2) NIE: This is the effect on IQ scores of changes in *Ascaris* infection that operate through hemoglobin levels and height-for-age z-scores (i.e. the dotted paths in Figure 7b). This represents the effect on IQ scores of changing the mediators from what they would be in the absence of the exposure to what they would be in the presence of the exposure, assuming that the exposure is set to some level.

$$E(NIE|c) = \beta_1^i\theta_2 + \beta_1^{ii}\theta_3$$

3) TE: This remains the overall effect of *Ascaris* infection on IQ scores and is the sum of the NDE and NIE (i.e. the dashed and dotted paths in Figure 7b).

$$E(TE|c) = \theta_1 + \beta_1^i\theta_2 + \beta_1^{ii}\theta_3$$

The covariates included in all regression models (i.e. the vector of C) included all measured confounders of the exposure-outcome relationship (Asc-IQ), the mediator-outcome relationships (M_{hb}-IQ and M_{haz}-IQ) and the exposure-mediator relationships (Asc-M_{hb} and Asc-M_{haz}). This methodology is robust to confounding between the two mediators [179]. Confounding was assessed separately for each relationship, according to the methodology described in Manuscript A. The final set of confounding variables included: socioeconomic status (i.e. maternal education (completed secondary school), cooks using gas, has a toilet with water and connection to public sewage in the home), baseline nutritional status (i.e. stunted), use of health care (i.e. number of healthy growth visits attended from birth to one year of age), baseline development scores (i.e. Bayley-III cognition raw score), number of years in preschool by five years of age and birth weight.

Interaction between the exposure and each mediator as well as interaction between the two mediators was assessed according to the methodology described by VanderWeele [181]. All interaction terms had point estimates with tight confidence intervals surrounding null values and allowing for interaction in the mediation analyses had no effect on the results; therefore, interaction was considered unimportant and subsequently ignored.

All analyses were conducted in the Bayesian framework. Analyses without adjustment for misclassification of *Ascaris* infection were compared to analyses with adjustment for misclassification of *Ascaris* infection using Bayesian latent class regression models, as described in Manuscript A. The latent class regression models were used to obtain exposure (*Ascaris*) misclassification-adjusted estimates of the coefficients in regression models 1.1-1.2 and 2.1-2.3 (above). These adjusted coefficients were used in the formulae to calculate the NDE, NIE and TE, adjusted for exposure misclassification. Diffuse, non-informative priors were used for all regression coefficients. Informative priors were specified for the sensitivity and specificity values of the Kato-Katz and direct smear diagnostic techniques for *Ascaris* infection (Table 6). The models were jointly estimated using the Gibbs sampler.

4.10. Ethics oversight

All research personnel conducted the study in an ethical manner, consistent with international principles of good research practice. During data collection, data were stored at local research facilities in Peru and were only accessible to personnel involved in the study. The original parent study was approved by the Research Ethics Boards of the McGill University Health Centre in Montreal, Canada; the Universidad Peruana Cayetano Heredia in Lima, Peru; and the Instituto Nacional de Salud in Lima, Peru. The original trial was registered with clinicaltrials.gov (NCT01314937). The extended follow-up study was approved by the Research Ethics Boards of the McGill University Health Centre and the Universidad Peruana Cayetano Heredia. (Note that approval from the Instituto Nacional de Salud in Lima, Peru was only necessary for clinical trials being conducted in Peru and therefore, was not necessary for the follow-up cohort study.) Specific approval for the current analyses was obtained from the Research Ethics Board of the McGill University Health Centre. Informed consent was obtained from parents/guardians of the participating children at the initiation of the parent study (for participation between one and two years of age) and again at the beginning of the extended follow-up (for participation between three and five years of age). According to Peruvian ethics guidelines for children participating in clinical trials, informed consent was obtained from both the mother and father of the participating child at the initiation of the parent study. In the event that the child only had one parent, or if either parent was absent for an indefinite period of time, the present parent signed a sworn declaration to declare the absence of the other parent, in accordance with Peruvian ethics

guidelines. For parents under the age of 18 years, assent was obtained and informed consent was requested from their parent or guardian over the age of 18 years.

5. Results

5.1. Preface to Manuscript A

The results of this thesis are summarized in three manuscripts. All manuscripts use data from a longitudinal cohort study conducted in Iquitos, Peru. The first manuscript, Manuscript A, outlines the results of thesis objective 1 (to determine the effect of the number of times found STH-infected between one and five years of age on child development at five years of age). Children were recruited at one year of age and followed-up annually to five years of age. STH infection was measured at all study visits and the number of times found STH-infected at each of the five study visits was summed and used as an indicator of cumulative STH infection. The effect of cumulative *Ascaris*, *Trichuris*, hookworm and any STH infection on total IQ score, verbal IQ score and performance IQ score at five years of age was examined. Analyses were performed under the Bayesian framework, using Bayesian latent class analysis to adjust for exposure misclassification.

Results from this manuscript have been presented at the following scientific conferences:

1. Blouin B, Casapia M, Gyorkos TW. Repeated soil-transmitted helminth infections reduce cognitive development scores in preschool-age children. *American Society for Tropical Medicine and Hygiene 65th Annual Meeting,* Atlanta, GA, November 13-17, 2016.
2. Blouin B, Casapia M, Gyorkos TW. Repeated soil-transmitted helminth infections reduce cognitive development scores in preschool-age children. *Séptima Conferencia Anual ASTMH Latinoamérica en Perú [7th annual Latin American ASTMH conference in Peru],* Lima, Peru, February 21, 2017
3. Blouin B, Casapia M, Gyorkos TW. Impaired child development in the Amazon region of Peru: Describing the current situation and identifying early-life targets for intervention. *10th European Congress on Tropical Medicine and International Health*. Antwerp, Belgium, October 17, 2017.

These results were also presented at a regional meeting organized by the Pan American Health Organization in Peru:

4. Blouin B, Casapia M, Gyorkos TW. Morbilidad atribuida a los helmintos transmitidos por el suelo (HTS): datos de un programa de investigación de largo plazo en el departamento de Loreto [Morbidity attributable to soil-transmitted helminths (STH): data from a long-term research program in Loreto]. *Reunión hacia una estrategia integral de abordaje integral de las geohelmintiasis en el Perú [Meeting towards a comprehensive strategy for integrated geohelminth control in Peru].* Organización Panamericana de la Salud [Pan American Health Organization], Lima, Peru: October 26, 2017.

This manuscript has been accepted for publication in the *International Journal of Epidemiology*. It follows the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting results from cohort studies.

5.2. Manuscript A: The effect of cumulative soil-transmitted helminth infections over time on child development: A four-year longitudinal cohort study in preschool children using Bayesian methods to adjust for exposure misclassification

The effect of cumulative soil-transmitted helminth infections over time on child development:

A four-year longitudinal cohort study in preschool children using Bayesian methods to adjust for exposure misclassification

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ABSTRACT

Background: Limited research has documented an association between soil-transmitted helminth (STH) infections and child development. This has recently been identified as an important knowledge gap.

Methods: A longitudinal cohort study was conducted in Iquitos, Peru between September 2011 and July 2016. A cohort of 880 children, recruited at one year of age, was followed-up to five years. STH infection was measured annually and child development was measured with the Wechsler Preschool and Primary Scale of Intelligence III (WPPSI-III) at five years. Linear regression models were used to investigate the effect of the number of detected STH infections between one and five years of age on WPPSI-III scores at five years of age. Bayesian latent class analysis was used to adjust for exposure misclassification.

Results: A total of 781 (88.8%) children were included in the analysis. In multivariable analysis, adjusted for STH misclassification using clinical prior specifications for the sensitivity of the diagnostic techniques, increasing numbers of *Ascaris*, *Trichuris,* hookworm and any STH infections were associated with lower WPPSI-III scores.

Conclusions: These results document an association between cumulative STH infection and lower child development. Adjusting for STH misclassification was key in providing robust estimates of association and will be essential in future research using data on STH infection. Improving STH diagnostic test parameters is also paramount. STH control in preschool children may contribute to lowering the disease burden associated with poor child development.

Keywords: soil-transmitted helminth, child health, cohort study, epidemiology

KEY MESSAGES

- This research documents a longitudinal association between cumulative STH infection and lower child development scores.
- This is the first longitudinal study conducted in preschool children specifically investigating the effect of STH infection on child development with appropriate adjustment for confounding variables.
- This is the first study regarding STH infection and child development that has adjusted for bias due to STH misclassification.
- This research quantifies the developmental impairment associated with STH infections and highlights the importance of accurate STH diagnostics.
- These results can be used to motivate Ministries of Health in STH-endemic countries to prioritize STH control strategies, including mass deworming campaigns, in preschool children.

INTRODUCTION

The soil-transmitted helminths (STHs) (i.e. *Ascaris lumbricoides*, *Trichuris trichiura* and the two hookworm species, *Ancylostoma duodenale* and *Necator americanus*) are parasites that live in the human gut. A total of 103 countries around the world are endemic for STH infections [1] and approximately two billion people are at risk of infection.[2] Several microscopic techniques are available for diagnosing STH infections from stool specimens; however, no gold standard exists and all techniques are limited by imperfect sensitivities.[3, 4]

It has been proposed that STH infections adversely affect child development primarily due to mediating factors including malnutrition and anemia.[5] Because brain plasticity is greatest during the first few years of life, perturbations during this critical time of development could have long-term effects on brain development.[6] It is therefore likely that the effect of STH infection on child development is more pronounced in preschool children compared to older children.[7] Several observational studies have documented associations between STH infection and child development.[8-20] The quality of this body of evidence, however, is limited by poor study designs,[8-10, 12-19] failure to adjust for critical confounding variables,[8-11, 19, 20] small sample sizes,[10, 15, 18, 20] and inappropriate statistical analyses.[10, 11, 14, 20] Most published research has been conducted in school-age children. No past research has adjusted for STH misclassification due to imperfect sensitivities of the diagnostic techniques used. The need for research to clarify the effects that STH infections may have on child development has recently been emphasized by both the STH and child development scientific communities.[5, 21]

The objective of the current research, therefore, was to determine the effect of the number of times a child was found STH-infected between one and five years of age on total IQ scores, verbal IQ scores and performance IQ scores at five years of age, while adjusting for bias due to STH misclassification.

METHODS

The original data source for this study was a parent randomized controlled trial (RCT) conducted in Iquitos, Peru between September 2011 and July 2013.[22] A population-based sample of 1760 children was recruited and randomized to one of four deworming strategies of different combinations of mebendazole and placebo. These children were followed-up at 18 and 24 months of age. Children were included in the trial regardless of their STH infection status. Following completion of the RCT, follow-up of the same children continued on an annual basis to five years of age. The current study uses data from the original RCT and the follow-up (i.e. from the one, two, three, four and five-year timepoints).

The current study is a longitudinal cohort study. Data collection took place in the rural and peri-urban communities surrounding the city of Iquitos in the Peruvian Amazon. Inclusion and exclusion criteria were specified in the parent RCT.[22] At each annual visit, a questionnaire was administered to the child's primary caregiver; the child's height and weight were measured; the child's cognitive development was assessed; and a stool specimen was obtained.

At the two to five-year visits, all stool specimens were analyzed using the Kato-Katz technique within 24 hours of collection. While this diagnostic technique has near perfect specificity, a

recent meta-analysis found the following sensitivity values from one stool specimen for *Ascaris*, *Trichuris* and hookworm infections, respectively: 63.8% (95% Credible Interval (CrI): 59.1-68.6%), 82.2% (95% CrI: 80.1-84.5%) and 59.5 (95% CrI: 56.9-62.2%).[3] All children were treated with mebendazole at the two-year visit and any child found infected at subsequent visits was referred for treatment.

Stool specimens collected at recruitment (when treatment allocation for the parent trial took place) required special processing because ethical guidelines required any individual found STH-infected to be treated. Due to sufficient clinical equipoise regarding the trial research question, it was deemed acceptable to collect stool specimens from all children participating in the RCT and only immediately analyzing those specimens from children who were randomized to receive deworming using the Kato-Katz technique (this ensured that those found infected would be treated). Stool specimens from children randomized to receive placebo were stored in 10% formalin until they were analyzed using the direct smear technique, following the two-year visit (i.e. when all children were treated). (Note that it is not possible to analyze preserved stool specimens with the Kato-Katz technique.) Recent sensitivity estimates of the direct smear technique for diagnosis of *Ascaris*, *Trichuris* and hookworm infections are: 52.1% (95% CrI: 46.6-57.7%), 62.8% (95% CrI: 56.9-68.9%) and 42.8% (95% CrI: 38.3-48.4%), respectively.[3]

Because no single high-quality scale of child development exists for both one and five-year-old children, two separate scales were used to measure child development in this study. The Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) was used up to three years of age and the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III)

was used at four and five years. These two scales are widely used internationally.[23] Both scales were administered by highly trained research assistants in a secluded area to avoid distractions. All research assistants were nurses or nurse-midwives with a minimum Bachelor's degree education. Research assistants were blinded to group assignment during the trial and were unaware of a child's current STH infection status during follow-up visits. The Bayley-III was administered to all 1760 participants at one and two years of age. For feasibility reasons, at the three, four and five-year visits, child development was measured in a random sample of 880 children.

Ethics

The study was approved by the Universidad Peruana Cayetano Heredia in Lima, Peru; the Instituto Nacional de Salud in Lima, Peru; and, the Research Ethics Board of the McGill University Health Centre in Montreal, Canada. Informed consent was obtained from parents/guardians of the participating children. Assent was obtained for any parent under 18 years, with informed consent requested from their parent or guardian over 18 years.

Statistical analyses

Double data entry was performed and data cleaning followed a comparison of the two databases. Summary statistics were generated to describe the study population at baseline.

Linear regression models were used to investigate the effect of cumulative STH infection on child development. Cumulative STH infection was defined as the number of times a child was found infected between one and five years of age and was categorized as: never infected,

infected once, infected twice, infected three times, infected four times and infected five times. Categories were combined, as necessary, in the case of sparse data. The effect of infection with any of the three STH infections (any STH infection), and with species-specific infections, on full scale IQ score, verbal IQ score and performance IQ score at five years of age was estimated. The covariates included in the multivariable models were chosen based on theoretical knowledge (variables that are thought to be associated with both the exposure and outcome of interest without being mediators of this relationship) and statistical criteria. Variables that were considered during model selection are listed in the Supplementary material. Univariable regression models were used to identify variables that were associated with the outcome and exposure. The final multivariable models included: socioeconomic status (i.e. maternal education (completed secondary school), cooks using gas, and has a toilet with water connected to public sewage in the home), baseline nutritional status (i.e. stunted), use of health care (i.e. number of healthy growth visits attended from birth to one year of age), baseline development scores (i.e. Bayley-III cognition raw scores) and number of years in preschool by five years. Missing exposure data were imputed using multiple imputation (details described in the Supplementary material).

Bayesian latent class linear regression models were used to estimate the exposure effects while adjusting for misclassification of the exposure due to imperfect sensitivity and specificity of the STH diagnostic techniques. This methodology has been fully described previously.[24] A brief description is included in the Supplementary material. Diffuse, non-informative priors were used for all regression coefficients. Informative priors were specified for the sensitivity and specificity values of the two diagnostic techniques (Table 1). Clinical priors, as well as optimistic and

pessimistic priors, were specified for comparison purposes and were assumed to follow a beta distribution. For comparison purposes, analyses were also run assuming that the sensitivity and specificity values were 100%. The prior ranges for the sensitivities and specificities of the direct smear and Kato-Katz techniques were developed based on previous research and expert opinion.[3]

(Table 1 here)

Sample size

A total of 880 of the original 1760 children were randomly sampled for this study. A difference in the mean IQ score of five points was considered the minimum clinically significant effect size. Assuming that the standard deviation of the outcome in all exposure groups would be 15,[23] and assuming that approximately 20% of the population is unexposed (never STH-infected) and that 20%, 25%, 25% and 10% of the population would have been found STH-infected one, two, three, and four or five times, respectively (based on preliminary data), a sample size of 880 is able to detect a difference of five points between each infection category and the never infected category, with a total 95% confidence interval width of 6.28, 5.95, 5.95 and 7.68 points for one, two, three and four-five times found infected, respectively.

Statistical analyses were performed in Stata, version 13.1 and WinBUGS version 1.4.3.

RESULTS

The study flowchart is presented in Figure 1. Of the 880 children, 795 (90.3%) completed the five-year visit and 781 (88.8%) had valid WPPSI-III measurements and were included in this

analysis. (WPPSI-III scores are considered invalid if a participant scores zero on two or more of the verbal or performance subtests.) Most children lost to follow-up moved outside the study area. The exposure variable, cumulative STH infection, was missing for 11 children. Of all stool specimens analysed throughout the study, 10.1% were analysed using the direct smear technique and 89.9% were analysed using the Kato-Katz technique.

Baseline characteristics of the trial and cohort populations were similar (Table 2). STH infection prevalence increased from 12.4% at one year of age to 50.8% at five years of age (Table 3). Hookworm infection was relatively uncommon with a prevalence of 0.34% at baseline and 4.7% at five years. Table 4 shows the frequencies of any STH and species-specific infection at each time point. At five years of age, WPPSI-III scores in this population were low. The mean (± sd) total IQ, verbal IQ and performance IQ scores were 77.1 (± 10.8), 77.0 (± 11.8) and 81.1 (± 11.9), respectively. At five years of age, the proportion of children considered to have below average development was 87.8% and the proportion of children considered to have extremely low development was 20.6%.

(Table 2 here)

(Table 3 here)

(Table 4 here)

In univariable analyses, children with increasing numbers of detected STH infections had lower total IQ scores (Table 5), verbal IQ scores (Table 6) and performance IQ scores (Table 7). In multivariable analyses, effects were attenuated (Table 5-7). The largest effect sizes were observed in the associations of any STH infection with verbal IQ scores. Compared to children

who were never found infected with any STH infection, the adjusted difference (β (95% confidence interval (CI))) in verbal IQ scores was: -1.64 (-4.00, 0.72) for children found infected one time; -3.31 (-5.71, -0.90) for children found infected two times, -4.22 (-6.77, -1.68) for children found infected three times; and, -2.57 (-5.24, 0.10) for children found infected four or five times. The effects for hookworm infection on IQ scores were inconclusive (Tables 5-7).

(Table 5 here)

(Table 6 here)

(Table 7 here)

Results from the Bayesian latent class analyses adjusted for misclassification of STH infection and confounding are presented in Table 8. For all species and for any STH infection, the misclassification adjusted results shift away from the null, suggesting that misclassification led to bias towards the null. Infection by all STH species was found to lead to lower developmental scores. In the analyses using clincal sensitivity and specificity priors (see Table 1), cumulative *Ascaris*, *Trichuris*, hookworm and any STH infection were associated with lower total IQ scores; cumulative *Ascaris*, *Trichuris*, and any STH infection were associated with lower verbal IQ scores; and, cumulative *Ascaris*, *Trichuris*, and any STH infection were associated with lower performance IQ scores. The largest effects found were for effects of any STH infection on verbal IQ score (β (95% Credible Interval (CrI)) for two, three and four or five detected infections compared to zero or one infections: -9.42 (-15.46, -3.04), -8.58 (-13.75, -3.90), and, -7.59 (-12.81, -2.75), respectively) and, for effects of *Ascaris* infection on verbal IQ score (β (95% CrI)) for two, three and four or five detected infections compared to zero or one infections: -8.27 (-13.85, -3.10), -6.69 (-12.05, -2.05), and, -5.06 (-10.75, 0.05), respectively). The

misclassification-adjusted results using optimistic and pessimistic priors for sensitivity of the Kato-Katz and direct smear techniques show how dependent the results are on these prior specifications. In general, assuming that the sensitivities of the diagnostic techniques are poorer led to larger main effect sizes.

(Table 8 here)

DISCUSSION

The results document an association between cumulative STH infection and lower child development in a cohort of preschool children. From the STH misclassification-adjusted results, infection by each STH species was associated with lower IQ scores, with *Ascaris* infection having the largest effect. Cumulative *Ascaris*, *Trichuris* and any STH infections were found to be associated with lower total, verbal and performance IQ scores. Hookworm infection was found to be associated with lower total IQ scores but its effect on verbal and performance IQ scores was inconclusive. The relatively wide 95% credible intervals for these effects, however, lead to some ambiguity regarding the exact size and clinical significance of these effects. In this study, we chose to look at the effect of the number of detected STH infections over a long time period instead of looking at one infection at one single time point, as in previous studies. It was consistently found that the largest effect on IQ scores was for being found infected two or three times. Compared to children who were never found infected or who were found infected one time, children found infected two or three times had IQ scores that ranged, on average, from one to nine points lower, depending on the STH species and the IQ construct.

Low WPPSI-III scores were found with over 85% of children scoring in the below average range. These results are consistent with regional indicators (e.g. poverty, malnutrition and illiteracy rates);[25] however, while the Spanish version of the WPPSI-III was validated in a Mexican population and used previously in Peru,[26] it has not been previously validated in this specific population and generalizability may be limited.

The results inform the current debate surrounding the benefits of deworming on child health. A recent Cochrane Review investigating the effect of deworming on cognitive development in children concluded that deworming was unlikely to have an effect on cognitive development.[27] Several researchers and public health experts have challenged this review [28] and one of the specific criticisms is that many of the reviewed trials only provided a single dose of deworming. Considering the high probability of rapid re-infection following treatment, single dose deworming is likely insufficient to produce an important effect on a potentially chronic condition.[28] While these results do not directly address the effect of deworming, they do show that a single STH infection at one time point may be unlikely to have an important long-term effect on child development and that recurrent infections over time may have important effects. Therefore, to obtain an accurate understanding of the true burden of STH infection, future research must focus on the health effects of long-term infection and of repeated doses of deworming provided over several years.

This study is unique. A large cohort of children recruited at one year of age was followed to five years, with annual measurements of STH infection and child development. This allowed for cumulative STH infections over time to be investigated, a unique and possibly more relevant

exposure that has not previously been studied. Additionally, to our knowledge, no previous study investigating the effect of STH infection on child development has adjusted for misclassification of STH infection. Since no gold standard diagnostic technique exists, all research conducted on STH infections suffers from such misclassification bias.

The results also show that the magnitude of the misclassification of STH infection can have an important effect on the adjusted results. There is little agreement regarding the sensitivity values of the Kato-Katz and other STH diagnostic techniques in the literature with different publications reporting different sensitivity values.[3, 4] The results highlight that it is important that additional research be conducted to determine the true sensitivity of the Kato-Katz and other STH diagnostic techniques.

This study has limitations. First, the exposure, cumulative STH infection over time, is a simple proxy measure. Ideally, stool specimens would be collected daily and the total amount of time that each child was STH-infected would be calculated. This is obviously infeasible. We believe, however, that the number of times detected infected annually is a reasonable estimate of the relative burden of STH infection between one and five years of age. Second, this is an observational study and, therefore, residual confounding may explain some of the observed associations. Such confounding, however, would need to be strong to explain the full magnitude of the observed associations.

CONCLUSION

These results provide important empirical evidence regarding the effect of STH infection on child development. It has been shown that, in preschool children, an association exists between cumulative STH infections up to five years of age, and lower development scores. The results also highlight the importance of adjusting for STH misclassification. Additional research on diagnostic technique parameters would enable researchers to obtain more precise and conclusive results from analyses adjusted for STH misclassification. The results, however, do suggest that prioritizing preschool children for STH interventions including deworming could lead to life-long improvements in cognition and may ultimately contribute to improving productivity in adulthood and reducing poverty levels in STH-endemic countries.

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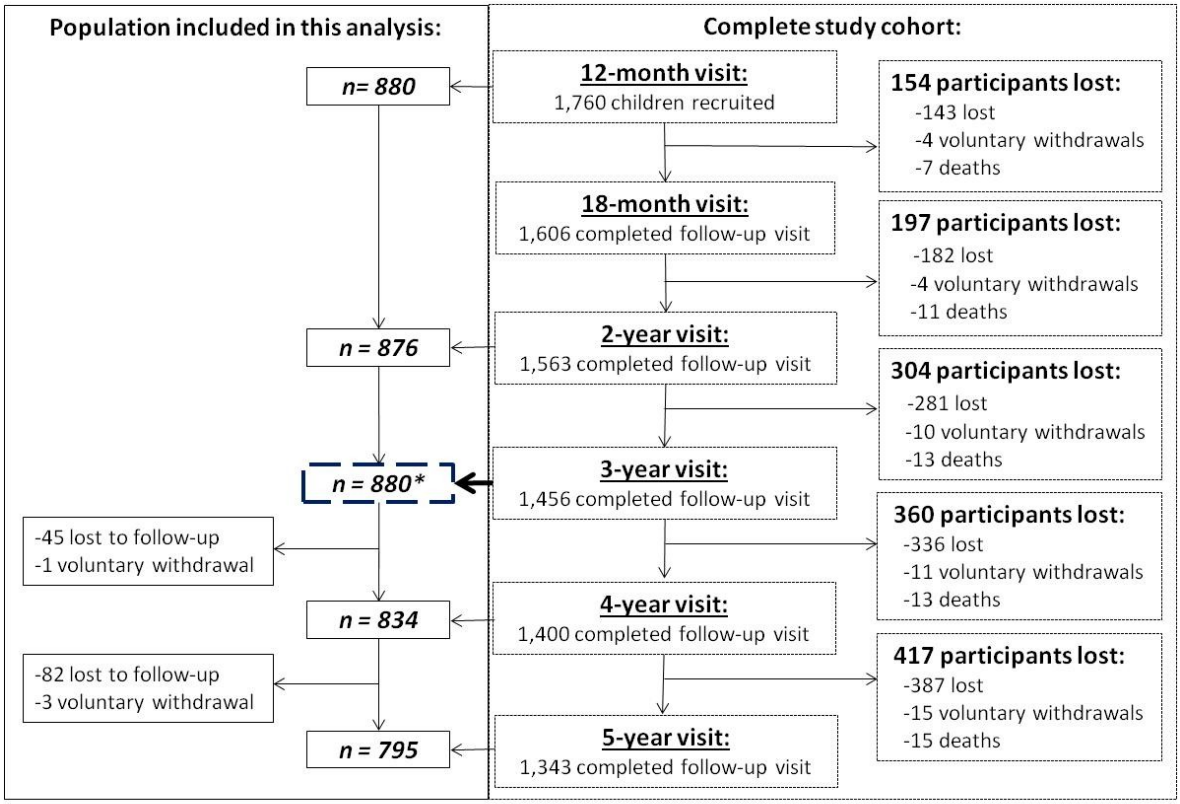
Figure 1: Study flowchart showing the entire study population from the parent trial (N=1760) and the random sample included in the follow-up study (n=880), Iquitos, Peru, September 2011 to July 2016.



* Random selection of the 880 participants who would complete the Bayley-III and WPPSI-III at 3, 4 and 5 years occurred at the 3-year visit

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Table 1: Probability ranges and coefficients of the beta prior densities for the sensitivities and specificities of the Kato-Katz and direct smear techniques used in the Bayesian latent class analyses to adjust for misclassification of STH infection

		Clinical priors			Optimistic priors			Pessimistic priors		
		Range*	Beta distribution coefficients		Range*	Beta distribution coefficients		Range*	Beta distribution coefficients	
			α	β		α	β		α	β
Kato-Katz	*Ascaris*:									
	Sensitivity	0.55-0.75	55.86	29.63	0.70-0.80	214.34	70.81	0.50-0.60	208.45	170.38
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Trichuris:									
	Sensitivity	0.75-0.90	77.85	15.75	0.85-0.95	116.06	12.05	0.70-0.80	214.34	70.81
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Hookworm:									
	Sensitivity	0.52-0.68	85.65	56.78	0.63-0.73	226.28	105.98	0.47-0.57	198.73	183.37
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
Direct smear	*Ascaris*:									
	Sensitivity	0.40-0.60	47.30	47.30	0.55-0.65	220.49	146.68	0.35-0.45	146.68	220.49
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Trichuris:									
	Sensitivity	0.55-0.75	55.86	29.63	0.70-0.80	214.34	70.81	0.50-0.60	208.45	170.38
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Hookworm:									
	Sensitivity	0.35-0.55	42.02	51.57	0.50-0.60	208.45	170.38	0.30-0.40	121.41	226.29
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26

* Prior probability ranges were developed based on previous research [3] and expert opinion.

Table 2: Baseline characteristics of the entire study population (N=1760) at 12 months of age and of the 880 children randomly selected for the follow-up study, Iquitos, Peru (between September 2011 and July 2016)

	Random sample [Mean or % (SD or n)]	Entire cohort [Mean or % (SD or n)]
N	880	1760
Child characteristics:		
Sex (males) [%, n]	51.9 (457)	52.3 (920)
Age (months) [mean, sd]	12.1 (0.28)	12.1 (0.30)
Birth weight (kg) [mean, sd]	3.13 (0.48)[a]	3.14 (0.47)[b]
Number of healthy growth visits from birth to 1 year of age [mean, sd]	7.6 (3.5)	7.6 (3.5)
Vaccinations up to date at 11 months of age [%, n][c]	74.3 (652)[d]	73.8 (1296)[e]
Taking iron supplementation [%, n]	35.7 (313)[f]	33.1 (583)[g]
Ever received deworming [%, n]	0.0 (0)[h]	0.0 (0)[i]
Hospitalized in the past [%, n]	9.6 (84)	9.3 (164)
Walks without support [%, n]	24.6 (216)	24.6 (433)[j]
Stunted [%, n]	24.0 (211)	24.2 (425)
Underweight [%, n]	7.5 (66)	8.6 (151)
Wasted [%, n]	1.7 (15)	2.3 (40)
Bayley-III[l]: cognition raw score [mean, sd]	42.6 (3.0)	42.5 (3.0)
Bayley-III [l]: receptive language raw score [mean, sd]	13.0 (1.6)	12.9 (1.6)
Bayley-III[l]: expressive language raw score [mean, sd]	13.6 (2.2)	13.5 (2.1)
Bayley-III[l]: fine motor raw score [mean, sd]	29.2 (1.5)	29.2 (1.5)
Maternal characteristics:		
Maternal age (years) [mean, sd]	27.1 (7.1)	26.5 (7.1)
Married or common-law [%, n]	81.9 (721)	80.9 (1423)
Maternal education		
Completed primary school [%, n]	85.7 (754)	85.9 (1511)
Completed secondary school [%, n]	32.5 (286)	31.1 (547)
Mother is employed [%, n]	18.7 (164)[j]	17.5 (307)[d]
Household characteristics:		
Number of persons living in the home [mean, sd]	6.6 (2.7)	6.6 (2.7)
Wood/earth house material [%, n]	75.7 (666)	76.9 (1354)
Cooks using:		
Gas [%, n]	31.4 (276)	31.5 (555)
Charcoal [%, n]	28.1 (247)	26.6 (468)
Firewood [%, n]	40.1 (353)	41.5 (730)
Other [%, n]	0.5 (4)	0.4 (7)
Electric energy in the home [%, n]	91.0 (801)	90.7 (1596)

Owns a working radio [%, n]	53.2 (468)	51.0 (897)
Owns a working television [%, n]	75.0 (660)	74.2 (1305)
Presence of running water in the home [%, n]	51.3 (451)	51.0 (898)
Type of toilet in the home		
Latrine or pit without drainage [%, n]	42.3 (372)	41.6 (732)
Toilet with water and connection to public sewage [%, n]	43.6 (384)	42.9 (755)
Latrine or silo with drainage [%, n]	10.0 (88)	11.5 (203)
Other [%, n][k]	4.1 (36)	4.0 (70)

[a] Missing data for 79 participants
[b] Missing data for 166 participants
[c] Up-to-date vaccinations includes having received all of the following vaccines: one dose of Bacille Calmette-Guérin (BCG), one dose of hepatitis B, three doses of polio, three doses of pentavalent, two doses of rotavirus and two doses of pneumococcal
[d] Missing data for two participants
[e] Missing data for three participants
[f] Missing data for four participants (respondent did not know)
[g] Missing data for five participants (respondent did not know)
[h] Missing data for 15 participants (respondent did not know)
[i] Missing data for 20 participants (respondent did not know)
[j] Missing data for one participant
[k] Other includes: Directly in river, Open-air, Septic tank, Other
[l] Bayley-III: Bayley Scales of Infant and Toddler Development, Third Edition

Table 3: STH prevalence and intensity at each study time point, in preschool children in Iquitos, Peru, September 2011 to July 2016.

	1 year [a]	2 years [b]	3 years [b]	4 years [b]	5 years [b]
Ascaris					
Prevalence [% (n)]	11.0 (97)[c]	30.7 (269)[e]	30.6 (269)[c]	28.3 (236)[f]	28.2 (224)[g]
Intensity (epg[h]) [mean (sd)]	329.9 (1416.6)[d]	2,304.2 (11 987.5)[e]	2,009.9 (8566.4)[c]	3,468.4 (12 923.5)[f]	6,157.3 (23 596.1)[g]
Prevalence of moderate/ heavy intensity infection [% (n)]	2.0 (9)[d]	9.6 (84)[e]	8.1 (71)[c]	13.3 (111)[f]	16.6 (132)[g]
Trichuris					
Prevalence [% (n)]	2.5 (22)[c]	21.8 (191)[e]	26.5 (233)[c]	34.4 (286)[f]	38.6 (307)[g]
Intensity (epg[h]) [mean (sd)]	26.5 (261.3)[d]	46.7 (225.5)[e]	66.4 (265.2)[c]	171.5 (1214.6)[f]	340.3 (1293.1)[g]
Prevalence of moderate/ heavy intensity infection [% (n)]	0.7 (3)[d]	0.7 (6)[e]	1.3 (11)[c]	2.8 (23)[f]	7.8 (62)[g]
Hookworm					
Prevalence [% (n)]	0.34 (3)[c]	1.37 (12)[e]	1.7 (15)[c]	2.8 (23)[f]	4.7 (37)[g]
Intensity (epg[h]) [mean (sd)]	1.7 (26.8)[d]	1.4 (17.6)[e]	1.25 (13.3)[c]	7.9 (72.3)[f]	9.8 (66.9)[g]
Prevalence of moderate/ heavy intensity infection [% (n)]	0 (0)[d]	0 (0)[e]	0 (0)[c]	0 (0)[f]	0 (0)[g]
Any STH					
Prevalence [% (n)]	12.4 (109)[c]	41.1 (360)[e]	42.7 (376)[c]	48.1 (401)[f]	50.8 (404)[g]

[a] At the one year of age visit, 449 (51%) stool specimens were analyzed using the Kato-Katz technique and 431 (49%) stool specimens were analyzed using the direct smear technique
[b] At the two to five years of age visits, all available stool specimens were analyzed using the Kato-Katz technique
[c] Data available for all 880 participants
[d] Data available for 449 participants (who had their stool specimen analyzed with the Kato-Katz technique)
[e] Data available for 876 participants
[f] Data available for 833 participants
[g] Data available for 795 participants
[h] Eggs per gram of stool

Table 4: Frequency of detected STH infections between one and five years of age for the 784 participants with complete STH data included in this analysis, Iquitos, Peru, September 2011 to July 2016.

	Ascaris	*Trichuris*	Hookworm	Any STH
No detected infections [% (n)]	31.4 (246)	40.6 (318)	91.5 (717)	19.9 (156)
One detected infection [% (n)]	31.1 (244)	22.5 (176)	6.9 (54)	21.7 (170)
Two detected infections [% (n)]	20.0 (157)	16.6 (130)	1.0 (8)	21.9 (172)
Three detected infections [% (n)]	12.2 (96)	12.4 (97)	0.6 (5)	18.8 (147)
Four detected infections [% (n)]	4.2 (33)	7.3 (57)	0.0 (0)	13.7 (107)
Five detected infections [% (n)]	1.0 (8)	0.8 (6)	0.0 (0)	4.1 (32)

*Complete data available for 784 participants

Table 5: Univariable and multivariable linear regression results for the effect of cumulative *Ascaris* infection, cumulative *Trichuris* infection, cumulative hookworm infection and cumulative any STH infection on Total IQ score, in preschool children in Iquitos, Peru, September 2011 to July 2016.

	Univariable [a] β (95% CI)	Multivariable with multiple imputation [b] β (95% CI)
# times found infected with *Ascaris*:		
0	REF	REF
1	-3.56 (-5.44, -1.69)	-1.45 (-3.19, 0.30)
2	-5.78 (-7.90, -3.66)	-2.50 (-4.50, -0.51)
3	-6.81 (-9.30, -4.32)	-3.05 (-5.40. -0.70)
4-5	-6.22 (-9.69, -2.75)	-0.89 (-4.13, 2.36)
Mother completed secondary school	7.95 (6.44, 9.47)	4.81 (3.24, 6.38)
Cooks using gas	6.03 (4.46, 7.60)	2.22 (0.64, 3.80)
Toilet with water and connection to public sewage in the home	5.81 (4.34, 7.29)	2.43 (0.92, 3.94)
Stunted at 1 year	-4.29 (-6.03, -2.56)	-2.48 (-4.09, -0.88)
Bayley-III[c] cognition raw score at 1 year	0.55 (0.31, 0.80)	0.28 (0.05, 0.51)
# healthy growth visits from birth to 1 year of age	0.58 (0.37, 0.79)	0.23 (0.04, 0.43)
# years in preschool by 5 years	4.05 (2.93, 5.17)	2.14 (1.05, 3.23)
# times found infected with *Trichuris*:		
0	REF	REF
1	-3.35 (-5.29, -1.41)	-1.40 (-3.20, 0.41)
2	-4.88 (-7.04, -2.71)	-2.08 (-4.13, -0.03)
3	-7.45 (-9.83, -5.07)	-3.19 (-5.48, -0.90)
4-5	-6.15 (-8.98, -3.33)	-1.62 (-4.34, 1.10)
Mother completed secondary school	7.95 (6.44, 9.47)	4.79 (3.23, 6.35)
Cooks using gas	6.03 (4.46, 7.60)	2.27 (0.69, 3.86)
Toilet with water and connection to public sewage in the home	5.81 (4.34, 7.29)	2.19 (0.65, 3.73)
Stunted at 1 year	-4.29 (-6.03, -2.56)	-2.46 (-4.08, -0.85)
Bayley-III[c] cognition raw score at 1 year	0.55 (0.31, 0.80)	0.29 (0.06, 0.52)
# healthy growth visits from birth to 1 year of age	0.58 (0.37, 0.79)	0.23 (0.03, 0.43)
# years in preschool by 5 years	4.05 (2.93, 5.17)	2.06 (0.98, 3.14)
# times found infected with hookworm:		
0	REF	REF
1-3	-3.49 (-6.20, -0.78)	-1.92 (-4.35, 0.51)
Mother completed secondary school	7.95 (6.44, 9.47)	5.03 (3.47, 6.58)
Cooks using gas	6.03 (4.46, 7.60)	2.34 (0.76, 3.93)
Toilet with water and connection to	5.81 (4.34, 7.29)	2.80 (1.30, 4.30)

public sewage in the home		
Stunted at 1 year	-4.29 (-6.03, -2.56)	-2.59 (-4.19, -0.98)
Bayley-III[c] cognition raw score at 1 year	0.55 (0.31, 0.80)	0.28 (0.05, 0.51)
# healthy growth visits from birth to 1 year of age	0.58 (0.37, 0.79)	0.25 (0.06, 0.45)
# years in preschool by 5 years	4.05 (2.93, 5.17)	2.09 (1.01, 3.18)
# times found infected with any STH:		
0	REF	REF
1	-2.26 (-4.54, 0.03)	-0.83 (-2.95, 1.28)
2	-5.39 (-7.68, -3.10)	-2.48 (-4.62, -0.33)
3	-7.11 (-9.49, -4.73)	-3.14 (-5.41, -0.86)
4-5	-7.23 (-9.66, -4.86)	-1.85 (-4.24, 0.54)
Mother completed secondary school	7.95 (6.44, 9.47)	4.75 (3.18, 6.32)
Cooks using gas	6.03 (4.46, 7.60)	2.26 (0.68, 3.85)
Toilet with water and connection to public sewage in the home	5.81 (4.34, 7.29)	2.35 (0.82, 3.88)
Stunted at 1 year	-4.29 (-6.03, -2.56)	-2.45 (-4.06, -0.84)
Bayley-III[c] cognition raw score at 1 year	0.55 (0.31, 0.80)	0.28 (0.05, 0.50)
# healthy growth visits from birth to 1 year of age	0.58 (0.37, 0.79)	0.24 (0.04, 0.44)
# years in preschool by 5 years	4.05 (2.93, 5.17)	2.10 (1.02, 3.18)

[a] Complete data available for 770 participants
[b] 781 participants were included in the analyses (complete data were available for 770 participants and number of times found infected with any STH infection and species- specific infections was imputed for 11 participants with missing data)
[c] Bayley III: Bayley Scales of Infant and Toddler Development, Third Edition

Table 6: Univariable and multivariable linear regression results for the effect of cumulative *Ascaris* infection, cumulative *Trichuris* infection, cumulative hookworm infection and cumulative any STH infection on Verbal IQ score, in preschool children in Iquitos, Peru, September 2011 to July 2016.

		Univariable [a] β (95% CI)	Multivariable with multiple imputation [b] β (95% CI)
# times found infected with *Ascaris*			
	0	REF	REF
	1	-5.30 (-7.36, -3.24)	-3.20 (-5.15, -1.25)
	2	-6.50 (-8.83, -4.17)	-3.21 (-5.46, -0.97)
	3	-7.17 (-9.91, -4.44)	-3.40 (-6.01, -0.78)
	4-5	-6.14 (-9.96, -2.33)	-0.84 (-4.47, 2.78)
Mother completed secondary school		7.67 (5.98, 9.36)	4.29 (2.53, 6.04)
Cooks using gas		6.28 (4.55, 8.00)	2.57 (0.80, 4.34)
Toilet with water and connection to public sewage in the home		5.78 (4.15, 7.41)	2.26 (0.58, 3.95)
Stunted at 1 year		-4.69 (-6.60, -2.79)	-2.79 (-4.58, -0.99)
Bayley-III[c] cognition raw score at 1 year		0.67 (0.40, 0.94)	0.40 (0.15, 0.65)
# healthy growth visits from birth to 1 year of age		0.57 (0.34, 0.80)	0.24 (0.02, 0.46)
# years in preschool by 5 years		3.67 (2.43, 4.91)	1.81 (0.59, 3.03)
# times found infected with *Trichuris*			
	0	REF	REF
	1	-3.08 (-5.23, -0.94)	-1.18 (-3.21, 0.84)
	2	-4.69 (-7.09, -2.30)	-1.99 (-4.30, 0.32)
	3	-7.44 (-10.07, -4.81)	-3.35 (-5.94, -0.75)
	4-5	-6.69 (-9.81, -3.57)	-2.19 (-5.24, 0.87)
Mother completed secondary school		7.67 (5.98, 9.36)	4.40 (2.64, 6.15)
Cooks using gas		6.28 (4.55, 8.00)	2.61 (0.83, 4.39)
Toilet with water and connection to public sewage in the home		5.78 (4.15, 7.41)	2.06 (0.32, 3.79)
Stunted at 1 year		-4.69 (-6.60, -2.79)	-2.76 (-4.58, -0.94)
Bayley-III[c] cognition raw score at 1 year		0.67 (0.40, 0.94)	0.41 (0.16, 0.67)
# healthy growth visits from birth to 1 year of age		0.57 (0.34, 0.80)	0.24 (0.01, 0.46)
# years in preschool by 5 years		3.67 (2.43, 4.91)	1.65 (0.44, 2.87)
# times found infected with hookworm			
	0	REF	REF
	1-3	-3.18 (-6.16, -0.20)	-1.37 (-4.11, 1.37)
Mother completed secondary school		7.67 (5.98, 9.36)	4.64 (2.89, 6.39)
Cooks using gas		6.28 (4.55, 8.00)	2.75 (0.97, 4.53)
Toilet with water and connection to		5.78 (4.15, 7.41)	2.69 (1.01, 4.38)

public sewage in the home			
Stunted at 1 year		-4.69 (-6.60, -2.79)	-2.92 (-4.73, -1.12)
Bayley-III[c] cognition raw score at 1 year		0.67 (0.40, 0.94)	0.40 (0.15, 0.66)
# healthy growth visits from birth to 1 year of age		0.57 (0.34, 0.80)	0.26 (0.04, 0.48)
# years in preschool by 5 years		3.67 (2.43, 4.91)	1.72 (0.50, 2.94)
# times found infected with any STH			
	0	REF	REF
	1	-2.96 (-5.48, -0.45)	-1.64 (-4.00, 0.72)
	2	-6.13 (-8.65, -3.61)	-3.31 (-5.71, -0.90)
	3	-7.99 (-10.61, -5.38)	-4.22 (-6.77, -1.68)
	4-5	-7.81 (-10.45, -5.16)	-2.57 (-5.24, 0.10)
Mother completed secondary school		7.67 (5.98, 9.36)	4.26 (2.50, 6.01)
Cooks using gas		6.28 (4.55, 8.00)	2.59 (0.82, 4.37)
Toilet with water and connection to public sewage in the home		5.78 (4.15, 7.41)	2.17 (0.46, 3.89)
Stunted at 1 year		-4.69 (-6.60, -2.79)	-2.72 (-4.52, -0.92)
Bayley-III[c] cognition raw score at 1 year		0.67 (0.40, 0.94)	0.40 (0.14, 0.65)
# healthy growth visits from birth to 1 year of age		0.57 (0.34, 0.80)	0.24 (0.02, 0.46)
# years in preschool by 5 years		3.67 (2.43, 4.91)	1.65 (0.44, 2.86)

[a] Complete data available for 770 participants
[b] 781 participants were included in the analyses (complete data were available for 770 participants and number of times found infected with any STH infection and species- specific infections was imputed for 11 participants with missing data)
[c] Bayley III: Bayley Scales of Infant and Toddler Development, Third Edition

Table 7: Univariable and multivariable linear regression results for the effect of cumulative *Ascaris* infection, cumulative *Trichuris* infection, cumulative hookworm infection and cumulative any STH infection on Performance IQ score, in preschool children in Iquitos, Peru, September 2011 to July 2016.

	Univariable [a] β (95% CI)	Multivariable with multiple imputation [b] β (95% CI)
# times found infected with *Ascaris:*		
0	REF	REF
1	-2.35 (-4.43, -0.27)	-0.18 (-2.17, 1.80)
2	-5.56 (-7.91, -3.20)	-2.29 (-4.58, -0.003)
3	-6.09 (-8.86, -3.33)	-2.53 (-5.21, 0.14)
4-5	-6.08 (-9.93, -2.23)	-1.18 (-4.98, 2.61)
Mother completed secondary school	8.10 (6.41, 9.79)	5.27 (3.49, 7.06)
Cooks using gas	5.72 (3.97, 7.46)	1.93 (0.13, 3.73)
Toilet with water and connection to public sewage in the home	5.93 (4.29, 7.56)	2.74 (1.02, 4.45)
Stunted at 1 year	-3.36 (-5.29, -1.43)	-1.66 (-3.49, 0.17)
Bayley-III[c] cognition raw score at 1 year	0.43 (0.16, 0.71)	0.16 (-0.09, 0.42)
# healthy growth visits from birth to 1 year of age	0.59 (0.36, 0.82)	0.26 (0.03, 0.48)
# years in preschool by 5 years	3.80 (2.55, 5.05)	1.82 (0.58, 3.06)
# times found infected with *Trichuris*		
0	REF	REF
1	-3.93 (-6.09, -1.78)	-1.92 (-3.98, 0.14)
2	-5.12 (-7.52, -2.71)	-2.46 (-4.80, -0.12)
3	-6.89 (-9.52, -4.25)	-2.78 (-5.38, -0.17)
4-5	-5.30 (-8.43, -2.17)	-0.77 (-3.89, 2.35)
Mother completed secondary school	8.10 (6.41, 9.79)	5.19 (3.41, 6.97)
Cooks using gas	5.72 (3.97, 7.46)	2.03 (0.23, 3.84)
Toilet with water and connection to public sewage in the home	5.93 (4.29, 7.56)	2.51 (0.76, 4.26)
Stunted at 1 year	-3.36 (-5.29, -1.43)	-1.64 (-3.48, 0.20)
Bayley-III[c] cognition raw score at 1 year	0.43 (0.16, 0.71)	0.17 (-0.08, 0.43)
# healthy growth visits from birth to 1 year of age	0.59 (0.36, 0.82)	0.26 (0.04, 0.49)
# years in preschool by 5 years	3.80 (2.55, 5.05)	1.78 (0.55, 3.01)
# times found infected with hookworm		
0	REF	REF
1-3	-3.35 (-6.33, -0.37)	-2.27 (-5.05, 0.50)
Mother completed secondary school	8.10 (6.41, 9.79)	5.42 (3.65, 7.19)
Cooks using gas	5.72 (3.97, 7.46)	2.03 (0.23, 3.82)
Toilet with water and connection to	5.93 (4.29, 7.56)	3.07 (1.36, 4.77)

public sewage in the home		
Stunted at 1 year	-3.36 (-5.29, -1.43)	-1.72 (-3.55, 0.11)
Bayley-III[c] cognition raw score at 1 year	0.43 (0.16, 0.71)	0.17 (-0.09, 0.43)
# healthy growth visits from birth to 1 year of age	0.59 (0.36, 0.82)	0.28 (0.05, 0.50)
# years in preschool by 5 years	3.80 (2.55, 5.05)	1.80 (0.57, 3.04)
# times found infected with any STH		
0	REF	REF
1	-2.01 (-4.55, 0.53)	-0.58 (-3.00, 1.84)
2	-5.48 (-8.02, -2.93)	-2.71 (-5.17, -0.25)
3	-6.26 (-8.90, -3.62)	-2.37 (-4.97, 0.24)
4-5	-6.63 (-9.30, -3.96)	-1.28 (-4.01, 1.45)
Mother completed secondary school	8.10 (6.41, 9.79)	5.20 (3.41, 6.99)
Cooks using gas	5.72 (3.97, 7.46)	2.01 (0.21, 3.82)
Toilet with water and connection to public sewage in the home	5.93 (4.29, 7.56)	2.68 (0.93, 4.42)
Stunted at 1 year	-3.36 (-5.29, -1.43)	-1.62 (-3.45, 0.22)
Bayley-III[c] cognition raw score at 1 year	0.43 (0.16, 0.71)	0.16 (-0.10, 0.42)
# healthy growth visits from birth to 1 year of age	0.59 (0.36, 0.82)	0.27 (0.05, 0.50)
# years in preschool by 5 years	3.80 (2.55, 5.05)	1.88 (0.65, 3.11)

[a] Complete data available for 770 participants
[b] 781 participants were included in the analyses (complete data were available for 770 participants and number of times found infected with any STH infection and species- specific infections was imputed for 11 participants with missing data)
[c] Bayley III: Bayley Scales of Infant and Toddler Development, Third Edition

Table 8: Results from Bayesian Latent Class models adjusted for misclassification due to imperfect sensitivity and specificity of the Kato-Katz and direct smear techniques for identifying STH infection, in preschool children (n=781) in Iquitos, Peru, September 2011 to July 2016.

	Perfect sensitivity/ specificity β (95% CrI)	Clinical priors for sensitivity/ specificity β (95% CrI)	Optimistic priors for sensitivity/ specificity β (95% CrI)	Pessimistic priors for sensitivity/ specificity β (95% CrI)
1) Outcome: Total IQ Score				
# times found infected with *Ascaris:*				
0-1	REF	REF	REF	REF
2	-1.83 (-3.58, -0.07)	-5.50 (-9.92, -1.32)	-3.68 (-6.57, -0.74)	-7.05 (-11.59, -1.84)
3	-2.26 (-4.38, -0.14)	-5.33 (-9.43, -1.75)	-3.46 (-6.36, -0.51)	-7.01 (-11.11, -3.10)
4-5	-0.07 (-3.13, 3.00)	-3.75 (-8.33, 0.39)	-1.35 (-4.95, 2.32)	-5.83 (-10.14, -1.64)
# times found infected with *Trichuris*:				
0	REF	REF	REF	REF
1	-1.36 (-3.14, 0.43)	-3.02 (-6.40, 0.36)	-2.39 (-5.02, 0.26)	-3.66 (-7.49, 0.52)
2	-2.10 (-4.11, -0.07)	-2.80 (-5.83, 0.16)	-2.26 (-4.87, 0.31)	-3.48 (-6.80, -0.17)
3	-3.18 (-5.44, -0.88)	-4.27 (-7.44, -1.08)	-3.96 (-6.81, -1.14)	-4.63 (-8.16, -1.09)
4-5	-1.60 (-4.28, 1.09)	-2.58 (-6.55, 1.31)	-1.70 (-5.48, 1.97)	-3.47 (-7.58, 0.57)
# times found infected with Hookworm:				
0	REF	REF	REF	REF
1-3	-1.80 (-4.20, 0.60)	-3.70 (-7.28, -0.20)	-3.63 (-7.19, -0.12)	-3.76 (-7.27, -0.24)
# times found infected with any STH:				
0-1	REF	REF	REF	REF
2	-2.04 (-3.84, -0.24)	-4.04 (-10.74, 10.80)	-3.60 (-7.11, 0.05)	-0.41 (-12.90, 13.52)
3	-2.71 (-4.64, -0.78)	-6.19 (-10.60, -1.60)	-4.52 (-7.34, -1.72)	-6.24 (-12.65, -0.18)
4-5	-1.30 (-3.34, 0.73)	-4.63 (-9.61, 1.54)	-2.83 (-5.57, -0.06)	-4.32 (-11.83, 3.18)
2) Outcome: Verbal IQ Score				
# times found infected with *Ascaris*:				
0-1	REF	REF	REF	REF
2	-1.55 (-3.52, 0.43)	-8.27 (-13.85, -3.10)	-4.27 (-7.47, -1.10)	-10.01 (-14.67, -4.92)
3	-1.63 (-4.02, 0.76)	-6.69 (-12.05, -2.05)	-3.03 (-6.24, 0.18)	-8.29 (-12.92, -3.92)
4-5	0.87 (-2.57, 4.31)	-5.06 (-10.75, 0.05)	-0.89 (-4.88, 3.10)	-6.99 (-11.63, -2.47)

# times found infected with *Trichuris*:				
0	REF	REF	REF	REF
1	-1.13 (-3.13, 0.88)	-2.67 (-6.91, 1.32)	-2.00 (-5.03, 0.97)	-3.57 (-8.44, 1.55)
2	-1.86 (-4.12, 0.42)	-2.09 (-5.48, 1.23)	-1.78 (-4.68, 1.12)	-2.55 (-6.32, 1.17)
3	-3.19 (-5.74, -0.66)	-3.95 (-7.59, -0.36)	-3.63 (-6.82, -0.45)	-4.36 (-8.39, -0.27)
4-5	-1.96 (-4.96, 1.05)	-3.27 (-7.75, 1.01)	-2.54 (-6.69, 1.48)	-4.14 (-8.80, 0.42)
# times found infected with Hookworm:				
0	REF	REF	REF	REF
1-3	-1.53 (-4.23, 1.18)	-3.43 (-7.55, 0.69)	-3.36 (-7.42, 0.71)	-3.59 (-7.76, 0.55)
# times found infected with any STH:				
0-1	REF	REF	REF	REF
2	-2.32 (-4.32, -0.31)	-9.42 (-15.46, -3.04)	-4.79 (-8.73, -0.75)	-11.84 (-17.18, -5.40)
3	-3.21 (-5.37, -1.06)	-8.58 (-13.75, -3.90)	-5.01 (-8.20, -1.88)	-10.63 (-15.68, -5.75)
4-5	-1.40 (-3.68, 0.90)	-7.59 (-12.81, -2.75)	-3.38 (-6.50, -0.31)	-9.96 (-14.55, -5.29)
3) Outcome: Performance IQ Score				
# times found infected with *Ascaris*:				
0-1	REF	REF	REF	REF
2	-2.36 (-4.36, -0.35)	-0.95 (-9.79, 16.11)	-3.84 (-7.26, -0.31)	3.03 (-11.67, 16.37)
3	-2.49 (-4.90, -0.07)	-4.86 (-9.42, -0.42)	-3.84 (-7.18, -0.43)	-5.17 (-11.07, -0.16)
4-5	-0.80 (-4.29, 2.69)	-2.37 (-7.94, 3.97)	-1.61 (-5.74, 2.64)	-2.07 (-9.77, 4.71)
# times found infected with *Trichuris*:				
0	REF	REF	REF	REF
1	-1.89 (-3.92, 0.15)	-4.03 (-8.05, -0.06)	-3.33 (-6.46, -0.28)	-4.53 (-9.08, 0.51)
2	-2.41 (-4.71, -0.08)	-3.85 (-7.57, -0.31)	-2.87 (-5.95, 0.16)	-4.77 (-8.75, -0.85)
3	-2.64 (-5.21, -0.06)	-4.34 (-8.18, -0.62)	-3.87 (-7.23, -0.57)	-4.76 (-8.88, -0.61)
4-5	-0.94 (-3.98, 2.09)	-1.69 (-6.21, 2.74)	-0.58 (-4.81, 3.66)	-2.55 (-7.19, 2.04)
# times found infected with Hookworm:				
0	REF	REF	REF	REF
1-3	-1.77 (-4.49, 0.97)	-3.36 (-7.37, 0.69)	-3.31 (-7.35, 0.69)	-3.41 (-7.40, 0.59)
# times found infected with any STH:				
0-1	REF	REF	REF	REF
2	-2.33 (-4.38, -0.28)	2.68 (-9.13, 14.95)	-3.46 (-7.65, 1.07)	10.61 (-4.16, 16.22)
3	-2.06 (-4.27, 0.14)	-5.16 (-9.67, -0.90)	-4.52 (-7.80, -1.26)	-4.31 (-8.82, -0.25)

4-5	-1.00 (-3.32, 1.33)	-1.70 (-7.42, 3.76)	-2.23 (-5.41, 0.89)	0.25 (-5.59, 4.48)

*All effect estimates are adjusted for: maternal education (completed secondary school), cooks using gas, has a toilet with water and connection to public sewage in the home, stunted at one year of age, Bayley-III cognition raw score at one year, number of healthy growth visits attended from birth to one year of age and, number of years in preschool by 5 years.

SUPPLEMENTARY MATERIAL

Additional details regarding statistical analyses

Confounding:

The variables investigated as potential confounders included: socioeconomic status (i.e. residential district, urban/rural status, mother's marital status (i.e. married/common-law vs single), maternal education (i.e. completed secondary school), mother employed, father or mother's partner employed, number of people living in the home, house material, cooks using gas, presence of electricity in the home, working radio ownership, working television ownership, water source, has a toilet with water and connection to public sewage in the home, and household income); sex; healthcare seeking behavior (i.e. number of healthy growth visits attended from birth to one year of age and vaccines up to date at baseline); hygiene (i.e. number of baths per day and use of soap for bathing); hospitalizations since birth; baseline anthropometry/ malnutrition (i.e. stunted, underweight, wasted, birth weight); baseline development scores (i.e. Bayley-III cognition raw score, Bayley-III receptive language raw score, Bayley-III expressive language raw score and, Bayley-III fine motor raw score); breastfeeding (i.e. exclusively breastfed to six months and continued breastfeeding at one year); and, number of years in preschool by five years.

Multiple imputation:

Missing exposure data were imputed using multiple imputation. Missing outcome data were not imputed as it has been shown that imputing missing outcome data does not usually prevent bias nor improve precision when estimating regression effects [1-3]. No missing covariate data were present. Multinomial regression models were used as the imputation models for cumulative STH infections. All covariates included in the outcome models were also included in the imputation models as well as other relevant covariates, with complete data, that predicted the missing data, as appropriate. These included: being infected with any STH infection at one year of age, and being infected with any STH at three years of age for imputing cumulative *Ascaris* infections; being infected with any STH infection at one year of age, being infected with *Ascaris* infection at three years of age, and residential district for imputing cumulative *Trichuris* infections; being

infected with *Trichuris* infection at one year of age, and infection with any STH infection at three years of age for imputing cumulative hookworm infections; and, always using shoes outside of the home for imputing cumulative any STH infections.

Bayesian Latent Class Analysis:

The latent class analysis allows for the adjustment for misclassification of the exposure with individual variation in sensitivity and specificity values due to the fact that, while the majority of stool specimens were analyzed with the Kato-Katz technique, some stool specimens were analyzed with the direct smear technique. Three separate models were specified: 1) *The outcome model* is a linear regression that models child development scores conditional on latent STH infection (i.e. the true, unmeasured exposure) and the confounding variables mentioned previously; 2) *The exposure models* are logistic regressions that model true latent STH infection status at each study time point, conditional on covariates that predict species-specific STH infection. These variables were chosen based on Bayesian information criterion (BIC). The exposure models allow for differences in the probabilities of being STH infected between various groups of children to be accounted for; and 3) *The misclassification models* predict the measured STH infection status at each of the five time points according to the true latent STH infection status at each time point and the sensitivity and specificity values of the diagnostic technique used (i.e. Kato-Katz technique or direct smear technique) at each time point, according to:

$$P(mSTH_i = 1) = \begin{matrix} S_{KK}(tSTH_i)(KK_i) + (1 - C_{KK})(1 - tSTH_i)(KK_i) + \\ S_{DS}\,(tSTH_i)(1 - KK_i) + \ (1 - C_{DS})(1 - tSTH_i)(1 - KK_i) \end{matrix}$$

Where:

i = study time points

$mSTH_i$ = Measured STH infection status at time point i (dichotomous variable)

$tSTH_i$ = Latent, true STH infection status at time point i (dichotomous variable)

KK_i= Indicator variable indicating technique used to analyze stool specimen at time point i (KK_i= 1 represents use of the Kato-Katz technique; KK_i= 0 represents use of the direct smear technique)

S_{KK}= Sensitivity of the Kato-Katz technique

C_{KK}= Specificity of the Kato-Katz technique

S_{DS}= Sensitivity of the direct smear technique

C_{DS}= Specificity of the direct smear technique

The different sensitivities of the two separate diagnostic techniques were incorporated into the measurement models to account for the lower sensitivity of the direct smear technique compared to the Kato-Katz technique. The models were jointly estimated using the Gibbs sampler, such that the latent true exposure was essentially imputed within the misclassification and exposure models, analogous to missing data techniques, and subsequently included in the outcome model.

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5.3 Preface to Manuscript B

The second manuscript, Manuscript B, addresses thesis objective 2 (to determine the effect of the number of detected STH infections during a critical window of development (i.e. between one and two years of age) on child development at two, three, four and five years of age). The focus of this manuscript is to examine whether STH infection, during the second year of life, affects child development trajectories. Specifically, the effect of cumulative *Ascaris*, *Trichuris* and any STH infections between one and two years of age on repeated measures of cognitive and verbal abilities at two, three, four and five years of age, are examined. The effect of hookworm infection was not examined because of the very low prevalence of hookworm between one and two years of age (i.e. < 2%). Analyses were performed under the Bayesian framework, using Bayesian latent class analysis to adjust for exposure misclassification.

The results from this manuscript have been presented at the following scientific conference:

1. Blouin B, Casapia M, Gyorkos TW. Assessing long-term effects of STH infection during a critical window of development: *Ascaris* infection and poor child development. *23rd Canadian Conference on Global Health*. Ottawa, Canada, October 30, 2017.

This manuscript has been formatted for, and is currently under review in, the journal *PLoS Neglected Tropical Diseases*. It follows the STROBE guidelines for reporting results from cohort studies.

5.4. Manuscript B: A longitudinal cohort study of soil-transmitted helminth infections during the second year of life and long-term consequences on cognitive and verbal abilities

A longitudinal cohort study of soil-transmitted helminth infections during the second year of life and long-term consequences on cognitive and verbal abilities

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Abstract

Background: Soil-transmitted helminth (STH) infection leads to malnutrition and anemia, and has been linked to impaired child development. Previous research on this topic is limited and mostly conducted in school-age children. The goal of this study was to determine the effect of the number of detected STH infections between one and two years of age on subsequent cognitive and verbal abilities, in a cohort of preschool children.

Methodology/Principal Findings: A longitudinal cohort study was conducted in 880 children in Iquitos, Peru between September 2011 and July 2016. Children were recruited at one year of age and followed up at 18 months and then annually between two and five years of age. STH infection was measured with the Kato-Katz technique or the direct smear technique. Child development was measured with the Bayley Scales of Infant and Toddler Development-III at the one to three-year visits and with the Wechsler Preschool and Primary Scale of Intelligence-III at the four and five-year visits. Bayesian latent class hierarchical multivariable linear regression models were used to account for the repeated outcome measures for each child and to adjust for STH misclassification. Children found infected with any STH infection between one and two years of age had lower cognitive scores between two and five years of age (between group score differences (95% credible intervals) for infected once, and infected two or three times, compared to never infected: -4.31 (-10.64, -0.14) and -3.70 (-10.11, -0.11), respectively). Similar results were found for *Ascaris* infection and for verbal scores.

Conclusions/Significance: An association was found between having been infected with *Ascaris* or any STH between one and two years of age and lower cognitive and verbal abilities

later in childhood. These results suggest that targeting children for STH control as of one year of age is particularly important.

Author summary

Intestinal worm infections are parasites that can have serious health consequences, including malnutrition. They affect over one billion people in low and middle income countries. It has been proposed that intestinal worm infections can have a negative effect on brain development in young children. This topic, however, has not yet been properly researched. The goal of this study, therefore, was to investigate the effect of worm infections on cognitive and verbal abilities in preschool children between one and five years of age. The study was conducted in the Amazon region of Peru. Children who were infected with these worms between one and two years of age had lower scores on tests of cognitive and verbal abilities between two and five years of age. These results provide empirical evidence of worm-attributable cognitive impairment in children living in endemic countries and provide support for mass deworming programs targeting preschool children.

Introduction

Investing in child health at a young age is the most effective and efficient strategy to optimize the health of children as they grow. During the first few years of life the brain develops rapidly and small perturbations in this process can have long-term effects on its structural and functional capacity [1]. The early childhood period is therefore considered to be the most important development period across the lifespan [2]. In low and middle-income countries (LMICs), poor child development is alarmingly common with over 200 million children at risk of not reaching their development potential due to the effects of poverty, including malnutrition and inadequate care [1, 3]. Interventions to prevent child malnutrition must occur during the first two years of life to prevent future growth and development deficits [4].

In LMICs, the soil-transmitted helminths (STHs), including *Ascaris lumbricoides*, *Trichuris trichiura* and the two hookworm species (*Necator americanus* and *Ancylostoma duodenale*), are a significant contributor to poor health and malnutrition [5]. The burden of disease attributable to STH infections can be even more pronounced when children are exposed to these infections at an early age. Worldwide, approximately 2 billion people are infected with STHs, of which an estimated 5-10% are children under two years of age [6, 7]. As the STHs are spread through contaminated soil, food and hands, children begin to acquire these infections as they become mobile and begin to explore the environment [8]. *Ascaris* and *Trichuris* are the predominant STH infections in early childhood [9] with infection documented even in the first year of life [10]. Although the World Health Organization (WHO) recommends that children as young as 12 months of age be included in deworming programs in endemic areas, many challenges still need to be overcome to effectively reach this age group in STH control programs [11, 12].

Due to the effects on malnutrition and anemia, the STHs are thought to have an effect on child development. Previous research on this topic has documented associations between STH infections and poor child development; however, the interpretation of this research is limited due to poor research designs [13-21], failure to adjust for important confounding variables [13, 15, 21-23], small sample sizes [15, 22, 24, 25], grouping STHs with other parasites in the analyses [18, 23] and inadequate and inappropriate statistical analyses [17-22, 24, 25]. Furthermore, no study has previously looked at the long term effect on development of STH infection specifically during the first two years of life – the most critical period for development across the lifespan. The objective of the current research, therefore, was to evaluate the long-term effect of STH infection between one and two years of age on repeated measures of child development between two and five years of age.

Methods

Study design and population

The general study methodology has been described in detail elsewhere [26]. Briefly, a longitudinal cohort study was conducted in the rural and peri-urban areas surrounding Iquitos, Peru between September 2011 and July 2016. A total of 1,760 children were recruited at 12 months of age and followed-up at 18 months and at two, three, four and five years of age. During recruitment, a sampling frame for the study was obtained from participating health centre records and from a door-to-door census conducted in the study area by the research team before initiation of the study. The study population consisted of eligible children living in the catchment areas of the twelve major health centres serving the three rural/peri-urban communities surrounding Iquitos (i.e. Nanay, Belén and San Juan), who, during study

recruitment, were between 12 and 14 months of age. Study inclusion criteria included: 1) children between 12 and 14 months of age at recruitment; 2) children attending one of the participating health centres for their 12-month routine healthy growth and development visit (note that the parent/guardian of any child who was identified as a potential participant from the sampling frame but who did not attend his/her routine visit at 12 months of age was contacted at home by a research assistant and encouraged to attend); 3) children who were not consulting medical advice for a suspected STH infection; 4) children who had not been dewormed in the six months prior to their recruitment into the study; and 5) children who did not have any serious congenital or chronic medical condition. Study exclusion criteria included: 1) children who lived outside of the identified study area; 2) children whose family planned to move outside of the study area in the year following recruitment; 3) children whose parents did not consent to participate in the study.

Exposure ascertainment

STH infection was measured at all study visits. At all visits, a parent or guardian of the participating child collected the stool specimen from the child within 24 hours prior to the scheduled study visit. One or two days before the study visit, the research assistant visited the participating child's parent or guardian in their home and provided the materials and instructions necessary to collect a fresh stool specimen. Stool specimens were transported to the study laboratory where they were analyzed by trained technologists. At the 12 and 18 months of age visits, half of the stool specimens were analysed by the Kato-Katz technique within 24 hours of deposition. The remaining stool specimens were stored in 10% formalin and analyzed by the direct smear technique after the child had completed their two-year study visit. The rationale for

this has been described in detail previously [26]. At all remaining study visits, all stool specimens were analysed with the Kato-Katz technique within 24 hours following deposition. Because no gold standard test exists for diagnosing STH infection, all currently available tests are limited by imperfect sensitivities. Recent sensitivity estimates with 95% credible intervals (CrI) for the Kato-Katz technique include: 63.8% (59.1, 68.6), 82.2% (80.1, 84.5) and 59.5% (56.9, 62.2) for *Ascaris*, *Trichuris* and hookworm infection, respectively [27]. Recent sensitivity estimates with 95% CrIs for the direct smear technique include: 52.1% (46.6, 57.7), 62.8% (56.9, 68.9) and 42.8% (38.3, 48.4) for *Ascaris*, *Trichuris* and hookworm infection, respectively [27].

Outcome ascertainment

Child development was measured with age-appropriate standardized scales at the one, two, three, four and five years of age study visits (child development was not measured at the 18-month visit). Because no single high quality test exists to measure child development over this entire age range, two different scales were used. At the one to three years of age visits, the cognitive, receptive language, expressive language and fine motor subtests of the Bayley Scales of Infant and Toddler Development, Third edition (Bayley-III) were used. The Bayley-III subtests were administered, at the one and two-year visits, to all children; and at the three-year visit, for feasibility reasons, to a random sample of 880 children of the original study population. In the event that a child who was randomly sampled to be administered the Bayley-III was lost to follow-up at the three-year visit, the next child scheduled to pass their study visit from the same health centre catchment area, who had not originally been randomly selected, was selected to be administered the Bayley-III. At the four and five years of age visits, the Spanish version of the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III) was used. The

seven subscales used to generate the full scale IQ score, the verbal IQ score and the performance IQ score were administered to the same children randomly sampled to be administered the Bayley-III at the three-year study visit. These subscales include: vocabulary, information, word reasoning, matrices, picture concepts, block design and coding. Both scales were administered by highly trained research assistants who had previous healthcare experience (e.g. nurses or nurse-midwives) with a minimum Bachelor's degree education. Research assistants were unaware of the child's STH status during the assessments. Scales were administrated in secluded areas to avoid distractions and, in the event that a child did not appear to be performing at his/her optimal ability, either a break was taken or the assessment was postponed to a future date. Both scales provide age-scaled composite scores for cognitive ability (i.e. the cognitive composite score from the Bayley-III and the performance IQ score from the WPPSI-III) and verbal ability (i.e. the language composite score from the Bayley-III and the verbal IQ score from the WPPSI-III) with a reference norm mean of 100 and standard deviation of 15.

Measurement of covariates

Child height and weight were measured at all study visits. Children were measured without clothes or shoes. Weight was measured with a portable electronic scale accurate to the nearest 0.01 kg in the sitting position for children under two years of age and in the standing position for children two years and older. Length/height was measured using a portable stadiometer accurate to the nearest millimeter. Child length (recumbent position) was measured for children two years of age and younger, and child height (standing position) was measured for children older than two years. A questionnaire was administered to the parent or guardian of participating

children at all study visits regarding relevant socio-demographic information and health and medical history.

Ethics

The study was conducted according to the principles of the Declaration of Helsinki. The study was approved by the Universidad Peruana Cayetano Heredia in Lima, Peru (11005, 12009); the Instituto Nacional de Salud in Lima, Peru (032-11); and, the Research Ethics Boards of the McGill University Health Centre in Montreal, Canada (10-242-PED, 12-026-PED). Written informed consent was obtained from parents/guardians of the participating children. In the event that a parent of the participating child was under the age of 18, written assent was obtained from the minor parent, with informed consent obtained from their parent or guardian over 18 years of age.

Statistical analyses

Anonymized data were stored on password-protected computers in locked offices. Double data entry was performed for all entered data and data cleaning followed a comparison of the two databases. Summary statistics of the study population included means and standard deviations for continuous variables and counts and proportions for binary and categorical data.

Hierarchical linear regression models were used to investigate the effect of the number of times a child was detected STH-infected between one and two years of age on repeated measures of development scores at two, three, four and five years of age. The effect of *Ascaris* infection, *Trichuris* infection and being infected with any of the three STH species (any STH infection) on

both cognitive and verbal scores was estimated. The specific effect of hookworm infection was not assessed due to the very low prevalence of hookworm in this age group. The exposure was categorized into found infected zero times, one time, two times and three times. Due to the low number of children who were found infected at all three time points between one and two years of age, the categories for being found infected two and three times were combined.

Both univariable and multivariable regression models are presented. The covariates included in the final models were chosen based on theoretical knowledge (i.e. confounding variables that are thought to be associated with both the exposure and outcome of interest without being mediators of this relationship) and statistical criteria. Baseline variables considered as potential confounders included: socioeconomic status (i.e. residential district, urban/rural status, mother's marital status (i.e. married/common-law vs single), maternal education (i.e. completed secondary school), mother employed, father or mother's partner employed, number of people living in the home, house material, cooks using gas, presence of electricity in the home, working radio ownership, working television ownership, water source, has a toilet with water and connection to public sewage in the home, and household income); sex; healthcare seeking behavior (i.e. number of healthy growth visits attended from birth to one year of age and vaccines up to date at baseline); hygiene (i.e. number of baths per day and use of soap for bathing); hospitalizations since birth; anthropometry/malnutrition (i.e. stunted, underweight, wasted, birth weight); baseline development scores (i.e. Bayley-III cognition raw score, Bayley-III receptive language raw score, Bayley-III expressive language raw score and, Bayley-III fine motor raw score); and breastfeeding (i.e. exclusively breastfed to six months and continued breastfeeding at one year). Univariable hierarchical linear regression models with an individualized intercept were used to

determine if each variable was associated with the outcome variables and univariable multinomial regression models were used to determine if each variable was associated with the exposure variables. Correlations and 2 x 2 tables were also used to observe relationships between the confounding variables. The final presented models include confounding variables that are associated with both the exposure and outcome and that affected the association between the exposure and outcome of interest. These include socioeconomic status (i.e. maternal education (completed secondary school), cooks using gas, has a toilet with water and connection to public sewage in the home), baseline nutritional status (i.e. stunted), use of health care (i.e. number of healthy growth visits attended from birth to one year of age), baseline development scores (i.e. Bayley-III cognition raw score) and age. An individualized intercept term was used to allow each child's baseline development score to be different and an individualized slope for age was used to allow children to develop at different rates. The age variable was centered to reduce posterior correlation between the individualized intercept and slope, in order to aid convergence of the Markov Chain Monte Carlo (MCMC) process.

Missing exposure and outcome data were imputed using multiple imputation. No covariate had missing data. Multinomial regression models were used as the imputation models for cumulative STH infections and linear regression models were used as the imputation models for development scores. All covariates included in the outcome models were also included in the imputation models as well as other relevant covariates, with complete data, that predicted the missing data, as appropriate.

To address exposure misclassification due to imperfect sensitivities and specificities of the diagnostic techniques used to measure STH infection, Bayesian latent class hierarchical regression models were used. This method was particularly useful in this context because it allows for individual variation in sensitivity and specificity values (here, due to the fact that, while the majority of stool specimens were analyzed with the Kato-Katz technique, some stool specimens were analyzed with the direct smear technique) and because a gold standard diagnostic technique for STH infection does not exist and therefore the sensitivity and specificity values are not exactly known. This method has been described in detail and used previously [28]. Briefly, three separate models were specified: 1) *The outcome model* is a hierarchical linear regression that models child development scores conditional on latent STH infection (i.e. the true, unmeasured exposure) and the confounding variables mentioned previously; 2) *The exposure models* are logistic regressions that model true latent STH infection status at 12, 18 and 24 months of age, conditional on covariates that predict species-specific STH infection. These covariates were chosen based on Bayesian information criterion (BIC). The exposure models allow for differences in the probabilities of being STH-infected between various groups of children to be accounted for; and 3) *The misclassification models* predict the measured STH infection status at each of the three time points according to the true latent STH infection status at each time point and the sensitivity and specificity values of the diagnostic technique used (i.e. Kato-Katz technique or direct smear technique) at each time point, according to:

$$P(mSTH_i = 1) = \begin{matrix} S_{KK}(tSTH_i)(KK_i) + (1 - C_{KK})(1 - tSTH_i)(KK_i) + \\ S_{DS}\,(tSTH_i)(1 - KK_i) + \ (1 - C_{DS})(1 - tSTH_i)(1 - KK_i) \end{matrix}$$

Where:

i = study time points

$mSTH_i$ = Measured STH infection status at time point i (dichotomous variable)

$tSTH_i$ = Latent, true STH infection status at time point *i* (dichotomous variable)

KK_i= Indicator variable indicating technique used to analyze stool specimen at time point *i* (KK_i= 1 represents use of the Kato-Katz technique; KK_i= 0 represents use of the direct smear technique)

S_{KK}= Sensitivity of the Kato-Katz technique

C_{KK}= Specificity of the Kato-Katz technique

S_{DS}= Sensitivity of the direct smear technique

C_{DS}= Specificity of the direct smear technique

Diffuse, non-informative priors were used for all regression coefficients in the outcome and exposure models. Informative priors were specified for the sensitivity and specificity values of the two diagnostic techniques (Table 1). Clinical priors representing the best summary of the information from the published literature and expert opinion, [27] as well as optimistic and pessimistic priors, were specified for sensitivity analyses. Optimistic priors are those assuming higher accuracy for the techniques than the "best estimate" clinical priors would suggest, and similarly, pessimistic priors assume lower accuracy than the clinical priors would suggest. All prior densities on the sensitivity and specificity parameters were assumed to follow a beta distribution. Although unrealistic, for comparison purposes, analyses were also run assuming that the sensitivity and specificity values were 100%. The models were jointly estimated using the Gibbs sampler, such that the latent true exposure was essentially imputed within the misclassification and exposure models, analogous to missing data techniques, and subsequently included in the outcome model. WinBUGS (version 1.4.3, MRC, Cambridge) was used to run the Markov Chain Monte Carlo (MCMC) process used for all inferences.

Table 1: Probability ranges and corresponding coefficients of the beta prior densities for the sensitivities and specificities of the Kato-Katz and direct smear techniques used in the Bayesian latent class analyses to adjust for misclassification of STH infection

		Clinical priors			Optimistic priors			Pessimistic priors		
		Range*	Beta distribution coefficients		Range*	Beta distribution coefficients		Range*	Beta distribution coefficients	
			α	β		α	β		α	β
Kato-Katz	*Ascaris*:									
	Sensitivity	0.55-0.75	55.86	29.63	0.70-0.80	214.34	70.81	0.50-0.60	208.45	170.38
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Trichuris:									
	Sensitivity	0.75-0.90	77.85	15.75	0.85-0.95	116.06	12.05	0.70-0.80	214.34	70.81
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Hookworm:									
	Sensitivity	0.52-0.68	85.65	56.78	0.63-0.73	226.28	105.98	0.47-0.57	198.73	183.37
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
Direct smear	*Ascaris*:									
	Sensitivity	0.40-0.60	47.30	47.30	0.55-0.65	220.49	146.68	0.35-0.45	146.68	220.49
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Trichuris:									
	Sensitivity	0.55-0.75	55.86	29.63	0.70-0.80	214.34	70.81	0.50-0.60	208.45	170.38
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26
	Hookworm:									
	Sensitivity	0.35-0.55	42.02	51.57	0.50-0.60	208.45	170.38	0.30-0.40	121.41	226.29
	Specificity	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26	0.95-0.99	231.95	6.26

* Prior probability ranges were developed based on previous research [27] and expert opinion.

Sample size

A sample size of 880 children with four measures of the outcome per child (i.e. at 2, 3, 4 and 5 years of age) was available for analysis. The primary outcome, cognitive score, was compared between children who were never found STH-infected to children who were found infected one time and two or three times. No estimate of the intraclass correlation coefficient (ICC) for repeated measures of cognitive scores of children between two and five years of age was found in the literature. Therefore, based on preliminary data, an ICC for cognitive scores was estimated to be 0.2. This corresponds to a design effect of 1.6 (design effect = 1 + (# observations per child – 1) × ICC). Assuming that the repeated observations were independent, a total sample size of 3,520 would be available (880 participants × 4 outcome measures per participant = 3,520 outcome measurements). Taking the correlation between repeated measures into account, the effective sample size is 2,200 (3,520 outcome measurements / 1.6 (the design effect) = 2,200). Based on expert opinion in child development, a difference in mean scores of 5 points (i.e. 1/3 of a standard deviation) was considered the minimum clinically significant effect size. Assuming that the standard deviation of cognitive scores is 15, and having known from preliminary data that 43% of the population is unexposed (never STH-infected) and that 36% and 21% of the population were found STH-infected one, and two or three times, respectively (based on preliminary data), an effective sample size of 2,200 would be able to detect a difference of 5 points in cognitive scores between never infected and infected once, and between never infected and infected two or three times, with total 95% confidence interval widths of 2.84 and 3.34, respectively. Therefore, the sample size provided sufficient precision for the planned comparisons.

Results

A description of the population included in this analysis at baseline, including the relationship between the original study population and the random sample, has been described previously [26]. The mean age at recruitment was 12.1 months with 51.9% of the population being male. At baseline, malnutrition was present with 24%, 7.5% and 1.7% of the population considered to be stunted, underweight and wasted, respectively. The study flowchart is shown in Figure 1. Missing STH infection data were present for four children at 18 months of age and for four children at 24 months of age due to loss to follow-up. Number of times found STH infected between one and two years of age was therefore missing for a total of eight children (< 1%). Development scores were missing for a total of four children at the two years of age visit due to loss to follow-up. At the three years of age visit, development scores were missing for two children due to a protocol violation (two children who should have been administered the Bayley-III were not, due to an error by a research assistant). At the four years of age visit, development scores were missing for a total of 63 children (7%): 46 due to loss to follow-up, 15 due to invalid WPPSI-III measurements (WPPSI-III measurements are considered invalid if a participant scores 0 on two or more performance subscales and/or verbal subscales) and two due to protocol violations. At the five-year visit, development scores were missing for a total of 99 children (11%): 85 due to loss to follow-up and 14 due to invalid WPPSI-III measurements. The majority of children who were lost to follow-up throughout the study moved homes between study visits and could not be located.

Figure 1: Study flowchart for 880 children randomly sampled at the 36-month visit to be included in this analysis in Iquitos, Peru, September 2011 to July 2016.

Tables 2 and 3 show the detected STH prevalence and intensity data at the 12 months, 18 months and 24 months of age study visits (unadjusted for misclassification). At one year of age the prevalence of being infected with at least one STH infection was 12.4%. This more than tripled by two years of age at which point the prevalence reached 41.1% (Table 2). *Ascaris* was the most common species with 46.6% of the population infected with *Ascaris* at least one time between one and two years of age (Table 3). Hookworm infection was uncommon with only 2% of the population harbouring a hookworm infection at some point between one and two years of age (Table 3). Raw, scaled and composite development scores from the Bayley-III and the WPPSI-III between one and five years of age are presented in Table 4. Overall, composite scores decreased over time with the highest scores obtained at the baseline visit and the lowest scores obtained at the final, five years of age visit.

Table 2: STH prevalence and intensity at the 12, 18 and 24 months of age study visits in preschool children in Iquitos, Peru, September 2011 to July 2016.

	12 months[a]	18 months[b]	24 months[c]
Ascaris			
Prevalence [% (n)]	11.0 (97)	23.3 (204)[e]	30.7 (269)[e]
Intensity (epg[g]) [mean (sd)]	329.9 (1,416.6)[d]	1,468.3 (7,661.6)[f]	2,304.2 (11,987.5)[e]
Prevalence of moderate/ heavy intensity infection [% (n)]	2.0 (9)[d]	5.6 (24)[f]	9.6 (84)[e]
Trichuris			
Prevalence [% (n)]	2.5 (22)	7.7 (67)[e]	21.8 (191)[e]
Intensity (epg[g]) [mean (sd)]	26.5 (261.3)[d]	45.0 (346.5)[f]	46.7 (225.5)[e]
Prevalence of moderate/ heavy intensity infection [% (n)]	0.7 (3)[d]	0.7 (3)[f]	0.7 (6)[e]
Hookworm			
Prevalence [% (n)]	0.34 (3)	0.57 (5)[e]	1.37 (12)[e]
Intensity (epg[g]) [mean (sd)]	1.7 (26.8)[d]	3.6 (48.3)[f]	1.4 (17.6)[e]
Prevalence of moderate/ heavy intensity infection [% (n)]	0 (0)[d]	0 (0)[f]	0 (0)[e]
Any STH			
Prevalence [% (n)]	12.4 (109)	27.9 (244)[e]	41.1 (360)[e]

[a] At the one year of age visit, 449 (51%) stool specimens were analyzed using the Kato-Katz technique and 431 (49%) stool specimens were analyzed using the direct smear technique
[b] At the 18 months of age visit, 428 (48.9%) stool specimens were analyzed using the Kato-Katz technique and 448 (51.1%) stool specimens were analyzed using the direct smear technique
[c] At the 24 months of age visit, all available stool specimens were analyzed using the Kato-Katz technique
[d] Data available for 449 participants (who had their stool specimen analyzed with the Kato-Katz technique)
[e] Data available for 876 participants
[f] Data available for 428 participants (who had their stool specimen analyzed with the Kato-Katz technique)
[g] Eggs per gram of stool

Table 3: Frequency of the number of detected STH infections between 12 and 24 months of age for the 880 children included in this analysis, Iquitos, Peru, September 2011 to July 2016.

	Ascaris	***Trichuris***	**Hookworm**	**Any STH**
No detected infections [% (n)]	53.4 (466)	74.0 (645)	97.9 (854)	44.5 (388)
One detected infection [% (n)]	30.4 (265)	20.4 (178)	1.8 (16)	33.5 (292)
Two detected infections [% (n)]	13.7 (119)	5.4 (47)	0.2 (2)	18.1 (158)
Three detected infections [% (n)]	2.5 (22)	0.2 (2)	0 (0)	3.9 (34)

*Complete data available for 872 participants

Table 4: Raw, scaled and composite scores from the Bayley Scales of Infant and Toddler Development (Bayley-III) and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III) at the 12, 24, 36, 48 and 60 months of age study visits, Iquitos, Peru, September 2011 to July 2016.

	12 months	24 months[a]	36 months[b]	48 months[c]	60 months[d]
Bayley-III: Cognition					
Raw [mean (sd)]	42.6 (3.0)	59.2 (3.2)	67.5 (3.1)	NA	NA
Scaled [mean (sd)]	11.0 (1.9)	8.1 (1.3)	7.4 (0.8)	NA	NA
Bayley-III: Receptive language					
Raw [mean (sd)]	13.0 (1.6)	23.9 (2.3)	31.6 (3.4)	NA	NA
Scaled [mean (sd)]	7.9 (1.9)	8.4 (1.2)	8.5 (1.3)	NA	NA
Bayley-III: Expressive language					
Raw [mean (sd)]	13.5 (2.2)	24.7 (3.4)	34.1 (3.8)	NA	NA
Scaled [mean (sd)]	8.9 (1.8)	7.4 (1.5)	7.9 (1.3)	NA	NA
Bayley-III: Fine Motor					
Raw [mean (sd)]	29.2 (1.5)	39.4 (2.1)	47.9 (3.3)	NA	NA
Scaled [mean (sd)]	10.0 (1.6)	10.3 (1.7)	9.6 (1.6)	NA	NA
WPPSI-III: Block design					
Raw [mean (sd)]	NA	NA	NA	18.0 (2.3)	20.1 (2.8)
Scaled [mean (sd)]	NA	NA	NA	10.0 (1.2)	8.1 (1.5)
WPPSI-III: Information					
Raw [mean (sd)]	NA	NA	NA	15.2 (3.9)	18.4 (3.7)
Scaled [mean (sd)]	NA	NA	NA	7.0 (2.5)	6.6 (2.4)
WPPSI-III: Matrices					
Raw [mean (sd)]	NA	NA	NA	3.7 (3.2)	7.5 (4.7)
Scaled [mean (sd)]	NA	NA	NA	6.4 (2.7)	6.7 (3.3)
WPPSI-III: Vocabulary					
Raw [mean (sd)]	NA	NA	NA	7.2 (4.3)	11.0 (4.8)
Scaled [mean (sd)]	NA	NA	NA	6.7 (2.9)	6.8 (2.6)
WPPSI-III: Picture concepts					
Raw [mean (sd)]	NA	NA	NA	4.1 (2.5)	6.0 (3.8)
Scaled [mean (sd)]	NA	NA	NA	7.7 (2.1)	6.3 (2.4)
WPPSI-III: Word reasoning					
Raw [mean (sd)]	NA	NA	NA	2.1 (2.3)	4.3 (3.7)
Scaled [mean (sd)]	NA	NA	NA	6.3 (1.7)	5.4 (2.1)
WPPSI-III: Coding					
Raw [mean (sd)]	NA	NA	NA	4.6 (5.1)	13.7 (6.2)
Scaled [mean (sd)]	NA	NA	NA	6.8 (2.2)	7.5 (2.0)
Verbal composite score [mean (sd)]	90.7 (9.0)	87.9 (6.6)	89.5 (6.4)	79.7 (11.9)	77.0 (11.8)
Cognitive composite score	105.1	90.5	86.8	87.4	81.1

[mean (sd)]	(9.7)	(6.6)	(4.0)	(9.5)	(11.9)

[a] Missing data for 4 participants
[b] Missing data for 2 participants
[c] Missing data for 63 participants
[d] Missing data for 99 participants
NA: scale was not administered at this study time point

Results from univariable and multivariable hierarchical linear regression models for the effects of STH infection between one and two years of age on cognitive and verbal scores between two and five years of age are shown in Tables 5 and 6, respectively. In univariable analyses, children found infected with *Ascaris*, *Trichuris* and any STH infection had cognitive scores between 1 and 2 points lower, on average, compared to children who were never found infected (Table 5). Children found infected with *Ascaris*, *Trichuris* and any STH infection had verbal scores between 2 and 3 points lower, on average, compared to children who were never found infected (Table 6). In multivariable analyses, adjusted for relevant confounding variables, results were attenuated. The effects (beta (95% confidence interval)) on cognitive scores of being infected with any STH infection one time and two or three times between one and two years of age, compared to never being detected infected were: -0.54 (-1.31, 0.23) and -0.52 (-1.41, 0.37), respectively. The same effects on verbal scores were -0.95 (-1.91, 0.01) and -0.75 (-1.87, 0.37), respectively.

Table 5: Univariable and multivariable linear regression results for the effect of cumulative *Ascaris* infection, cumulative *Trichuris* infection, and cumulative any STH infection on cognitive scores in preschool children in Iquitos Peru, September 2011 to July 2016.

	Univariable β (95% CI)	**Multivariable with multiple imputation** [b] β (95% CI)
# times found infected with *Ascaris*:		
0	REF [a]	REF
1	-1.45 (-2.29, -0.61) [a]	-0.64 (-1.40, 0.12)
2-3	-1.98 (-3.03, -0.94) [a]	-0.63 (-1.59, 0.32)
Mother completed secondary school	4.03 (3.29, 4.78)	2.89 (2.14, 3.64)
Cooks using gas	2.75 (1.97, 3.53)	1.22 (0.46, 1.98)
Toilet with water and connection to public sewage in the home	2.43 (1.69, 3.16)	0.78 (0.06, 1.51)
Stunted at 1 year	-2.49 (-3.34, -1.64)	-1.43 (-2.22, -0.64)
Bayley-III[c]: cognition raw score at 1 year	0.10 (0.06, 0.14)	0.06 (0.02, 0.09)
# healthy growth visits from birth to 1 year of age	0.30 (0.20, 0.40)	0.15 (0.05, 0.25)
Age	-2.76 (-3.04, -2.49)	-2.75 (-3.02, -2.48)
# times found infected with *Trichuris*:		
0	REF [a]	REF
1	-1.07 (-1.99, -0.15) [a]	-0.14 (-0.99, 0.71)
2-3	-1.94 (-3.55, -0.33) [a]	-0.30 (-1.76, 1.17)
Mother completed secondary school	4.03 (3.29, 4.78)	2.88 (2.12, 3.64)
Cooks using gas	2.75 (1.97, 3.53)	1.26 (0.50, 2.03)
Toilet with water and connection to public sewage in the home	2.43 (1.69, 3.16)	0.83 (0.10, 1.57)
Stunted at 1 year	-2.49 (-3.34, -1.64)	-1.43 (-2.23, -0.63)
Bayley-III[c]: cognition raw score at 1 year	0.10 (0.06, 0.14)	0.06 (0.02, 0.09)
# healthy growth visits from birth to 1 year of age	0.30 (0.20, 0.40)	0.14 (0.05, 0.24)
Age	-2.76 (-3.04, -2.49)	-2.74 (-3.02, -2.47)
# times found infected with any STH:		
0	REF [a]	REF
1	-1.46 (-2.31, -0.62) [a]	-0.54 (-1.31, 0.23)
2-3	-1.98 (-2.94, -1.02) [a]	-0.52 (-1.41, 0.37)
Mother completed secondary school	4.03 (3.29, 4.78)	2.86 (2.11, 3.61)
Cooks using gas	2.75 (1.97, 3.53)	1.26 (0.50, 2.03)
Toilet with water and connection to public sewage in the home	2.43 (1.69, 3.16)	0.76 (0.03, 1.49)
Stunted at 1 year	-2.49 (-3.34, -1.64)	-1.42 (-2.21, -0.63)

Bayley-III[c]: cognition raw score at 1 year	0.10 (0.06, 0.14)	0.06 (0.02, 0.09)
# healthy growth visits from birth to 1 year of age	0.30 (0.20, 0.40)	0.15 (0.05, 0.24)
Age	-2.76 (-3.04, -2.49)	-2.73 (-3.00, -2.46)

[a] 872 participants included in the analysis (number of times found infected with any STH infection and species-specific infections were missing for 8 participants)
[b] 880 participants were included in the analyses (complete data available for 754 participants; number of times found infected with any STH infection and species-specific infections was imputed for 8 participants; and cognitive score was imputed for 4, 2, 63 and 99 participants at the 2, 3, 4 and 5 year visit, respectively)
[c] Bayley III: Bayley Scales of Infant and Toddler Development, Third Edition

Table 6: Univariable and multivariable linear regression results for the effect of cumulative *Ascaris* infection, cumulative *Trichuris* infection, and cumulative any STH infection on verbal scores in preschool children in Iquitos Peru, September 2011 to July 2016.

		Univariable β (95% CI)	**Multivariable with multiple imputation** [b] β (95% CI)
# times found infected with *Ascaris*:			
	0	REF [a]	REF
	1	-2.03 (-3.10, -0.96) [a]	-0.92 (-1.88, 0.03)
	2-3	-2.10 (-3.43, -0.77) [a]	-0.20 (-1.40, 1.00)
Mother completed secondary school		4.77 (3.81, 5.73)	2.92 (1.98, 3.85)
Cooks using gas		3.69 (2.69, 4.68)	1.61 (0.65, 2.57)
Toilet with water and connection to public sewage in the home		3.74 (2.82, 4.67)	1.64 (0.73, 2.55)
Stunted at 1 year		-3.71 (-4.80, -2.63)	-2.31 (-3.30, -1.32)
Bayley-III[c]: cognition raw score at 1 year		0.16 (0.11, 0.20)	0.09 (0.05, 0.14)
# healthy growth visits from birth to 1 year of age		0.48 (0.35, 0.61)	0.27 (0.15, 0.39)
Age		-4.28 (-4.55, -4.01)	-4.25 (-4.52, -3.98)
# times found infected with *Trichuris*:			
	0	REF [a]	REF
	1	-2.07 (-3.24, -0.90) [a]	-0.64 (-1.69, 0.41)
	2-3	-2.77 (-4.81, -0.72) [a]	-0.24 (-2.09, 1.60)
Mother completed secondary school		4.77 (3.81, 5.73)	2.87 (1.93, 3.82)
Cooks using gas		3.69 (2.69, 4.68)	1.61 (0.64, 2.57)
Toilet with water and connection to public sewage in the home		3.74 (2.82, 4.67)	1.66 (0.74, 2.58)
Stunted at 1 year		-3.71 (-4.80, -2.63)	-2.28 (-3.28, -1.28)
Bayley-III[c]: cognition raw score at 1 year		0.16 (0.11, 0.20)	0.10 (0.05, 0.14)
# healthy growth visits from birth to 1 year of age		0.48 (0.35, 0.61)	0.27 (0.15, 0.39)
Age		-4.28 (-4.55, -4.01)	-4.25 (-4.52, -3.97)
# times found infected with any STH:			
	0	REF [a]	REF
	1	-2.24 (-3.31, -1.17) [a]	-0.95 (-1.91, 0.01)
	2-3	-2.88 (-4.10, -1.66) [a]	-0.75 (-1.87, 0.37)
Mother completed secondary school		4.77 (3.81, 5.73)	2.89 (1.95, 3.84)
Cooks using gas		3.69 (2.69, 4.68)	1.63 (0.67, 2.60)
Toilet with water and connection to public sewage in the home		3.74 (2.82, 4.67)	1.58 (0.67, 2.50)
Stunted at 1 year		-3.71 (-4.80, -2.63)	-2.29 (-3.28, -1.29)

Bayley-III[c]: cognition raw score at 1 year	0.16 (0.11, 0.20)	0.09 (0.05, 0.14)
# healthy growth visits from birth to 1 year of age	0.48 (0.35, 0.61)	0.27 (0.15, 0.39)
Age	-4.28 (-4.55, -4.01)	-4.25 (-4.52, -3.97)

[a] 872 participants included in the analysis (number of times found infected with any STH infection and species-specific infections were missing for 8 participants)
[b] 880 participants were included in the analyses (complete data available for 754 participants; number of times found infected with any STH infection and species-specific infections was imputed for 8 participants; and verbal score was imputed for 4, 2, 63 and 99 participants at the 2, 3, 4 and 5 year visit, respectively)
[c] Bayley III: Bayley Scales of Infant and Toddler Development, Third Edition

Table 7 shows the results from the Bayesian latent class hierarchical models, adjusted for exposure misclassification using the different prior specifications for the sensitivities of the Kato-Katz and direct smear techniques (prior specifications listed in Table 1). Results were dependent on prior specifications for the sensitivities of the diagnostic techniques. For *Ascaris* infection and any STH infection, point estimates tended to be further from the null when prior specifications for the sensitivities of the diagnostic techniques were lower (i.e. using pessimistic priors). The effect (β (95% CrI)) on cognitive scores between two and five years of age of being infected with *Ascaris* infection one time between one and two years of age compared to never being infected using: a) priors with perfect sensitivities and specificities, b) optimistic priors, c) clinical priors and d) pessimistic priors, was: a) -0.68 (-1.46, 0.10), b) -1.27 (-2.66, 0.12), c) -3.44 (-10.73, 0.19), and d) -4.26 (-9.13, 0.03), respectively. The effect (β (95% CrI) on cognitive scores of being infected with *Ascaris* infection two or three times compared to never being infected using: a) priors with perfect sensitivities and specificities, b) optimistic priors, c) clinical priors and d) pessimistic priors, was: a) -0.63 (-1.60, 0.35), b) -0.99 (-2.23, 0.27), c) -3.08 (-10.32, -0.003), and d) -3.68 (-8.33, -0.40), respectively. Similar effects of the prior specifications were found for the effects of any STH infection. Using the clinical prior specifications, the effects (β (95% CrI)) on cognitive scores of being infected with any STH infection one time and two or three times compared to never being infected were: -4.31 (-10.64, -

0.14), and -3.70 (-10.11, -0.11), respectively. Results for *Trichuris* infection were inconclusive but suggest that either no effect, or very small effects, exist. Similar results were found with respect to the effects on verbal scores (Table 7).

Table 7: Results from Bayesian latent class hierarchical models for the effect of STH infection between one and two years of age on child development scores between two and five years of age, adjusted for misclassification of STH infection in preschool children in Iquitos Peru, September 2011 to July 2016.

	Perfect sensitivity/ specificity β (95% CrI)	**Clinical priors for sensitivity/ specificity β (95% CrI)**	**Optimistic priors for sensitivity/ specificity β (95% CrI)**	**Pessimistic priors for sensitivity/ specificity β (95% CrI)**
1) Outcome: Cognitive score				
# times found infected with *Ascaris:*				
0	REF	REF	REF	REF
1	-0.68 (-1.46, 0.10)	-3.44 (-10.73, 0.19)	-1.27 (-2.66, 0.12)	-4.26 (-9.13, 0.03)
2-3	-0.63 (-1.60, 0.35)	-3.08 (-10.32, -0.003)	-0.99 (-2.23, 0.27)	-3.68 (-8.33, -0.40)
# times found infected with *Trichuris*:				
0	REF	REF	REF	REF
1	-0.10 (-0.96, 0.75)	-0.08 (-1.23, 1.11)	-0.06 (-1.18, 1.06)	-0.14 (-1.35, 1.11)
2-3	-0.27 (-1.77, 1.23)	0.05 (-2.61, 2.87)	-0.06 (-2.75, 2.73)	0.16 (-2.49, 3.04)
# times found infected with any STH:				
0	REF	REF	REF	REF
1	-0.58 (-1.36, 0.20)	-4.31 (-10.64, -0.14)	-1.42 (-2.86, 0.01)	-5.68 (-9.47, -1.07)
2-3	-0.49 (-1.40, 0.40)	-3.70 (-10.11, -0.11)	-0.94 (-2.21, 0.31)	-4.84 (-8.60, -1.04)
2) Outcome: Verbal score				
# times found infected with *Ascaris*:				
0	REF	REF	REF	REF
1	-0.90 (-1.89, 0.08)	-2.81 (-6.05, 0.14)	-1.75 (-3.55, 0.07)	-4.34 (-7.77, 0.002)
2-3	-0.10 (-1.33, 1.13)	-1.41 (-3.88, 0.69)	-0.53 (-2.07, 1.03)	-2.59 (-5.25, 0.31)
# times found infected with *Trichuris*:				
0	REF	REF	REF	REF
1	-0.67 (-1.74, 0.40)	-0.90 (-2.46, 0.61)	-0.93 (-2.43, 0.54)	-0.89 (-2.53, 0.75)
2-3	-0.33 (-2.21, 1.57)	-0.16 (-3.56, 3.17)	-0.003 (-3.46, 3.43)	-0.31 (-3.68, 3.06)
# times found infected with any STH:				
0	REF	REF	REF	REF

1	-0.92 (-1.91, 0.05)	-3.07 (-6.03, -0.24)	-2.17 (-3.97, -0.37)	-4.53 (-7.82, -0.13)
2-3	-0.73 (-1.87, 0.42)	-1.77 (-4.07, 0.30)	-0.95 (-2.54, 0.62)	-2.97 (-5.63, 0.06)

All results are adjusted for maternal education (completed secondary school), cooks using gas, has a toilet with water and connection to public sewage in the home, stunted at one year of age, Bayley-III cognition raw score at one year, number of healthy growth visits attended from birth to one year of age and age. All hierarchical models include an individualized intercept term and an individualized slope for age.

Discussion

The results, adjusted for exposure misclassification, have shown that infection with *Ascaris* and any STH infection during the critical window of development between one and two years of age can have small effects on cognitive and verbal abilities between two and five years of age. On average, children infected with *Ascaris* between one and two years of age had cognitive and verbal scores between one and four points lower compared to children who were never found infected with *Ascaris*. Children infected with any STH infection between one and two years of age had cognitive and verbal scores between one and six points lower, on average, compared to children who were never found infected between one and two years of age. A lack of precision and general uncertainty regarding the sensitivities of the STH diagnostic techniques led to some uncertainty regarding the exact effect sizes and clinical significance of some results.

As in any study whose results rely on data from imperfect diagnostic techniques, the results are dependent on what has been assumed about the unknown properties of the tests. Here, prior specification about the sensitivities of the STH diagnostic techniques used plays a key role. For example, the results obtained assuming optimistic priors about the test properties are quite different than those obtained using pessimistic priors. For the effect of any STH infection on cognitive scores, the effect of being found infected one time using optimistic priors was -1.42 (-2.86, 0.01) and the same effect using the pessimistic priors was -5.68 (-9.47, -1.07). Due to the lack of a gold standard for diagnosing STH infection, determining the true sensitivities of the diagnostic techniques available is not straight forward. Even limiting our search to studies that used Bayesian methods to account for a lack of a gold standard, we found very different values of sensitivities reported in the literature [27, 29]. The relative uncertainty regarding the true

sensitivity values for the STH diagnostic techniques was the rationale for using and comparing a range of prior specifications. The prior specifications were developed based on the research available to date; however, our models suggested that the true sensitivity values may, in fact, be even lower than the pessimistic priors used. This uncertainty regarding the true sensitivity values of the STH diagnostic techniques is problematic and limits researchers' abilities to obtain accurate and informative misclassification-adjusted results for any research question involving STH infections. This is a topic that requires much more research attention.

Despite lacking high precision in estimating effects of STH infections, our results have clinical relevance. If we assume that the true sensitivity values of the STH diagnostic techniques are at least as low as the range of the clinical priors used, we found that being infected with *Ascaris* infection two or three times and being infected with any STH infection one time and two or three times during the second year of life are associated with lower cognitive scores between two and five years of age. We also found that being infected with any STH infection one time during the second year of life is associated with lower verbal scores between two and five years of age. These results are consistent with research that suggests that the first two years of life is a particularly critical time period for brain development across the lifespan [2]. In STH-endemic areas, children as young as eight months of age are being found infected [10] and our results suggest that infection during this critical window of development may have long term consequences on brain development. WHO now recommends that children as young as 12 months of age be included in STH control programs [11]; however, large efforts are still required to obtain the goal coverage rates set by WHO of 75% coverage in endemic countries by 2020 [12]. Political motivation to reach preschool children with appropriate STH control programs

continues to be a major challenge [30] and this is, in part, due to a lack of rigorous evidence quantifying the disease burden of STH infection in this age group. Our results contribute to the body of research evidence documenting the burden of STH infection in young children and highlight the importance of targeting children as of 12 months of age in STH control and prevention approaches.

In the last decade considerable attention has been given to the concept of life course epidemiology and several models and structured approaches have been developed to relate exposures over time to a later health outcome [31, 32]. The current analysis involves a combination of two theoretical approaches to quantify the effect of multiple binary exposure measurements collected over time. A critical period framework approach was used by specifically focussing on the effect of STH infection during a particularly critical window of development, the second year of life. Additionally, an accumulation framework approach was also used by summing indicators of binary variables (i.e. STH infection) over time (i.e. between one and two years of age). We make the assumptions here that STH infection between one and two years of age only is important, irrespective of STH infections at other time points and also make the assumption that the specific timing of infection within the second year of life is unimportant [31].

This study has several strengths and limitations. The longitudinal design allowed us to investigate cumulative STH infections during a particular time window on long term, repeated measures of child development. This allowed us to establish temporality with regard to the timing of the exposure and outcome relationship and allowed us to look at development

trajectories as opposed to development scores at one single time point. This is the first study to specifically investigate the effect of STH infection during the second year of life, the most critical period for brain development across the lifespan, on child development. Our adjustment for STH misclassification allowed us to address an important bias due to measurement error. Since no gold standard diagnostic technique exists for STH infection, all research involving STH infection is limited by this bias and no previous research on this specific topic has yet adjusted for this bias. Among the limitations of the research include our proxy measure for amount of time spent STH-infected. Because it was unfeasible to collect daily stool specimens and to determine how long each child was infected during their second year of life, we used the number of times found infected at the three scheduled study visits during this period as a proxy measure for amount of time infected. Furthermore, we did not have data regarding STH infection during the first year of life which may be important. Additional limitations include non-verifiable assumptions of our regression models including correct model specification and correct prior specifications for the sensitivity and specificity values used in the analyses adjusted for STH misclassification.

In conclusion, this study has documented associations between *Ascaris* and any STH infection and lower cognitive and verbal scores of child development. A lack of precision led to some uncertainty regarding some of the effect sizes and relative clinical significance of the results. Nonetheless, these results contribute to the body of evidence regarding the burden of STH infection and specifically highlight the importance of STH control and prevention in young children two years of age and younger. While this population group isn't necessarily the primary target for STH control, we have shown that STH infections at this age may have important and

irreversible effects on child development. These results are generalizable to the 103 LMICs considered endemic for STH infections and provide evidence that can contribute to reducing global inequities in both child development and poverty.

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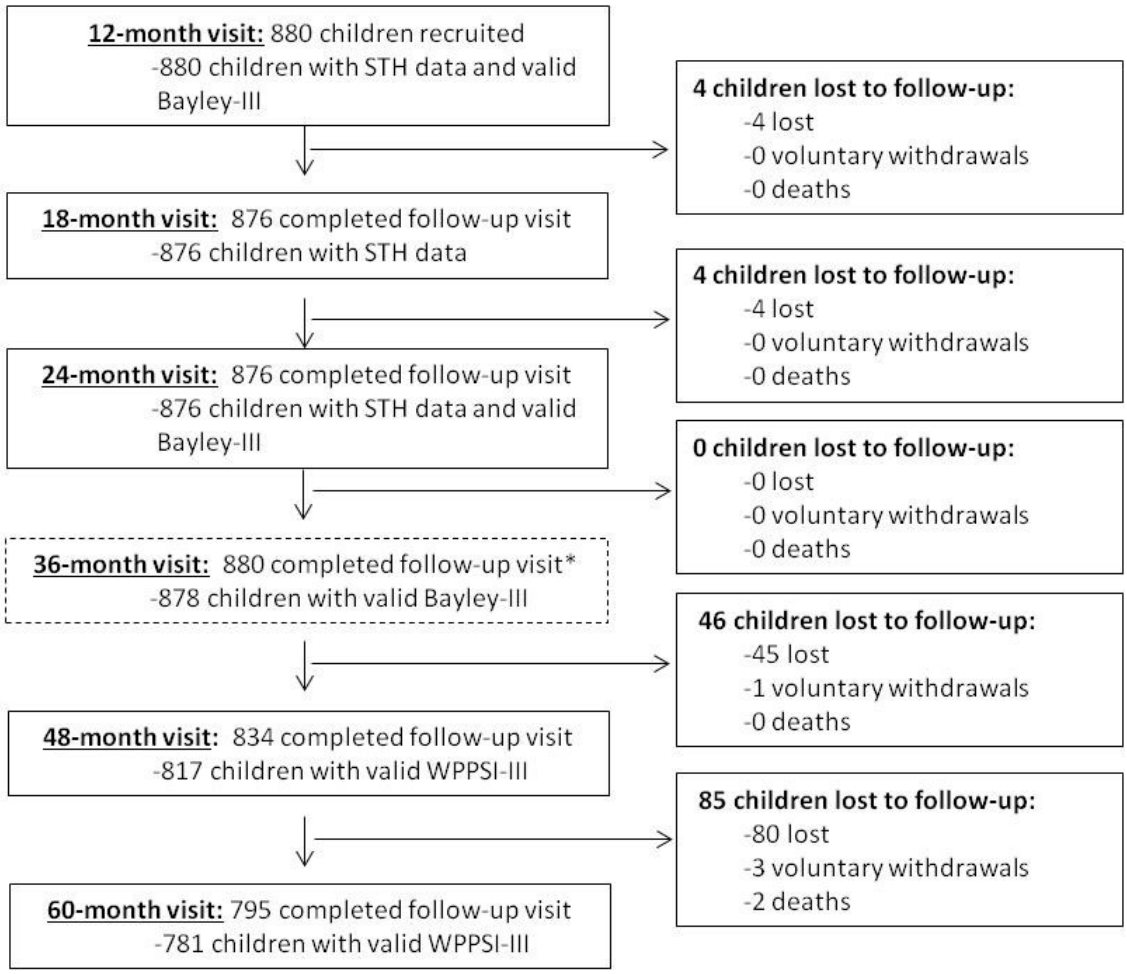
Figure 1: Study flowchart for 880 children randomly sampled at the 36-month visit to be included in this analysis in Iquitos, Peru, September 2011 to July 2016.



*Random sample selected at 36 month study visit

5.5: Preface to Manuscript C

The third and final manuscript addresses thesis objective 3 (to investigate the role of hemoglobin levels and malnutrition as potential mediators of the relationship between *Ascaris* infection between one and five years of age and child development at five years of age). This manuscript follows from the results in Manuscript A and decomposes the total effects of *Ascaris* infection on total IQ score that are presented in Manuscript A. In this Manuscript C, both hemoglobin levels and malnutrition are investigated as mediators of the relationship between *Ascaris* infection and total IQ scores. The total effects are decomposed into the natural direct effects and the natural indirect effects. This manuscript also shows that adjustment for exposure misclassification can be easily performed in mediation analyses under the Bayesian framework, using Bayesian latent class analysis. Investigating mediators of the relationship between *Ascaris* infection and IQ scores, specifically, was chosen because it was found, in Manuscript A, that *Ascaris* had the strongest and most consistent effect on IQ scores. Additional investigation of mediators of the relationship between *Trichuris* infection and IQ scores, and of the relationship between hookworm infection and IQ scores, is the focus of future research.

Results from this manuscript have been presented at the following scientific conference:

1. Blouin B, Casapia M, Gyorkos TW. The effect of soil-transmitted helminth infections on child development: is it mediated by hemoglobin levels? *American Society for Tropical Medicine and Hygiene 66th Annual Meeting*. Baltimore, MD, November 8, 2017.

This manuscript has been formatted for submission to the journal *Epidemiology*.

5.6. Manuscript C: Using Bayesian latent class analysis to adjust for exposure misclassification in a mediation analysis with multiple mediators: The role of hemoglobin levels and malnutrition in the association between *Ascaris* infection and IQ scores

Using Bayesian latent class analysis to adjust for exposure misclassification in a mediation analysis with multiple mediators: The role of hemoglobin levels and malnutrition in the association between *Ascaris* infection and IQ scores

Running head: A mediation analysis adjusted for exposure misclassification

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Abstract

Background: Soil-transmitted helminth (STH) infections have been found to adversely affect child development. The objective was to investigate hemoglobin levels and malnutrition as mediators of an observed association between *Ascaris* infection and IQ scores in children.

Methodology: A longitudinal cohort study was conducted in Iquitos, Peru between September 2011 and July 2016. Children were recruited at one year of age and followed-up annually to five years. *Ascaris* infection and malnutrition were measured at each study visit and hemoglobin levels were measured between three and five years. The exposure was defined as the number of detected *Ascaris* infections between one and five years. IQ scores were measured at five years of age with the Wechsler Preschool and Primary Scale of Intelligence-III. Bayesian latent class regression models were used to correct for exposure misclassification.

Results: A total of 781 children were included in the analysis. In *Ascaris* misclassification adjusted results, mean hemoglobin levels mediated the association between *Ascaris* infection and IQ scores (the natural direct effects (95% CrI) and natural indirect effects (95% CrI) were, compared to 0 or 1 infection: -0.91 (-4.63, 2.82) and -4.25 (-6.92, -1.59) for the effect of 2 infections; -1.41 (-3.79, 0.98) and -1.17 (-1.95, -0.43) for the effect of 3 infections; and, -0.39 (-3.21, 2.41) and -2.65 (-4.32, -0.99) for the effect of 4 or 5 infections).

Conclusion: Hemoglobin levels appear to mediate the association between *Ascaris* infection and IQ scores. Including iron-enhancing interventions in STH control programs should be considered to help reduce STH-attributable morbidity.

Keywords: *Ascaris*, Intelligence Quotient, hemoglobin, malnutrition, exposure misclassification, Bayesian latent class analysis, mediation

Introduction

The soil-transmitted helminth (STH) infections (*Ascaris lumbricoides*, *Trichuris trichiura* and the hookworm species *Ancylostoma duodenale* and *Necator americanus*) are intestinal parasites that have been associated with a number of adverse health consequences. STH-attributable morbidity is generally thought to be due to nutritional impairment caused by intestinal bleeding, nutrient malabsorption, competition for micronutrients, reduction of food intake and diarrhea.[1, 2] Large quantities of *Ascaris* worms in the small intestine, for example, can cause abdominal distension and pain, lactose intolerance and malabsorption of nutrients.[3, 4]

It has been proposed that STH infections adversely affect child development,[5, 6] primarily theorized to be due to mediating factors including malnutrition and anemia. Several studies have documented associations between STH infection and child development;[7-22] however, only two studies have attempted to identify the mediating mechanisms responsible for this association.[11, 16] Both of these studies performed simple mediation analyses (i.e. comparing multivariable regression models with and without possible mediators included) and the study population in both studies was school-age children. One study concluded that hemoglobin may be a mediator of the relationship between *Trichuris* infection and verbal fluency.[11] The other study concluded that hemoglobin may mediate the relationship between *Trichuris* and school performance and also between *Ascaris* and school performance.[16] This latter study also identified malnutrition (height-for-age and body mass index (BMI)) as a potential mediator of the relationship between *Trichuris* and school performance.[16] Both of these analyses investigated each mediator individually and therefore made an intrinsic assumption that malnutrition and hemoglobin levels are independent and do not affect one another, an assumption that is unlikely to be true.

Additionally, both analyses used imperfect STH diagnostic techniques without adjusting for STH misclassification.

While formal mediation analyses were introduced in the 1980s,[23] new advances in causal inference epidemiology has spurred further development of mediation analyses under the counterfactual framework.[24] This has allowed for the decomposition of total effects into direct and indirect effects where investigators can assess the extent to which the effect of an exposure on an outcome is due to a mediating variable. This framework has also been extended to allow for the assessment of multiple mediators along the pathway from the exposure to the outcome.[25] The objective of the current research, therefore, was to apply modern mediation analysis methodology to assess the extent to which a documented association between *Ascaris* infection and IQ scores[20] is mediated by hemoglobin levels and malnutrition, in preschool children. Furthermore, we aimed to show that, by performing mediation analyses within the Bayesian framework, adjustment for misclassification bias can be easily performed.

Methods

The data come from a longitudinal cohort study conducted in the peri-urban and rural areas surrounding Iquitos, Peru between September 2011 and July 2016.[20] A total of 1760 children, who were between 12 and 14 months of age and living in the study area (defined as the catchment area of the 12 major health centers serving the area), were recruited into the study and subsequently followed up annually to five years of age. Details of the study population, recruitment procedures and study design have been described previously.[20] At all annual study visits, in addition to child assessments, a questionnaire was administered to the child's parent or

guardian to obtain information regarding socio-demographic characteristics and the child's health in the previous year.

Exposure ascertainment

Ascaris infection was measured at all study visits from stool specimens with either the Kato-Katz or direct smear techniques (rationale described previously).[20] The majority of the stool specimens (89.9%) were analysed with the Kato-Katz technique, which is the technique currently recommended by the World Health Organization (WHO) for STH diagnosis in large-scale deworming programs.[26] The remaining stool specimens were analysed with the direct smear technique. While no perfect diagnostic technique exists for STH infection, both the Kato-Katz and direct smear techniques are limited by imperfect sensitivities. Recent sensitivity estimates for detecting *Ascaris* infection using the Kato-Katz and direct smear techniques include 63.8% (95% credible interval (CrI): 59.1-68.6%) and 52.1% (95% CrI: 46.6-57.7%), respectively.[27] The exposure, cumulative *Ascaris* infection, was defined as the number of times that each child was found infected with *Ascaris* across the five study visits.

Outcome ascertainment

At the five years of age visit, child development was measured with the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III) – Spanish version.[28] At the three years of age visit, for feasibility purposes, a random sample of 880 children was selected to have development assessed at subsequent visits. Therefore, the children who were administered the WPPSI-III at the five-year visit included children who were randomly sampled at the three-year visit, and who, at the five-year visit, were not lost to follow-up. The WPPSI-III was

administered in a secluded area to avoid distractions, by highly trained research assistants, all of whom had a minimum bachelor's degree level of education and were trained healthcare professionals (i.e. nurses or nurse mid-wives). The outcome variable was defined as the total IQ score from the WPPSI-III at the five years of age visit.

Mediator ascertainment

Child hemoglobin levels were measured at three, four and five years of age. Hemoglobin levels were measured by research assistants using a portable HemoCue® machine, accurate to within 1.5% of the gold standard reference.[29] Blood for this test was drawn from finger-prick using disposable lancets. The first two drops of blood were wiped away and the third drop of blood was used for the analysis. The first mediator variable was defined as each child's mean hemoglobin levels between three and five years of age.

Anthropometry was measured at all study visits. Height-for-age z-score was chosen as the measure of malnutrition because of previous research showing that short child length/height is a strong indicator of chronic malnutrition and is therefore more highly correlated with child development compared to other anthropometric measures.[30] Length/height was measured using a portable stadiometer accurate to the nearest millimeter (Seca Corp., Baltimore, MD, USA). Children were measured with clothes and shoes removed. Child length (recumbent position) was measured in children two years of age and younger and child height (standing position) was measured in children three years of age and older. Height-for-age z-scores were calculated using the WHO Child Growth Standards Stata igrowup package for children up to five years of age[31] and using the WHO Anthro plus software for children older than five.[32] The second mediator

variable was defined as each child's mean height-for-age z-score between one and five years of age.

Ethics

The study was approved by research ethics boards from the Universidad Peruana Cayetano Heredia in Lima, Peru; the Instituto Nacional de Salud in Lima, Peru; and the McGill University Health Centre in Montreal, Canada. Informed consent was obtained from parents/guardians of the participating children. Assent was obtained for any parent under 18 years, with informed consent requested from their parent or guardian over 18 years.

Statistical analyses

Descriptive statistics, including means and standard deviations for continuous variables and counts and proportions for dichotomous and categorical data, were calculated to describe the study population at baseline.

The two directed acyclic graphs (DAGs) used to conceptualize the mediation analysis are presented in Figure 1. The two mediators were assessed sequentially according to the methodology for assessing multiple mediators when the sequential order can be hypothesized.[25] First, the contribution of hemoglobin as a mediator was assessed according to the DAG in Figure 1a to examine the portion of the effect mediated through hemoglobin levels.[25] Two regression models were fit: 1.1) a linear regression model, modeling total IQ scores at five years of age (IQ), conditional on the number of times found infected with *Ascaris* between one and five years

of age (Asc), mean hemoglobin levels between three and five years of age (M_{hb}), and other covariates (C); and 1.2) a linear regression model, modeling M_{hb} conditional on Asc and C:

1.1 $E(IQ|Asc, M_{hb}, C) = \theta_0 + \theta_1 Asc + \theta_2 M_{hb} + \theta_4 C$

1.2 $E(M_{hb}|Asc, C) = \beta_0 + \beta_1 Asc + \beta_2 C$

From these regression models the natural direct effect (NDE) is defined by θ_1. This represents the effect of *Ascaris* infection on IQ scores when mean hemoglobin levels are fixed to the value they would be when the number of times found *Ascaris*-infected is 0 or 1 (i.e. the dashed path in Figure 1a). The natural indirect effect (NIE) is defined by $\theta_2\beta_1$ and represents the effect on IQ scores of changes in *Ascaris* infection that operate through hemoglobin levels (i.e. the dotted path in Figure 1a). This represents the effect on IQ scores of changing mean hemoglobin from what it would be in the absence of the exposure to what it would be in the presence of the exposure, assuming that the exposure is set to some level. The total effect (TE) is the overall effect of *Ascaris* infection on IQ scores and is simply the sum of the NDE and NIE. The number of *Ascaris* infections was treated as a categorical variable and included in the regression models as dummy variables.[33]

Second, the additional mediating contribution of mean height-for-age z-scores was assessed according to the DAG in Figure 1b. Three regression models were fit: 2.1) the same linear regression model as 1.1 but also including mean height-for-age z-score between one and five years of age (M_{haz}) as a covariate; 2.2) the same linear regression model as 1.2; and, 2.3) a linear regression model, modeling M_{haz} conditional on Asc and C:

2.1 $E(IQ|Asc, M_{hb}, C) = \theta_0 + \theta_1 Asc + \theta_2 M_{hb} + \theta_3 M_{haz} + \theta_4 C$

2.2 $E(M_{hb}|Asc, C) = \beta_0^i + \beta_1^i Asc + \beta_2^i C$

2.3 $E(M_{haz}|Asc, C) = \beta_0^{ii} + \beta_1^{ii}Asc + \beta_2^{ii}C$

From these regression models, the NDE is still represented by θ_1(i.e. the dashed path in Figure 1b) and the NIE is represented by ($\beta_1^i\theta_2 + \beta_1^{ii}\theta_3$). The NIE now represents the effect on IQ scores of changes in *Ascaris* infection that operate through hemoglobin levels and height-for-age z-scores (i.e. the dotted paths in Figure 1b). The TE remains the overall effect of *Ascaris* infection on IQ scores and is the sum of the NDE and NIE.

The covariates included in all regression models (i.e. the vector of C) include all measured confounders of the exposure-outcome relationship (Asc-IQ), the mediator-outcome relationships (M_{hb}-IQ and M_{haz}-IQ) and the exposure-mediator relationships (Asc-M_{hb} and Asc-M_{haz}). This analysis is robust to confounding between the two mediators.[25] Confounding was assessed based on theoretical knowledge and statistical criteria (i.e. covariates associated with both variables of each relationship without being mediators of the relationship). The following variables were assessed as confounders of each relationship: socioeconomic status (i.e. residential district, urban/rural status, mother's marital status, maternal education (completed secondary school), mother employed, father or mother's partner employed, number of people living in the home, house material, cooks using gas, presence of electricity in the home, working radio ownership, working television ownership, water source, has a toilet with water and connection to public sewage in the home, and household income); sex; healthcare seeking behavior (i.e. number of healthy growth visits attended between birth and one year and vaccines up to date at baseline); hygiene (i.e. number of baths per day and use of soap for bathing); hospitalizations since birth; baseline nutrition (i.e. stunted, underweight, wasted, birth weight); baseline development scores (i.e. cognition raw score, receptive language raw score, expressive language raw score and, fine

motor raw score); breastfeeding (i.e. exclusively breastfed to six months and continued breastfeeding at one year); and number of year in preschool by five years of age. Univariable linear and multinomial regression models were used to determine if each variable was associated with the outcome variable, mediator variables and exposure variable. Correlations and 2 x 2 tables were also examined to observe relationships between the confounding variables. The final set of confounding variables included socioeconomic status (i.e. maternal education (completed secondary school), cooks using gas, has a toilet with water and connection to public sewage in the home), baseline nutritional status (i.e. stunted), use of health care (i.e. number of healthy growth visits attended between birth and one year), baseline development scores (i.e. cognition raw scores), years of preschool attended by five years of age and birth weight.

Missing exposure data, missing birth weight data, missing hemoglobin data and missing height-for-age z-scores were imputed using multiple imputation. Missing outcome data were not imputed as it has been shown that imputing missing outcome data does not usually prevent bias nor improve precision when estimating regression effects.[34-36] A multinomial regression model was used as the imputation model for cumulative *Ascaris* infection and linear regression was used as the imputation models for birth weight, hemoglobin levels and height-for-age z-scores. All covariates included in the outcome models were also included in the imputation models as well as other relevant covariates, with complete data, that predicted the missing data, as appropriate. Interaction between the exposure and each mediator, as well as interaction between the two mediators, was assessed according to the methodology described by VanderWeele.[37] All interaction terms had point estimates with tight confidence intervals surrounding null values and

allowing for interaction in the mediation analyses had no effect on the results; therefore, interaction was considered unimportant and subsequently ignored.

All analyses were conducted in the Bayesian framework. Analyses without adjustment for exposure (i.e. *Ascaris*) misclassification were compared to analyses with adjustment for exposure misclassification using Bayesian latent class regression models, which have been described previously.[20, 38] The latent class regression models were used to obtain exposure misclassification-adjusted estimates of the coefficients in regression models 1.1-1.2 and 2.1-2.3 (above). These adjusted coefficients were used in the formulae to calculate the NDEs, NIEs and TEs, adjusted for exposure misclassification. Diffuse, non-informative priors were used for all regression coefficients. Informative priors were specified for the sensitivity and specificity values of the Kato-Katz and direct smear diagnostic techniques for *Ascaris* infection (Table 1). Since specificity of the diagnostic techniques is known to be near perfect, specificity values of the diagnostic techniques were always assumed to be between 95-99%. For the sensitivity values of the diagnostic techniques, clinical priors representing the best summary of the information from the past literature and expert opinion,[27] as well as optimistic and pessimistic priors were specified for sensitivity analyses. Optimistic priors are those assuming higher accuracy for the techniques than the "best estimate" clinical priors would suggest, and similarly, pessimistic priors assume lower accuracy than the clinical priors would suggest. All prior densities on the sensitivity and specificity parameters were assumed to follow a beta distribution. The models were jointly estimated using the Gibbs sampler. WinBUGS (version 1.4.3, MRC, Cambridge) was used to run the Markov Chain Monte Carlo (MCMC) process used for all inferences.

Results

Of the 880 children who were randomly sampled to have their development measured as of three years of age, 880 (100%), 876 (99.5%), 880 (100%), 834 (94.8%) and 795 (90.3%) completed the one, two, three, four and five years of age visits, respectively. The majority of children lost to follow-up moved homes and/or were impossible to locate. Therefore, missing data were assumed to be random. A total of 781 children (88.8%) had valid IQ score measurements at five years of age. Of these, the number of detected *Ascaris* infections was missing for a total of 11 (1.4%) children; mean hemoglobin levels were missing for 7 (0.9%) children; mean height-for-age z-scores were missing for 11 (1.4%) children; and birth weight was missing for 74 (9.5%) children.

A description of the study population is presented in Table 2. The mean age at recruitment was 12.1 months and 51.9% of the study population was male. *Ascaris* prevalence, hemoglobin levels and height-for-age z-scores at each study visit are presented in Table 3. *Ascaris* prevalence increased from 11% to 30.7% between one and two years of age and remained at approximately 30% from two to five years of age. Mean hemoglobin levels were between 11.0 g/dL and 11.3 g/dL at the three to five years of age visits. Mean height-for age z-scores were low at all study visits with a range of -1.9 (at the two-year visit) to -1.2 (at the five-year visit).

The results from the mediation analysis corresponding to DAG 1a, investigating hemoglobin levels alone as a mediator of the relationship between *Ascaris* infection and IQ scores, are shown in Table 4. The results, unadjusted for misclassification of *Ascaris,* suggest that hemoglobin

levels do not act as a mediator of the association between *Ascaris* infection and IQ scores. This is represented by the near zero NIEs for all comparisons.

The results adjusted for misclassification of *Ascaris* infection, however, suggest that hemoglobin levels do, in fact, act as an important mediator of the relationship between the number of detected *Ascaris* infections and IQ scores. Using the clinical priors, the NDEs (95% CrI) and NIEs (95% CrI), compared to 0 or 1 infection are: -0.91 (-4.63, 2.82) and -4.25 (-6.92, -1.59) for the effect of 2 infections; -1.41 (-3.79, 0.98) and -1.17 (-1.95, -0.43) for the effect of 3 infections; and, -0.39 (-3.21, 2.41) and -2.65 (-4.32, -0.99) for the effect of 4 or 5 infections. The total effects of two, three and four or five infections appear to be attributable to mediation through hemoglobin levels. The total effect of three infections is, to some extent, attributable to an indirect effect mediated through hemoglobin levels but may also be attributable to pathways that do not involve hemoglobin levels. Similar results were found using the optimistic and pessimistic priors; however, using priors that suggested lower sensitivity values tended to lead to larger point estimates for the NDEs, NIEs and TEs.

The results from the mediation analysis corresponding to DAG 1b, investigating hemoglobin levels and height-for-age z-scores as sequential mediators of the relationship between *Ascaris* infection and IQ scores, are shown in Table 5. The results, unadjusted for misclassification of *Ascaris* infection, suggest that a small amount of mediation is due to height-for-age z-scores. While the misclassification unadjusted results for the analysis of hemoglobin levels alone as a mediator suggested no mediation of hemoglobin, the results including both hemoglobin and

height-for-age z-scores suggest that a modest amount of mediation exists, which can therefore be attributed to the inclusion of height-for-age z-scores as a mediator.

Similar to the analysis corresponding to DAG 1a, the *Ascaris*-misclassification adjusted results from the analysis of hemoglobin levels and height-for-age z-score as mediators suggest that mediation is indeed present. Using the clinical priors, the NDEs (95% CrI) and NIEs (95% CrI), compared to 0 or 1 infection, are: -0.49 (-3.68, 2.72) and -3.29 (-4.90, -1.78) for the effect of 2 infections; -3.97 (-7.24, -0.49) and 0.03 (-0.99, 1.02) for the effect of 3 infections; and, -1.27 (-4.70, 2.19) and -2.41 (-3.90, -1.18) for the effect of 4 or 5 infections. A lack of precision leading to wide confidence intervals, however, precludes any meaningful comparisons between results that only include hemoglobin levels as a mediator and those that include both hemoglobin levels and height-for-age z-scores as mediators. The NDE, NIE and TE results from the two analyses are similar, and confidence intervals overlap, perhaps suggesting that no additional mediating contribution of height-for-age exists. However, the wide confidence intervals also allow for differences to exist. Therefore, the results for determining the additional mediating contribution of mean height-for-age z-scores (apart from those on the same pathway as hemoglobin levels) are inconclusive. A summary of the main results (NDEs, NIEs and TEs from analyses using the clinical priors) is presented in Figure 2.

Discussion

The results suggest that hemoglobin levels act as an important mediator of the relationship between *Ascaris* infection and IQ scores. These results are consistent with a previous study that found that hemoglobin levels may mediate the relationship between *Ascaris* infection and school

performance.[16] While decreased hemoglobin levels are most commonly associated with *Trichuris* and hookworm infections,[39-41] we have shown that *Ascaris* infection may also have an important effect on hemoglobin levels. It has been shown that the presence of *Ascaris* in the small intestine can cause erosion of the intestinal villi responsible for nutrient absorption leading to malabsorption of various nutrients, including iron.[3, 4, 42] A congruent body of evidence exists documenting the importance of iron for healthy brain development during the early years of life.[5] Iron deficiency can cause brain abnormalities because iron is essential for proper neurogenesis and for the differentiation of certain brain cells and regions.[43-45] Based on animal models, it has been suggested that iron deficiency impairs myelination, dendritogenesis, synaptogensis and neurotransmission – processes that are highly dependent on iron-containing enzymes and hemoproteins.[46, 47]

While the results suggest that hemoglobin levels appear to play an important role in the relationship between *Ascaris* and IQ scores, they also show that hemoglobin levels are unlikely to be the only variable responsible for this relationship. The results suggest that it is likely that other pathways exist between *Ascaris* infection and IQ scores that do not involve hemoglobin levels or height-for-age z-scores. Several theories have been proposed as alternative mediation pathways. One theory suggests that inflammation caused by STH infection leads to specific cytokine actions that could affect brain function and behaviour.[48] STH infections may produce substances that interfere with neuronal transmission and the immunological mechanism involved in combating STH infections may affect brain function.[48, 49] It has also recently been proposed that possible effects of helminths on cognitive development may be due to helminth-induced changes to gut microbiota composition and diversity, especially in young children.[50] We were

unable to determine if malnutrition (independent of hemoglobin levels) plays an important role. Mediation analyses conducted with a larger sample size may be able to detect smaller changes and may be able to disentangle the effects of hemoglobin levels and malnutrition.

The mediation analyses conducted required several assumptions. First, it was assumed that there is no residual confounding of: 1) the exposure-outcome relationship; 2) the mediator-outcome relationship; and 3) the exposure-mediator relationship. Confounding was assessed for each of the three relationships separately with a variety of possible confounding variables and all observed confounders of the three relationships were included in the set of covariates. However, as with all analyses using observational data, this assumption is unverifiable. Second, it was assumed that there is no confounder of the mediator-outcome relationship that is affected by the exposure. All of the confounders of the mediator-outcome relationship were measured before the exposure. Therefore, due to temporality, it can be assumed that the exposure did not affect these covariates. Finally, it was assumed that there is no interaction between the exposure and mediator variables and between the two mediators. Interaction was assessed between *Ascaris* and hemoglobin levels, *Ascaris* and height-for-age z-scores, and between hemoglobin and height-for-age z-scores. No evidence of deviation from additivity was found for any of these relationships.

This research has several limitations. First, hemoglobin levels were only measured as of the three-year visit. Therefore, important effects of hemoglobin levels between one and three years of age may have been missed. Second, both the exposure (number of detected *Ascaris* infections) and the mediators (mean hemoglobin levels and mean height-for-age z-scores) are

expressed as summary measures over the same time period (i.e. between one and five years of age). Therefore, temporal ordering of the exposure and the mediators cannot be established. This could lead to reverse causation bias. It is, however, biologically very unlikely that low hemoglobin levels or malnutrition would cause *Ascaris* infection. Finally, the sample size was not ideal for obtaining highly precise estimates or for detecting small differences between the models, especially for those analyses that are adjusted for exposure misclassification. This prevented any firm conclusions regarding the additional effect of malnutrition as a mediator to be made.

This is the first study to conduct a modern mediation analysis of the relationship between *Ascaris* infection and child development. We have used recently developed methodology to investigate both hemoglobin levels and height-for-age z-scores as mediators and also used Bayesian methods to adjust for exposure misclassification throughout the analysis. These results contribute additional evidence of a possible effect of STH infection on child development attributable to reduced hemoglobin levels. We have shown however, that other factors may also be involved. Furthermore, we have shown that misclassification of STH infection is an important bias that, if not taken into account, can lead to biased results and unsubstantiated conclusions. Our results contribute to the evidence base that suggests that STH control programs targeted to young children may be beneficial in terms of child development. Research into the added value of nutritional interventions to improve iron levels within STH control programs, like iron-containing micronutrient supplements or nutrition education programs, would be useful.

Table 1: Probability ranges and corresponding coefficients of the beta prior densities for the sensitivities and specificities of the Kato-Katz and direct smear techniques used in the Bayesian latent class analyses to adjust for misclassification of *Ascaris* infection

	Kato-Katz		Direct smear	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
Clinical priors:				
Range* (%)	55-75	95-99	40-60	95-99
Beta distribution α coefficient	55.86	231.95	47.30	231.95
Beta distribution β coefficient	29.63	6.26	47.30	6.26
Optimistic priors:				
Range* (%)	70-80	95-99	55-65	95-99
Beta distribution α coefficient	214.34	231.95	220.49	231.95
Beta distribution β coefficient	70.81	6.26	146.68	6.26
Pessimistic priors:				
Range* (%)	50-60	95-99	35-45	95-99
Beta distribution α coefficient	208.45	231.95	146.68	231.95
Beta distribution β coefficient	170.38	6.26	220.49	6.26

* Prior probability ranges were developed based on previous research[27] and expert opinion.

Table 2: Description of the 880 preschool children included in this study population, Iquitos, Peru, September 2011 to July 2016

Variable	Mean (sd) or % (n)
Sex [% male (n)]	51.9 (457)
Age at recruitment (in months) [mean (sd)]	12.1 (0.3)
Maternal education (completed secondary school) [% (n)]	32.5 (286)
Cooks using gas [% (n)]	31.4 (276)
Toilet with water and connection to public sewage in the home [% (n)]	43.6 (384)
Stunted at 1 year [% (n)]	24.0 (211)
Bayley-III cognition raw score at 1 year [mean (sd)]	42.6 (3.02)
# healthy growth visits from birth to 1 year [mean (sd)]	7.6 (3.5)
# years in preschool by 5 years [mean (sd)][a]	1.2 (0.7)
Birth weight (in kg) [mean (sd)][b]	3.1 (0.5)
Total IQ score at 5 years [mean (sd)][c]	77.1 (10.8)

[a] Missing data for 86 participants; [b] Missing data for 79 participants; [c] Missing data for 99 participants

Table 3: *Ascaris* prevalence, hemoglobin levels and height-for-age z-scores of the 880 preschool children included in the study population at each study visit, Iquitos, Peru, September 2011 to July 2016

	Ascaris prevalence [% (n)]	Hemoglobin levels [mean (sd)]	Height-for-age z-score [mean (sd)]
1 year	11.0 (97)[a]	--	-1.33 (0.97)[a]
2 years	30.7 (269)[b]	--	-1.90 (0.94)[b]
3 years	30.6 (269)[a]	11.0 (1.1)[a]	-1.64 (0.85)[a]
4 years	28.3 (236)[c]	11.2 (1.0)[d]	-1.39 (0.86)[d]
5 years	28.2 (224)[e]	11.3 (1.0)[e]	-1.23 (0.80)[f]
Composite*	1.3 (1.2)[g]	11.2 (0.7)[h]	-1.50 (0.77)[g]

[a] Missing data for 0 participants; [b] Missing data for 4 participants; [c] Missing data for 47 participants; [d] Missing data for 46 participants; [e] Missing data for 85 participants; [f] Missing data for 86 participants; [g] Missing data for 96 participants; [h] Missing data for 92 participants; * Refers to mean number of detected *Ascaris* infections between one and five years of age, mean hemoglobin levels between three and five years of age, and mean height-for-age z-score between one and five years of age.

Table 4: Linear regression results (i.e. beta values and 95% credible intervals) with NDEs, NIEs and TEs from the mediation analysis corresponding to DAG 1a investigating the contribution of mean hemoglobin levels as a mediator of the association between number of *Ascaris* infections and total IQ score at five years of age, in preschool children in Iquitos, Peru (September 2011-July 2016). Unadjusted and adjusted (for misclassification of *Ascaris* infection using the three sets of priors described in Table 1) results are presented.

	No adjustment for misclassification Predicted change to IQ score (95% CrI)	**Adjustment using clinical priors** Predicted change to IQ score (95% CrI)	**Adjustment using optimistic priors** Predicted change to IQ score (95% CrI)	**Adjustment using pessimistic priors** Predicted change to IQ score (95% CrI)
Outcome model (1.1):				
# of detected *Ascaris* infections:				
2	-1.77 (-3.53, -0.01)	-0.91 (-4.63, 2.82)	-1.37 (-4.21, 1.46)	-3.31 (-7.49, 0.75)
3	-2.30 (-4.44, -0.18)	-1.41 (-3.79, 0.98)	-3.97 (-6.76, -1.11)	-5.53 (-8.35, -2.56)
4-5	-0.10 (-3.17, 2.97)	-0.39 (-3.21, 2.41)	-1.22 (-4.48, 2.09)	-3.23 (-6.46, 0.13)
Mean hemoglobin levels	1.78 (0.84, 2.74)	1.91 (0.72, 3.10)	2.12 (0.97, 3.26)	2.11 (0.92, 3.30)
Mother completed secondary school	4.86 (3.29, 6.44)	4.87 (3.33, 6.41)	4.60 (3.05, 6.16)	4.82 (3.27, 6.37)
Cooks using gas	1.94 (0.36, 3.50)	2.13 (0.54, 3.73)	1.80 (0.20, 3.39)	1.91 (0.29, 3.50)
Toilet with water and connection to public sewage in the home	2.67 (1.17, 4.15)	2.85 (1.32, 4.37)	2.53 (0.98, 4.08)	2.78 (1.24, 4.31)
Stunted at 1 year	-1.94 (-3.60, -0.25)	-1.95 (-3.67, -0.21)	-1.77 (-3.48, -0.05)	-1.70 (-3.43, 0.02)
Raw cognition score at 1 year	0.25 (0.02, 0.47)	0.28 (0.06, 0.49)	0.26 (0.04, 0.47)	0.28 (0.06, 0.50)
# healthy growth visits from birth to 1 year	0.23 (0.04, 0.43)	0.24 (0.05, 0.44)	0.21 (0.01, 0.41)	0.21 (0.01, 0.41)
# years in preschool by 5 years	2.08 (0.98, 3.16)	2.12 (1.04, 3.19)	1.98 (0.88, 3.07)	2.10 (1.02, 3.16)
Birth weight	1.76 (0.27, 3.23)	1.60 (-0.27, 3.49)	1.91 (0.10, 3.73)	1.80 (-0.07, 3.66)
Mediation model (1.2):				
# of detected *Ascaris* infections:				
2	-0.01 (-0.14, 0.12)	-2.23 (-2.37, -2.06)	-1.06 (-1.19, -0.92)	-2.05 (-2.38, -1.11)
3	-0.06 (-0.22, 0.10)	-0.61 (-0.71, -0.51)	0.22 (-0.01, 0.44)	-0.44 (-0.65, 0.15)
4-5	-0.0002 (-0.23, 0.23)	-1.39 (-1.50, -1.27)	-0.54 (-0.76, -0.32)	-1.28 (-1.52, -0.59)

Mother completed secondary school	0.08 (-0.03, 0.20)	0.002 (-0.08, 0.09)	0.05 (-0.07, 0.17)	0.002 (-0.10, 0.13)
Cooks using gas	0.14 (0.02, 0.25)	0.23 (0.14, 0.32)	0.19 (0.08, 0.31)	0.22 (0.10, 0.32)
Toilet with water and connection to public sewage in the home	-0.04 (-0.15, 0.07)	-0.22 (-0.30, -0.13)	-0.15 (-0.26, -0.03)	-0.18 (-0.28, -0.07)
Stunted at 1 year	-0.05 (-0.17, 0.08)	-0.44 (-0.54, -0.35)	-0.27 (-0.40, -0.14)	-0.41 (-0.53, -0.24)
Raw cognition score at 1 year	0.008 (-0.01, 0.02)	-0.003 (-0.01, 0.01)	0.01 (-0.01, 0.02)	0.001 (-0.01, 0.02)
# healthy growth visits from birth to 1 year	0.003 (-0.01, 0.02)	0.002 (-0.01, 0.01)	0.01 (-0.01, 0.02)	0.01 (-0.01, 0.02)
# years in preschool by 5 years	0.07 (-0.01, 0.15)	-0.03 (-0.10, 0.03)	0.06 (-0.02, 0.14)	0.04 (-0.03, 0.11)
Birth weight	-0.15 (-0.26, -0.04)	-1.00 (-1.08, -0.91)	-0.68 (-0.80, -0.55)	-0.92 (-1.05, -0.64)
Natural direct effects:				
# of detected *Ascaris* infections:				
2	-1.77 (-3.53, -0.01)	-0.91 (-4.63, 2.82)	-1.37 (-4.21, 1.46)	-3.31 (-7.49, 0.75)
3	-2.30 (-4.44, -0.18)	-1.41 (-3.79, 0.98)	-3.97 (-6.76, -1.11)	-5.53 (-8.35, -2.56)
4-5	-0.10 (-3.17, 2.97)	-0.39 (-3.21, 2.41)	-1.22 (-4.48, 2.09)	-3.23 (-6.46, 0.13)
Natural indirect effects:				
# of detected *Ascaris* infections:				
2	-0.02 (-0.27, 0.23)	-4.25 (-6.92, -1.59)	-2.24 (-3.53, -1.01)	-4.32 (-7.20, -1.66)
3	-0.10 (-0.42, 0.18)	-1.17 (-1.95, -0.43)	0.46 (-0.02, 1.10)	-0.91 (-1.79, 0.32)
4-5	-0.0003 (-0.44, 0.43)	-2.65 (-4.32, -0.99)	-1.14 (-2.01, -0.45)	-2.68 (-4.52, -0.95)
Total effects:				
# of detected *Ascaris* infections:				
2	-1.79 (-3.57, -0.03)	-5.16 (-8.10, -2.21)	-3.61 (-6.02, -1.20)	-7.62 (-11.42, -2.86)
3	-2.40 (-4.56, -0.27)	-2.58 (-4.99, -0.16)	-3.51 (-6.37, -0.58)	-6.44 (-9.47, -2.86)
4-5	-0.10 (-3.17, 2.99)	-3.04 (-5.55, -0.53)	-2.36 (-5.55, 0.87)	-5.92 (-9.08, -1.91)

Table 5: Linear regression results (i.e. beta values and 95% credible intervals) with NDEs, NIEs and TEs from the mediation analysis corresponding to DAG 1b investigating the joint contribution of mean hemoglobin levels and mean height-for-age z-scores as mediators of the association between number of *Ascaris* infections and total IQ score at five years of age, in preschool children in Iquitos, Peru (September 2011-July 2016). Unadjusted and adjusted (for misclassification of *Ascaris* infection using the three sets of priors described in Table 1) results are presented.

	No adjustment for misclassification Predicted change to IQ score (95% CrI)	**Adjustment using clinical priors** Predicted change to IQ score (95% CrI)	**Adjustment using optimistic priors** Predicted change to IQ score (95% CrI)	**Adjustment using pessimistic priors** Predicted change to IQ score (95% CrI)
Outcome model (2.1):				
# of detected *Ascaris* infections:				
2	-1.79 (-3.48, -0.08)	-0.49 (-3.68, 2.72)	-0.72 (-3.93, 2.25)	-3.09 (-6.84, 0.56)
3	-1.80 (-3.87, 0.30)	-3.97 (-7.24, -0.49)	-3.28 (-6.05, -0.46)	-3.83 (-7.19, -0.60)
4-5	-0.01 (-3.03, 2.98)	-1.27 (-4.70, 2.19)	-0.58 (-3.80, 2.70)	-4.31 (-7.89, -0.87)
Mean hemoglobin levels	1.44 (0.49, 2.38)	2.39 (1.21, 3.57)	2.14 (0.99, 3.33)	1.99 (0.76, 3.21)
Mean height-for-age z scores	3.31 (2.17, 4.45)	3.09 (1.91, 4.27)	3.10 (1.93, 4.28)	3.11 (1.95, 4.27)
Mother completed secondary school	4.33 (2.79, 5.86)	4.03 (2.47, 5.59)	4.16 (2.62, 5.69)	3.97 (2.42, 5.53)
Cooks using gas	1.76 (0.21, 3.31)	1.42 (-0.19, 3.02)	1.59 (0.01, 3.17)	1.35 (-0.27, 2.97)
Toilet with water and connection to public sewage in the home	2.23 (0.75, 3.69)	2.10 (0.54, 3.66)	2.21 (0.68, 3.73)	1.75 (0.19, 3.30)
Stunted at 1 year	1.06 (-0.86, 3.01)	1.40 (-0.66, 3.45)	1.29 (-0.74, 3.33)	1.15 (-0.93, 3.23)
Raw cognition score at 1 year	0.18 (-0.04, 0.40)	0.21 (-0.01, 0.43)	0.22 (0.01, 0.44)	0.26 (0.05, 0.47)
# healthy growth visits from birth to 1 year	0.16 (-0.03, 0.35)	0.14 (-0.06, 0.33)	0.14 (-0.05, 0.34)	0.16 (-0.04, 0.35)
# years in preschool by 5 years	1.81 (0.74, 2.88)	1.71 (0.63, 2.78)	1.74 (0.66, 2.81)	1.63 (0.55, 2.71)
Birth weight	0.95 (-0.51, 2.43)	1.92 (0.12, 3.74)	1.63 (-0.21, 3.48)	1.30 (-0.59, 3.17)
Mediation model (2.2):				
# of detected *Ascaris* infections:				
2	-0.01 (-0.14, 0.12)	-1.13 (-1.28, -0.98)	-0.99 (-1.19, 0.09)	1.04 (0.90, 1.19)
3	-0.06 (-0.21, 0.10)	0.20 (-0.02, 0.41)	0.27 (-0.23, 0.49)	-0.50 (-0.67, -0.32)

4-5	0.001 (-0.23, 0.23)	-0.65 (-0.92, -0.43)	-0.67 (-0.83, -0.07)	0.49 (0.33, 0.66)
Mother completed secondary school	0.08 (-0.03, 0.20)	0.07 (-0.05, 0.19)	0.06 (-0.06, 0.17)	0.03 (-0.09, 0.15)
Cooks using gas	0.14 (0.02, 0.25)	0.20 (0.08, 0.31)	0.20 (0.07, 0.32)	0.20 (0.09, 0.32)
Toilet with water and connection to public sewage in the home	-0.04 (-0.15, 0.07)	-0.12 (-0.23, -0.0001)	-0.13 (-0.24, -0.001)	-0.14 (-0.25, -0.02)
Stunted at 1 year	-0.05 (-0.17, 0.08)	-0.31 (-0.44, -0.19)	-0.29 (-0.44, -0.01)	-0.35 (-0.48, -0.22)
Raw cognition score at 1 year	0.01 (-0.01, 0.02)	0.01 (-0.01, 0.02)	0.01 (-0.01, 0.02)	-0.0003 (-0.02, 0.02)
# healthy growth visits from birth to 1 year	0.003 (-0.01, 0.02)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.02)	-0.0002 (-0.02, 0.01)
# years in preschool by 5 years	0.07 (-0.01, 0.15)	0.06 (-0.02, 0.14)	0.07 (-0.01, 0.15)	0.01 (-0.07, 0.09)
Birth weight	-0.14 (-0.25, -0.03)	-0.71 (-0.82, -0.59)	-0.67 (-0.83, -0.07)	-0.79 (-0.92, -0.67)
Mediation model (2.3):				
# of detected *Ascaris* infections:				
2	0.007 (-0.10, 0.11)	-0.19 (-0.37, -0.01)	-0.12 (-0.31, 0.29)	0.14 (-0.03, 0.31)
3	-0.15 (-0.28, -0.02)	-0.15 (-0.38, 0.07)	-0.14 (-0.42, 0.06)	-0.08 (-0.27, 0.10)
4-5	-0.02 (-0.21, 0.16)	-0.28 (-0.53, -0.04)	-0.21 (-0.45, 0.15)	-0.02 (-0.22, 0.18)
Mother completed secondary school	0.17 (0.08, 0.27)	0.15 (0.05, 0.24)	0.15 (0.06, 0.25)	0.15 (0.06, 0.25)
Cooks using gas	0.07 (-0.03, 0.17)	0.06 (-0.04, 0.16)	0.06 (-0.03, 0.16)	0.08 (-0.02, 0.17)
Toilet with water and connection to public sewage in the home	0.13 (0.04, 0.22)	0.09 (-0.01, 0.19)	0.10 (0.01, 0.20)	0.11 (0.01, 0.20)
Stunted at 1 year	-0.91 (-1.01, -0.81)	-0.94 (-1.05, -0.84)	-0.93 (-1.04, -0.82)	-0.96 (-1.06, -0.85)
Raw cognition score at 1 year	0.02 (0.01, 0.04)	0.02 (0.01, 0.03)	0.02 (0.01, 0.04)	0.02 (0.01, 0.03)
# healthy growth visits from birth to 1 year	0.02 (0.01, 0.04)	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)
# years in preschool by 5 years	0.09 (0.02, 0.16)	0.08 (0.01, 0.15)	0.09 (0.02, 0.15)	0.08 (0.02, 0.15)
Birth weight	0.24 (0.15, 0.33)	0.17 (0.06, 0.27)	0.19 (0.07, 0.37)	0.13 (0.03, 0.24)
Natural direct effects:				
# of detected *Ascaris* infections:				
2	-1.79 (-3.48, -0.08)	-0.49 (-3.68, 2.72)	-0.72 (-3.93, 2.25)	-3.09 (-6.84, 0.56)

3	-1.80 (-3.87, 0.30)	-3.97 (-7.24, -0.49)	-3.28 (-6.05, -0.46)	-3.83 (-7.19, -0.60)
4-5	-0.01 (-3.03, 2.98)	-1.27 (-4.70, 2.19)	-0.58 (-3.80, 2.70)	-4.31 (-7.89, -0.87)
Natural indirect effects:				
# of detected *Ascaris* infections:				
2	0.01 (-0.40, 0.42)	-3.29 (-4.90, -1.78)	-2.55 (-4.26, 1.00)	2.51 (1.06, 4.03)
3	-0.58 (-1.14, -0.09)	0.03 (-0.99, 1.02)	0.16 (-1.63, 1.15)	-1.24 (-2.19, -0.38)
4-5	-0.08 (-0.81, 0.64)	-2.41 (-3.90, -1.18)	-1.69 (-3.07, 0.67)	0.92 (-0.02, 1.98)
Total effects:				
# of detected *Ascaris* infections:				
2	-1.79 (-3.53, -0.01)	-3.78 (-6.60, -0.96)	-3.26 (-5.73, -0.54)	-0.59 (-3.79, 2.51)
3	-2.39 (-4.53, -0.24)	-3.93 (-7.23, -0.52)	-3.12 (-6.06, -0.19)	-5.07 (-8.38, -1.87)
4-5	-0.08 (-3.17, 2.97)	-3.67 (-7.09, -0.30)	-2.28 (-5.52, 1.21)	-3.39 (-6.75, -0.14)

Figure 1: Directed acyclic graphs (DAGs) used to conceptualize the mediation analysis

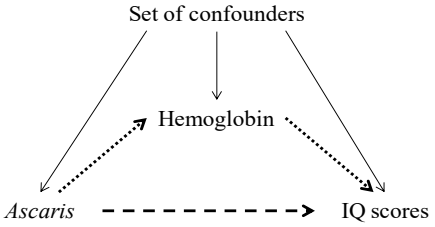


Figure 1a: DAG for analysis only considering hemoglobin as a mediator of the relationship between *Ascaris* infection and IQ scores

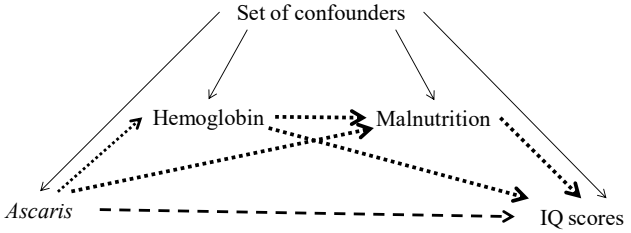
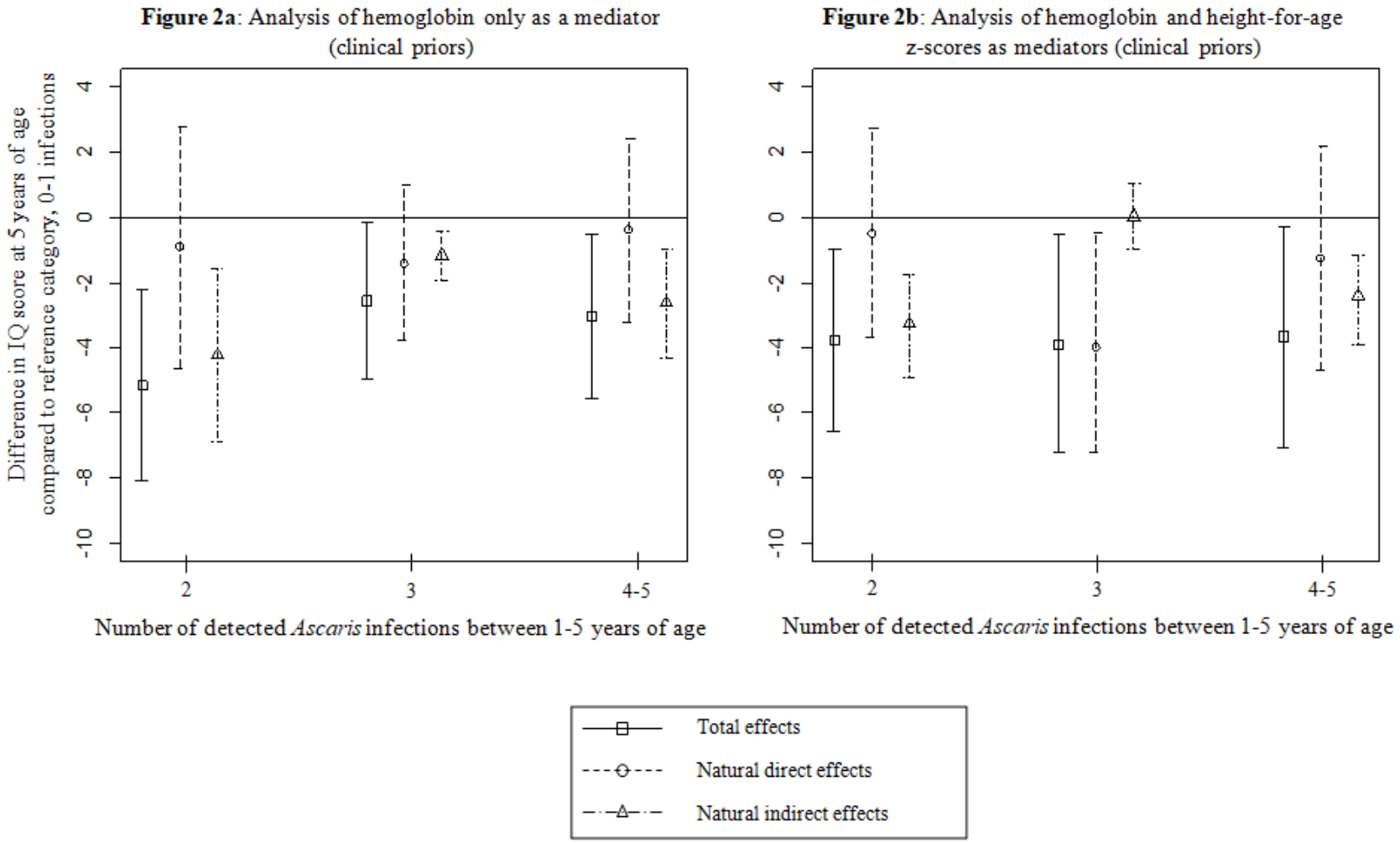


Figure 1b: DAG for analysis considering both hemoglobin levels and malnutrition jointly as mediators of the relationship between *Ascaris* infection and IQ scores

Direct effects

Indirect effects

Figure 2: Total effects, natural direct effects and natural indirect effects with 95% credible intervals from the mediation analyses investigating hemoglobin levels alone as a mediator of the relationship between number of *Ascaris* infections and IQ scores and investigating hemoglobin levels and height-for-age z-scores as mediators, in preschool children in Iquitos, Peru (September 2011-July 2016). Results are adjusted for misclassification of *Ascaris* infection using clinical priors.



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6. Discussion

6.1. Summary of results

This research has documented important associations between STH infections and lower child development in preschool children using rigorous epidemiological methods. Although causation cannot necessarily be implied from these results, the mediation analysis allowed for a mechanism of effect to be identified which supports the idea that the association observed between STH infections and child development may be causal. First, it was found that children with a higher number of cumulative *Ascaris* infections, *Trichuris* infections, hookworm infections and any STH infection, between one and five years of age, were more likely to have lower IQ scores at five years of age. Second, when the window of exposure was narrowed to the second year of life (a particularly critical time of development), a child's cognitive and verbal abilities between two and five years of age were also found to have been affected by having had a higher number of cumulative *Ascaris* infections or any STH infection. On average, the point estimates from the analysis investigating the effect of STH infection between one and two years of age were smaller than those from the analysis investigating the effect of STH infection between one and five years of age. This suggests that, while infection between one and two years of age can have important long term effects on child development, the effects of infection during the preschool years (i.e. up to five years of age) accumulate over time with continued or repeated infections. However, a lack of precision leading to wide confidence intervals prevented meaningful comparisons between these two sets of results. Finally, hemoglobin levels were identified as an important mediator of the relationship between *Ascaris* infection between one and five years of age and total IQ scores at five years of age. The results from the mediation analysis also suggest that factors other than hemoglobin levels may be responsible for the observed association between *Ascaris* and IQ scores.

These results are consistent with previous research that has documented associations between *Ascaris* infection and lower child development [17, 18, 21, 22], between *Trichuris* and lower child development [17, 18, 20-23], between hookworm and lower child development [13, 18, 24] and between any STH infection and lower child development [14-16, 19, 25]. The results are also consistent with a previous study that found that hemoglobin levels acted as a mediator of the relationship between *Ascaris* infection and lower child development [22].

Although a small association was found between hookworm infection between one and five years of age and lower total IQ scores, no significant effects were observed on verbal or performance IQ scores. This was likely due to the low prevalence of hookworm infection in the study population. The prevalence of hookworm infection at baseline was only 0.34%. While this increased steadily over the four years of follow-up, it only reached 4.7% at five years of age. On average, children found to be infected with hookworm at least once between one and five years of age had slightly lower verbal and performance IQ scores at five years compared to children who were never found infected; however, the wide confidence intervals (likely due to the relatively low prevalence of infection) included the possibility that no effect exists or that a small effect in the opposite direction may even exist. Due to the very low prevalence of hookworm infection between one and two years of age, it was not possible to investigate the effect of hookworm infection on child development during this critical time period.

In the last decade considerable attention has been given to the concept of life course epidemiology and several models and structured approaches have been developed to relate exposures over time to a later health outcome [182, 183]. Throughout this research, the effect of the number of detected STH infections over time was used as the exposure instead of looking at one infection at one single time point, as in previous studies. While it may be unlikely that one single STH infection will have serious long term effects, the effect of cumulative infections over time may be a more relevant and realistic exposure. In Manuscript A, an accumulation hypothesis is tested by summing indicators of binary variables (i.e. STH infection) over time (i.e. between one and five years of age). In this analysis, the assumption made is that cumulative STH infection between one and five years of age affects child development irrespective of the specific timing of infection [182]. Manuscript B combines an accumulation hypothesis with a critical period hypothesis. The goal was to determine if a more precise time point during the first five years of life exists when STH infections may have a particularly important and irreversible effect on child development. A critical period framework approach was used by specifically focussing on the effect of STH infection during a particularly critical window of development (i.e. the second year of life). Additionally, the accumulation framework approach was also used by summing indicators of binary variables (i.e. STH infections) over time (i.e. between one and two years of age). In this analysis, the assumption was made that it is STH infection between

one and two years of age that is important, irrespective of STH infections at other time points. Furthermore, the assumption was made that the specific timing of infection, within the second year of life, is unimportant [182, 184].

6.2. Adjustment for STH misclassification

A unique aspect of the current research is the correction for STH misclassification, especially in the mediation analysis. While previous research has been dedicated to measuring the diagnostic test parameters of the current techniques available to diagnose STH infections [123, 185], no previous study investigating the effect of STH infection on child development has adjusted for misclassification of STH infection. One previous study investigating the association between STH infection and *Schistosoma japonicum* infection compared their results with and without adjustment for misclassification of STH infection (diagnosed with the Kato-Katz technique) [178]. The authors of this study concluded that results that ignored STH misclassification were biased towards the null [178]. Since no gold standard technique exists for diagnosing STH infection, all previous research conducted regarding STH infections suffers from misclassification bias. Ignoring this can lead to unpredictable bias in the reported results [186]. Compared to misclassification-unadjusted results, the effect sizes from our analyses adjusted for misclassification were shifted away from the null, suggesting that failing to take misclassification of STH infection into account can lead to a bias towards the null.

As in any study whose results rely on data from imperfect diagnostic tests, the results are dependent on what has been assumed about the unknown properties of the tests. The results show that the magnitude of the misclassification of STH infection can have large effects on the adjusted results and that correct prior specification of the sensitivity and specificity values of the diagnostic techniques is important. Due to the lack of a gold standard for diagnosing STH infection, determining the true sensitivities of the diagnostic techniques available is not straight forward. Even limiting a literature search to published studies that used Bayesian methods to account for a lack of a gold standard, very different values of sensitivities were found to have been reported in the literature [123, 185]. This uncertainty regarding true sensitivity values for STH diagnostic techniques leads to the specification of relatively wide and imprecise priors and was the rationale for using and comparing a range of prior specifications. The prior

specifications were developed based on the research available to date; however, our models suggested that the true sensitivity values may, in fact, be even lower than the pessimistic priors used. This uncertainty regarding the true sensitivity values of the STH diagnostic techniques is problematic and limits researchers' abilities to obtain accurate and informative misclassification-adjusted results for any research question involving STH infections. Molecular diagnostic methods (e.g. quantitative polymerase chain reaction (qPCR)) have recently been developed and offer an optimistic alternative for STH diagnosis in the future. These methods have been found to have markedly increased sensitivities compared to the currently available microscopic techniques [125]; however, they have not frequently been used in STH research. Currently, the cost associated with these techniques makes them unfeasible for widespread use in STH-endemic settings. Use of these improved diagnostic techniques in research settings, however, would be expected to substantially reduce the bias associated with STH misclassification. Furthermore, they could be used as the gold standard in research studies investigating the diagnostic parameters of the more field-friendly microscopic techniques currently available. If the sensitivities and specificities of the microscopic techniques can, at least, be accurately identified, this would significantly improve adjustment for STH misclassification. Future research and attention is needed to make these types of highly sensitive techniques for STH diagnosis more cost-effective and accessible for common use worldwide.

6.3. Implications for deworming research

These results may inform the current debate surrounding the benefits of deworming on child health. Periodic deworming has been recognized as the most effective and efficient public health intervention that currently exists to combat STH infections [86]. A recent Cochrane Review summarized RCTs and quasi-RCTs investigating the effect of deworming on cognitive development in children and concluded that deworming was unlikely to have an effect on cognitive development [156]. Several researchers and public health experts have challenged this review [167, 168]. One of the specific criticisms is that many of the trials included in the Cochrane Review only provided a single dose of deworming. WHO recommends regular periodic deworming on an annual or bi-annual basis, depending on the baseline prevalence of STH infection [134] and considering the high probability of rapid re-infection following treatment, single dose deworming is likely not sufficient to cause an important effect on a

potentially chronic condition [167]. While our results do not directly address the issue of deworming, they do show that a single infection at one time point is unlikely to have an important long-term effect on child development and that recurrent or cumulative infections over time may have important effects. Therefore, to obtain an accurate understanding of the true burden of STH infection, future research should focus on the health effects of long-term infection and of repeated doses of deworming provided over several years.

It is also worth mentioning the differences and implications between studying the effects of STH infections and studying the effects of deworming. Conducting research on the effects of an intervention, such as deworming, is favorable because an intervention can be highly controlled, highly specific and, most importantly, randomized. This allows for the counterfactual scenario to be mimicked as closely as possible, allowing for causal inference to be made. The debate surrounding STH infections and deworming have arisen because, despite the fact that some observational research has found that STH infection can have negative effects on child growth and development, RCTs investigating the effects of deworming medicines have not, so far, found beneficial effects on growth or development. This has caused some doubt as to the true morbidity caused by STH infections and whether results from observational research are biased. It is crucial to understand, however, that treating an STH infection with deworming is not the same as preventing the infection from being acquired in the first place. This means that individuals randomized to the deworming arm in an RCT will not be representative of individuals who were not found STH-infected in an observational study. Morbidity caused by STH infection begins when larvae migrate through the body, before infection can even be diagnosed [187]. The effects of infection on growth and development caused by damage to the intestines leading to bleeding, malabsorption of nutrients, etc. will not be reversed immediately following ingestion of a deworming tablet. Deworming is intended to kill the worms in the intestine and will obviously prevent additional negative effects to the host, but has no effect on repairing the damage already done. Depending on the duration and intensity of infection, some of this damage may be permanent [6]. The importance of research investigating the effects of STH infection (and not just deworming), therefore, should not be underestimated. It will never be possible to randomize individuals to become STH-infected; therefore, determining the true

morbidity associated with these infections will only be achieved with the highest quality observational research.

6.4. Strengths and limitations

The research included in this thesis has several strengths. The longitudinal design with a long follow-up period (four years) allowed for the investigation of cumulative STH infections over time. This type of cumulative measure for STH infection is likely a more relevant exposure than infection at one single time point. The longitudinal design also allowed for temporality to be established with regard to the timing of the exposure and outcome relationship and also for development trajectories to be examined. This research was conducted in preschool children, arguably the most relevant population group for this research question and for which very little research evidence exists. This the first time that the effect of STH infection, during the second year of life (the most critical period for brain development across the lifespan), on child development has been studied. Furthermore, this is the first time that a modern mediation analysis of the relationship between *Ascaris* infection and child development has been conducted. Finally, the adjustment for STH misclassification throughout all analyses allowed for an important bias to be addressed. Since no gold standard diagnostic technique is available for STH infection, all research involving STH infection is limited by misclassification bias and no previous research on this specific topic has yet adjusted for this bias.

The results of this research must, however, be interpreted considering its limitations. First, the exposure variable, the number of times detected STH-infected throughout the study, is a simple proxy measure. Ideally, stool specimens would have been collected daily and the total amount of time that each child was found to be STH-infected between one and five years of age and between one and two years of age would have been calculated. This is obviously not feasible to do on a large scale over many years. The number of times detected infected at the study visits, however, is a reasonable estimate of the relative burden of STH infection during the specific timeframes used. Furthermore, data regarding STH infection during the first year of life were not available. Although previous research indicates that infection during the first year of life is rare in this population [127], infection during this time may be important. Second, this is an observational study and, therefore, residual confounding cannot be disregarded as a possible

explanation of the observed associations. The covariates included in the final models were chosen based on theoretical knowledge (i.e. variables that are thought to be associated with both the exposure and outcome of interest without being mediators of this relationship) and statistical criteria. Many variables were considered during model selection but not included in the final model because they were either not independently associated with the exposure, outcome or mediators (as appropriate) and/or did not improve model fit, having no effect on the association between the exposure and outcome of interest. Residual confounding would need to be strong to explain the entire magnitude of the observed associations and it is unlikely that this is present outside of the scope of variables that were considered in this analysis. For the mediation analysis, hemoglobin levels were only measured as of the three-year visit. The summary measure of the exposure, *Ascaris* infection, spans the one to five years of age timeframe; however, the measure of hemoglobin levels only spans the three to five years of age timeframe. Therefore, important effects of hemoglobin levels between one and three years of age may have been missed. Additionally, both the exposure (number of detected *Ascaris* infections) and the mediators (mean hemoglobin levels and mean height-for-age z-scores) are expressed as summary measures over the same time period (i.e. between one and five years of age). Therefore, temporal ordering of the exposure and the mediators cannot be established. This could lead to reverse causation bias. It is, however, biologically very unlikely that low hemoglobin levels or malnutrition would cause *Ascaris* infection. Throughout all analyses, the sample size was not ideal for obtaining highly precise estimates or for detecting small or subtle effects, especially for the analyses that are adjusted for STH misclassification. This prevented conclusive interpretations of the results from being made regarding the effect of hookworm infection and regarding malnutrition as a mediator. The lack of precision led to some uncertainty regarding the exact effect sizes and subsequently, the clinical relevance of some results. Finally, an additional limitation is the non-verifiable assumptions of the regression models including correct model specification and, especially, correct prior specifications for the sensitivity and specificity values used in the analyses adjusted for STH misclassification.

6.5. Future research

This research investigated the effect of cumulative STH infections over time using STH prevalence data. It would be valuable for future research to examine the effect of cumulative

infection with moderate or heavy intensity STH infections (e.g. the number of moderate or heavy intensity infections detected over time). Consideration of infection intensity would be useful because STH-related morbidity is usually associated with moderate or heavy intensity infections and less commonly with light intensity infections. This type of analysis, however, will likely suffer from bias due to measurement error that will be difficult to adjust for as estimates of the measurement error associated with identifying STH intensity do not currently exist.

Additional research is also needed to develop and make accessible a gold standard diagnostic technique for STH infections and to precisely identify the sensitivities and specificities of the commonly used STH diagnostic techniques that are currently available. The uncertainty surrounding the true sensitivities of the diagnostic techniques most commonly used greatly limits researchers' ability to adjust for bias due to STH misclassification. Furthermore, research regarding the measurement error associated with quantifying the intensity of STH infection (i.e. from egg counts) would be useful to allow for bias due to measurement error to be adjusted for in analyses regarding the intensity of infection.

Finally, research conducted in areas with higher hookworm prevalence would be useful to precisely determine the effect of hookworm infection on child development in preschool children. Hookworms burrow into the intestinal wall and inhibit host coagulation leading to significant bleeding and hemoglobin digestion [97, 98]. For this reason, hookworm infection has been repeatedly associated with reduced iron levels and anemia [89] and is therefore hypothesized to contribute to impaired child development. The current research suggests that hookworm infection may have negative effects on child development but the very low prevalence of this infection in the study population limited the analyses and interpretation of results. Research regarding the mechanisms of effect of *Trichuris* infection and hookworm infection on child development, using modern mediation analysis methodology, would also make a valuable contribution.

6.6. Clinical relevance

These results provide important empirical evidence regarding the effect of STH infection on child development, a topic that has received inadequate attention over the decades but which has

recently been highlighted as an important gap in knowledge [27-31, 188]. Despite some inconclusive results, it has been shown that, in preschool children, an association does exist between cumulative STH infections over time and lower development scores. The results also suggest that hemoglobin levels play an important role in this association but that other factors are also likely at play. The results also highlight the importance of considering and adjusting for misclassification of STH infection.

WHO now recommends that children as young as 12 months of age be included in STH control programs [134]; however, large efforts are still required to obtain the goal coverage rates set by WHO of 75% coverage in endemic countries by 2020 [189]. Political motivation to reach preschool children with appropriate STH control programs continues to be a major challenge [139] and this is, in part, due to a lack of rigorous evidence quantifying the disease burden of STH infection in this age group. The current research results contribute to the body of research evidence documenting the burden of STH infection in young children and highlight the importance of targeting children as of 12 months of age in STH control and prevention programs. Including nutritional interventions to improve iron levels within these programs, like iron-containing micronutrient supplements or different types of nutrition education initiatives, would likely lead to improved outcomes. Prioritizing preschool children, as of one year of age, for STH control could lead to life-long improvements in cognition and education and may ultimately contribute to improving productivity in adulthood and reducing poverty levels.

7. Conclusion

Cumulative STH infection between one and five years of age was found to be associated with lower IQ scores at five years of age and this association was, in part, mediated by hemoglobin levels. Furthermore, *Ascaris* infection, specifically during the second year of life, was associated with lower cognitive and verbal abilities between two and five years of age. These results contribute to the body of evidence regarding the interactions between STH infection, malnutrition and child development. While preschool children may not harbor the highest prevalence or intensity of STH infection, because they are in such a critical time for growth and development, infection during this time period can have important long-term effects. Preschool children, therefore, need to be prioritized for STH control, including deworming. Increased efforts and investments need to be made to reach the WHO target deworming coverage rates of 75% in preschool children living in STH-endemic settings. Improving child development around the world requires an integrated approach with a variety of complementary interventions. STH control, coupled with nutrition interventions, in young children as of one year of age, should be considered among these interventions which will ultimately contribute to achieving healthy childhood development ensuring the future health, well-being and productivity of all children.

8. References

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Appendices

Appendix 1: Preliminary data used for original sample size calculations showing number and frequency of children with 0, 1, 2 and 3 STH infections between 12 and 36 months of age (summing detected infections over the 12, 24 and 36-month time points for each child).

# infections	Any STH		*Ascaris*		*Trichuris*		Hookworm	
	n	%	n	%	n	%	n	%
0	511	36%	677	48%	849	60%	1359	96%
1	470	33%	473	34%	370	26%	50	4%
2	349	25%	219	16%	180	13%	1	0%
3	80	6%	41	3%	11	1%	0	0%

Appendix 2: Sample size calculations for Objective 1 to determine the total width of the 95% confidence interval for the difference between two means (i.e. for each category compared to the reference category of no infections). Estimated frequencies of infection counts were derived based on preliminary data presented in Appendix 1.

Species	Infection counts	Estimated frequency [n, (%)]	n2/n1[a]	Sample size available[b]	SD for pop 1 and pop 2	Total width of 95% CI[c]
Any STH	0	176 (20%)	REF	REF	REF	REF
	1	176 (20%)	1	352	15	6.28
	2	220 (25%)	1.25	396	15	5.95
	3	220 (25%)	1.25	396	15	5.95
	4-5	88 (10%)	0.5	264	15	7.68
Ascaris	0	264 (30%)	REF	REF	REF	REF
	1	202 (23%)	0.77	466	15	5.50
	2	176 (20%)	0.67	440	15	5.73
	3	176 (20%)	0.67	440	15	5.73
	4-5	62 (7%)	0.23	326	15	8.37
Trichuris	0	352 (40%)	REF	REF	REF	REF
	1	308 (35%)	0.88	660	15	4.60
	2	352 (40%)	1	704	15	4.44
	3-5	132 (15%)	0.38	484	15	6.01
Hook-worm	0	660 (75%)	REF	REF	REF	REF
	1	176 (20%)	0.27	836	15	4.99
	2-5	44 (5%)	0.07	704	15	9.16

[a] n2 refers to sample size available in comparison group (i.e. 1 count, 2 counts, etc.) and n1 refers to the sample size available in reference group (i.e. 0 counts)

[b] Sample size available = n1 + n2

[c] The minimum clinically significant effect size is 5; therefore, a total CI width **less than 10** would not cross the null effect (i.e. 0).

Appendix 3: Sample size calculations for Objective 2, Aim 2.1 to determine the total width of the 95% confidence interval for the difference between mean cognitive scores (i.e. for each category compared to the reference category of no infections). Frequencies of infection counts were calculated from data already collected.

Species	**Infection counts**	**Frequency [n, (%)]**	**Effective sample size[b]**	**n1/n2[a]**	**SD for pop 1 and pop 2**	**Total width of 95% CI[c]**
Any STH	0	379 (43%)	946	REF	REF	REF
	1	314 (36%)	792	1.194	15	2.84
	2-3	188 (21%)	462	2.048	15	3.34
Ascaris	0	466 (53%)	1,166	REF	REF	REF
	1	282 (32%)	704	1.656	15	2.81
	2-3	132 (15%)	330	3.533	15	3.67
Trichuris	0	642 (73%)	1,606	REF	REF	REF
	1	194 (22%)	484	3.318	15	3.05
	2-3	44 (5%)	110	15	15	5.88

[a] n2 refers to sample size available in comparison group (i.e. 1 count, 2-3 counts.) and n1 refers to the sample size available in reference group (i.e. 0 counts)

[b] The effective sample size = Observations available assuming independent data / design effect; design effect = 1+(# repeated measures -1) × ICC = 1+(4-1) × 0.2 = 1.6

[c] The minimum clinically significant effect size is 5; therefore, a total CI width **less than 10** would not cross the null effect (i.e. 0).

Appendix 4: Sample size calculations for Objective 2, Aim 2.2 to determine the total width of the 95% confidence interval for the difference between mean verbal scores (i.e. for each category compared to the reference category of no infections). Frequencies of infection counts were calculated from data already collected.

Species	**Infection counts**	**Frequency [n, (%)]**	**Effective sample size[b]**	**n1/n2[a]**	**SD for pop 1 and pop 2**	**Total width of 95% CI[c]**
Any STH	0	379 (43%)	797	REF	REF	REF
	1	314 (36%)	667	1.195	15	3.09
	2-3	188 (21%)	389	2.049	15	3.64
Ascaris	0	466 (53%)	982	REF	REF	REF
	1	282 (32%)	593	1.656	15	3.06
	2-3	132 (15%)	278	3.532	15	4.00
Trichuris	0	642 (73%)	1353	REF	REF	REF
	1	194 (22%)	408	3.316	15	3.33
	2-3	44 (5%)	93	14.548	15	6.31

[a] n2 refers to sample size available in comparison group (i.e. 1 count, 2-3 counts.) and n1 refers to the sample size available in reference group (i.e. 0 counts)

[b] The effective sample size = Observations available assuming independent data / design effect; design effect = 1+(# repeated measures -1) × ICC = 1+(4-1) × 0.3 = 1.9

[c] The minimum clinically significant effect size is 5; therefore, a total CI width **less than 10** would not cross the null effect (i.e. 0).

Appendix 5: Sample size calculations for Objective 2, Aims 2.1 and 2.2 to determine the total width of the 95% confidence interval for the difference between mean cognitive and verbal scores (i.e. for each category compared to the reference category of no infections), assuming an ICC of 0.9 (worst case scenario). Frequencies of infection counts were calculated from data already collected.

Species	Infection counts	Frequency [n, (%)]	Effective sample size[b]	n1/n2[a]	SD for pop 1 and pop 2	Total width of 95% CI[c]
Any STH	0	379 (43%)	409	REF	REF	REF
	1	314 (36%)	342	1.196	15	4.33
	2-3	185 (21%)	200	2.045	15	5.09
Ascaris	0	466 (53%)	504	REF	REF	REF
	1	282 (32%)	304	1.658	15	4.29
	2-3	132 (15%)	143	3.524	15	5.59
Trichuris	0	642 (73%)	694	REF	REF	REF
	1	194 (22%)	209	3.321	15	4.65
	2-3	44 (5%)	48	14.458	15	8.80

[a] n2 refers to sample size available in comparison group (i.e. 1 count, 2-3 counts.) and n1 refers to the sample size available in reference group (i.e. 0 counts)

[b] The effective sample size = Observations available assuming independent data / design effect; design effect = 1+(# repeated measures -1) × ICC = 1+(4-1) × 0.9 = 3.7

[c] The minimum clinically significant effect size is 5; therefore, a total CI width **less than 10** would not cross the null effect (i.e. 0).