IMPROVING EARLY CHILDHOOD GROWTH AND DEVELOPMENT IN LOW- AND MIDDLE-INCOME COUNTRIES: A RANDOMIZED CONTROLLED TRIAL OF DEWORMING INCORPORATED INTO ROUTINE CHILD HEALTH CARE IN PERU

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SHORT TITLE

A randomized controlled trial of mebendazole in early preschool-age children

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ABSTRACT

BACKGROUND The World Health Organization recommends mass deworming in soil-transmitted helminth (STH)-endemic areas as of 12 months of age; however, evidence of benefits in children under two years of age is limited and coverage remains suboptimal. This age corresponds to a critical window to intervene and prevent poor health and malnutrition in the short- and long-term. Therefore, the objective of this study was to determine the effect of a deworming intervention, including optimal timing and frequency, on growth and development in children from 12 to 24 months of age.

METHODS A double-blind randomized controlled trial of deworming was conducted in Iquitos, Peru. Children were enrolled during their routine 12-month clinic visits in study health centres and followed-up at their 18- and 24-month visits. Random assignment was to one of four groups: 1) deworming at the 12-month visit, placebo at the 18-month visit; 2) placebo at the 12-month visit, deworming at the 18-month visit; 3) deworming at both the 12- and 18-month visits; or 4) placebo at both the 12- and 18-month visits. Weight, length, STH infection, and socio-demo-epi information were ascertained at all visits. Development was assessed at baseline and the 24-month visit. One-way ANOVA analyses used an intention-to-treat approach with multiple imputation for missing values. Adjusted, per-protocol, complete case and subgroup analyses were also conducted.

RESULTS A total of 1760 children were enrolled between September 2011 and June 2012. At baseline, the prevalence of any STH was 14.5%, stunting was 24.2% and underweight was 8.6%. A total of 1563 (88.8%) children attended the 24-month visit. Between 12 and 24 months, STH infection prevalence rose to 42.6%, stunting increased to 46.8% and underweight increased to 10.2%. The greatest gains in weight and length were observed in the deworming-at-12-months-only group. No group had gains in growth or development statistically significantly higher than the placebo group; however, there was a statistically significantly greater improvement in weight gain (unadjusted difference in kg

(95% CI): 0.12 (0.01, 0.23)) and length gain (unadjusted difference in cm (95% CI): 0.31 (0.04, 0.58)) in children receiving deworming at the 12-month visit compared to the 18-month visit.

CONCLUSION Overall, there was no statistically significant benefit of deworming on growth in this population of preschool-age children. However, the results do indicate that, for children between 12 and 24 months of age, once-yearly deworming at 12 months of age provides the greatest growth benefits compared to later or more frequent deworming. A greater benefit may be apparent in areas of higher prevalence or intensity of infection. These results contribute to WHO policy and recommendations on deworming targeting preschool-age children in the over 100 STH-endemic areas of the world. They also contribute to providing practical guidance to governments in integrating deworming into early childhood health care.

RÉSUMÉ

CONTEXTE L'Organisation mondiale de la santé (OMS) recommande le déparasitage de masse à partir de l'âge de 12 mois dans les zones où l'infestation par les helminthes transmis par le sol (HTS) est endémique. Par contre, les données sur les avantages pour les enfants âgés de moins de deux ans sont limitées et la couverture n'est pas optimale. Cet âge correspond à une période critique pour l'intervention afin de promouvoir la santé et prévenir la malnutrition à court terme ainsi qu'à long terme. Par conséquent, l'objectif de cette étude était de démontrer l'effet d'une intervention de déparasitage, y compris la fréquence et les moments optimaux, et sur la croissance et le développement des enfants âgés entre 12 et 24 mois.

MÉTHODES Un essai contrôlé randomisé à double insu a été mené à Iquitos, au Pérou, sur le déparasitage. Des enfants de 12 mois ont été inscrits aux centres de santé pendant leurs visites de routine et des suivis ont eu lieu lors de leurs visites à 18 mois et à 24 mois. L'assignation aléatoire était faite à l'un des quatre groupes d'interventions : 1) déparasitage à 12 mois et placébo à 18 mois ; 2) placébo à 12 mois, déparasitage à 18 mois ; 3) déparasitage à 12 mois et à 18 mois ; ou 4) placébo à 12 mois et à 18 mois. À chaque visite, le poids, la longueur, la présence d'infection HTS, et l'information sociodémographique et épidémiologique ont été notés. Le développement a été évalué à l'inclusion et à la visite à 24 mois. Des analyses de variance ANOVA ont été utilisées avec une approche intention de traiter, et l'imputation multiple pour les valeurs manquantes. Des analyses ajustées, 'complete case', 'per protocol' et de sous-groupes ont également été menées.

RÉSULTATS Entre septembre 2011 et juin 2012, 1760 enfants ont été inclus. À l'inclusion, la prévalence de HTS était de 14,5%, le retard de croissance était de 24,2%, et l'insuffisance pondérale était de 8,6%. Un total de 1563 enfants (88,8%) ont assisté à la visite de 24 mois. Entre 12 et 24 mois, la prévalence d'infection HTS a augmenté jusqu'à 42,6%, le retard de croissance a augmenté à 46,8%, et l'insuffisance pondérale a augmenté à 10,2%.

Le groupe de déparasitage à 12 mois seulement a démontré les plus grands gains de poids et de longueur. Aucun groupe n'avait de gains en croissance ou en développement plus élevés de manière significative que le groupe placébo à 12 mois et à 18 mois. Par contre, pour les enfants qui ont été déparasités à 12 mois, comparé aux enfants déparasités à 18 mois, il y avait un effet statistiquement significatif sur le gain de poids (différence non-ajustée en kg (IC95) : 0,12 (0,01, 0,23)) et de longueur (différence non-ajustée en cm (IC95) : 0,31 (0,04, 0,58)).

CONCLUSION En conclusion, il n'y avait aucun avantage statistiquement significatif de déparasitage sur la croissance de cette population d'enfants d'âge préscolaire. Cependant, les résultats indiquent que, pour les enfants âgés entre 12 et 24 mois, un déparasitage unique à 12 mois offre les plus grands bénéfices de la croissance par rapport à plus tard ou de déparasitage plus fréquente. Une amélioration des effets peut être apparente dans les zones de prévalence ou l'intensité de l'infection plus élevé. Ces résultats contribuent aux données probantes à l'appui des politiques et recommandations de l'OMS sur le déparasitage d'enfants d'âge préscolaire dans les zones endémiques. Ils contribuent aussi des renseignements pratiques pour guider les gouvernements qui se chargent d'intégrer le déparasitage dans leurs programmes de santé de l'enfant.

ACKNOWLEDGEMENTS

Never would I have imagined that attending a McGill seminar in May 2003, while on a summer break from my MSc studies at Dalhousie University, would change the course of my life. My chance encounter with Theresa Gyorkos has led me down such an incredible path to where I am today. Her faith in my abilities to realize my full potential provided me with the confidence to make the transition from MSc student to Research Coordinator to PhD Candidate and independent researcher. My immersion in the world of global health introduced me to people, places and topics that I have become so passionate about over the years. Theresa has provided me not only with exceptional mentoring in an academic sense, but also in terms of developing valuable life skills. It has been wonderful to always have an advocate, teacher, friend and the most enthusiastic cheerleader in my corner.

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value on and fund research relevant to improving the health of populations in developing countries.

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DEDICATION

This thesis is dedicated to the more than one billion people who suffer from neglected tropical diseases in low- and middle-income countries.

PREFACE AND CONTRIBUTION OF CO-AUTHORS

Contributions of co-authors

Serene A. Joseph MSc (SAJ) (first author on all manuscripts) was the on-site Project Director of the trial. She was responsible for developing the research question and study design, obtaining project funding and ethics approvals, developing study instruments, training research assistants, overseeing all data collection and quality control activities in the field, analyzing the data, preparing the first draft of all manuscripts, revising and finalizing manuscripts and disseminating results. **Theresa W. Gyorkos PhD (TWG)** (last author on all manuscripts) was the Principal Investigator and oversaw all academic, scientific, ethical and logistical aspects of the trial. She provided input into the research question, study design, data analysis and results interpretation. She edited all manuscripts before input from other co-authors and assisted in finalizing manuscripts. **Martín Casapía MD, MSc (MC)** (co-author on all manuscripts) was a trial co-investigator, and the director of the Peruvian partner NGO, Asociación Civil Selva Amazónica, where the study took place. He provided local expertise into the study design and epidemiological and logistical issues arising during data collection, key access to officials at the local Ministry of Health and participating health centres, input into obtaining ethics approval and other authorizations in Peru, interpreting the results and revising the manuscripts. **Grace Marquis PhD (GM)** (co-author on Manuscript B) was a co-investigator of the trial. She provided nutritional expertise based on valuable previous experience working in Peru and other developing countries. She assisted in study design and methodology related to nutritional outcomes, as well as results interpretation and manuscript revision. **Antonio Montresor MD (AM)** (co-author on Manuscript B) was a co-investigator of the trial and a liaison with WHO. He provided input into the research question, study design and methodology, results interpretation and revising the manuscripts. He provided substantive expertise on global public health and clinical issues related to deworming and soil-transmitted helminth infection. **Elham Rahme PhD (ER)** (co-author on all manuscripts) was a trial co-investigator who provided biostatistical expertise and methodological advice on

trial conduct. She was responsible for input into the study design and methodology, data analysis, results interpretation and revising the manuscripts. **Brian Ward MDCM, MSc (BW)** (co-author on Manuscript B) was a trial co-investigator who provided expertise on infectious diseases, tropical medicine, and randomized controlled trials. He provided input into the study design and methodology, results interpretation and manuscript revision. **Brittany Blouin MSc (BB)** (co-author on all manuscripts) served as the Canadian-based coordinator during the timeline of data collection. She provided input into operational and epidemiological aspects of fieldwork, and contributed to data analysis, interpretation and revision of manuscripts. **Mathieu Maheu-Giroux MSc (MMG)** (co-author on Manuscripts A and B) served as the Canadian-based coordinator during the timeline of study design and grant application submissions. He provided input into the research question, study design and study methodology, as well as data analysis, results interpretation and revision of manuscripts. **Lidsky Pezo BSc (LP)** (co-author on Manuscript B) was the local Project Coordinator in Peru. She provided input into operational aspects of study design and fieldwork, assisted in training and supervision of research assistants, and interfaced with local collaborators in Peru. She contributed to the interpretation of results and manuscript revision. **Fabiola Lazarte BSc (FL)** (co-author on Manuscript C) was a Peruvian consultant on the study. She provided her expertise in child development from a Peru-based perspective. She assisted in adapting and translating the development instrument for local use in Iquitos. She was involved in training research assistants and overseeing quality control activities related to developmental assessment. She assisted in results interpretation and manuscript revision.

Originality Statement

The research presented in this thesis is the result of an original research project developed and executed primarily by SAJ. After years of experience working in Peru and in high-risk populations of children and reproductive-age women, SAJ noticed a clear knowledge gap related to deworming in early preschool-age children. From a review of the literature, the soil-transmitted helminth (STH) parasite burden in the younger age groups appeared to be higher than had previously been thought. The nutrition literature demonstrated the unique and critical window of growth and development in children under two years of age. Linking the two disciplines, it seemed likely that STH infection could exacerbate growth and development delays in already vulnerable young children. The World Health Organization (WHO) first started recommending the inclusion of children between 12 and 24 months in deworming interventions after an Informal Consultation in 2002. Despite these recommendations, many national deworming programs and Ministry of Health guidelines, including in Peru, continue to exclude children in this age group. Clinical equipoise also persists with limited evidence available from well-conducted and age-appropriate clinical trials. With increasing deworming coverage in older children, and countries revisiting their deworming policies, it seemed to be a timely moment to provide new evidence on the benefits of deworming in children under two years of age. In developing this project, SAJ's goal, therefore, was to fill this knowledge gap in an academic sense, and to contribute to policy and practice in STH-endemic areas. This is the only deworming trial to be conducted exclusively in children in the second year of life. Other trials that included preschool-age children had methodological limitations and/or were limited by sample sizes too small to detect age-disaggregated differences. This is also the first trial in preschool-age children to examine the effects of deworming on cognitive, language and fine motor skills, and the first to use the rigorous Bayley Scales of Infant Development. Overall, this project contributes new evidence to the field of neglected tropical diseases, soil-transmitted helminths and growth and development in children between one and two years of age.

ACRONYMS AND ABBREVIATIONS

ALB	albendazole
AM	arithmetic mean
ANOVA	analysis of variance
BAYLEY-III	Bayley Scales of Infant and Toddler Development, Third Edition
BCG	Bacille Calmette-Guérin
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CIHR	Canadian Institutes of Health Research
CRED	Crecimiento y Desarrollo (Growth and Development)
CR	Cure rate
CS	Centro de Salud (Health Centre)
DALY	Disability-adjusted life years
DIRESA	Dirección Regional de Salud (Regional Ministry of Health)
DPT	Diphtheria, Pertussis and Tetanus
EESS	Establecimiento de Salud (Health Establishment)
EPG	eggs per gram
ERR	egg reduction rate
GM	geometric mean
ICDS	Integrated Child Development Services
INS	Instituto Nacional de Salud (National Institutes of Health)
ITT	Intention-to-treat
LAZ	length-for-age z-score
LMIC	low- and middle-income country
MDG	Millennium Development Goals
MBD	mebendazole
MINSA	Ministerial de Salud (Ministry of Health)
MMR	Measles, Mumps and Rubella
NCHS	National Center for Health Statistics
NS	non-significant
NTD	neglected tropical disease

PAHO	Pan American Health Organization
PBO	placebo
PC	Preventive chemotherapy
PS	Puesto de Salud (Health post)
RA	Research Assistant
RCT	randomized controlled trial
RR	Rate (risk) ratio
SD	standard deviation
SES	socioeconomic status
STH	soil-transmitted helminth
WAZ	Weight-for-age z-score
WLZ	Weight-for-length z-score
WHO	World Health Organization
YLD	Years lived with disability

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1 INTRODUCTION

Improving early childhood health in low- and middle-income countries (LMICs) is a priority theme in global health research and a focus of international efforts, including the Millennium Development Goals (MDGs) and Canada's Muskoka Initiative. Children under two years of age are at a particularly critical stage of growth and development (Victora et al. 2010). Interventions to target poor health, malnutrition and developmental delays in this age group include promotion of breastfeeding, timely introduction of appropriate foods, micronutrient supplementation, vaccinations, optimal mother-child interaction and child stimulation (Victora et al. 2008, Victora et al. 2010, Walker et al. 2011, Grantham-McGregor et al. 2014).

Early acquisition of infectious pathogens may also contribute to short- and long-term adverse health in LMICs. The soil-transmitted helminth (STH) disease cluster includes *ascariasis*, *trichuriasis* and hookworm disease. STHs are transmitted in contaminated food, water and the environment in warm, tropical and subtropical climates. They are widespread in areas of extreme poverty with poor sanitation and limited access to potable water. STH infection can lead to poor growth, anemia, loss of appetite, developmental delays and decreased productivity, among others. The World Health Organization (WHO) recognizes the importance of preventive chemotherapy (PC) (i.e. deworming) as a public health intervention in areas of STH infection prevalence over 20% (WHO 2002, WHO 2006, WHO 2014). The Copenhagen Consensus in 2012 ranked deworming as the fourth best intervention to address the most pressing global challenges. PC consists of large-scale anthelminthic treatment without screening targeted to high-risk populations, including: 1) women of reproductive-age, particularly pregnant women; 2) school-age children (i.e. 5 to 14 years of age); and 3) preschool-age children (i.e. 1 to 4 years of age). Prior to 2002, children under 24 months of age were excluded from deworming programs largely because of a paucity of evidence and regulations from drug manufacturers (Montresor et al. 2002). WHO convened an Informal Consultation in 2002 at which time all available published

and unpublished evidence was reviewed, and deworming guidelines were modified to include 12 to 24-month old children (Montresor et al. 2002, WHO 2002, Montresor et al. 2003). These modifications were based primarily on STH prevalence, drug safety and toxicity, and cost-benefit considerations in young age groups, and extrapolation from older age groups (the inclusion of children under 12 months of age continues to be contraindicated based on limited evidence of safety). Despite changes to WHO policy, many countries still exclude children under two years of age in national deworming policies and global deworming coverage remains suboptimal.

The evidence on STH burden and deworming benefits also continues to be limited in the 12 to 24-month old age group. Only two deworming trials have focused exclusively on growth in preschool-age children (i.e. 1 to 4 years of age); however, one excluded children under 18 months of age (Awasthi et al. 2000) and the other included children as of six months of age (Awasthi and Pande 2001). Five other trials included the 12 to 24 month age group in their study populations, but participants ranged from six months to eight years of age (Kloetzel et al. 1982, Stoltzfus et al. 2004, Alderman et al. 2006, Awasthi et al. 2008, Awasthi et al. 2013). Only two of these studies were double-blind, placebo-controlled trials (Kloetzel et al. 1982, Stoltzfus et al. 2004), but sample sizes were too small to detect age-disaggregated differences in the 12 to 24 month group. Overall, results have been contradictory, and there are methodological and other limitations which prevent the extrapolation of results to early preschool-age children. No trial has looked at the effect of deworming on cognitive, language and fine motor development in early preschool-age children.

Therefore, this double-blind, randomized, placebo-controlled trial was designed to examine the overall effect, and optimal timing and frequency, of deworming on growth and development in children between 12 and 24 months of age, living in an STH-endemic area of the Peruvian Amazon.

2 LITERATURE REVIEW

2.1 Soil-transmitted helminth (STH) infection

The soil-transmitted helminth (STH) disease cluster includes *ascariasis* (i.e. roundworm infection, caused by *Ascaris lumbricoides*), *trichuriasis* (i.e. whipworm infection, caused by *Trichuris trichiura*) and hookworm disease (caused by *Necator americanus* or *Ancylostoma duodenale*). It is common in warm, tropical and subtropical climates in low- and middle-income countries (LMICs). STHs are nematodes with a free-living and parasitic stage. STH eggs can live for years in the soil under moist and shady conditions (Cox 1982, Crompton 1994). *Ascaris* and *Trichuris* are transmitted through ingestion of larvae contained within eggs (i.e. infective eggs) in contaminated food and water. In the case of hookworm, larvae can be swallowed, or can directly penetrate the skin. All STH larvae migrate through the body, settle in the intestines and develop into adult parasitic worms after one to three months (Cox 1982). Eggs are released by adult female worms in the feces of infected individuals. In areas of poverty with poor sanitation and waste management practices and an inadequate water supply, the environment remains in a permanent state of contamination and the cycle of infection continues. Based on overlapping geography and transmission patterns, co-infection of more than one STH species often occurs within the same human host (Cox 1982).

STH infection is considered to be one of the most important Neglected Tropical Diseases (NTDs), named as such based on the vulnerable populations they affect, and the lack of research and funding attention directed towards them. Recent estimates indicate that STH infection affects over 1.45 billion people in over 100 endemic countries (Pullan et al. 2014). Mortality from STH infection is low and mainly results from intestinal obstruction from high worm load; however, morbidity can be high. It is estimated that STH infection contributes 4.98 million years lived with disability (YLD) and 5.18 million disability-adjusted life years (DALYs) (Pullan et al. 2014). By disrupting normal nutrient intake, excretion and utilization in their hosts, and by causing blood loss, these intestinal parasites have a direct and indirect adverse impact on nutritional status. This includes poor

growth, anemia, loss of appetite, developmental delays and decreased productivity, among others, beginning in childhood and persisting into adulthood (Stephenson 1987, Crompton 2000, Stephenson et al. 2000, Crompton and Nesheim 2002) (Figure 1). The degree of morbidity associated with STH infection is related primarily to the parasite burden. The World Health Organization (WHO) has set species-specific thresholds of STH infection intensity to categorize the parasite burden as light, moderate or heavy, based on egg count per gram of feces (i.e. eggs per gram (epg)) (WHO 1998). Light intensity infection is often asymptomatic. The chronic nature of the infection, and host factors, such as nutritional demands and age, can also contribute to morbidity (Crompton and Nesheim 2002). The prevalence and intensity of *Ascaris* and *Trichuris* infections are thought to peak in childhood, whereas hookworm infection peaks in late adolescence or early adulthood (Anderson 1986).

2.2 Treatment for STH infection

Treatment for STH infection includes one of four anthelminthic drugs (i.e. deworming): 1) albendazole (ALB); 2) mebendazole (MBD); 3) pyrantel pamoate; or 4) levamisole (WHO 1996). Albendazole and mebendazole are the most common treatments as they are safe, low-cost, single-dose (in 400 mg and 500 mg doses, respectively), and easily administered (even by non-medical personnel). They are from the class of drugs known as benzimidazoles, which act selectively on the adult parasite in the intestine, killing some or all of the adult worms, and decreasing worm load and secretion of eggs into the environment. Benzimidazoles are poorly absorbed by the body, and released within 24 hours, leading to infrequent occurrence of adverse events from their use. Adverse events, when reported, tend to be mild and transitory, and primarily include gastrointestinal upset, diarrhea, vomiting and headaches (Hall et al. 2008). Owing to the potential benefits of not just curing, but reducing worm load, efficacy of deworming is considered both in terms of treatment cure rates (CR) and egg reduction rates (ERR) (WHO 2013). Studies have demonstrated high efficacy of single-dose albendazole and mebendazole against *Ascaris* (CR of 88% and 95%,

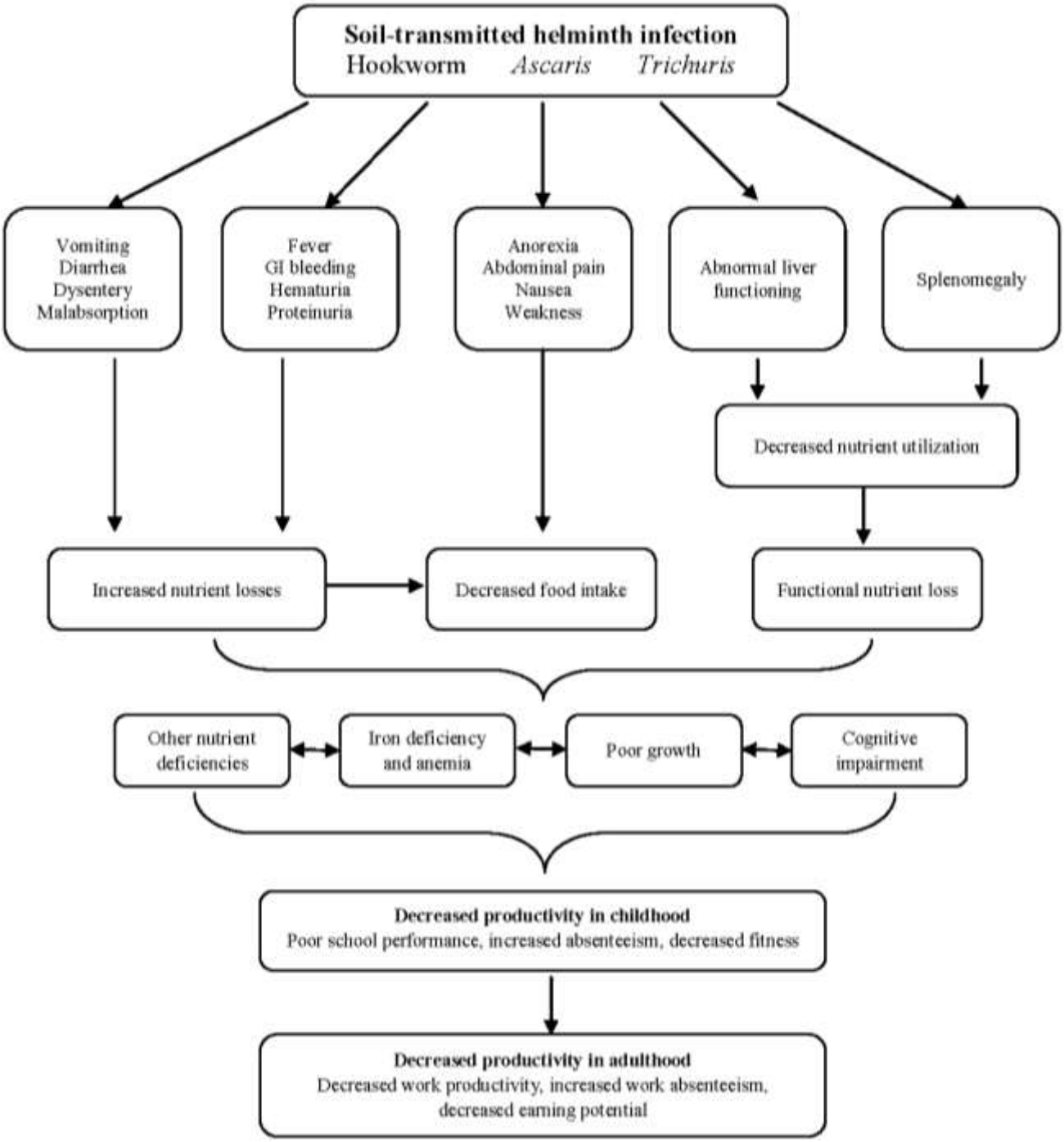

Figure 1. Conceptual framework illustrating the short- and long-term health, nutritional and developmental consequences of soil-transmitted helminth infection. Adapted from Crompton & Nesheim, 2002; Stephenson & Holland, 1987; and Stephenson, 2000.

respectively, and ERR of 87-100% and 96-100%, respectively) (Keiser and Utzinger 2008, Keiser and Utzinger 2010). Lower and more variable efficacy have been observed for albendazole and mebendazole against hookworm (CR of 72% and 15%, respectively, and ERR of 64-100% and 0-98%, respectively) and *Trichuris* (CR of 28% and 36%, respectively, and ERR of 0-90% and 81-93%, respectively) (Keiser and Utzinger 2008, Keiser and Utzinger 2010). Despite the suboptimal CRs and ERRs, particularly for *Trichuris,* albendazole and mebendazole continue to be the most widely used deworming treatments as appropriate alternatives are not yet available. Although drug resistance has been observed in veterinary public health, this has not yet been observed in practice in human populations (Keiser and Utzinger 2010, WHO 2013).

Based on the low-cost of deworming, the high prevalence and morbidity of STH infection, and the safety of available medications, WHO recommends large-scale preventive chemotherapy (PC) in STH-endemic areas. This involves targeting deworming interventions towards specific high-risk groups regardless of infection status (i.e. without prior screening), rather than identifying infected individuals through screening (WHO 2006). The Copenhagen Consensus in 2012 also stressed the importance of PC by ranking it the fourth best intervention to address the most pressing global challenges (Copenhagen Consensus Center 2012). WHO recommends once-yearly deworming in low-risk areas where prevalence is between 20% and 50%. In high-risk areas where prevalence is above 50%, deworming twice (or three times if resources are available) is recommended (WHO 2005, WHO 2006). PC is targeted to high-risk groups, including: 1) women of reproductive age, including pregnant women after the first trimester; 2) school-age children (i.e. 5 to 14 years of age); and 3) preschool-age children (i.e. 1 to 4 years of age) (WHO 1996, WHO 2006). Most research attention and deworming efforts have been focused primarily on school-age children, due to the high STH infection prevalence and morbidity in this age group. School-based programs are also easy to implement, especially as deworming can be provided by non-health care personnel, such as teachers. Donation programs targeted to school-age populations have been recently established, and provide albendazole

or mebendazole free of charge to eligible governments (WHO 2014). This has resulted in increasing coverage of deworming in school-age populations, currently estimated to be 36% worldwide (WHO 2011, WHO 2014). A target of reaching at least 75% of at-risk school-age children was set by the World Health Assembly Resolution in 2001 (WHO 2001). This target has been increasingly used for preschool-age children.

2.3 STH infection and preventive chemotherapy in early preschool-age children

As scaling-up of these school-based programs continues and coverage increases, preschool-age children may lag behind as preschool programs remain a challenge. WHO recommends piggybacking deworming in early childhood onto vaccination or supplementation programs or campaigns, child health days, or programs for the elimination of lymphatic filariasis (WHO 2014). The global proportion of at-risk preschool-age children receiving deworming in 2012 was estimated to be on the order of 25%, with a low of 5% coverage in Europe and a high of 35% in the South-East Asia region (WHO 2014). This coverage has decreased since previous reports (WHO 2011).

Coverage in early preschool-age children may be even more insufficient as their inclusion in PC has been more recent. Prior to 2002, children under two years of age had been excluded from deworming interventions. In this age group, the safety profile was not well established and treatment was contraindicated according to drug manufacturer regulations (Montresor et al. 2002). Traditionally, the occurrence of STH infection had also been perceived to be low in this younger age group. However, there has been increasing empirical evidence which shows that the opposite is true, and that children begin to acquire these infections as they become mobile and begin to explore the environment (Allen et al. 2002, Albonico et al. 2008). In Belén, a community of extreme poverty in the Peruvian Amazon, while the prevalence of *Ascaris* or *Trichuris* was only 4% in children at seven to nine months of age, it rose to almost 30% by 12 to 14 months of age (Gyorkos et

al. 2011). In a cohort of preschool-age children in Ecuador, over 20% suffered from *Ascaris* or *Trichuris* infection at least once in the first two years of life, with infection first appearing around seven months of age (Menzies et al. 2014). Although *Ascaris* and *Trichuris* are the predominant STH infections in early childhood (Hall et al. 2008), there is also evidence to suggest that hookworm infection may be high in early preschool-age children. A study by Stoltzfus et al (2004) in Zanzibar demonstrated that 31.3% of children under 30 months of age were infected with hookworm (Stoltzfus et al. 2004). Overall, global estimates indicate that 5-10% of those infected with STH are children under two years of age (WHO 2002, de Silva et al. 2003).

In 2002, WHO convened an informal consultation of experts, and subsequently recommended the inclusion of children as of 12 months of age in deworming activities in endemic areas (Allen et al. 2002, WHO 2002, Montresor et al. 2003). The WHO recommendations were based primarily on animal studies, toxicity data and other safety data. The expert panel recommended that deworming, either in a reduced dose of 200 mg of albendazole, or in the usual dose of 500 mg of mebendazole, could be safely given to children 12 to 24 months of age (Allen et al. 2002). As there was some concern that deworming pills could pose a choking hazard to younger children, WHO recommended that the pill be crushed and mixed with water or juice when administered to the youngest age groups (Albonico et al. 1996). Deworming in children under 12 months of age continues to be contraindicated based on cost-benefit considerations and limited evidence of safety.

2.4 The critical growth and development window in early childhood

Despite the WHO recommendations, many countries still exclude children under 24 months of age in their national deworming programs and coverage remains suboptimal. This is of concern, as children in this early preschool-age group are at an important stage for rapid growth and development. Early childhood before the age of two years is a particularly critical time for malnutrition (i.e. stunting,

underweight and wasting). After one year of age, children in developing countries continue to suffer from growth faltering and deviate even further from normal growth standards (Victora et al. 2010). This window of time corresponds to a reduction or cessation of breastfeeding and the introduction of liquid and solid foods which are not always of optimal nutritional quality in poorer populations. As mobility increases, this is also a time for early acquisition of some infectious pathogens in the environment, including STHs. A study reviewing data from 54 countries confirmed that interventions to prevent child malnutrition must occur during the first two years of life to prevent further growth deficits (Victora et al. 2010). Interventions in these first '1000 days' (which also includes pregnancy) are essential to prevent both short- and longer-term adverse effects (Bryce et al. 2008, Victora et al. 2008, Victora et al. 2010, Black et al. 2013). There has also been some evidence that stunting may be irreversible after 36 months of age (Bhutta et al. 2008).

The new WHO Child Growth Standards were established to improve estimates of malnutrition worldwide in children up to five years of age. They include a population of children from Brazil, Ghana, India, Norway, Oman and the USA (WHO Multicentre Growth Reference Study Group 2006). The WHO standards improve upon the previously used National Center for Health Statistics (NCHS) reference and the Centers for Disease Control and Prevention (CDC) reference, not only for including a more representative international population, but also for being a standard, rather than a reference, for how children should grow. This standard can be used to classify continuous measures of weight and height (length in children less than two years of age) as categorical measures of malnutrition. Z-scores are calculated based on the standard, and take into account age and sex of the child. Malnutrition can be categorized as stunted (i.e. low height-for-age z-score), underweight (i.e. low weight-for-age z-score) and wasted (i.e. low weight-for-height z-score). Mild malnutrition is based on a z-score between -1 and -2 deviations (SD), moderate malnutrition is based on a z-score between -2 and -3 SD, and severe malnutrition is based on a z-score -3 SD or more, from the

standard. Moderate-to-severe levels (<-2 SD) are most commonly used to classify malnutrition prevalence in populations (de Onis and Blossner 1997).

Evidence from the WHO Child Growth Standards demonstrates that, with appropriate nutrition and health interventions, all children have a similar potential for healthy growth, provided these interventions are given at an opportune time (WHO Multicentre Growth Reference Study Group 2006, de Onis et al. 2009, Onyango 2009); however, children living in areas of greatest poverty suffer the most from health and social inequities due to increased disease burden and lack of access to necessary health interventions and services (Belizán et al. 2007).

Poverty is also a major underlying cause of developmental deficits, primarily through increased nutritional deficiencies and infection, an inadequate home environment and stimulation, and low parental education (Grantham-McGregor et al. 2007). Independently and interactively, these risk factors can impact brain development and thus cognitive, language and motor functioning in early life, and later school achievement and productivity in adulthood (Victora et al. 2008, Martorell et al. 2010). Thus, appropriate and integrated interventions must be provided to improve early childhood growth and development, reduce health inequities, and provide those most vulnerable populations an opportunity to escape the vicious cycle of poverty (Grantham-McGregor et al. 2007). Improving the health of the youngest children has been a focus of many international efforts, including the Millennium Development Goals (MDGs) (UN Millennium Project 2005). These are a set of eight objectives which aim to reduce poverty worldwide. The first MDG specifically targets poverty and hunger, including reducing the prevalence of underweight in children under five years of age. The 2015 deadline to achieve the MDGs is quickly approaching, and the focus is now shifting to the post-MDG agenda. Canada's Muskoka Initiative also focuses on reducing malnutrition and infectious diseases in children living in developing countries.

2.5 Deworming benefits in children in the second year of life

Interventions that can help reduce malnutrition in early childhood include exclusive breastfeeding, micronutrient supplementation and vaccinations (Bhutta et al. 2008). Targeting the social components linked to poverty, such as mother-child interactions and child stimulation are also necessary to improve child development and health (Engle et al. 2011, Walker et al. 2011, Grantham-McGregor et al. 2014). However, the evidence-base on including deworming as one of the essential early childhood interventions in the critical growth and development window before two years of age is limited.

A Cochrane review was published in 2007, and again in 2012, looking at the effects of targeted deworming (i.e. targeted to high-risk groups of school-age and preschool-age children) on growth and school performance (Taylor-Robinson et al. 2007, Taylor-Robinson et al. 2012). These included 34 and 41 trials, respectively. A meta-analysis was also published by Hall et al (2008) examining the effects of STH infection and deworming on growth and nutrition of children up to 18 years of age. It included 19 trials, the number being restricted to those conducted in areas where the prevalence of STH was over 50%. An additional independent literature search was conducted using the keywords "deworming" OR "de-worming" OR "anthelminthic" OR "anthelmintic" AND "trial" OR "RCT" AND "preschool" OR "pre-school". From these three sources, a total of seven studies (described below and summarized in Table 1 on page 19), were considered to be comparable as they: 1) included children between 12 and 24 months of age in their child study populations; 2) were conducted in healthy children as opposed to exclusively malnourished children (i.e. the most likely to be included in PC programs, and not treated in clinical visits); 3) examined growth (e.g. weight, height/length, stunting or underweight); and 4) were randomized controlled trials which compared either albendazole or mebendazole to placebo or usual care.

Kloetzel et al (1982) conducted a double-blind randomized controlled trial including 337 children between one and eight years of age in Brazil. Children were randomly assigned to single regimens of mebendazole (100 mg, twice a day for three days) (n=165) or placebo (n=172), and followed up for 10 months. Growth indicators which were measured included weight, length, head, chest and mid-arm circumference, and tricipital skin fold. Outcomes were expressed as improvement, deterioration or no change in nutritional status, based on the percent change in reference weight-for-age (for children up to 60 months of age) or reference weight-for-length (for children 60 months of age and older). No significant differences were seen between intervention groups. This study is limited by a wide age range of children included, a small sample size, particularly to detect age-disaggregated differences (e.g. for 12 to 24 month olds), and non-standard outcome measurements (i.e. improvement or deterioration in nutritional status).

Awasthi et al (2000) performed a quasi-randomized controlled trial of albendazole (600 mg) (n=610) vs. placebo (calcium powder) (n=451). The study population included children aged 1.5 to 3.5 years from 32 slums attending 'Anganwadi Centers' (where health services can be provided by Integrated Child Development Services (ICDS)) in North India. Allocation was based on the last digit of serial numbers assigned to children in each cluster. The intervention was provided every six months for a total of two years. Research assistants providing treatment were not blinded. Authors specifically state that research assistants 'occasionally' switched the treatment type to which the participant had been originally assigned. Over 70% of children took the intervention in the presence of research staff, with the option to take the medication at home. Outcome measurements included a change in underweight and stunting (using the NCHS reference). Follow-up was high with 601 children and 444 children attending the last follow-up at two years in the albendazole and placebo groups, respectively. The proportion of stunting in the albendazole group was statistically significantly lower than in the placebo group after two years (difference of 9.4%; 95% CI: 6.0% to 12.8%). However, in terms of weight and length changes over two years, no significant differences

were seen between groups. Although the age group is more narrow, it is limited by inadequate allocation concealment and blinding, non-standard dosage of albendazole, and the allowance for taking the assigned intervention in the home and not solely in the presence of research personnel. The authors also state that the sample size was based on the categorical outcomes of stunting and underweight, and was not sufficiently powered to detect weight or length gains. Children between 12 and 17 months were specifically excluded as STH infection prevalence was assumed to be low in this group.

Awasthi and Pande (2001) used a cluster design to randomly assign urban slums to vitamin A and albendazole (400 mg) (n=63 slums, 988 children) or vitamin A only (n=61 slums, n=1022), every six months. Children 6 months to 12 months of age were enrolled and followed-up for 1.5 years. Outcome measurements included weight and height on a total of 832 children in the albendazole and vitamin A group and 840 children in the vitamin A only group. Overall, mean weight gain was statistically significantly greater in the group receiving albendazole. No statistically significant difference in height gain was apparent. The trial was open-label and outcome assessors were not blinded to treatment status. The study population also included children under 12 months of age for whom deworming is contraindicated. Additionally, a non-standard dose of albendazole for children under 24 months of age was used (i.e. 400 mg instead of the recommended dose of 200 mg). Although authors state that they used an intention-to-treat approach, the final results included complete cases only, which could have led to biased results. Clustering was also not taken into account in the analyses.

Stoltzfus et al (2004) looked at the effects of mebendazole on the primary outcome of anemia using a double-blind, randomized controlled trial design. The study population included children from six to 71 months of age living in a malaria-endemic area of Pemba Island, Zanzibar. Those with severe anemia (< 70 g/L) were not eligible for the study. A factorial design was used to randomly allocate children to mebendazole (500 mg) and iron (10 mg daily) (n=170),

mebendazole and iron placebo (n=176), mebendzole placebo and iron (n=170) or mebendazole placebo and iron placebo (n=172). Treatment was provided every three months for a total of 12 months. Growth was included as a secondary outcome. Weight and length were measured and categorized as moderate-to-severe stunting and mild wasting (calculated as <-1 SD and included as moderate-to-severe wasting was uncommon), using the CDC reference. A total of 459 children completed the trial. Results were disaggregated by age less than 30 months (n=184), or 30 months and older (n=275). In children less than 30 months of age, mebendazole was found to have a significant reduction on both mild wasting and small arm circumference. There was no significant difference in stunting. It is not clear if any effect of underweight was examined. This study provides some information on potential benefits in the younger age groups, but again is limited by a small sample size, which was calculated for the primary outcome of anemia, to detect age-disaggregated differences (e.g. for 12 to 24 month olds) and the use of non-standard outcomes (i.e. mild wasting, no mention of underweight, use only of categorical outcomes), and frequency of treatment (i.e. three times per year).

Alderman et al (2006) conducted a cluster-randomized controlled trial in children between one and seven years of age in Uganda. Fifty parishes were randomized (by a coin flip) to receive albendazole (400 mg) (provided only to healthy children) and usual care or usual care without albendazole during child health days. Usual care consisted of vaccinations and vitamin A supplementation. Forty-eight parishes were included in the final study (i.e. 24 per group). A total of five child health days took place in the study time (between November 2000 and June 2003). The frequency of deworming and time between follow-up visits varied based on the dates and number of health days attended by the child (i.e. not randomly assigned, but self-selected). Children were eligible to enter into the study at any child health day. Body weight was measured at least twice in 14,940 children in the albendazole group and 13,055 in the control group. Weight gain was calculated as the change in weight between the first and last child health day attended. A significant benefit of deworming twice a year was found, with a 10%

gain in weight in those in the albendazole group compared to the control group. The cluster design, although allowing for a large sample size, prevented the possibility of blinding, and was not taken into account in the analyses. The age range was also wide and results may not be generalizable to the under two year old population.

Awasthi et al (2008) used a similar open-label cluster-randomized trial design to look at the potential effects of deworming on child malnutrition. Children were enrolled between one to five years of age in urban slums in North India using ICDS infrastructure. Slums were randomized to albendazole (400 mg) and usual care (n=25 slums) or usual care without albendazole (n=25 slums), provided twice-yearly. Usual care included vitamin A supplementation. Follow-up was for two years. Complete length/height and weight information was available for 1852 children in the deworming group and 1860 children in the control group. There was no significant difference in height gain over two years, but weight gain was significantly greater in the albendazole group at both one and two year follow-up visits (a difference of 0.36 kg and 1.0 kg, respectively). The trial was open-label and outcome assessors were not blinded to treatment status. Age-disaggregated data were also not provided to detect the effect in the 12 to 24 month age group. No information on adverse events was collected.

Awasthi et al (2013) conducted an additional open-label cluster-randomized trial in North India. This included over one million children attending ICDS programs from six months to six years of age. A factorial design was used to randomly assign clusters to albendazole (400 mg) and vitamin A supplementation, individually or in combination, or usual care. Treatment was every 6 months. The primary outcome was mortality, with weight and length/height measured in a subgroup of 5165 children. No improvement in weight or height was seen in the albendazole group after two years of follow-up. No age-disaggregated information was provided. Children chosen for complete measurements were not randomly selected, but rather were chosen by ICDS workers, which could have introduced bias.

Overall, these studies have provided contradictory evidence on the nutritional benefits of deworming in child populations which include those in the 12 to 24 month old age group. There are individual methodological limitations to these studies, as well as substantial limitations to extrapolating their results to children under two years of age. Of the previous studies, none have examined the effect exclusively in children in the second year of life or provided pertinent age-disaggregated data. With wide age ranges of children included in the study populations (down to six months and up to eight years of age), previous studies may have been underpowered to detect statistically significant effects of deworming in any particular age subgroup. No studies have determined the most appropriate time for deworming in children less than two years of age. Considering that STH prevalence is generally lower at 12 months of age (Gyorkos et al. 2011), but can increase rapidly by 18 months of age (Gyorkos, unpublished data), this information is necessary to determine the time at which deworming can have maximum health and nutrition benefits. An additional limitation is that most studies used albendazole for the dewormed group; however, in the younger age groups who have more *Ascaris* and *Trichuris* infection, and negligible hookworm infection (Anderson 1986), deworming with mebendazole, rather than albendazole, may be more efficacious and produce a greater impact (Keiser and Utzinger 2008). Those that have used albendazole have not reported using a reduced dose for those under two years of age, as recommended by WHO (Allen et al. 2002). Lastly, as these younger children are undergoing rapid growth, appropriate and standard indicators need to be measured to demonstrate the additional benefit that deworming may have, over and above the normal growth which takes place at this stage.

The effect of providing deworming on development outcomes has been studied almost exclusively in school-age children. Some observational studies and randomized controlled trials (RCTs) have shown a benefit of deworming on cognition, measured directly through psychometric tests, or indirectly through school indicators such as school performance and attendance (Nokes et al. 1992, Nokes and Bundy 1993, Sakti et al. 1999, Ezeamama et al. 2012). This has been

mainly seen through a reduction in hookworm or *Trichuris* infection. However, the combined evidence is mixed, and the Cochrane review, which also examined development outcomes, was unable to detect an overall significant benefit of deworming on cognition in school-age children (Taylor-Robinson et al. 2012).

The evidence base in preschool-age children is even more limited. Oberhelman (1998) used a cross-sectional design to look at risk factors for 'suspect' scores on four scales of the Denver Developmental Screening Test II in children living in rural Nicaragua (Oberhelman et al. 1998). There was some evidence for a link between intestinal parasite infections (not limited to STH) and deficient development scores; however this relationship did not persist in multivariable analysis nor in a subgroup analysis of children under 24 months of age. Two trials that looked at the effects of deworming on growth in preschool-age children also examined the secondary outcome of development. Awasthi et al (2000) used the revised prescreening Denver Questionnaire to assess development in the population of 1.5 to 3.5 year old children in North India. The questionnaire was self-administered by literate mothers and administered by research assistants to illiterate mothers. Development was assessed at baseline and two years later, and was classified as normal or questionable depending on the number of tasks the child could perform. No significant difference was detected between the deworming and control groups. Stoltzfus et al (2001) also examined language and gross motor skills in two subgroups of children who were age-appropriate for the tests used (i.e. n=255 between 12-36 months for language and n=359 between 12-48 months for gross motor skills). A variety of sources had been used to develop the assessment instrument, including the Griffiths and McCarthy scales. The most rapid increases in scores were seen in the 12-24 month age group. Although not statistically significant, there was a trend towards a benefit of deworming on both developmental measures.

The Cochrane review, the meta-analysis and other publications have highlighted the limitations of previous studies (e.g. inadequate allocation concealment and blinding, high loss-to-follow-up, insufficient adjustment for clustering of

observations, inadequate data analyses, etc.) and recommended that better-designed RCTs are necessary to provide appropriate evidence on the benefits of deworming on multiple health and health-related outcomes (Taylor-Robinson et al. 2007, Hall et al. 2008, Bundy et al. 2009). In particular, evidence on the benefits for preschool-age children was deemed to be necessary for future intervention planning (Hall et al. 2008). Providing evidence on the benefits of deworming in the younger age group between one and two years of age is essential, as the burden of disease attributable to STH infections may be even more pronounced when children are exposed to these infections at an early age. It is becoming increasingly recognized that STH infection in early childhood may have important adverse effects on health and nutrition as the parasites take up a greater proportion of the body in younger children (Hall et al. 2008). Considering these unique nutritional demands and growth patterns of younger children, aggregated results from older children do not provide an appropriate indication of the potential benefit of deworming on growth, nutrition and development in younger age groups in this critical window. Based on the scarcity and limitations of previous studies, and their contradictory results, it is clear that methodologically sound research is needed to provide evidence on the benefit of deworming interventions in children in their second year of life.

Table 1. Published randomized controlled trials examining the effect of deworming (albendazole or mebendazole) on growth, including children between 12 and 24 months of age in their child study populations.

Author, date, setting	Study design	Age (years)	Intervention groups; Frequency	Sample size	Follow-up time	Deworming-attributable results
Kloetzel, 1982 Brazil	Double-blind, randomized controlled trial	1 to 8	mebendazole (100mg, 2x/day for 3 days) placebo Single regimen	Enrolled: not specified Analyzed: 337	10 months	No difference in weight or length, or head, chest or mid-arm circumference
Awasthi, 2000 India	Single-blind, cluster-randomized controlled trial	1.5 to 3.5	albendazole (600 mg) and usual care placebo and usual care 6-monthly	Enrolled: 1061 Analyzed: 1045	24 months	Significant reduction in proportion of stunted children No difference in underweight, wasting, weight or length gain
Awasthi, 2001 India	Open-label, cluster-randomized trial	0.5 to 1	albendazole (400 mg) and vitamin A vitamin A only (provision of usual care not clear) 6-monthly	Enrolled: 2010 Analyzed: 1672	18 months	Significant improvement in weight gain No difference in height gain
Stoltzfus, 2004 Tanzania	Double-blind, randomized controlled trial	0.5 to 5	mebendazole (500 mg) mebendazole and iron (10 mg/day) placebo and iron placebo and placebo 3-monthly	Enrolled: 684 Analyzed: 459	12 months	Significant reduction in mild wasting (in subgroup of children < 30 months at baseline) No difference in stunting
Alderman, 2006 Uganda	Open-label, cluster-randomized trial	1 to 7	albendazole (400mg) and usual care usual care only Up to five treatments, at child health days	Enrolled: not specified Analyzed: 27995	Variable (average 16.6 months)	Significant increase in weight gain
Awasthi, 2008 India	Open-label, cluster-randomized trial	1 to 5	albendazole (400 mg) and usual care usual care only 6-monthly	Enrolled: 3935 Analyzed: 3712	24 months	Significant increase in weight gain No significant difference in height gain
Awasthi, 2013 India	Open-label, cluster randomized factorial trial	0.5 to 6	albendazole (400 mg) and usual care albendazole, vitamin A and usual care vitamin A and usual care usual care only 6-monthly	Enrolled: estimated one million Analyzed: 5165	Variable (24 to 60 months)	No difference in absolute weight or height

3 OBJECTIVES

The principal objective of this study was to determine the effect of a deworming intervention, in terms of overall benefit, and optimal timing and frequency, on weight gain in children between 12 and 24 months of age.

The secondary objectives were to determine the effect of a deworming intervention, in terms of overall benefit, and optimal timing and frequency, on length gain and cognitive, language and fine motor development, and on the prevalence and intensity of STH infection, children between 12 and 24 months of age.

4 METHODS

4.1 Research design

The effect of a deworming intervention was evaluated in children 12 to 24 months old using a double-blind randomized controlled trial design. The intervention was incorporated into health services during routine childhood growth and development ('Crecimiento y Desarollo' or CRED) visits in a highly STH-endemic area of the Peruvian Amazon. Children were randomized to one of four interventions: Group 1: usual care and deworming treatment at the 12-month CRED visit and usual care and placebo at the 18-month CRED visit; Group 2: usual care and placebo at the 12-month CRED visit and usual care and deworming at the 18-month CRED visit; Group 3: usual care and deworming at both the 12 and 18-month CRED visits; and Group 4: usual care and placebo at both the 12 and 18-month CRED visits. Changes in weight, length, cognitive and motor development and STH infection were evaluated between the 12 and 24-month visits.

4.2 Ethics approval and trial monitoring

The trial received ethics approval in Peru from the Comité Institucional de Ética of the Universidad Peruana Cayetano Heredia (UPCH) in Lima. As it was a randomized controlled trial, it received additional ethics approval from the National Institute of Health (Instituto Nacional de Salud (INS)) in Lima. The local Ministry of Health office (Dirección Regional de Salud (DIRESA) Loreto) in Iquitos also approved the study. In Canada, ethics approval was obtained from the Research Ethics Board of the Research Institute of the McGill University Health Centre in Montréal, Québec. All ethics approvals were renewed and kept up-to-date throughout the study. An independent Data Safety and Monitoring Committee (DSMC) was set up with three members, from Canada, the U.S., and Peru, to review all adverse events occurring in study participants at three time points: at the halfway point of recruitment; following completion of recruitment; and during follow-up. Continuation of the study was approved at each review. The trial was registered with ClinicalTrials.gov (NCT01314937).

4.3 Study population

4.3.1 Study setting

The trial was conducted in neighbouring districts in and around the city of Iquitos, the capital of the Loreto region in the north-eastern area of Peru (Manuscript A, Figure 1). Iquitos is the largest city in the Amazon with a population of approximately half a million people. It is located at the confluence of the Amazon, Nanay and Itaya rivers and is only accessible by boat or plane. It has a tropical climate, characterized by a rainy season between November and May. The periurban and rural zones along the rivers are densely populated. As these areas are prone to flooding during the rainy season, the houses are built on stilts or on floating platforms. The climatic and geographic challenges have resulted in inadequate access to potable water, proper sanitation and waste management practices. This has led to a vulnerable population living in extreme poverty and an environment of permanent fecal contamination. Malaria is endemic in Loreto, but is not common in Iquitos.

The study area included four districts (Belén, Iquitos, Punchana and San Juan) where poverty is widespread, STH infections are highly endemic and malnutrition prevalence is high. The prevalence of STH infection in children 12 to 14 months of age was recently estimated to be 29% (Gyorkos et al. 2011). The STH prevalence doubles by 18 months of age (Gyorkos, unpublished data) and reaches over 85% in school-age children (5-14 years of age) (Casapía et al. 2006). Previous research in the area has shown a significant association between STH infection and poor growth in preschool- and school-age children (Casapía et al. 2006, Casapía et al. 2007, Gyorkos et al. 2011). Both STH infection and malnutrition have been identified as priority concerns by stakeholders in the community (Casapía et al. 2007). At present, there is no routine deworming for preschool-age children (i.e. children between one and four years of age, according to WHO definitions for deworming programs) (WHO 2006). The Peruvian Ministry of Health guidelines recommend deworming only as of two years of age (MINSA 2011).

Preschool-age children attend routine government-sponsored CRED visits (similar to well baby clinics) at health centres in Peru once-monthly from birth to 11 months of age (with two visits before one month of age), and every two months from 12 to 24 months of age (with less frequent visits thereafter to school age). Each health centre has one to two nurses who are responsible for CRED check-ups. During routine CRED visits, anthropometric measurements (e.g. weight and length/height) are taken, developmental milestones are recorded (e.g. fine and gross motor skills), and children receive routine age-appropriate immunizations and supplements. Parents also receive nutrition and other health counselling for their child (MINSA 2011).

4.3.2 Study population

The study population included children attending their routine 12-month CRED visit in the study area. Using information provided by the Peruvian Ministry of Health on health centre location and attendance, 12 study health centres ("Centros de Salud" (C.S.) and "Puestos de Salud" (P.S.)) were identified in the study area. These included: 1) P.S. America; 2) C.S. Belen; 3) C.S. Bellavista Nanay; 4) C.S. Cardozo; 5) P.S. 1 de Enero; 6) C.S. 6 de Octubre; 7) C.S. 9 de Octubre; 8) P.S. Masusa; 9) P.S. Porvenir; 10) C.S. Progreso; 11) C.S. San Juan; and 12) P.S. Tupac Amaru.

Inclusion criteria for participation in the trial were: 1) children attending any one of the study health centres for their 12- month CRED visit (for feasibility and to ensure recruitment of children from STH-endemic areas); and 2) children living in Belén, Iquitos, Punchana or San Juan districts (to ensure recruitment of children from STH-endemic areas and anticipated high follow-up).

Exclusion criteria, preventing participation in the study, were: 1) children attending the clinic for suspected STH infection (as they may require immediate, specific treatment); 2) children who received deworming treatment in the six

months prior to randomization (as the effect of previous treatment may interfere with the effect of the randomized treatment); 3) children whose families planned to move outside of the study area within the next 12 months (to maximize follow-up attendance); 4) children under 12 months of age (as treatment is contraindicated in this age group), or 14 months of age or older (to ensure participants were at a comparable growth and developmental stage); and 5) children with any serious congenital or chronic medical condition (as they may require more individualized treatment and follow-up by the health centres).

4.4 Recruitment

4.4.1 Pre-recruitment census

A community-wide census in all health centre jurisdictions was undertaken between April 2011 and August 2011 prior to recruitment. In households where any child under 12 months of age was present, information was recorded on the child's date of birth, address, and CRED attendance history. This database of children was cross-referenced with lists of CRED attendance from each health centre to establish a list of children who would be potentially eligible to participate in the study based on place of residence and age of the child.

4.4.2 Recruitment – home visit

Trained and dedicated research assistants (RAs) were assigned to one or two health centres each to recruit study participants in the respective communities and health centres. RAs were all healthcare personnel (e.g. nurse or nurse-midwife) with a minimum Bachelor's degree education. Nurse-technicians were also trained and paired with RAs to assist in recruitment. Potential participants were identified from the pre-recruitment lists and contacted in their homes. For those who agreed, eligibility was determined (Appendix 1), and an informed consent form was administered to the parent/legal guardian (Appendix 2). An evaluation of understanding of the consent form was also assessed (Appendix 3). Any incorrect responses were clarified, and all correct responses were reinforced.

As both parents' written signatures were required according to INS regulations in Peru, an additional visit was scheduled if both parents were not present during the initial meeting. In the case that one parent was absent (due to separation, travel, etc.), a sworn declaration was signed by the parent present to confirm the motive of the absence (Appendix 4). If the parent was a minor, a direct blood relative (e.g. grandmother or grandfather of the participating child) provided consent, and the parent provided assent for participating in the study. In the case of a mother/father who was physically or mentally incapacitated, a legal guardian or direct blood relative served as a witness for consent.

For all children who were deemed to be eligible, and for whom written consent was provided, a household questionnaire, which included questions on socio-demographic and health information about the child and family, was then administered to that parent who was the primary caregiver (Appendix 5). At this time, parents were also provided with the information and materials needed to collect a stool specimen from the child. This included information on the correct consistency and size of the specimen. Parents were then given an appointment at the health centre, at which time they would deposit the stool specimen and the child's anthropometric measures and development would be ascertained. All forms and questionnaires were returned to the local research office at the end of each work day, and reviewed by the Project Director, local Study Coordinator, and, when needed, by the local Principal Investigator, to confirm the eligibility of each child. All eligible children were given a date to attend the second recruitment visit at the health centre to meet with the RA and complete outcome measurements.

Additional steps were taken to identify potentially eligible children in the health centres during pre-recruitment and recruitment. For eligible children whose parents were able to provide informed consent without a home visit (i.e. both parents were present, or one parent was permanently absent), the household questionnaire was administered in the health centre. Children under 12 months of

age who were identified in health centres were provided with information on the study. If they agreed, they were contacted at the time of their 12-month birthday to participate in the study.

4.4.3 Recruitment – health centre visit

Outcome measurements were ascertained by RAs at the second recruitment visit which was scheduled one to two days after the home visit. Outcome measurements included anthropometry (i.e. weight and length), development (i.e. cognition, language and motor skills), and STH infection. The quality of the stool specimen was first verified. If no specimen or an inadequate specimen (i.e. liquid specimen and/or insufficient quantity) was provided, then anthropometry was ascertained and a subsequent visit was scheduled to arrange for another stool specimen. If any child was discovered to be ill on the day of his or her health centre visit, the visit was postponed until the child had recovered.

After verification of the quality and quantity of the stool specimen, the child was undressed and weighed (in duplicate) using a portable electronic scale, accurate to the nearest 0.01 kg (Seca 334, Seca Corp., Baltimore, MD, USA). Length (i.e. the recommended measurement of height in children less than two years of age) was measured as recumbent crown-heel length on a flat surface using a stadiometer (Seca 210, Seca Corp., Baltimore, MD, USA), accurate to the nearest millimetre.

Development was assessed using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) (Pearson Education Inc, Texas). The Bayley-III is a rigorous instrument which is used to assess developmental functioning in preschool-age children (Bayley 2006). It has been adapted for use in international research settings (Aboud et al. 2013, Manji et al. 2014, Yousafzai et al. 2014) and previous versions have been used in Peruvian populations (Colombo et al. 2014). Subtests which were translated into Spanish and adapted for local use were cognitive, receptive language, expressive language, and fine motor. Each subtest consisted of items which were administered by RAs in a play-based manner in the presence of a maximum of two caregivers. All attempts were

made to complete the assessment in one visit, including taking breaks (e.g. for feedings, etc.). However, to ensure the child was performing under optimal conditions, a second visit was scheduled if needed. The test was administered as recommended during the health centre visit (Bayley 2006); however, some modifications were required:

a) if a child did not answer all first three items correctly, the RA would reverse in blocks of three items at a time (i.e. rather than to the previous age starting point) until three correct responses was achieved (i.e. the basal). The RA would then continue in a forward manner from the first unadministered item until the stopping point was reached (i.e. incorrect responses to five sequential items).

b) for items where verbal instructions were suggested but not explicitly stated, instructions were developed and a maximum number of times that they could be repeated was specified, in order to standardize practices among RAs.

c) adaptation of words and images in some test items, including all pictures in the Picture Book and some pictures in the Stimulus Book, was required to ensure cultural appropriateness. The age-appropriateness of both the image and the accompanying word were considered when adapting the items. All modifications to images and objects were pre-tested in children of the same target age of the trial, as well as in older children.

The gross motor subtest was not included as these skills were thought to be less variable in the age group of children studied; however, the WHO gross motor milestones (i.e. walking alone, standing alone, walking with assistance, hands and knees crawling, and standing with assistance) were assessed by observation (WHO Multicentre Growth Reference Study Group 2006).

4.4.4 Intervention groups

Following confirmation of eligibility, informed consent, and all baseline outcome assessments in the health centres, children were randomized into one of the following four intervention groups.

Group 1 (MBD/PBO): Usual care and deworming at the 12-month visit and usual care and placebo at the 18-month visit.

Group 2 (PBO/MBD): Usual care and placebo at the 12-month visit and usual care and deworming at the 18-month visit.

Group 3 (MBD/MBD): Usual care and deworming at both the 12 and 18-month visits.

Group 4 (PBO/PBO): Usual care and placebo at both the 12 and 18-month visits.

Deworming consisted of a single-dose mebendazole tablet (500 mg) (manufactured by Janssen Pharmaceuticals Inc.; donated by INMED Peru). The placebo was identical to the deworming tablet in terms of size, colour and markings (manufactured and purchased from Laboratorios Hersil, Peru). Tablets were crushed and mixed with juice to minimize any potential choking hazard (Albonico et al. 2008). The crushed tablet was administered by research assistants at the end of each visit after all other study procedures had been completed. All children received deworming at the end of the one-year follow-up (i.e. at the 24-month visit), according to Peruvian Ministry of Health guidelines (MINSA 2011). Children received usual care interventions and services from health centre personnel according to Peruvian Ministry of Health guidelines (MINSA 2011). This included the administration of measles, mumps and rubella (MMR) vaccination at the 12-month visit, and diphtheria, pertussis and tetanus (DPT) vaccine booster at the 18-month visit. Vitamin A supplementation was not routinely provided.

4.4.5 Sample size

Sample size calculations were based on detecting the smallest meaningful difference among intervention groups in mean weight gain over 12 months. Weight gain was chosen as the primary outcome as it was thought to be more modifiable in a short time period (compared to length), and also for comparability with other studies. From pilot data, the anticipated mean weight gain ± standard deviation between 12 and 24 months in the group receiving usual care and placebo at both time points was approximately 2.0 kg ± 0.8 kg. Based on previous research (Awasthi et al. 2008) an improvement in weight gain of 0.5 kg in the group receiving deworming at the 18-month visit (with estimated STH infection prevalence of 50%) was expected, compared to the group receiving usual care and placebo at both the 12 and 18-month visits (i.e. 2.5 kg). An improvement in weight gain of 0.7 kg was estimated for the group receiving deworming at both the 12 and 18-month visits (i.e. 2.7 kg). These differences attempted to take into account effect dilution from treating infected children (who would benefit directly from the deworming treatment) and non-infected children (who may not benefit directly from the deworming treatment). The mean weight gain in those receiving usual care and deworming at the 12-month visit was estimated to be half of the gain in the group receiving usual care and deworming at the 18-month visit (i.e. 2.25 kg).

In order to have 80% power to detect a minimum difference of 0.2 kg (i.e. the difference between 2.7 kg and 2.5 kg) in mean weight gain among intervention groups, assuming a common standard deviation of 0.8, and using a one-way ANOVA which accounts for pair-wise multiple comparisons using the Tukey correction, the estimated sample size per group was 366 children. The required sample size was increased to 440 children per group (1760 in total), to take into account potential loss-to-follow-up of 20% after 12 months (based on attrition rates from previous studies in the area by the research team (Larocque et al. 2006, Blouin et al. 2013)) (MC4G Software©, GP Brooks, Ohio University, 2008).

4.4.6 Randomization and masking

Computer-generated randomly ordered blocks of eight and twelve were used to randomly allocate children to each intervention group. This blocking sequence was used to ensure a balance in the number of children assigned to each group, as well as to ensure that the randomization sequence would not be predictable to any trial personnel or participants. Research personnel not directly involved in the trial prepared small envelopes containing the randomly assigned intervention. These were numbered from 1 to 1760, with each number corresponding to one of the four intervention groups. Randomization was conducted once, but separate envelopes were prepared for the intervention at 12 months before baseline enrolment, and again for the intervention at 18 months before the first follow-up visit. Envelopes were stored in a temperature-regulated pharmacy at the research facility, and distributed by the Project Director (SAJ) or the local Study Coordinator (LP) in sequential order to research assistants until the sample size was achieved. All health centre and research personnel, including assistants, laboratory technicians, collaborators and co-investigators, and parents of participants were blinded to intervention status.

4.4.7 Analysis of stool specimens

After the intervention was administered, the stool specimen was labeled with a unique number between 1 and 1760, corresponding to the randomly assigned treatment code for the deworming trial.

Stool specimens from all participants were transferred to the laboratory at the local research facility (Asociación Civil Selva Amazónica) to be read by one of two experienced laboratory technologists. Stool specimens from participants who were subsequently randomized to receive active deworming treatment were analyzed immediately by the Kato-Katz method, as recommended by WHO, (within 24 hours of initial collection, as a fresh specimen is required for this technique) to determine both prevalence and intensity of STH infection (WHO

2004, WHO 2011). For a one-stool specimen, sensitivity and specificity are over 96% for *Ascaris* and over 91% for *Trichuris* (Tarafder et al. 2010). There is lower sensitivity and specificity for hookworm; however, hookworm infection is generally uncommon in very young children in this study area.

This procedure of immediately analyzing stool specimens only of those randomly allocated to the intervention groups receiving active deworming treatment takes into account the ethical imperative of treating those who would be found to have positive results. Stool specimens of those receiving inactive placebo tablets were stored in 10% formalin and examined by the direct method upon completion of the trial, at which time all participants received deworming treatment. To maintain blinding, each specimen code was replaced with a laboratory code by the local study coordinator for use by the laboratory technologists. Laboratory technologists were provided with a list of those laboratory codes which would be analyzed and those which were to be stored. Each list was kept on a password-protected computer, one in the coordinator's office and one in the lab accessible only to the laboratory supervisor. A master list linking all information was stored at the research office in Canada (Research Institute of the McGill University Health Centre).

4.5 Follow-up visits

Participants were followed-up every 6 months, at their 18 and 24-month CRED visit in the health centre. Assessment of weight, length and STH infection were repeated at both follow-up visits. At the 18-month visit the second randomly assigned intervention was administered. The Bayley-III was administered again at the 24-month visit. Development was not assessed at the 18-month visit, due to small anticipated differences at the midpoint assessment. Follow-up visits were scheduled during the previous visit, and confirmed by telephone and in person in the week preceding the scheduled visit. The 24-month visit was scheduled 6 months after the 18-month visit. In the case that a participant did not attend their 18-month visit, he/she remained eligible for the 24-month visit, which was

scheduled 12 months after initial enrolment. In instances where participants were not located prior to the day of their anticipated 18 or 24-month visit, or a scheduled date was missed, a minimum of four additional attempts were made to locate the study participant. Participants remained eligible for their 18-month visit up to three months after their anticipated date of follow-up, and until trial completion for the 24-month visit. All children received mebendazole at the 24-month visit according to Peruvian Ministry of Health guidelines (MINSA 2011).

4.6 Adverse event reporting

Information on minor and severe adverse events was obtained through passive reporting (i.e. dependent on reporting by parents or health care personnel) at follow-up visits or in between visits. Severe adverse events were based on WHO definitions and included: 1) death; 2) life-threatening conditions; 3) in-patient hospitalization or prolongation of an existing hospitalization; 4) persistent or significant disability/incapacity; 5) cancer; or 6) overdose (accidental or intentional)(WHO 2006). Minor adverse events included all reported illnesses that did not meet the definition of a serious adverse event. All minor and serious adverse events were reported to ethics committees. Summary reports of adverse events were also provided to the Data Safety and Monitoring Commitee (DSMC).

4.7 Standardization and quality control

Prior to commencing recruitment, in-depth practical training of the research assistants took place according to WHO guidelines (de Onis et al. 2004, WHO Multicentre Growth Reference Study Group 2006) to ensure accurate outcome assessment, and standardization among research assistants. Inter and intra-rater reliability of over 95% was achieved for weight and length assessments, which are considered acceptable levels for anthropometric measurements (Ulijaszek and Kerr 1999, de Onis et al. 2004).

Extensive training of RAs and pretesting of the adapted instrument took place for two months prior to the start of the baseline and follow-up. Adaptation and training of the Bayley-III was performed by FL and SAJ. On-site supervision, video recordings, and re-training, were used to ensure consistency of administration and scoring throughout the trial.

The consistency of egg count assessments was evaluated among the laboratory technicians using standard quality control methods (Montresor et al. 1998). The laboratory supervisor read 10% of the slides of each microscopist without prior knowledge of the result. In the case of a discrepancy larger than 10%, a discussion took place between the laboratory supervisor and the microscopist to resolve the discrepancy and further slides were examined to avoid repeated errors.

Data collection activities during fieldwork were regularly supervised by SAJ and LP. All completed forms were checked immediately after data collection at the end of each day. Additional quality control of questionnaires was undertaken at the time of data entry. Questionnaires were reviewed and data were double-entered by two separate trained assistants. Any inconsistencies were verified by reviewing the original questionnaires. All data cleaning was performed by SAJ.

4.8 Analyses

4.8.1 Variable classification and definitions

To classify child anthropometric measurements (i.e. length and weight) into categories of stunting, underweight and wasting, WHO Anthro software (Version 3, 2011) was used to calculate length-for-age z scores (LAZ), weight-for-age z scores (WAZ), and weight-for-length z scores (WLZ), respectively. Z scores are calculated taking into account a child's sex and age and are based on a comparison to a WHO international standard population. Moderate-to-severe categories of stunting, underweight and wasting are based on LAZ, WAZ and WLZ of <-2SD. Severe stunting, underweight and wasting are defined as LAZ, WAZ and WLZ of <-3SD, respectively (de Onis and Blossner 1997, WHO Multicentre Growth Reference Study Group 2009).

Categories of STH infection intensity were determined from established WHO guidelines (WHO 2002). For *Ascaris* infection, light, moderate and heavy intensity are based on egg counts per gram of feces (epg) of 1-4999, 5000-49999 and 50000 and greater, respectively. For *Trichuris* infection, the categories for light, moderate and heavy intensity infection are an epg of 1-999, 1000-9999 and 10000 and greater, respectively. Light, moderate and heavy intensity hookworm infection are based on epgs of 1-1999, 2000-3999 and 4000 and greater, respectively. Both arithmetic and geometric mean epgs were calculated and reported.

Development scores were calculated separately for each scale. The raw score was calculated as the total number of correct responses between the basal and the stopping point, added to the total number of unadministered items prior to the basal. Scaled scores were calculated based on the total raw score in each scale, scaled between 1 and 19 based on the child's age in months and days and the specific subtest (Bayley 2006). Scaled scores were used to make comparisons within the trial and not as an indication of development delays or deficits compared to other populations. The WHO gross motor milestone of walking alone was categorized as yes, if the child was able to walk without any assistance or support, regardless of the other milestones achieved; and no, if the child could not walk without assistance, but achieved at least one of the other gross motor milestones (i.e. standing alone, walking with assistance, hands and knees crawling, and/or standing with assistance).

Principal Component Analysis was used to create an asset-based index for socioeconomic status (SES) to be included in multivariable analyses (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Variables included in the index were house material, type of cooking fuel, television ownership, radio ownership and electricity in the home (Filmer and Pritchett 2001, Gyorkos et al. 2013). The SES index explained 40.1% of the variance and was divided into quartiles for subsequent analyses.

For ease of interpretation and comparison with previous published studies, both p values and confidence intervals are reported for the analyses described below. All statistical analyses were performed using the Statistical Analysis Systems statistical software package version 9.3 (SAS Institute, Cary, NC, USA).

4.8.2 Baseline associations

Associations with the outcomes of stunting and underweight at baseline were examined initially in univariable analyses. Variables that were significant at the $p < 0.20$ level, or that were deemed to be important from previous published research, were included in multivariable modelling to determine the most parsimonious model. If variables were highly correlated, the most informative variable (i.e. with more variation, more accurate measurements and/or important factors in previous literature) was chosen to be included in multivariable model building. Multivariable associations with stunting and underweight were examined using a generalized linear model with a log link, a Poisson distribution, and a robust variance estimator to estimate the risk ratio for the dichotomous outcomes of moderate-to-severe stunting and moderate-to-severe underweight, where no and mild categories of stunting, and no and mild categories of underweight, respectively, comprised the reference groups (Spiegelman and Hertzmark 2005, Wilber and Fu 2010). Analyses were first restricted to children whose stool specimens were examined by the WHO-recommended Kato-Katz method (WHO 2011). Analyses were then performed including all children in the study population.

4.8.3 Effect of deworming on growth

The primary outcome of the trial was mean weight gain (in kilograms) between the baseline 12-month visit and the 24-month follow-up visit (i.e. after 12 months). Mean weight gain (kg) was compared between the four intervention groups using the one-way ANOVA procedure. Additional analyses were

conducted to examine differences between intervention groups in terms of derived weight indices (i.e. mean weight-for-age z score) and length and derived length indices (mean length gain and mean length-for-age Z score). Multivariable linear regression was also conducted adjusting for age, sex, SES and continued breastfeeding at 12 months of age.

All analyses were first expressed using an intention-to-treat (ITT) approach such that participants were analyzed according to their assigned intervention group. Multiple imputation, using a Markov Chain Monte Carlo (MCMC) model with five imputations, was used to impute weight and length measurements for those who did not attend the 18-month and/or 24-month follow-up visits (i.e. for whom follow-up measurements were missing). Variables related to the outcome, and hypothesized to be related to missing the follow-up visit(s) were used to impute the missing weight and length outcomes. These included baseline weight, length, socioeconomic status, continued breastfeeding at 12 months, sex, and age. Analyses were then repeated 1) using a complete case approach on all participants who had attended the final follow-up visit, 2) using a per-protocol approach only including those participants who attended all three visits and who did not report having received deworming outside of the trial at any time between baseline and the final follow-up visit and 3) selected analyses restricted to STH-infected children. The overall benefit of deworming was determined by comparing growth outcomes (i.e. weight gain, WAZ change, length gain and LAZ change) between each intervention group and the control group.

To explore the effect of the timing of deworming, growth outcomes in Group 1 (i.e. receiving deworming once at the 12-month visit) were compared to Group 2 (i.e. receiving deworming once at the 18-month visit). To explore the effect of the frequency of deworming, growth outcomes in Group 1 and Group 2 (i.e. receiving deworming once at the 12-month or 18-month visit, respectively) were each compared to Group 3 (i.e. receiving deworming twice at both the 12 and 18-month CRED visits).

4.8.4 Effect of deworming on development

The effect of deworming on development was examined separately for each scale in unadjusted intention-to-treat analysis using one-way ANOVA. Developmental outcomes included absolute raw scores and scaled scores at the 24-month visit, and the change in raw and scaled scores from baseline to the 24-month visit. Multivariable linear regression was conducted to adjust for baseline anthropometry, baseline development score (in the case of absolute score outcomes), age, sex, breastfeeding to 12 months of age and SES. For children who were missing their 24-month visit, multiple imputation using a Markov Chain Monte Carlo model was used to impute development scores at follow-up (e.g. based on baseline values of age, sex, anthropometry and SES).

The relationship between child, maternal and household factors at baseline and development scores at the 24-month visit was also examined using multivariable linear regression analyses. Variables that were significant at $p < 0.20$ in univariable analyses were included in further multivariable model building. The final model included all significant variables at $p < 0.05$, as well as adjustment for age, intervention group, and the RA who performed the assessment.

4.9 Other considerations

The integrity of the trial with respect to blinding was ensured in the following way: only one member of the research team, not involved in outcome ascertainment, was unblinded to prepare the selected stool specimens for examination. All other members of the research team remained blinded. Results of stool examinations were available to parents at the end of the study.

Children were assigned an identification number for the duration of the study to ensure the confidentiality of the results. All original documents, including questionnaires and informed consent forms, were kept in a locked cabinet and room. Preserved stool specimens were marked with a corresponding lab code and kept in the locked laboratory. All electronic information (e.g. databases) was

stored on a password-protected computer in a locked room. Access to original documents, electronic information and preserved stool specimens was restricted to the Project Director, Principal Investigator and Project Coordinator, and to data entry staff when needed.

There were no costs associated with participation in this study as visits took place during routine health centre visits according to the Peruvian Ministry of Health schedule. Study treatments and testing of stool specimens were free of charge for the duration of the study. The cost of travel for all visits was reimbursed to encourage participation and follow-up during the course of the study.

4.10 Funding

This study was supported by grants from the Thrasher Research Fund and the Canadian Institutes of Health Research (CIHR) (MOP-110969) (Principal Investigator: Theresa W. Gyorkos). Ms. Joseph also received personal and project support from CIHR (Vanier Canada Graduate Scholarship and Michael Smith Foreign Study Supplement), the Fonds de Recherche du Québec – Santé and the Research Institute of the McGill University Health Centre. Results dissemination activities were supported by a CIHR Planning and Dissemination Grant (Principal Investigator: Serene A. Joseph).

PREFACE TO MANUSCRIPT A

For the randomized controlled trial of deworming in early preschool-age children in Iquitos, Peru, a pre-recruitment visit was conducted with children 12 and 13 months of age to assess eligibility and, if inclusion criteria were met, to obtain parental informed consent. At this time, an extensive household questionnaire was administered to collect baseline characteristics of the study population. This included questions on child and maternal health, household characteristics, and socioeconomic indicators. A second visit was scheduled in the health centre, at which time anthropometric measurements were taken (i.e. length and weight), the Bayley Scales of Infant and Toddler Development® was applied (i.e. measurement of cognition, language and motor skills), and a stool specimen was collected to be analyzed for the presence of soil-transmitted helminth (STH) eggs.

The following manuscript (Manuscript A) comprises the first results chapter of the thesis. It is a description of study enrolment methods, socio-demo-epi characteristics of the study population, and risk factors for malnutrition in participating children at baseline. Further detail on the trial methodology and its results including the effect of deworming on anthropometric outcomes is found in Manuscript B. A separate manuscript (Manuscript C) provides full detail on the methodology and effect of deworming on the secondary outcome of cognitive, language and motor development. Supplementary analyses can be found in Appendix 10.

Selected baseline results (in Manuscript A) have been presented by SAJ at scientific meetings. This includes 1) the use of a community-based pre-recruitment census to identify potential participants for the trial (presented at the Global Health Conference, Montréal, Canada, November 2011); 2) baseline associations between malnutrition and STH infection (presented at the European Congress of Tropical Medicine and International Health, Copenhagen, Denmark, September 2013); and 3) baseline characteristics of vaccination use and implications for integrated delivery with deworming (presented at the Canadian

Conference on Global Health, Ottawa, Canada, October 2013). SAJ also gave an invited presentation at the 1st International Meeting of Tropical Medicine Institutes in Lima, Peru, June 2013, which included details of study enrolment and baseline characteristics (see Appendix 9 for copies of abstracts).

This manuscript is currently under review in the journal of the Public Library of Science Neglected Tropical Diseases (PLoS NTD).

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This study was supported by grants from the Thrasher Research Fund and the Canadian Institutes of Health Research (CIHR) (MOP-110969) (Principal Investigator: Dr. Theresa W. Gyorkos). Ms. Joseph also received personal and project support from CIHR (Vanier Canada Graduate Scholarship and Michael Smith Foreign Study Supplement), the Fonds de Recherche du Québec – Santé and the Research Institute of the McGill University Health Centre.

5 MANUSCRIPT A

Risk factors associated with malnutrition in one-year-old children living in the Peruvian Amazon

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ABSTRACT

Background: Children under two years of age are in the most critical window for growth and development. As mobility increases, this time period also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical and sub-tropical environments. The association between malnutrition and STH infection, however, has been understudied in this vulnerable age group.

Methodology/Principal Findings: A survey was conducted in 12 and 13-month old children participating in a deworming trial in Iquitos, an STH-endemic area of the Peruvian Amazon. An extensive socio-demo-epi questionnaire was administered to the child's parent. Length and weight were measured, and the Bayley Scales of Infant and Toddler Development was administered to measure cognition, language and fine motor development. Stool specimens were collected to determine the presence of STH. The association between malnutrition (i.e. stunting and underweight) and STH infection, and other child, maternal and household characteristics, was analyzed using multivariable Poisson regression. A total of 1760 children were recruited between September 2011 and June 2012. Baseline data showed a prevalence of stunting and underweight of 24.2% and 8.6%, respectively. In a subgroup of 880 randomly-allocated children whose specimens were analyzed by the Kato-Katz method, the prevalence of any STH infection was 14.5%. Risk factors for stunting in these 880 children included infection with at least one STH species (aRR = 1.37; 95% CI 1.01, 1.86) and a lower development score (aRR = 0.97; 95% CI: 0.95, 0.99). A lower development score was also a significant risk factor for underweight (aRR = 0.92; 95% CI: 0.89, 0.95).

Conclusions: The high prevalence of malnutrition, particularly stunting, and its association with STH infection and lower developmental attainment in early preschool-age children is of concern. Emphasis should be placed on determining the most cost-effective, integrated interventions to reduce disease and malnutrition burdens in this vulnerable age group.

AUTHOR SUMMARY

Malnutrition, including stunting and underweight, is one of the leading causes of morbidity and mortality in preschool-age children. Children under two years of age are at a particularly critical period for growth and development, and for first exposure to worm infections in tropical and subtropical environments. The association between malnutrition and worm infection, however, is not well understood in this age group. A survey was therefore conducted between September 2011 and June 2012 in 1760 children 12 and 13 months of age living in a worm-endemic area of the Peruvian Amazon. Length, weight, development (i.e. cognitive, language and motor development), worm infection, and socio-demographic information were obtained. Results showed a high prevalence of stunting, and a significant association with worm infection and lower development. Overall, these adverse effects have the potential to negatively impact short-term and long-term health and nutrition, and educational and social achievement, into school-age and adulthood. Emphasis is needed on determining the most appropriate and effective interventions to reduce poor health and nutrition outcomes in this age group.

INTRODUCTION

Malnutrition is the leading cause of mortality in preschool-age children (i.e. children under five years of age) in low- and middle-income countries (LMICs). Over 150 million children suffer from one or more forms of malnutrition, including stunting, underweight and/or wasting [1,2]. Malnutrition also predisposes to infection, creating a vicious infection-malnutrition cycle that contributes to over 35% of the disease burden of early childhood [1,3]. Infection and poor quality and low availability of food, resulting in micronutrient and other deficiencies, are the primary causes of malnutrition in childhood [4]. Early childhood before the age of two years is a particularly critical time for growth faltering [5]. This window of time corresponds to weaning and the introduction of complementary foods, and, as mobility increases, for early acquisition of certain infectious pathogens. The soil-transmitted helminths (STHs), or worm infections, are one such pathogen cluster that is transmitted through contaminated food, water and/or the environment in warm, tropical and subtropical climates. The STH disease cluster includes three diseases, *ascariasis* (caused by the roundworm *Ascaris lumbricoides*), *trichuriasis* (caused by the whipworm *Trichuris trichiura*) and *ancylostomiasis* or hookworm disease (caused either by *Ancylostoma duodenale* or *Necator americanus*). The geographical distribution of these three diseases is overlapping, mainly in areas of poverty with poor sanitation and limited access to potable water. STHs are one of the most important Neglected Tropical Diseases (NTDs) and one of the most common infections worldwide, with recent estimates indicating that 1.45 billion people are infected in over 100 endemic countries [6]. It is estimated that they contribute 4.98 million years lived with disability (YLD) and 5.18 million disability-adjusted life years (DALYs) [6]. STHs are a significant contributor to poor health and nutritional status in all age groups, and especially in childhood.

Traditionally, the occurrence of STH infection had been perceived to be low in children under two years of age. However, there has been increasing empirical evidence which shows that the opposite is true [7]. In Belén, a community of

extreme poverty in the Peruvian Amazon, while the prevalence of *Ascaris* or *Trichuris* was only 4% in children at seven to nine months of age, it rose to almost 30% at 12 to 14 months of age [8]. In a cohort of preschool-age children in Ecuador, over 20% suffered from *Ascaris* or *Trichuris* infection at least once in the first two years of life, with infection first appearing around seven months of age [9]. There is also evidence to suggest that hookworm infection may be high in early preschool-age children, as demonstrated in a study by Stoltzfus et al (2004) in Zanzibar, in which 31.3% of children under 30 months of age were infected with hookworm [10].

It is becoming increasingly recognized that STH infection in early childhood may have important adverse effects on health and nutrition as the parasites take up a greater proportion of the body in younger children [11]. However, the importance of STH infection and its link with malnutrition in preschool-age children has been inadequately studied. Few studies have included preschool-age children in their study population, and even fewer provide age-disaggregated data to examine differing effects and sequelae in the critical growth window before two years of age. Evidence from the World Health Organization (WHO) Child Growth Standards demonstrates that, with appropriate nutrition and health interventions provided early in life, all children have a similar potential for healthy growth and development [12-14]; however, children living in areas of greatest poverty suffer the most from health and social inequities due to increased disease burden and lack of access to necessary health interventions and services [15]. Improving the health of the youngest children has been a focus of many international efforts, including Canada's Muskoka Initiative, and the Millennium Development Goals (MDGs) which aim to reduce poverty worldwide by 2015. With focus now shifting to the post-2015 MDG agenda, it is imperative to fill in knowledge gaps on the burden of disease and risk factors in early childhood to improve health in the short and the long term [16].

The principal objective of this study was to determine the association between malnutrition (i.e. stunting and underweight) and soil-transmitted helminth

infection and other child, maternal and household characteristics in 12 and 13-month old children, living in an area of extreme poverty in the Peruvian Amazon.

METHODS

Ethics approval

This study received ethics approval in Peru from the Comité Institucional de Ética of the Universidad Peruana Cayetano Heredia and the Instituto Nacional de Salud, in Lima, and the local Ministry of Health office (Dirección Regional de Salud Loreto) in Iquitos. Ethics approval was obtained in Canada from the Research Ethics Board of the Research Institute of the McGill University Health Centre in Montréal, Québec. Written informed consent was obtained by the parents or guardian of each child that participated in the study.

Study population

This study was conducted in neighbouring districts in and around the city of Iquitos, the capital of the Loreto region in the Peruvian Amazon (Figure 1). The study area included four districts (Belén, Iquitos, Punchana and San Juan) where poverty is widespread, STH infections are highly endemic and malnutrition prevalence is high. Both malnutrition and STH prevalence have been identified as priority concerns by stakeholders in the community [17].

The study population included children attending their routine 12-month growth and development ("Crecimiento y Desarrollo" or CRED) clinic visit in the study area, and whose parents had agreed to their participation in a randomized controlled trial to determine the benefit of deworming (mebendazole) on growth and development (ClinicalTrials.gov #NCT01314937). The current survey describes information obtained at the baseline 12-month CRED visit for the randomized controlled trial. Preschool-age children are scheduled to attend routine government-sponsored CRED visits (similar to well baby clinics) at health clinics in Peru once-monthly from birth to 11 months of age (with two visits before one month of age), and every two months from 12 to 24 months of age

(with less frequent visits thereafter to school age). During routine CRED visits, anthropometric measurements (e.g. length and weight) are taken, developmental milestones are recorded, and children receive routine age-appropriate vaccinations and supplements. Parents also receive nutrition and other health counselling for their child [18].

Using information provided by the Peruvian Ministry of Health on health centre location and attendance, 12 study health centres ("Centros de Salud" (C.S.) and "Puestos de Salud" (P.S.)) were identified in the study area. These included: 1) P.S. America; 2) C.S. Belen; 3) C.S. Bellavista Nanay; 4) C.S. Cardozo; 5) P.S. 1 de Enero; 6) C.S. 6 de Octubre; 7) C.S. 9 de Octubre; 8) P.S. Masusa; 9) P.S. Porvenir; 10) C.S. Progreso; 11) C.S. San Juan; and 12) P.S. Tupac Amaru.

Inclusion criteria for participating in the study were: 1) children attending any one of the study health centres for their 12-month CRED visit; and 2) children living in Belén, Iquitos, Punchana or San Juan districts. Exclusion criteria preventing participation in the study were: 1) children attending the health centre for suspected STH infection; 2) children who had received deworming treatment in the six months prior to the study; 3) children whose families planned to move outside of the study area within the next 12 months; 4) children under 12 months of age or 14 months of age or older; and 5) children with any serious congenital or chronic medical condition. All inclusion and exclusion criteria were based on considerations related to participation in the deworming trial.

Sample size

The sample size of the study was based on detecting a minimum difference of 0.20 kg in mean weight gain among different deworming interventions in the randomized controlled trial (3 intervention groups, and 1 control group). The required sample size was estimated to be 1760 children, or 440 children per group (MC4G Software©, GP Brooks, Ohio University, 2008).

Recruitment

A community-wide census in all health centre jurisdictions was undertaken between April 2011 and August 2011 prior to recruitment. In households where any child under 12 months of age was present, information was recorded on the child's date of birth, address, and CRED attendance history. This database of children was cross-referenced with lists of CRED attendance from each health centre to establish a list of children who would be potentially eligible to participate in the study based on place of residence and age of the child.

Trained research assistants, primarily nurses and nurse-midwives, were assigned to one or two health centres each to recruit study participants in the respective communities and health centres. For parents of eligible children, an informed consent form was administered and signed. A household questionnaire, which included questions on socio-demographic and health information about the child and family, was then administered to that parent who was the primary caregiver. At this time, parents were also provided with the information and materials needed to collect a stool specimen from the child. Parents were then given an appointment at the health centre, at which time they would deposit the stool specimen and the child's anthropometric measures and development would be ascertained. All forms and questionnaires were returned to the study offices at the end of each work day, and reviewed by the Project Director, the local Study Coordinator, and, when needed, by the local Principal Investigator, to confirm the eligibility of each child.

During the visit at the health centre, the quality of the stool specimen was first verified. If no specimen or an inadequate specimen (i.e. liquid specimen and/or insufficient quantity) was provided, then anthropometry was ascertained and a subsequent visit was scheduled to arrange for another stool specimen. If any child was discovered to be ill on the day of his or her health centre visit, the visit was postponed until the child had recovered. After verification of the quality and quantity of the stool specimen, the child was undressed and weighed (in duplicate) using a portable electronic scale (Seca 334, Seca Corp., Baltimore, MD, USA).

Length (i.e. the recommended measurement for height in children less than two years of age) was measured (in duplicate) as recumbent crown-heel length on a flat surface using a stadiometer (Seca 210, Seca Corp., Baltimore, MD, USA). Cognition, receptive and expressive communication (i.e. language) and fine motor development were assessed using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) (Pearson Education Inc, Texas, 2006). The latter instrument was translated into Spanish and adapted for local cultural appropriateness and validity. In place of the Bayley-III Gross Motor subtest, the WHO gross motor milestones (i.e. walking alone, standing alone, walking with assistance, hands and knees crawling, and standing with assistance) were assessed by observation [19]. Upon completion of all baseline outcome measurements and the provision of an adequate stool specimen, participants were enrolled into the deworming trial and randomly assigned to one of three intervention groups or the control group. The stool specimen was labeled with a unique number between 1 and 1760, corresponding to the randomly assigned treatment code for the deworming trial.

Stool specimens from all participants were transferred to the laboratory at the local research facility (Asociación Civil Selva Amazónica) to be read by one of two experienced laboratory technologists. Stool specimens from participants who were subsequently randomized to receive active deworming treatment were analyzed immediately by the Kato-Katz method, as recommended by WHO, (within 24 hours of initial collection, as a fresh specimen is required for this technique) to determine both prevalence and intensity of STH infection [20,21]. This procedure of immediately analyzing stool specimens only of those randomly allocated to the intervention groups receiving active deworming treatment takes into account the ethical imperative of treating those who would be found to have positive results. Stool specimens of those receiving inactive placebo tablets were stored in 10% formalin and examined by the direct method upon completion of the trial, at which time all participants received deworming treatment. To maintain blinding, each specimen code was replaced with a laboratory code by the local study coordinator for use by the laboratory technologists. Laboratory

technologists were provided with a list of those laboratory codes which would be analyzed and those which were to be stored. Each list was kept on a password-protected computer, one in the coordinator's office and one in the lab accessible only to the laboratory supervisor. A master list linking all information was stored at the research office in Canada (Research Institute of the McGill University Health Centre). Quality control was conducted on 10% of all Kato-Katz slides to ensure agreement in species identification and egg counts between laboratory technologists.

Statistical analyses

To classify child anthropometric measurements (i.e. length and weight) into categories of stunting, underweight and wasting, WHO Anthro software (Version 3, 2011) was used to calculate length-for-age z scores (LAZ), weight-for-age z scores (WAZ), and weight-for-length z scores (WLZ), respectively. Z scores are calculated taking into account a child's sex and age and are based on a comparison to a WHO international standard population. Moderate-to-severe categories of stunting, underweight and wasting are based on LAZ, WAZ and WLZ of <-2SD. Severe stunting, underweight and wasting are defined as LAZ, WAZ and WLZ of <-3SD, respectively [22].

Categories of STH infection intensity were determined from established WHO guidelines [23]. For *Ascaris* infection, light, moderate and heavy intensity are based on egg counts per gram of feces (epg) of 1-4999, 5000-49999 and 50000 and greater, respectively. For *Trichuris* infection, the categories for light, moderate and heavy intensity infection are an epg of 1-999, 1000-9999 and 10000 and greater, respectively. Light, moderate and heavy intensity hookworm infection are based on epgs of 1-1999, 2000-3999 and 4000 and greater, respectively. Both arithmetic and geometric mean epg were calculated and reported.

The development score was calculated as the mean crude score for each subtest of the Bayley-III, as well as a composite score of all four subtests combined. The range of possible scores was 0 to 91 for cognition, 0 to 49 for receptive communication, 0 to 48 for expressive communication and 0 to 66 for fine motor skills. Scaled scores were also calculated for descriptive purposes. These were derived from scaling the total raw score in each individual subtest to a metric between 1 and 19 according to the subtest and age of the child in months and days [24,25]. The WHO gross motor milestone of walking alone was categorized as yes if the child was able to walk without any assistance or support, regardless of the other milestones achieved, and no if the child could not walk without assistance, but achieved at least one of the other gross motor milestones (i.e. standing alone, walking with assistance, hands and knees crawling, and/or standing with assistance).

Principal Component Analysis was used to create an asset-based index for socioeconomic status (SES) to be included in multivariable analyses (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Variables included in the index were house material, type of cooking fuel, television ownership, radio ownership and electricity in the home [26,27]. The socioeconomic status index explained 40.1% of the variance and was divided into quartiles for subsequent analyses.

All associations with the outcomes of stunting and underweight were examined initially in univariable analyses. Variables that were significant at the $p < 0.20$ level, or that were deemed to be important from previous published research, were included in multivariable modelling to determine the most parsimonious model. If variables were highly correlated, the most informative variable (i.e. with more variation, more accurate measurements and/or important factors in previous literature) was chosen to be included in multivariable model building. Multivariable associations with stunting and underweight were examined using a generalized linear model with a log link, a Poisson distribution, and a robust

variance estimator to estimate the risk ratio for the dichotomous outcomes of moderate-to-severe stunting and moderate-to-severe underweight, where no and mild categories of stunting, and no and mild categories of underweight, respectively, comprised the reference groups [28,29]. Analyses were first restricted to children whose stool specimens were examined by the WHO-recommended Kato-Katz method [21]. Analyses were then performed including all children in the study population. All statistical analyses were performed using the Statistical Analysis Systems statistical software package version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Baseline characteristics of the study population

Between September 2011 and June 2012, parents of 2297 children 12 to 13 months of age were approached to participate in the study in order to meet the sample size requirements of 1760 eligible children. Three-hundred and eighty-five children did not meet the inclusion criteria, 126 children declined to participate, and 26 children were recruited but the sample size was reached before they were enrolled in the study. Anthropometric measurements and stool specimens were obtained from all 1760 enrolled children. All children were also administered the Bayley-III. Most children (90.1%) were 12 months of age, and 52.3% were male (Table 1). Previous attendance at CRED was not a requirement for participation in the study; however, less than 4% of children had no previous CRED attendance (n=62). The average number of CRED visits before enrolment in the study (i.e. from birth to 11 months, inclusive) was 7.6 (± 3.5). Only 25.5% (n=447) had all vaccinations up-to-date according to Peruvian Ministry of Health guidelines (i.e. one dose of Bacille Calmette-Guérin (BCG), one dose of hepatitis B, three doses of polio, three doses of pentavalent, two doses of rotavirus, three doses of pneumococcal and one dose of measles, mumps and rubella (MMR) vaccines) [18]; however, as MMR vaccine and the third dose of pneumococcal vaccine are scheduled at the 12-month CRED visit, many children had not yet received these

latter vaccinations. Including only vaccinations scheduled prior to 12 months, coverage of up-to-date vaccinations reached 80.3% (n=1410). In terms of family and household characteristics, 80.9% of mothers were married or in a common-law relationship and the average maternal age was 26.5 (± 7.1) years. The majority of families (88.6%) lived in peri-urban or rural areas, and only 51.0% had potable water access in their home. The average number of people living in the household was 6.6 (± 2.7). Sixty-nine percent of children had one or more siblings. The majority of children (89.5%) were still being breastfed, and roughly half (50.1%) had received liquids (other than water and water-based drinks) or food before the age of six months. Baseline socio-demographic and epidemiological characteristics were similar in the 880 children whose stool specimens were examined by the Kato-Katz method compared to the entire study population of children (n=1760) (results not shown).

Study population profile of malnutrition, STH infection and development

Twenty-five percent of the study population suffered from one or more forms of malnutrition. Prevalence of moderate-to-severe underweight, stunting and wasting were 8.6%, 24.2% and 2.3%, respectively (Table 2). Co-morbidity with two or three concurrent forms of malnutrition was present in 8.3% (n=146) of participants. Mean z scores were below the average (i.e. below 0) for all three indices. Severe malnutrition (i.e. a z score of < -3 SD for length-for-age, weight-for-age or weight-for-length) affected 5.5% (n=96) of the population.

The overall prevalence of any STH infection in children whose stool specimens were analyzed by the Kato-Katz method was 14.5% (n=219) (Table 3). The prevalence of infection was 11.5% for *Ascaris* (n=101), 4.5% for *Trichuris* (n=40) and 0.6% for hookworm (n=5). Eighteen children (2.1%) were infected with two STH species, but none with all three. For those who had their stool specimens stored and analyzed by the direct method, the prevalence was lower for all three STH species (i.e. 9.5%, 0.9% and 0.1% for *Ascaris, Trichuris,* and hookworm, respectively). Using the Kato-Katz method as the gold standard, and assuming equal STH prevalence in the two groups due to randomization, the direct method,

therefore, underestimated *Ascaris* infection by 17.4%, *Trichuris* infection by 80.0%, hookworm infection by 83.3%, and any STH prevalence by 29.0%. For the 880 children whose stool specimens were examined using the Kato-Katz method and who were found to be STH positive, most were found to have low intensity infection, with 86.1%, 92.5% and 100% harbouring light infections of *Ascaris*, *Trichuris* and hookworm, respectively (Table 4). There were no cases of heavy intensity infection of any STH species.

In terms of developmental functioning in all 1760 children, the mean composite development score on the Bayley-III was 98.1 (± SD 6.0) with a range between 73 and 123 points. On individual subtests, the mean score was 42.5 (± 3.0) for cognition, 12.9 (± 1.6) for receptive communication, 13.5 (± 2.1) for expressive communication and 29.2 (± 1.5) for fine motor skills. This translated to a mean scaled score of 9.9 (± 1.84), 7.2 (± 1.9), 8.1 (± 1.7) and 9.2 (± 1.5) for the cognitive, receptive language, expressive language and fine motor subtests, respectively. The mean scores were slightly higher for 13-month old children compared to 12-month old children (i.e. 43.2 vs. 42.5 for cognition, 13.3 vs. 12.9 for receptive communication, 13.8 vs. 13.4 for expressive communication, and 29.4 vs. 29.2 for fine motor skills, respectively). Twenty-three percent and 35.6% of 12 and 13-month old children, respectively, were able to walk without support.

Risk factors for stunting and underweight

In determining the risk factors for malnutrition in the group of children whose specimens were analyzed by the Kato-Katz method, stunting was found to be statistically significantly associated with the presence of any STH infection, male sex, older age (i.e. 13 months old), one or more hospitalizations since birth, lower SES, and lower birth weight in both unadjusted and adjusted analysis (Table 5). The crude score of each individual Bayley-III subtest was significantly associated with stunting in univariable analyses. The overall composite development score

was included in the multivariable model, with a lower score associated with an increased risk of stunting in the adjusted model (aRR 0.97; 95% CI: 0.95, 0.99).

Risk factors for underweight in unadjusted and adjusted analyses included lower birth weight, lower development score, and lower SES (Table 5). Continued breastfeeding at one year of age was associated with a decreased risk of underweight in unadjusted and adjusted analyses. No statistically significant association was found between underweight and any STH infection in either unadjusted or adjusted analyses.

No independent associations were found between malnutrition and up-to-date vaccinations, vitamin A supplementation, walking alone, maternal employment outside of the home, place of residence, place of delivery or antenatal care attendance (Table 5). The timing of introduction of liquids and foods was not associated with stunting or underweight in either unadjusted or adjusted analyses. STH infection was not associated with wasting in either unadjusted or adjusted analyses (results not shown).

Multivariable results for stunting, underweight and wasting were similar when analyses were extended to include participants with specimens analyzed by both the Kato-Katz and the direct method (results not shown).

DISCUSSION

This baseline assessment in 1760 preschool-age children aged 12 and 13 months in a community of extreme poverty in the Peruvian Amazon demonstrates an important association between malnutrition and child infection and developmental deficits. Previous studies in the area of Belen have found similar associations between malnutrition and STH infection in a wider age range of preschool-age children [8,30]. In contrast to previous studies, however, this association was apparent even with low intensity STH infection [8]. The current study updates previous estimates and provides in-depth data for that critical time period around

one year of age when interventions are likely to be considered to be integrated into vaccination programs or well baby clinics. Consistent with previous studies, lower socioeconomic status and older child age were associated with an increased risk of malnutrition [8,30,31]. Nonetheless, the latter result is somewhat unexpected, as the age range was quite restricted in the present study. This finding, along with a greater number of children who were walking alone at 13 months of age, support the concept of a critical window in which children are rapidly developing and growing before two years of age [5]. This has the potential to translate to an even greater impact of parasite infection and nutritional deficits on child health in this time period.

An interesting finding in this study was that STH and malnutrition prevalence were lower compared to previous work in the area [8]. The current study was embedded within the existing health infrastructure of routine growth and development clinic visits. Although previous attendance was not an inclusion criterion, there may have been higher-risk populations with low CRED attendance that would not have been easily reached, but who may have been included in the previous community-based surveys. We attempted to solve this problem by conducting a community census prior to enrolment to identify all children in the eligible jurisdictions, not only those who had had the opportunity to access health services previously. An increase in research attention and community-based health and nutrition campaigns may also explain some of the improvements. In particular, deworming campaigns directed towards school-age children, may have contributed to a reduction in overall environmental contamination in the area. This could have resulted in lower infection rates in younger children not directly targeted by campaigns, as has been shown in other settings [32]. A recent study also demonstrated a decrease in the prevalence of stunting in preschool-age children in Peru from 1991 to 2011, possibly due to economic growth and an increased emphasis on pro-poor social programs [33]. However, overall prevalence of stunting has remained unacceptably high, with children between 12 and 23 months, those living in the Amazon or Andean region and those of lower SES suffering disproportionately from malnutrition [33]. Prevalence of stunting

was also higher in males compared to females under the age of 36 months, which is consistent with our findings. Despite the positive trends in a reduction in stunting and STH infection in this and other studies, the current results demonstrate that even low STH prevalence and intensity of infection can be associated with poor growth in children in this vulnerable age group.

This study benefits from a large sample size of children, representative of the wider population of children living in the STH high-risk flooding areas of Iquitos. This representativity was helped in part by the community-wide census and by the inclusion of health centres from a wide catchment area. Nevertheless, hard-to-reach and hidden populations of children suffering from severe malnutrition or other chronic illnesses may be under-represented in the study. An additional strength of the study is the focus on children of a narrow age range in the critical growth window. Other studies have included populations of children at heterogeneous growth and development stages and have been unable to disaggregate differences by age. In-depth nutritional information was also collected to ensure that the impact of feeding behaviours was taken into account in all analyses. The ascertainment of when liquids and foods were first introduced and the age of weaning may have been limited by recall bias; however, the collection of information on the age of introduction of specific local foods (e.g. animal products, vegetables, fruits, purées, non-human milk, water and water-based liquids, etc.), and a 24-hour recall were used to increase validity of the responses. This study also incorporated comprehensive developmental testing. To our knowledge, this is the first study that has incorporated the Bayley-III, one of the most rigorous development tests available for preschool-age children, in conjunction with STH infection.

The study was limited by the fact that, for ethical reasons, the Kato-Katz technique could only be used to analyze half of the specimens from randomly-allocated participants (i.e. those who were randomly assigned to receive active deworming treatment), and therefore intensity data were not available for all participants. The higher STH prevalence in specimens analyzed by the Kato-Katz

technique suggests that the direct method likely underestimated STH prevalence, due to lower sensitivity and specificity, and/or the storage of specimens. In those with intensity data, a low prevalence of moderate-to-heavy intensity infection restricted the ability to detect differences in malnutrition risk according to the intensity of STH infection. In addition, although the malnutrition-infection association is known to be cyclical in nature [1,3], the direction of the associations between various risk factors and malnutrition cannot be established due to the cross-sectional nature of the baseline survey.

Overall, this study demonstrates an important association between stunting, low birth weight, SES, STH infection and cognitive, language and motor development in early preschool-age children in the most critical growth window. The results provide further evidence of the importance of determining the most cost-effective, integrated and multi-sectoral interventions to target this vulnerable age group, reduce health inequities, and prevent growth and development deficits in both the short and long-term.

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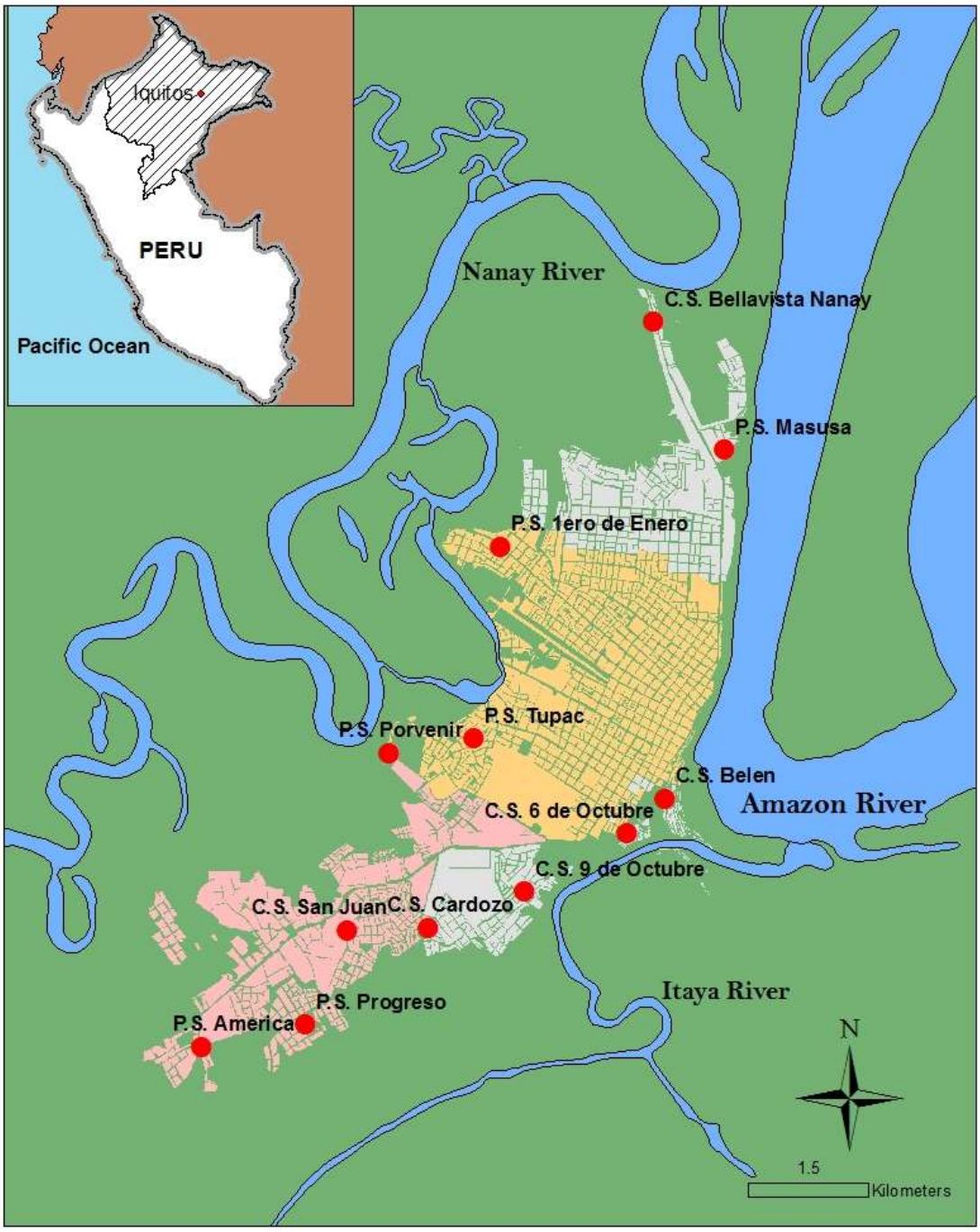

Figure 1. Map of the study area and location of the 12 participating health centres, Iquitos, Loreto, Peru. Enlarged area shows the city of Iquitos.

Table 1. Prevalence of stunting and underweight in 12 and 13-month old children, by child, maternal and household characteristics, Iquitos, Peru, September 2011 to June 2012 (n=1760).

	n	%	Prevalence of stunting (%)	Prevalence of underweight (%)
Child characteristics				
Age				
12 months	1586	90.1	23.1	8.6
13 months	174	9.9	33.9	8.6
Sex				
Male	920	52.3	28.8	10.2
Female	840	47.7	19.2	6.3
Birth weight*				
Low (< 2500 g)	122	7.7	38.5	15.6
Normal (≥ 2500 g)	1472	92.4	22.5	7.5
Continued breastfeeding at 1 year				
Yes	1575	89.5	23.6	8.3
No	185	10.5	29.7	10.5
Up-to-date vaccinations*				
Yes**	1410	80.3	23.3	7.7
No	347	19.8	27.7	12.4
Received vitamin A in previous year				
Yes	921	52.3	21.9	6.7
No	839	47.7	26.7	10.7
Any hospitalizations since birth				
Yes	163	9.3	35.6	8.6
No	1597	90.7	23.0	8.6

Cognitive development scaled score†				
0-9	677	38.5	31.8	14.0
10-19	1083	61.5	19.5	5.3
Receptive communication scaled score†				
0-9	1533	87.1	25.1	9.7
10-19	227	12.9	18.5	1.8
Expressive communication scaled score†				
0-9	1494	84.9	25.9	9.5
10-19	266	15.1	14.7	3.8
Fine motor skills scaled score†				
0-9	1050	59.7	29.0	10.7
10-19	710	40.3	17.2	5.6
Walking alone				
Yes	433	24.6	14.3	3.5
No	1324	75.4	27.4	10.3
Maternal characteristics				
Marital status				
Married/common-law	1423	80.9	24.2	8.9
Single	337	19.2	24.0	7.7
Highest level of education*				
Secondary incomplete	1205	68.5	27.6	10.1
Secondary complete	554	31.5	16.8	5.4
Employment outside of home				
Yes	179	10.2	31.3	6.2
No	1581	89.8	23.4	8.9

Place of delivery				
Health establishment	1591	90.4	24.1	8.1
Home	169	9.6	24.9	13.6
Attended any antenatal care				
Yes	1658	94.2	24.0	8.1
No	102	5.8	27.5	17.7
Household characteristics				
Socioeconomic status (SES)				
First quartile (i.e. lowest)	423	24.0	28.1	11.6
Second quartile	458	26.0	25.1	10.9
Third quartile	419	23.8	27.5	7.9
Fourth quartile (i.e. highest)	460	26.1	16.7	4.4
District				
Urban	200	11.4	21.5	6.0
Peri-urban/Rural	1560	88.6	24.6	9.0

* Totals do not sum to 1760 due to missing responses on birth weight (n=166 missing), vaccinations (n=3 missing) and maternal education (n=1 missing)

**Up-to-date vaccinations include those scheduled between birth and 11 months of age (i.e. 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)

*** STH=soil-transmitted helminth. See Table 3 for species-specific prevalences and intensities

† Scaled development scores are derived from the total raw score in each individual subtest, scaled between 1 and 19 (with a mean of 10) according to the subtest and the age of the child in months and days [24]

Table 2. Nutritional indicators in 12 and 13-month old children, Iquitos, Peru, September 2011 to June 2012 (n=1760).

Nutritional Indicator	Mean (SD*) or Frequency (%)	
Weight (kg) and derived indices**		
Weight [mean (SD)]	8.72	(0.98)
Weight-for-age Z score [mean (SD)]	-0.73	(0.96)
Moderate-to-severe underweight [# (%)]	152	(8.6)
Severe underweight [# (%)]	25	(1.4)
Length (cm) and derived indices**		
Length [mean (SD)]	72.13	(2.44)
Length-for-age Z score [mean (SD)]	-1.36	(0.96)
Moderate-to-severe stunting [# (%)]	426	(24.2)
Severe stunting [# (%)]	86	(4.9)
Weight (kg)/length (cm) and derived indices**		
Mean weight-for-length Z score	-0.10	(0.92)
Moderate-to-severe wasting [# (%)]	40	(2.3)
Severe wasting [# (%)]	3	(0.2)

*SD=standard deviation

** Using WHO international growth standards [12]

Table 3. Soil-transmitted helminth (STH) prevalence in 12 and 13-month old children, Iquitos, Peru, September 2011 to June 2012, by stool examination method.

		Prevalence			
		Kato-Katz*[1] (n=880)		Direct method*[2] (n=880)	
		# (%)		# (%)	
Ascaris	Infected	101	(11.5)	84	(9.5)
	Not infected	779	(88.5)	796	(90.5)
Trichuris	Infected	40	(4.5)	8	(0.9)
	Not infected	840	(95.5)	872	(99.1)
Hookworm	Infected	5	(0.6)	1	(0.1)
	Not infected	875	(99.4)	879	(99.9)
Any STH	Infected	128	(14.5)	91	(10.3)
	Not infected	752	(85.5)	789	(89.7)

*[1] The Kato-Katz method was used to analyze fresh stool specimens of those receiving deworming at baseline. *[2] The direct method was used to analyze stored stool specimens of those receiving placebo at baseline (i.e. average of 17 months later).

Table 4. Soil-transmitted helminth (STH) intensity in 12 and 13-month old children, Iquitos, Peru, September 2011 to June 2012, using the Kato-Katz method (n=880).

	Intensity*						Mean epg**			
	Light		Moderate		Heavy		AM***[1] (95% CI)		GM***[2] (95% CI)	
Ascaris	87	(86.1)	14	(13.9)	0	(0)	288.3	(195.9, 380.8)	2.2	(1.9, 2.6)
Trichuris	37	(92.5)	3	(7.5)	0	(0)	18.1	(5.5, 30.6)	1.3	(1.2, 1.3)
Hookworm	5	(100.0)	0	(0)	0	(0)	2.0	(0.8, 3.3)	1.0	(1.0, 1.1)

* Intensity data available only for those receiving deworming at baseline (i.e. n=880 specimens analyzed by the Kato-Katz method).

**epg=eggs per gram. The calculation of mean epg includes infected and uninfected individuals.

***[1] AM=arithmetic mean; ***[2] GM=geometric mean. A value of 1 was added to each observation to calculate the geometric mean.

Table 5. Risk factors for stunting and underweight in 12 and 13-month old children in Iquitos, Peru, September 2011 to June 2012 (n=796*).

Child, maternal and household characteristics	Stunting**		Underweight**	
	Crude RR (95% CI)	Adjusted RR‡[1] (95% CI)	Crude RR (95% CI)	Adjusted RR‡[2] (95% CI)
Any STH infection (yes vs. no)	1.32 (0.99, 1.76)	1.37 (1.01, 1.86)	1.24 (0.73, 2.10)	1.15 (0.65, 2.03)
Sex (male vs. female)	1.36 (1.07, 1.71)	1.35 (1.07, 1.72)	1.52 (1.00, 2.31)	NS‡
Age (13 vs. 12 months)	1.45 (1.06, 2.00)	1.52 (1.09, 2.12)	0.47 (0.18, 1.26)	NS
Birth weight (per kg increase)	0.44 (0.36, 0.55)	0.48 (0.38, 0.59)	0.38 (0.26, 0.54)	0.43 (0.30, 0.63)
Continued breastfeeding at 12 months (no vs. yes)	1.32 (0.96, 1.81)	NS	1.51 (0.87, 2.61)	1.73 (1.00, 2.98)
Vaccinations up-to-date† (no vs. yes)	1.27 (0.98, 1.64)	NS	1.67 (1.08, 2.59)	NS
Vitamin A supplementation in previous year (no vs. yes)	1.27 (1.01, 1.59)	NS	1.87 (1.24, 2.84)	NS
Any hospitalization since birth (yes vs. no)	1.77 (1.32, 2.37)	1.54 (1.12, 2.11)	NS	NS
Mean development score § (per 1 point increase)	0.96 (0.94, 0.97)	0.97 (0.95, 0.99)	0.91 (0.89, 0.94)	0.92 (0.89, 0.95)
Walking alone (yes vs. no)	0.63 (0.46, 0.85)	NS	0.46 (0.26, 0.84)	NS
First vs. fourth SES‖ quartile	2.01 (1.38, 2.92)	1.62 (1.11, 2.35)	3.89 (1.83, 8.28)	2.85 (1.28, 6.32)
Second vs. fourth SES quartile	1.75 (1.19, 2.56)	1.60 (1.10, 2.32)	3.30 (1.53, 7.10)	2.58 (1.18, 5.65)
Third vs. fourth SES quartile	2.01 (1.38, 2.92)	1.59 (1.10, 2.30)	2.55 (1.15, 5.66)	2.17 (0.99, 4.78)
Maternal employment outside of home (no vs. yes)	1.24 (0.90, 1.71)	NS	NS	NS
Place of residence (periurban/rural vs. urban)	NS	NS	2.31 (0.96, 5.58)	NS
Place of delivery (home vs. hospital)	NS	NS	1.87 (1.10, 3.16)	NS
Antenatal care attendance (no vs. yes)	NS	NS	2.07 (1.11, 3.87)	NS

*The analysis was restricted to the 880 children whose stool specimens were analyzed by the Kato-Katz method. The adjusted models include a sample size of 796 due to 84 missing responses on birth weight

**Reference group includes mild or no stunting (i.e. LAZ ≥ -2 SD) and mild or no underweight (i.e. WAZ ≥ -2 SD)

ǂRR = risk ratio: [1]RR for stunting adjusted for any STH infection, sex, age, birth weight, any hospitalizations since birth, development score and socioeconomic status; [2]RR for underweight adjusted for any STH infection, birth weight, continued breastfeeding, development score and socioeconomic status.

‡NS = not statistically significant (significance level of $p<0.20$ in crude analysis, and $p<0.05$ in adjusted analysis)

† Up-to-date vaccinations include those scheduled between birth and 11 months of age (i.e. 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 vaccines)

§Mean development score is the combined sum of the raw scores of each individual subtest of the Bayley-III

‖SES = socioeconomic status (lowest quartile = lowest SES; highest quartile = highest SES)

PREFACE TO MANUSCRIPT B

For the randomized controlled trial of deworming in early preschool-age children in Iquitos, Peru, children were first enrolled at their routine 12-month growth and development (CRED) visit in participating health centres. All children were followed up 6 and 12 months later (at their 18 and 24-month CRED visits, respectively). At all visits, a questionnaire was administered to collect socio-demo-epi information, anthropometric measurements were taken (i.e. length and weight), and a stool specimen was collected to be analyzed for the presence of soil-transmitted helminth infections. At the 12 and 24-month CRED visits, the Bayley Scales of Infant and Toddler Development (Third Edition) was applied to assess cognitive, language and motor development. All children received deworming treatment at the 24-month CRED visit.

The following manuscript (Manuscript B) comprises the second results chapter of the thesis. It is a description of the trial's design and methodology, baseline characteristics of the study population by intervention group, and results of the effect of the deworming interventions on the primary outcome of weight gain and on secondary growth outcomes. The previous manuscript (Manuscript A) provided details on enrolment procedures and the association between characteristics of the study population, including STH infection and malnutrition, at baseline. A separate manuscript (Manuscript C) is devoted to the secondary outcome of cognitive, language and motor development. Supplementary analyses can be found in Appendix 10.

Manuscript B has been submitted to The Lancet Infectious Diseases and is currently under review. It conforms to the Consort 2010 guidelines for reporting parallel group randomized trials. SAJ gave an invited presentation of the trial protocol at the "McGill-PAHO Workshop on integrating deworming intervention into preschool health packages in the Americas" in Washington DC, USA, March 2011. In addition, selected results from this manuscript have also been presented, including 1) ethical issues related to deworming trials in preschool-age children

(presented at the 3rd North American Congress of Epidemiology, Montréal, Canada, June 2011); 2) a review of previous deworming trials and identification of research gaps in 12- to 24-month old children (presented at the European Congress of Tropical Medicine and International Health, Barcelona, Spain, October 2011); and 3) main trial results on the effect of the deworming intervention on growth (presented at the International Congress of Parasitology, Mexico City, Mexico, August 2014). An abstract on the main trial results has also been accepted for oral presentation at the American Society of Tropical Medicine and Hygiene Meeting in New Orleans, LA, USA in November 2014 (see Appendix 9 for copies of abstracts).

A sub-study on the efficacy of mebendazole, using the new WHO guidelines, was conducted from June to August 2013. A sample of 89 STH-positive children at the 24-month visit provided an additional stool specimen three weeks after treatment. Egg reduction rates for *Ascaris*, *Trichuris* and hookworm were 100%, 74.4% and 71.1%, respectively, meeting the WHO cut-offs for satisfactory drug efficacy (WHO 2013). Preliminary results were presented at the American Society of Tropical Medicine and Hygiene Meeting (Washington DC, USA, November 2013).

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6 MANUSCRIPT B

The effect of deworming timing and frequency on growth in one-year-old children living in an STH-endemic area of Peru: a randomized controlled trial

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ABSTRACT

Background: Appropriate health and nutrition interventions to prevent long-term adverse effects in children are necessary before two years of age. One such intervention may include deworming, recommended as of 12 months of age by the World Health Organization in soil-transmitted helminth (STH)-endemic areas; however, the benefit of deworming has been understudied in early preschool-age children and coverage remains suboptimal.

Methods: A randomized, double-blind, placebo-controlled trial was conducted to determine the benefit, and optimal timing and frequency, of deworming (mebendazole) on growth in 12-24 month old children in Iquitos, Peru. Children were enrolled during their routine 12-month growth and development clinic visit, and followed up at their 18 and 24-month visits. Children were randomly allocated to: Group 1: deworming at the 12-month visit and placebo at the 18-month visit; Group 2: placebo at the 12-month visit and deworming at the 18-month visit; Group 3: deworming at both 12 and 18-month visits; or Group 4: placebo at both 12 and 18-month visits (i.e. control group). Differences in the primary outcome of weight gain 12 months post-deworming were analyzed using an intention-to-treat approach. The trial is registered with ClinicalTrials.gov (NCT01314937).

Findings: A total of 1760 children (i.e. 440 children per group) were enrolled between September 2011 and June 2012. Follow-up at the 24-month visit was completed by July 2013, with final attendance of 88.8% of children (n=1563). STH prevalence rose from 14.5% at baseline to 42.6% at the 24-month visit. Respective weight gains (mean kg (95% CI)) between 12 and 24 months were: Group 1: 2.05 (1.98, 2.13); Group 2: 1.94 (1.85, 2.02); Group 3: 2.04 (1.97, 2.11); and Group 4: 2.00 (1.93, 2.06). There was no statistically significant difference in weight gain in any of the deworming intervention groups compared to the control group. There was, however, a statistically significant improvement in weight gain in those receiving deworming once at the 12-month visit compared to those

receiving deworming once at the 18-month visit (unadjusted difference in kg (95% CI): 0.12 (0.01, 0.23)). Deworming at both time points was not associated with a significant improvement in weight gain over and above the once-yearly deworming at the 12-month visit.

Interpretation: Overall, there was no statistically significant benefit of deworming on growth in this population of preschool-age children. However, the results do indicate that, for children between 12 and 24 months of age, once-yearly deworming at 12 months of age provides the greatest growth benefits compared to later or more frequent deworming. A greater benefit may be apparent in areas of higher prevalence or intensity of infection. These results contribute to WHO policy and recommendations on deworming targeting preschool-age children in the over 100 STH-endemic areas of the world. They also contribute to providing practical guidance to governments in integrating deworming into early childhood health care.

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INTRODUCTION

The soil-transmitted helminth (STH) disease cluster includes *ascariasis*, *trichuriasis* and hookworm disease. It is considered to be one of the most common Neglected Tropical Diseases (NTD), affecting an estimated 1.45 billion people worldwide.[1] STHs are transmitted in contaminated food, water and the environment in areas of poverty in low- and middle-income countries. These intestinal parasites have a direct and indirect adverse impact on nutritional status by disrupting normal nutrient intake, excretion and utilization in their hosts and by causing blood loss and loss of appetite.[2, 3]

WHO recommends large-scale preventive chemotherapy programs, using anthelminthic treatment (i.e. deworming), for the high-risk groups of women of reproductive age, especially pregnant women, school-age children (i.e. 5 to 14 years of age), and preschool-age children (i.e. 1 to 4 years of age) in STH-endemic areas.[4] Adverse effects from deworming are infrequent, and when reported, are mild and transitory, including gastrointestinal upset and diarrhea.[5] Deworming interventions are often school-based in order to reach both enrolled and non-enrolled children. In preschool-age children, deworming is often piggybacked onto vaccination or supplementation programs, child health days, or programs for the elimination of lymphatic filariasis.[6] However, preschool-age children lag behind their school-age counterparts as scaling-up of school-based programs continues while that of preschool programs remains a challenge.[6] The global proportion of at-risk preschool-age children receiving deworming in 2012 was estimated to be on the order of 25%.[6] This coverage has decreased since previous reports.[7]

Prior to 2002, children under two years of age had been excluded from deworming interventions as the burden of STH infection was perceived to be low in this age group and the safety profile of available anthelminthics was not well established. In 2002, WHO convened an informal consultation of experts, and subsequently recommended the inclusion of children between 12 and 24 months

of age in deworming activities using single-dose albendazole (in a reduced dose of 200 mg) or mebendazole (in the usual dose of 500 mg).[8] These recommendations were based on animal studies, toxicity data and other safety data.[9] Despite the WHO recommendations and increasing evidence of the occurrence of STH infection in early preschool-age children,[9-14] many countries still exclude children under 24 months of age from their national deworming programs. Providing evidence on the benefits of deworming in the younger age group between one and two years of age is essential. Adverse effects of STH infection may be more pronounced in children during this critical time period and may extend well beyond childhood. A study reviewing data from 54 countries confirmed that preventive interventions must occur during the first two years of life to prevent growth deficits, such as stunting and underweight.[15] Interventions at this time are essential to prevent both short- and longer-term adverse health effects.[16] The evidence-base on including deworming as one of the essential early childhood interventions in this critical window is, however, limited. Randomized controlled trials conducted exclusively in school-age children or in both preschool-age and school-age children have provided mixed evidence on deworming benefits on growth and development.[5, 17, 18] Few studies have focused exclusively on the preschool-age population.[11, 19, 20]

Considering the unique nutritional demands and growth patterns of younger children, aggregated results from older children do not provide a clear indication of the potential benefit of deworming on growth and nutrition in younger age groups. To fill this research gap, we therefore conducted a randomized controlled trial on the benefit, and optimal timing and frequency, of a deworming intervention incorporated into routine child health services at one year of age, to improve growth by two years of age.

METHODS

Ethics approval and trial monitoring

This study received ethics approval in Peru from the Comité Institucional de Ética of the Universidad Peruana Cayetano Heredia and the Instituto Nacional de Salud, in Lima, and the local Ministry of Health office (Dirección Regional de Salud (DIRESA) Loreto) in Iquitos. Ethics approval was obtained in Canada from the Research Ethics Board of the Research Institute of the McGill University Health Centre in Montréal, Québec. An independent Data Safety and Monitoring Committee (DSMC) was established with three members, from Canada, the U.S., and Peru, to review all adverse events and approve continuation of the trial at three time points.

Study design and enrolment procedures

We conducted a randomized, double-blind, placebo-controlled trial of a deworming intervention incorporated into routine growth and development ('Crecimiento y Desarrollo" or CRED) visits in Iquitos, an STH-endemic area of the Peruvian Amazon. Details on baseline enrolment methodology and the study population have been described elsewhere.[13] Briefly, children were enrolled into the trial in their homes or participating health centres. Inclusion criteria were: 1) children attending any one of the 12 participating health centres for their 12-month CRED visit; and 2) children living in Belén, Iquitos, Punchana or San Juan districts. Exclusion criteria were: 1) children attending the health centre for suspected STH infection; 2) children who had received deworming treatment in the six months prior to the trial; 3) children whose families planned to move outside of the study area within the next 12 months; 4) children under 12 months of age or 14 months of age or older; and 5) children with any serious congenital or chronic medical condition.

Eligibility was assessed, and an informed consent form was signed by both parents (or guardian(s)) of the child. A baseline socio-demo-epi questionnaire was administered in the home or health centre to the primary caregiver of the child, and baseline outcome measurements, including weight, length and the provision

of a stool specimen, were ascertained in a subsequent visit in the health centre. All procedures were performed by dedicated, trained research assistants.

Intervention groups

Following confirmation of eligibility, informed consent and all baseline outcome assessments in the health centres, children were randomized into one of four intervention groups:

Group 1 (MBD/PBO): Usual care and deworming at the 12-month CRED visit and usual care and placebo at the 18-month CRED visit.

Group 2 (PBO/MBD): Usual care and placebo at the 12-month CRED visit and usual care and deworming at the 18-month CRED visit.

Group 3 (MBD/MBD): Usual care and deworming at both the 12 and 18-month CRED visits.

Group 4 (PBO/PBO): Usual care and placebo at both the 12 and 18-month CRED visits.

Deworming consisted of a single-dose mebendazole tablet (500 mg) (manufactured by Janssen Pharmaceuticals Inc.; donated by INMED Peru). The placebo was identical to the deworming tablet in terms of size, colour and markings (manufactured and purchased from Laboratorios Hersil, Peru). Tablets were crushed and mixed with juice for ease of administration and safety.[21] The crushed tablet was administered by research assistants at the end of each visit after all outcome assessments had been completed. All children received deworming at the 24-month visit according to Peruvian Ministry of Health guidelines.[22] Children received usual care interventions and services from health centre personnel.[22] This included the administration of measles, mumps and rubella (MMR) vaccination at the 12-month visit, and diphtheria, pertussis and tetanus - (DPT) vaccine booster at the 18-month visit.

Sample size

Sample size calculations were based on detecting the smallest meaningful difference among intervention groups in mean weight gain over 12 months, and took into account potential effect dilution from treating infected and non-infected children. From pilot data, mean weight gain ± standard deviation between 12 and 24 months in untreated children was estimated to be 2.0 kg ± 0.8 kg.

In order to have 80% power to detect a minimum difference of 0.20 kg in mean weight gain among intervention groups, assuming a common standard deviation of 0.8, and using a one-way ANOVA which accounts for pair-wise multiple comparisons using the Tukey correction, the estimated sample size per group was 366 children. The required sample size was increased to 440 children per group (1760 in total), to take into account potential loss-to-follow-up of 20% after 12 months (based on attrition rates from previous studies in the area by the research team[23, 24]) (MC4G Software©, GP Brooks, Ohio University, 2008).

Randomization and masking

Computer-generated randomly ordered blocks of eight and twelve were used to randomly allocate children to each intervention group. Blocking ensured that the randomization sequence would not be predictable and that the number of children assigned to each group would be balanced. Research personnel not directly involved in the trial prepared small envelopes containing the randomly assigned intervention for each visit. These were numbered from 1 to 1760, with each number corresponding to one of the four intervention groups. Envelopes were stored in a temperature-regulated pharmacy at the research facility, and distributed by the Project Director (SAJ) or the local Study Coordinator (LP) in sequential order to research assistants until the sample size was achieved. All health centre and research personnel, and parents of participants were blinded to intervention status.

Follow-up visits

Children were followed-up at their 18 and 24-month visit in the health centre, at which time all outcome ascertainments were repeated. At the 18-month visit the second randomly assigned intervention was administered. Each visit was scheduled six months after the previous visit. In the case that a participant did not attend their 18-month visit, children remained eligible for the 24-month visit, which was scheduled 12 months after initial enrolment. If participants were not located prior to the day of their anticipated follow-up visit, or a scheduled date was missed, a minimum of four additional attempts were made to locate them. Participants remained eligible for their 18-month visit up to three months after their anticipated date of follow-up, and until trial completion for the 24-month visit. A monetary reimbursement was provided to cover travel costs for each visit.

Outcome measurements

Prior to commencing recruitment, in-depth practical training of the research assistants took place according to WHO guidelines[25, 26] to ensure accurate outcome assessment and standardization. Inter and intra-rater reliability of over 95% was achieved for weight and length assessments, which are considered acceptable levels for anthropometric measurements.[25, 27]

Methods used for outcome measurements are described elsewhere.[13] Briefly, weight was measured using a portable electronic scale, accurate to the nearest 0.01 kg (Seca 334, Seca Corp., Baltimore, MD, USA). Additional secondary measurements included length, and the prevalence and intensity of STH infections. Length (i.e. the recommended measurement of height in children less than two years of age) was measured in duplicate as recumbent crown-heel length on a flat surface using a stadiometer (Seca 210, Seca Corp., Baltimore, MD, USA), accurate to the nearest millimetre. Stool specimens were collected to assess STH infection prevalence and intensity. For ethical reasons, only specimens from children receiving deworming treatment were immediately examined by trained laboratory technologists at the local research facility using the Kato-Katz method for the presence and intensity of STH infection (e.g. *Ascaris*, *Trichuris* and

hookworm).[28] Specimens from those children receiving placebo at 12 months and/or 18 months were stored in 10% formalin and analyzed by the direct method upon trial completion. This approach ensured that children found to be infected were treated. The Kato-Katz method is the recommended technique for assessment of the prevalence and intensity of intestinal parasitic infection in fresh stool.[28] For a one-stool specimen, sensitivity and specificity are over 96% for *Ascaris* and over 91% for *Trichuris*.[29] There is lower sensitivity and specificity for hookworm; however, hookworm infection is generally uncommon in very young children. Lower sensitivity to detect STH infection from storage and later analysis of specimens by the direct method was also anticipated[13].

Information on minor and severe adverse events was obtained through passive reporting at follow-up visits or in between visits. Severe adverse events were based on WHO definitions [30] and included: 1) death; 2) life-threatening conditions; 3) in-patient hospitalization or prolongation of an existing hospitalization; 4) persistent or significant disability/incapacity; 5) cancer; or 6) overdose (accidental or intentional).[30] Minor adverse events included all reported illnesses that did not meet the definition of a serious adverse event. All adverse events were reported to ethics committees. Summary reports of adverse events were also provided to the DSMC.

Data collection activities during fieldwork were regularly supervised by the Project Director (SAJ) and local Project Coordinator (LP). The consistency of egg count assessments was evaluated among the laboratory technologists using standard quality control methods.[28] The laboratory supervisor read 10% of the slides of the laboratory technologists without prior knowledge of the result to ensure quality control.

Analyses

Weight-for-age z scores (WAZ) and length-for-age z scores (LAZ) were calculated using WHO Anthro software (Version 3, 2011). WHO categories were

used to classify STH intensity according to species-specific counts of eggs per gram of feces (epg).[31] Both arithmetic and geometric mean epg were calculated.

The primary outcome of the trial was mean weight gain (in kilograms (kg)) between the baseline 12-month visit and the 24-month follow-up visit (i.e. after 12 months). Mean weight gain (kg) was compared between the four intervention groups using unadjusted one-way ANOVA procedure. Additional analyses were conducted to examine differences between intervention groups in terms of derived weight indices (i.e. mean WAZ) and length and derived length indices (mean length gain and mean LAZ). Multivariable analyses were also conducted adjusting for age, sex, socioeconomic status (based on an asset-based proxy index)[32, 33] and continued breastfeeding at 12 months of age.

All analyses were first expressed using an intention-to-treat (ITT) approach such that participants were analyzed according to their assigned intervention group. Multiple imputation, using a Markov Chain Monte Carlo (MCMC) model with five imputations, was used for those who did not attend the 18-month and/or 24-month follow-up visits. Variables related to the outcome, and hypothesized to be related to missing the follow-up visit(s) were used to impute missing weight and length measurements. These included baseline weight, length, socioeconomic status, continued breastfeeding at 12 months, sex, and age. Analyses were repeated 1) using a complete case approach on all participants who had attended the final follow-up visit, 2) using a per-protocol approach including those participants who attended all three visits and who did not report having received deworming outside of the trial between baseline and the final follow-up visit and 3) restricted to STH-infected children. The benefit of deworming was determined by comparing growth outcomes between each intervention group and the control group.

To explore the effect of the timing of deworming (i.e. at the 12-month visit or at the 18-month visit), growth outcomes in Group 1 were compared to Group 2. To

explore the effect of the frequency of deworming (i.e. provided once or twice), growth outcomes in Group 1 and Group 2 were each compared to Group 3.

All statistical analyses were performed using the Statistical Analysis Systems statistical software package version 9.3 (SAS Institute, Cary, NC, USA).

Role of the funding source

The funding agencies had no role in study design, data collection, data analysis, data interpretation, manuscript writing, or the decision to submit the manuscript for publication. The corresponding author had full access to the data and final responsibility for the decision to submit the manuscript for publication.

RESULTS

Participant flow

Between September 2011 and June 2012, the parents of 2297 children were approached to participate in the trial. Five-hundred and thirty-seven children were excluded as they did not meet the inclusion criteria (n=385), declined to participate (n=126), or were approached but not enrolled once the sample size was reached (n=26). A total of 1760 children were randomized to the four groups (Figure 1). All children received the assigned intervention at baseline. A total of 1606 children (91.2%) attended their first follow-up at the 18-month visit. Due to parental refusal, three children did not receive their randomly allocated intervention. The average time between the baseline and first follow-up visit was 6.3 months (± 0.41) and between the first follow-up visit and the second follow-up visit was 6.3 months (± 0.47). The average time between the baseline and second follow-up visit was 12.6 months (± 0.7).

Compliance

A total of 1517 children (86.2%) attended all three visits. Of those who did not attend all three visits, 108 (6.1%) attended the first visit only, 89 children (5.1%) attended the first and second visits and 46 children (2.6%) attended the first and

last visits. The proportion of children reported to have received deworming outside of the trial was 25.7% in Group 1; 26.8% in Group 2; 26.3% in Group 3; and 30.3% in Group 4.

Characteristics of the study population

Baseline characteristics of the study population by intervention group are found in Table 1. Groups were similar in terms of baseline weight (kg) and length (cm), age (months), birth weight (kg) and length (cm), continued breastfeeding, up-to-date vaccinations and hospitalizations since birth. There were small differences in the proportion of girls in each group and vitamin A supplementation in the previous year. In terms of maternal and household characteristics, groups were similar in the proportion of mothers who were married or common-law, the level of maternal education, and access to potable water in the home. Small differences were found in maternal employment outside of the home and area of residence. Baseline characteristics were similar between children who attended the final follow-up visit and those who missed their final visit (results not shown).

At baseline, the prevalence of any STH infection was 14.5% in the two groups whose specimens were analyzed by the Kato-Katz method (Table 2). At the 18-month visit, any STH prevalence was 28.5%. At the 24-month visit, at which time all specimens were analyzed by the Kato-Katz method, the overall prevalence of any STH increased to 42.6%. Prevalence of *Ascaris*, *Trichuris* and any STH infection was moderately lower in the groups which received deworming at the 18-month visit. Hookworm infection remained negligible. As expected due to lower sensitivity, STH prevalence in children whose stool specimens were analyzed by the direct method at 12 and 18 months was moderately lower (i.e. 10.5% and 24.5%, respectively). Certain sensitivity analyses were therefore conducted in subgroups of children found to be STH-positive 1) by both the direct and Kato-Katz methods and 2) only by the Kato-Katz method. Despite potential misclassification of STH infection status in children whose specimens were analyzed by the direct method, this strategy allowed for maximum comparison among all groups. Infection was predominantly low intensity for *Trichuris* and

hookworm infection at all three time points; however, moderate and heavy intensity *Ascaris* infection increased over the one-year follow-up period (Table 2).

The prevalence of stunting and underweight increased from 24.2% and 8.6% at baseline to 46.8% and 10.2%, respectively, at the 24-month visit.

Overall benefit of deworming on primary and secondary anthropometric outcomes

The greatest changes in all growth outcomes between the 12- and 24-month visits were seen in Group 1 (Table 3). When comparing the outcomes in each of the deworming intervention groups to the control group, however, no statistically significant benefit was detected in unadjusted or adjusted ITT analysis (Table 2). No statistically significant difference in any intervention group compared to the control group was seen in complete case analysis, per-protocol analysis or in analysis restricted to only those children who were positive for STH infection at baseline (results not shown).

Effect of deworming timing on primary and secondary anthropometric outcomes

In examining the effect of the timing at which deworming was administered, a statistically significant improvement was seen in Group 1 compared to Group 2, in terms of weight gain, length gain, WAZ change, and LAZ change between baseline and the final follow-up visit in both unadjusted and adjusted analyses (Table 4). These results remained significant in complete case analysis and in per-protocol analyses. In analyses restricted to children positive for STH infection at baseline, no significant differences were observed between groups (results not shown).

Effect of deworming frequency on primary and secondary anthropometric outcomes

In comparing the difference in anthropometric outcomes between Group 1, receiving deworming once yearly, and Group 3, receiving deworming twice yearly, no additional benefit on weight or length was apparent for twice-yearly

deworming in unadjusted or adjusted analyses (Table 5). Results remained consistent in complete case analysis, per-protocol analyses, and in restricted analyses to children infected with STH at baseline (results not shown). A statistically significant benefit, however, was observed in Group 3 compared to Group 2, in terms of weight gain and WAZ change. These results remained significant for both weight gain and WAZ change when adjusting for baseline characteristics, in complete case and per-protocol analyses, and for WAZ change in unadjusted and adjusted analyses restricted to those infected at any time during the follow-up.

Adverse events

From baseline until the end of follow-up, 38 minor adverse events were reported and were similarly distributed among groups (i.e. Group 1: 7; Group 2: 10; Group 3: 12; and Group 4: 9). There were 18 serious adverse events reported, which included deaths and hospitalizations (i.e. Group 1: 7; Group 2: 1; Group 3: 5; and Group 4: 5). None of these serious adverse events were deemed to be related to the deworming intervention.

DISCUSSION

This is the largest double-blind, randomized, placebo-controlled trial of deworming to our knowledge that has been conducted exclusively in children during the second year of life. This is the age at which WHO first recommends mass deworming programs, and it is also a time of rapid growth, development and STH acquisition. We were not able to demonstrate an overall benefit of deworming on growth in any of the intervention groups compared to the control group after one year of follow-up in intention-to-treat analysis or in further sensitivity analyses. The short follow-up time, particularly in the group receiving deworming only at 18 months, may have limited the potential to detect a benefit. It is clear that this age-group has not yet reached a steady state of STH infection (e.g. as evidenced by the over threefold increase in STH prevalence from 12 to 24

months of age) or growth (e.g. as evidenced by a negative deviation of WAZ and LAZ compared to the international WHO growth standard over 12 months). Benefits of the deworming intervention may be apparent only with a longer follow-up time. We were able to demonstrate safety of the deworming intervention in this age group, similar to results from previous studies.[21, 34]

Our results are consistent with a recent cluster-randomized trial of albendazole (administered every six months to children from six months to six years of age) conducted in north India where light intensity STH infection was also predominant.[20] Our findings do, however, contradict other trials in preschool-age children that found a positive effect of deworming on growth indicators.[11, 19] The lack of benefit in our study compared to these other studies could be due to differing deworming schedules and drugs, compliance, follow-up times, age group of the study populations, prevalence and intensity of infection, outcome definitions, and trial designs.

This trial was unique in using a multiple group design to look additionally at differences in the timing and frequency among the groups that received deworming. Our results demonstrate that, if deworming is provided, there is a significant benefit of providing earlier deworming on growth in this study population. Our results also demonstrate that deworming just once at 12 months of age is sufficient to improve growth, with no added benefit from an additional dose provided at 18 months of age. These results were consistent in unadjusted and adjusted analysis, as well as in sensitivity analyses, for multiple growth indicators.

This finding is somewhat surprising, in light of the low STH prevalence at baseline, and the similarly high STH infection prevalence in Group 1 compared to the control group at the 24-month visit. It is clear that one-round of deworming at 12 months alone does not prevent STH re-infection over this short and physiologically-dynamic period of time. Complementary health, nutrition, educational and environmental interventions would likely be needed to reduce acquisition of new infection, prevent re-infection, and impact STH prevalence

over the long-term. Nevertheless, our results suggest that reducing even low prevalence and intensity of STH infection between 12 and 18 months of age can improve growth. These improvements are apparent even after STH infection prevalence has returned to the level expected in the absence of treatment. The results are consistent with the concept of the critical growth and development window before two years of age[15], and in particular, providing interventions as early as possible in this window. Such considerations of timing are important in operationalizing deworming interventions in this age group. As deworming is contraindicated before one-year of age, at-risk children should be dewormed as soon as possible after their first birthday. This is quite feasible by piggybacking deworming onto existing child health interventions, using health centre or community-based infrastructure; for example, through MMR vaccination, Vitamin A supplementation, or other interventions provided at 12 months of age.[6]

Strengths of this study include the randomized controlled design, which minimized confounding and the influence of external factors. We were also able to maintain a high follow-up rate, despite a highly mobile population and environmental challenges such as flooding which displaced many participants in the study area. The consistency of results from intention-to-treat, complete case and per-protocol analyses demonstrate that results from children attending the final visit are likely generalizable to the original study population.

Limitations of the study include difficulties with compliance as over 25% of children received deworming at least once outside of the assigned intervention group. The report of receiving deworming outside of the trial protocol was likely underestimated as it was by self-report at each follow-up visit (i.e. for the previous six months). The actual receipt of deworming outside of the trial differed among groups, with the highest non-compliance in the control group. This non-compliance would likely have reduced the effect size between treated and untreated groups. Although deworming in children under 24 months of age is not recommended by the Ministry of Health in Peru, deworming is readily available without a prescription in pharmacies and through community-based campaigns.

During the study period, there was an increase in mass deworming campaigns due to the unusual severity of flooding.

The study is also limited by the fact that, for ethical reasons, we did not immediately analyze specimens from children randomized to placebo at the 12 or 18-month visits. This meant that accurate STH prevalence and intensity were only available for those receiving mebendazole at the 12-month and/or the 18-month visit (i.e. Group 1 (MBD/PBO) and Group 3 (MBD/MBD) at 12 months, and Group 2 (PBO/MBD) and Group 3 (MBD/MBD) at 18 months). Although there may have been a greater benefit of deworming in STH-infected children, we do not have accurate STH infection status in groups that could have served as appropriate controls (i.e. STH-infected children in Groups 2 and 4 at baseline or Groups 1 and 4 at 18 months). Any subgroup analyses including children whose specimens were analysed by the direct method would have been affected by misclassification of infection status.

The baseline prevalence of STH infection in the study population is also lower than had been anticipated based on prior studies conducted in the area.[35] The number of children who could have potentially benefited from deworming in the trial was therefore reduced, resulting in a greater dilution of the effect size than had been anticipated.

Overall, this is the first trial to provide evidence on the effect of deworming, including optimal timing and frequency, on growth exclusively in children in the critical window in the second year of life. Our results are relevant to WHO deworming policy for children as of one year of age in the over 100 STH-endemic areas worldwide. This trial also demonstrates the feasibility of incorporating deworming into routine growth and development health clinics along with other essential early childhood interventions. Further studies, including cost-effectiveness of integrating deworming with other health, nutritional and environmental interventions, are needed in this age group. Continued observational follow-up of the trial cohort is currently taking place, and will

provide evidence of the longer-term benefits of deworming provided before two years of age on growth throughout the preschool ages.

CONTRIBUTORS

MC, MMG, TWG, SAJ, AM, GSM, ER and BJW designed the study. BB, TWG, SAJ and LP were involved in data collection activities. BB, TWG, SAJ, and ER were involved in data analysis. All authors were involved in data interpretation and manuscript writing or revision, and approved the final version for submission.

DECLARATION OF INTERESTS

We declare that we have no competing interests.

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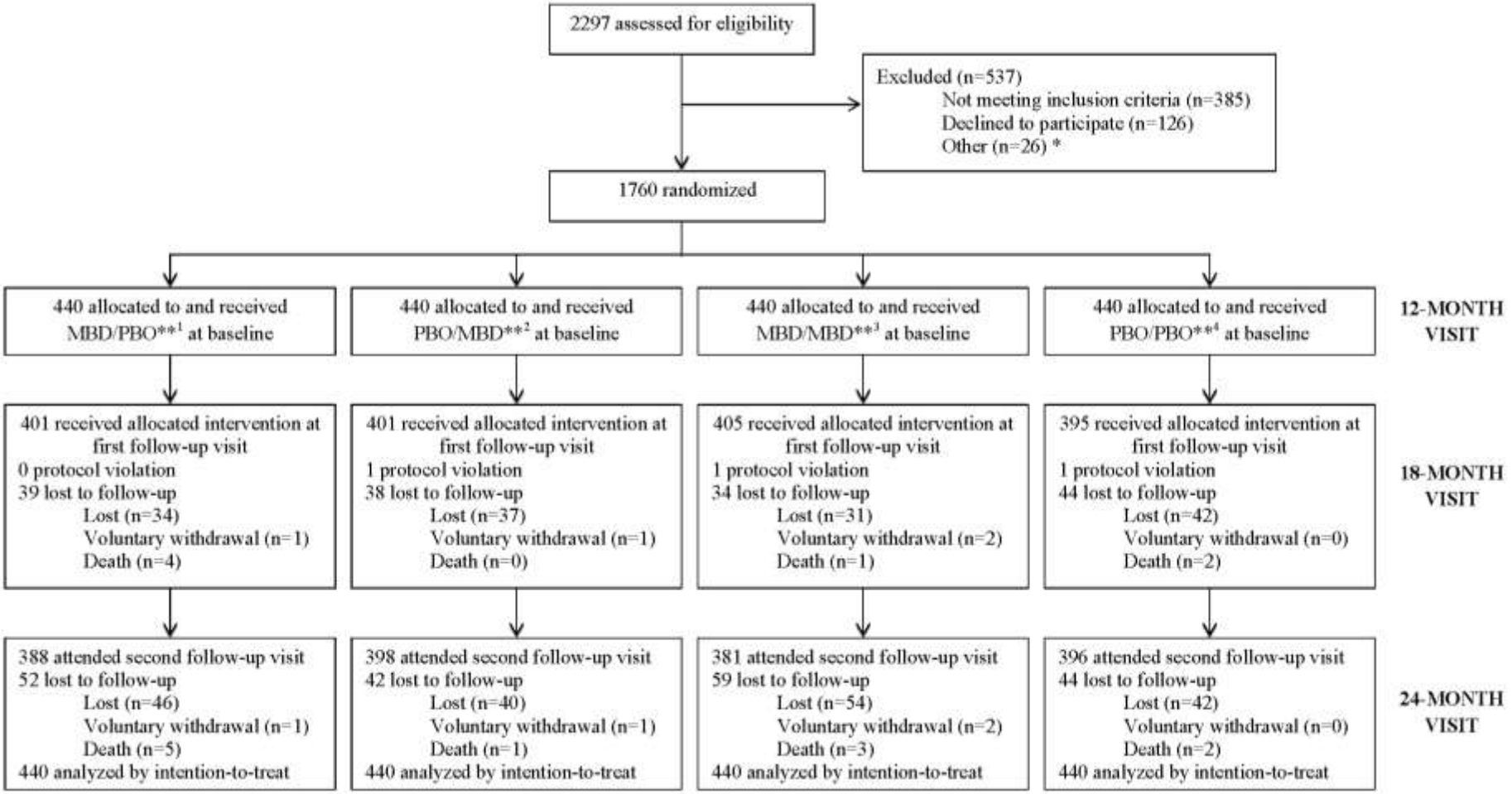

Figure 1. Trial profile

*26 participants were screened but were not enrolled once the sample size was met.

**[1] Group 1 (MBD/PBO)=mebendazole (12 months)/placebo (18 months); [2] Group 2 (PBO/MBD)=placebo (12 months)/mebendazole (18 months); [3] Group 3 (MBD/MBD)=mebendazole (12 months)/mebendazole (18 months); [4] Group 4 (PBO/PBO)=placebo (12 months)/placebo (18 months)

Table 1. Baseline characteristics of the study population (N=1760) by intervention group, Iquitos, Loreto, Peru (September 2011-July 2013).

	MBD/PBO*[1] (n=440)	PBO/MBD *[2] (n=440)	MBD/MBD *[3] (n=440)	PBO/PBO*[4] (n=440)
Child characteristics				
Weight (kg)	8.6 (1.0)	8.8 (1.0)	8.7 (1.0)	8.7 (0.9)
Length (cm)	71.9 (2.4)	72.3 (2.4)	72.1 (2.5)	72.2 (2.5)
Age (months)	12.5 (0.4)	12.5 (0.5)	12.5 (0.4)	12.5 (0.5)
Birth weight (kg)	3.1 (0.5)	3.2 (0.5)	3.2 (0.5)	3.2 (0.5)
Birth length (cm)	49.2 (2.5)	49.5 (2.5)	49.4 (2.3)	49.5 (2.7)
Sex (female)	215 (48.9)	222 (50.5)	203 (46.1)	200 (45.5)
Continued breastfeeding at 12 months	394 (89.6)	395 (89.8)	394 (89.6)	392 (89.1)
Up-to-date vaccinations**	346 (78.8)	351 (79.8)	358 (81.6)	355 (80.9)
Received vitamin A in previous year	213 (48.4)	241 (54.8)	251 (57.1)	216 (49.1)
Hospitalizations since birth	402 (91.4)	397 (90.2)	402 (91.4)	396 (90.0)
Walking without support	111 (25.2)	104 (23.7)	117 (26.6)	101 (23.1)
Maternal characteristics				
Married or common-law	358 (81.4)	351 (79.8)	357 (81.1)	357 (81.1)
Secondary education completed	142 (32.4)	140 (31.8)	133 (30.2)	139 (31.6)
Employment outside the home	47 (10.7)	45 (10.2)	50 (11.4)	37 (8.4)
Household characteristics				
Peri-urban or rural residence	382 (86.8)	391 (88.9)	388 (88.2)	399 (90.7)
Potable water in home	230 (52.3)	218 (49.6)	230 (52.3)	220 (50.0)
Earth or wood house material	342 (77.7)	342 (77.7)	338 (76.8)	332 (75.5)

Results are expressed as means (SD) or frequency (%)

*[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD) = mebendazole at the 12 and 18-month visit; [4]Group 4 (PBO/PBO) = placebo at the 12 and 18-month visit

**Up-to-date vaccinations include those scheduled between birth and 11 months of age (i.e. 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)

Table 2. Soil-transmitted helminth (STH) infection prevalence and intensity at the a) 12-month (n=880)*[1], b) 18-month (n=807)*[2] and c) 24-month (n=1563)*[3] follow-up visits by intervention group, Iquitos, Loreto, Peru (September 2011-July 2013).

	a) 12-month visit		b) 18-month visit	
	MBD/PBO‡[1] (n=440)	MBD/MBD‡[3] (n=440)	PBO/MBD‡[2] (n=401)	MBD/MBD‡[3] (n=405)
ASCARIS LUMBRICOIDES				
Prevalence (#, %)	48 (10.9)	52 (11.8)	93 (23.2)	82 (20.2)
Intensity				
No (#, %)	392 (89.1)	388 (88.2)	308 (76.8)	323 (79.8)
Light (#, %)	40 (9.1)	46 (10.4)	73 (18.2)	56 (13.8)
Moderate (#, %)	8 (1.8)	6 (1.4)	17 (4.2)	25 (6.2)
Heavy (#, %)	0 (0.0)	0 (0.0)	3 (0.8)	1 (0.2)
AM† (95% CI)	321.2 (171.4, 471.1)	253.9 (144.6, 363.2)	1524.6 (668.2, 2381.1)	1227.7 (687.9, 1767.5)
GM§ (95% CI)	2.2 (1.8, 2.8)	2.2 (1.8, 2.8)	5.4 (3.9, 7.3)	4.5 (3.3, 6.1)
TRICHURIS TRICHIURA				
Prevalence (#, %)	17 (3.9)	22 (5.0)	55 (13.7)	44 (10.8)
Intensity				
No (#, %)	423 (96.2)	418 (95.0)	346 (86.3)	361 (89.2)
Light (#, %)	16 (3.6)	20 (4.6)	52 (13.0)	41 (10.1)
Moderate (#, %)	1 (0.2)	2 (0.4)	3 (0.7)	3 (0.7)
AM (95% CI)	18.0 (-4.4, 40.5)	15.1 (3.8, 26.4)	41.5 (9.7, 73.2)	30.8 (10.6, 51.0)
GM (95% CI)	1.2 (1.1, 1.3)	1.3 (1.2, 1.4)	1.9 (1.6, 2.2)	1.7 (1.5, 2.0)
HOOKWORM				
Prevalence (#, %)	3 (0.7)	2 (0.5)	1 (0.3)	6 (1.5)
Intensity				
No (#, %)	437 (99.3)	438 (99.5)	400 (99.8)	399 (98.5)
Light (#, %)	3 (0.7)	2 (0.5)	1 (0.2)	6 (1.5)
AM (95% CI)	1.4 (-0.9, 3.7)	0.7 (-0.5, 1.8)	1.5 (-1.4, 4.4)	3.6 (-0.7, 7.9)
GM (95% CI)	1.03 (1.0, 1.1)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	1.1 (1.0, 1.1)
ANY STH INFECTION				
Prevalence (#, %)	60 (13.6)	67 (15.2)	123 (30.7)	107 (26.4)

c) 24-month visit	MBD/PBO‡[1] (n=388)	PBO/MBD‡[2] (n=398)	MBD/MBD‡[3] (n=381)	PBO/PBO‡[4] (n=396)
ASCARIS LUMBRICOIDES				
Prevalence (#, %)	128 (33.0)	127 (31.9)	117 (30.7)	128 (32.3)
Intensity				
No (#, %)	260 (67.0)	271 (68.1)	264 (69.3)	268 (67.7)
Light (#, %)	85 (21.9)	88 (22.1)	82 (21.5)	88 (22.2)
Moderate (#, %)	40 (10.3)	37 (9.3)	33 (8.7)	38 (9.6)
Heavy (#, %)	3 (0.8)	2 (0.5)	2 (0.5)	2 (0.5)
AM (95% CI)	2246.7 (1491.9, 3001.4)	2442.5 (948.3, 3936.7)	2205.5 (1179.9, 3231.2)	1952.0 (1238.8, 2665.2)
GM (95% CI)	12.1 (8.3, 17.4)	10.7 (7.5, 15.2)	9.5 (6.7, 13.5)	10.3 (7.3, 14.6)
TRICHURIS TRICHIURA				
Prevalence (#, %)	100 (25.8)	83 (20.9)	68 (17.9)	103 (26.0)
Intensity				
No (#, %)	288 (74.2)	315 (79.1)	313 (82.2)	293 (74.0)
Light (#, %)	97 (25.0)	82 (20.6)	66 (17.3)	100 (25.3)
Moderate (#, %)	3 (0.8)	1 (0.3)	2 (0.5)	3 (0.7)
AM (95% CI)	57.5 (31.0, 84.0)	26.4 (16.8, 35.9)	34.1 (16.5, 51.8)	55.6 (36.8, 74.3)
GM (95% CI)	3.3 (2.7, 4.1)	2.5 (2.1, 2.9)	2.2 (1.9, 2.7)	3.4 (2.8, 4.2)
HOOKWORM				
Prevalence (#, %)	4 (1.0)	6 (1.5)	5 (1.3)	9 (2.3)
Intensity				
No (#, %)	384 (99.0)	392 (98.5)	376 (98.7)	387 (97.7)
Light (#, %)	4 (1.0)	6 (1.5)	5 (1.3)	9 (2.3)
AM (95% CI)	1.4 (-0.6, 3.4)	1.6 (-0.2, 3.3)	2.1 (-0.4, 4.7)	7.5 (-2.2, 17.2)
GM (95% CI)	1.0 (1.0, 1.1)	1.1 (1.0, 1.1)	1.1 (1.0, 1.1)	1.1 (1.0, 1.2)
ANY STH INFECTION				
Prevalence (#, %)	175 (45.1)	163 (41.0)	149 (39.1)	179 (45.2)

* STH results at all visits include only children whose specimens were analyzed by the Kato-Katz method (i.e. [1]Group 1 and Group 3 at 12-month visit; [2]Groups 2 and 3 at 18-month visit (results were not available for 73 children who were lost to follow-up); [3]All groups at the 24-month visit (results were not available for 197 children who were lost to follow-up))

ǂ[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD) = mebendazole at the 12 and 18-month visit; [4]Group 4 (PBO/PBO) = placebo at the 12 and 18-month visit

†AM = arithmetic mean eggs per gram; §GM = geometric mean eggs per gram. A value of 1 was added to each observation to calculate the geometric mean.

Table 3. Overall benefit of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analyses (N=1760*), Iquitos, Loreto, Peru (September 2011 – July 2013).

	MBD/PBO**[1] (n=440)	PBO/MBD**[2] (n=440)	MBD/MBD**[3] (n=440)	PBO/PBO**[4] (n=440)
Outcome				
Weight gain, kg	2.05	1.93	2.04	2.00
(95% CI)	(1.98, 2.13)	(1.85, 2.02)	(1.97, 2.11)	(1.93, 2.06)
Unadjusted difference	0.05	-0.07	0.04	*reference*
(95% CI)	(-0.05, 0.16)	(-0.17, 0.04)	(-0.06, 0.14)	
p value	0.322	0.217	0.442	
Adjusted‡ difference	0.06	-0.06	0.05	*reference*
(95% CI)	(-0.05, 0.17)	(-0.16, 0.04)	(-0.05, 0.15)	
p value	0.289	0.259	0.318	
Length gain, cm	9.84	9.53	9.67	9.61
(95% CI)	(9.64, 10.05)	(9.33, 9.74)	(9.50, 9.85)	(9.41, 9.81)
Unadjusted difference	0.23	-0.08	0.06	*reference*
(95% CI)	(-0.07, 0.53)	(-0.38, 0.23)	(-0.21, 0.34)	
p value	0.129	0.626	0.651	
Adjusted difference	0.26	-0.06	0.12	*reference*
(95% CI)	(-0.04, 0.55)	(-0.36, 0.25)	(-0.15, 0.39)	
p value	0.087	0.714	0.390	
WAZ†[1] change	-0.23	-0.36	-0.24	-0.28
(95% CI)	(-0.30, -0.16)	(-0.43, -0.29)	(-0.30, -0.18)	(-0.34, -0.22)
Unadjusted difference	0.05	-0.08	0.04	*reference*
(95% CI)	(-0.05, 0.14)	(-0.17, 0.01)	(-0.05, 0.13)	
p value	0.321	0.090	0.359	
Adjusted difference	0.05	-0.07	0.04	*reference*
(95% CI)	(-0.04, 0.15)	(-0.16, 0.02)	(-0.05, 0.13)	
p value	0.277	0.126	0.339	
LAZ†[2] change	-0.51	-0.64	-0.56	-0.59
(95% CI)	(-0.58, -0.44)	(-0.71, -0.57)	(-0.62, -0.49)	(-0.66, -0.52)
Unadjusted difference	0.07	-0.05	0.03	*reference*
(95% CI)	(-0.02, 0.17)	(-0.15, 0.05)	(-0.06, 0.13)	
p value	0.132	0.328	0.474	
Adjusted difference	0.09	-0.04	0.04	*reference*
(95% CI)	(-0.01, 0.18)	(-0.14, 0.06)	(-0.05, 0.13)	
p value	0.083	0.454	0.377	

Results are expressed as mean (95% Confidence Interval)

* Intention-to-treat analysis includes data from 1563 children for whom final outcome information was available, and 197 children who were lost to follow-up and whose outcome information was estimated using multiple imputation

**[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD) = mebendazole at the 12 and 18-month visit; [4]Group 4 (PBO/PBO) = placebo at the 12 and 18-month visit

ǂ Adjusted models include age, sex, socioeconomic status and continued breastfeeding at 12 months of age

†[1]WAZ=weight-for-age z score; [2]LAZ=length-for-age z score. Z scores were derived using WHO international growth standards [36]

Table 4. The effect of the timing of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analyses (n=880*), Iquitos, Loreto, Peru (September 2011 – July 2013).

	MBD/PBO**[1] (n=440)	PBO/MBD**[2] (n=440)
Outcome		
Weight gain, kg	2.05	1.93
(95% CI)	(1.98, 2.13)	(1.85, 2.02)
Unadjusted difference	0.12	*reference*
(95% CI)	**(0.01, 0.23)**	
p value	0.033	
Adjusted‡ difference	0.12	*reference*
(95% CI)	**(0.01, 0.23)**	
p value	0.035	
Length gain, cm	9.84	9.53
(95% CI)	(9.64, 10.05)	(9.33, 9.74)
Unadjusted difference	0.31	*reference*
(95% CI)	**(0.04, 0.58)**	
p value	0.026	
Adjusted difference	0.31	*reference*
(95% CI)	**(0.05, 0.58)**	
p value	0.021	
WAZ†[1] change	-0.23	-0.36
(95% CI)	(-0.30, -0.16)	(-0.43, -0.29)
Unadjusted difference	0.13	*reference*
(95% CI)	**(0.03, 0.23)**	
p value	0.009	
Adjusted difference	0.12	*reference*
(95% CI)	**(0.03, 0.22)**	
p value	0.011	
LAZ†[2] change	-0.51	-0.64
(95% CI)	(-0.58, -0.44)	(-0.71, -0.57)
Unadjusted difference	0.12	*reference*
(95% CI)	**(0.03, 0.21)**	
p value	0.007	
Adjusted difference	0.12	*reference*
(95% CI)	**(0.03, 0.21)**	
p value	0.007	

Results are expressed as mean (95% Confidence Interval)

* Intention-to-treat analysis includes data from 1563 children for whom final outcome information was available, and 197 children who were lost to follow-up and whose outcome information was estimated using multiple imputation

**[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit

ǂ Adjusted models include age, sex, socioeconomic status and continued breastfeeding at 12 months of age

†[1]WAZ=weight-for-age z score; [2]LAZ=length-for-age z score. Z scores were derived using WHO international growth standards [36]

Table 5. The effect of the frequency of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analyses (n=1320*), Iquitos, Loreto, Peru (September 2011 – July 2013).

	MBD/PBO**[1] (n=440)	PBO/MBD**[2] (n=440)	MBD/MBD**[3] (n=440)
Outcome			
Weight gain, kg	2.05	1.93	2.04
(95% CI)	(1.98, 2.13)	(1.85, 2.02)	(1.97, 2.11)
Unadjusted difference	0.02	-0.10	*reference*
(95% CI)	(-0.09, 0.12)	**(-0.20, -0.01)**	
p value	0.777	0.039	
Adjusted‡ difference	0.01	-0.11	*reference*
(95% CI)	(-0.10, 0.12)	**(-0.21, -0.01)**	
p value	0.891	0.029	
Length gain, cm	9.84	9.53	9.67
(95% CI)	(9.64, 10.05)	(9.33, 9.74)	(9.50, 9.85)
Unadjusted difference	0.17	-0.14	*reference*
(95% CI)	(-0.10, 0.44)	(-0.41, 0.13)	
p value	0.219	0.313	
Adjusted difference	0.14	-0.18	*reference*
(95% CI)	(-0.13, 0.40)	(-0.44, 0.09)	
p value	0.309	0.199	
WAZ†[1] change	-0.23	-0.36	-0.24
(95% CI)	(-0.30, -0.16)	(-0.43, -0.29)	(-0.30, -0.18)
Unadjusted difference	0.01	-0.12	*reference*
(95% CI)	(-0.09, 0.10)	**(-0.21, -0.03)**	
p value	0.892	0.007	
Adjusted difference	0.01	-0.12	*reference*
(95% CI)	(-0.09, 0.11)	**(-0.20, -0.03)**	
p value	0.849	0.010	
LAZ†[2] change	-0.51	-0.64	-0.56
(95% CI)	(-0.58, -0.44)	(-0.71, -0.57)	(-0.62, -0.49)
Unadjusted difference	0.04	-0.08	*reference*
(95% CI)	(-0.05, 0.13)	(-0.18, 0.01)	
p value	0.383	0.072	
Adjusted difference	0.04	-0.08	*reference*
(95% CI)	(-0.05, 0.13)	(-0.17, 0.01)	
p value	0.337	0.086	

Results are expressed as mean (95% Confidence Interval).

* Intention-to-treat analysis includes data from 1563 children for whom final outcome information was available, and 197 children who were lost to follow-up and whose outcome information was estimated using multiple imputation.

**[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD) = mebendazole at the 12 and 18-month visit.

ǂ Adjusted models include age, sex, socioeconomic status and continued breastfeeding at 12 months of age

†[1]WAZ=weight-for-age z score; [2]LAZ=length-for-age z score. Z scores were derived using WHO international growth standards [36]

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PREFACE TO MANUSCRIPT C

For the randomized controlled trial of deworming in early preschool-age children in Iquitos, Peru, children were first enrolled at their routine 12-month growth and development (CRED) visit in participating health centres. All children were followed up 6 and 12 months later (at their 18 and 24-month CRED visits, respectively) at which time a socio-demo-epi questionnaire was administered, anthropometric measurements were taken, and a stool specimen was collected to detect soil-transmitted helminth infections. At both the 12 and 24-month visits, the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) was applied to assess child development in terms of cognitive, language and fine motor skills.

The following manuscript (Manuscript C) comprises the third and final results chapter of the thesis. It focuses on the effect of the deworming intervention on the secondary outcome of child development. Previous manuscripts provided details on enrolment procedures and the association between characteristics of the study population and malnutrition at baseline (Manuscript A), and on the trial's design and methodology, baseline characteristics of the study population by intervention group, and results of the effect of the deworming interventions on the primary outcome of weight gain and on secondary growth outcomes (Manuscript B).

Manuscript C has been submitted to Pediatrics and is currently under review. It conforms to the Consort 2010 guidelines for reporting parallel group randomized trials. Selected results will be presented by SAJ at the American Society of Tropical Medicine and Hygiene Meeting in New Orleans LA, USA, November 2014 (see Appendix 9 for a copy of the abstract).

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7 MANUSCRIPT C

The effect of deworming on early childhood development in Peru: a randomized controlled trial

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Short Title: Deworming and development in early childhood

Abbreviations: STH – soil-transmitted helminth; SES – socioeconomic status; RA – research assistant; RCT – randomized controlled trial; LMIC – low- and middle-income countries

Key Words: deworming, cognition, language, fine motor skills, low- and middle-income countries, randomized controlled trial

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What's Known on This Subject

Reducing soil-transmitted helminth infection may improve development in school-age children in low- and middle-income countries. No large-scale trial has been conducted to determine the potential benefit in children in the critical window before two years of age.

What This Study Adds

This is the first trial to examine the effect of deworming on cognitive, language and fine motor development exclusively in children in the second year of life. Overwhelming concomitant poverty and malnutrition may obscure the true effect of deworming.

ABSTRACT

Background: There is a knowledge gap on the effect of early childhood deworming on development in low- and middle-income countries. This evidence is important in the critical window of growth and development before two years of age.

Methods: A randomized controlled trial of the benefit, and optimal timing and frequency, of deworming on development was conducted in Iquitos, Peru. Children were enrolled during routine 12-month growth and development visits and randomly allocated to: 1) deworming at the 12-month visit and placebo at the 18-month visit; 2) placebo at the 12-month visit and deworming at the 18-month visit; 3) deworming at the 12 and 18-month visits; or 4) placebo at the 12 and 18-month visits. The Bayley Scales of Infant Development III was used to assess cognitive, language and motor skills at the 12 and 24-month visits. One-way ANOVA analyses used an intention-to-treat approach.

Results: Between September 2011 and June 2012, 1760 children were enrolled. Attendance at the 24-month visit was 88.8% (n=1563). Raw scores on all subtests increased over 12 months; however, cognitive and expressive language scaled scores decreased. There was no statistically significant benefit of deworming, or effect of timing or frequency, on any of the development scores.

Conclusions: After 12 months of follow-up, an overall benefit of deworming on cognition, language or fine motor development was not detected. Additional child and maternal interventions, including sustained periodic deworming, should be considered to prevent malnutrition and poverty which impact the accumulation of developmental deficits in this critical period.

INTRODUCTION

Evidence from low- and middle-income countries (LMICs) has highlighted the importance of ensuring optimal conditions in early childhood, and in particular, the first two years of life, for healthy development in the short and long-term [1-4]. Poverty is a major underlying cause of developmental deficits, through increased nutritional deficiencies and infection, an inadequate home environment and stimulation, and low parental education[1]. These risk factors can impact brain development and thus cognitive functioning in early life, and later school achievement and productivity in adulthood[2,3]. Thus, appropriate and integrated interventions must be provided to improve early child development, reduce health inequities, and provide those most vulnerable populations an opportunity to escape the vicious cycle of poverty[1].

Interventions to improve child development include micronutrient supplementation and breastfeeding, and targeting the social components linked to poverty, such as mother-child interactions and child stimulation[5-7]. There has been less evidence on the potential benefits of interventions for infections in early childhood on short or long-term development. The soil-transmitted helminth (STH) disease cluster (i.e. *Ascaris*, *Trichuris* and hookworm) is common in the most vulnerable populations in LMICs. STHs persist in contaminated environments with poor sanitation and limited access to improved water sources. The impact of providing single-dose anthelminthic treatment (i.e. deworming) on cognition has been studied almost exclusively in school-age children. Some observational studies and randomized controlled trials (RCTs) have shown a benefit of deworming (mainly through a reduction in hookworm or *Trichuris* infection) on cognition, measured directly through psychometric tests, or indirectly through school indicators such as school performance and attendance[8-11]. The combined evidence is mixed, and a recent Cochrane review was unable to detect an overall significant benefit of deworming on cognition in school-age children[12]. The evidence base in preschool-age children is even more limited.

One cross-sectional study found some evidence for a link between intestinal parasite infections (not limited to STH) and deficient scores on the Denver Developmental Screening Test II in children living in rural Nicaragua[13]. This relationship did not persist in multivariable analysis nor in a subgroup analysis of children under 24 months of age. Stoltzfus et al (2001) conducted the only RCT on deworming and development in preschool-age children[14]. Although not statistically significant, there was a trend towards a benefit of deworming on language and gross motor development.

With little research attention and challenges in measuring developmental outcomes in younger children, a large research gap exists as to the potential benefits of deworming in early preschool-age children. We therefore conducted a randomized controlled trial on the effects of a deworming intervention provided at 12 months of age on child development at 24 months of age, measured by cognitive, language and fine motor skills.

METHODS

Ethics approval and trial monitoring

Ethics approval for this trial was obtained both in Peru, from the Comité Institucional de Ética of the Universidad Peruana Cayetano Heredia and the Instituto Nacional de Salud, in Lima; and in Canada, from the Research Ethics Board of the Research Institute of the McGill University Health Centre in Montréal, Québec. Additional authorization was granted from the local Ministry of Health (Dirección Regional de Salud (DIRESA) de Loreto) office in Iquitos. An independent and international Data Safety and Monitoring Committee (DSMC) reviewed all adverse events during the course of the trial, and approved continuation of the study. Both parents (or guardian(s)) provided a signed informed consent form to confirm participation of their child in the study.

Details on the trial have been described elsewhere[15,16]. Briefly:

1) Study design and enrolment procedures: We conducted a randomized, double-blind, placebo-controlled trial of deworming at 12 and/or 18 months of age in children living in Iquitos, a soil-transmitted helminth (STH)-endemic area of the Peruvian Amazon. Children were enrolled during their routine 12-month growth and development visits in participating health centres and followed-up to their 24-month visit. Children were eligible to participate in the trial if they were: 1) living in the study area; and 2) attending one of the 12 participating study health centres for their 12-month growth and development visit. Children were not eligible to participate if they: 1) were attending the clinic for suspected STH infection; 2) had received deworming in the six months prior to enrolment in the trial; 3) had plans to move outside of the study area in the next year; 4) were younger than 12 months of age or 14 months of age or older; or 5) suffered from serious congenital or chronic medical conditions.

2) Outcome measurements and follow-up visits: The primary outcome of the trial was weight gain over 12 months of follow-up. Additional growth outcomes included length gain and derived indices (i.e. weight-for-age and length-for-age z scores). A secondary outcome was the effect of deworming on child development (as defined below). A socio-demo-epi questionnaire was administered to the primary caregiver of the child at the 12-month visit. Baseline outcome measurements, including weight, length and STH infection, were ascertained in a subsequent visit in the health centre. These measurements were repeated at the 18 and 24-month visits. All measurements were assessed by trained research assistants (RAs).

Development was assessed at the 12 and 24-month visits using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). The Bayley-III is a rigorous instrument that is used to assess developmental functioning in children under 42 months of age[17]. It has been adapted for use in international settings[18-20], and previous versions have been used in Peruvian populations [21]. Subtests that

were included in the current trial were cognitive, receptive language, expressive language, and fine motor. The gross motor subtest was not included as these skills were thought to be less variable in the age group of children studied; however, the World Health Organization (WHO) gross motor milestones were used to assess the age at which the child began to walk without support[22]. Each subtest consisted of items which were administered by trained RAs in the presence of one or two caregivers. RAs were all healthcare personnel (e.g. nurse or nurse-midwife) with a minimum Bachelor's degree education. All attempts were made to complete the assessment in one visit, including taking breaks for feedings. To ensure the child was performing under optimal conditions, a second visit was scheduled if needed. The test was administered as recommended during the health centre visit[17]. Some modifications of items and test administration were required:

a) if a child did not answer all first three items correctly, the RA would reverse in blocks of three items at a time (i.e. rather than to the previous age start point) until three correct responses were achieved (i.e. the basal). The RA would then continue in a forward manner from the first unadministered item until the stopping point was reached (i.e. incorrect responses to five sequential items).

b) for items where verbal instructions were not specified, we developed specific instructions and a maximum number of times that they could be repeated to standardize practices among RAs.

c) adaptation of words and images in some items, including all pictures in the Picture Book and some pictures in the Stimulus Book, was required. The age-appropriateness of both the image and the accompanying word were considered when adapting the items. All modifications were pre-tested in children of the same target age of the trial and in older children.

Extensive training of RAs and pretesting of the adapted instrument took place for two months prior to the start of the 12 and 24-month visits. Adaptation and

training of the Bayley-III was performed by FL and SAJ. On-site supervision, video recordings, and re-training were used to ensure consistency of administration and scoring throughout the trial. All data collection activities were regularly supervised by SAJ and LP.

3) Intervention groups: After the completion of all baseline outcome measurements, participating children were randomly allocated to:

Group 1 (MBD/PBO): Deworming (i.e. 500 mg single-dose mebendazole) at the 12-month visit and placebo at the 18-month visit.

Group 2 (PBO/MBD): Placebo at the 12-month visit and deworming at the 18-month visit.

Group 3 (MDB/MBD): Deworming at both the 12 and 18-month visits.

Group 4 (PBO/PBO): Placebo at both the 12 and 18-month visits.

Usual care interventions and services (e.g. vaccinations) were provided by health centre personnel, according to Peruvian Ministry of Health guidelines.[23] The deworming tablet was manufactured by Janssen Pharmaceuticals Inc. and donated by INMED Peru. The identical placebo tablet was manufactured and purchased from Laboratorios Hersil, Peru. Tablets were crushed and mixed with juice, and administered by RAs upon completion of all other visit procedures.

4) Sample size: A total sample size of 1760 was estimated (i.e. 440 children per group), based on detecting a minimum difference of 0.20 kg in the primary outcome of weight gain over one year among the different deworming intervention groups [16]. The sample size took into account 80% power, a common standard deviation of 0.8, estimated loss-to-follow-up of 20% over 12 months, and the Tukey correction for multiple comparisons (MC4G Software©, GP Brooks, Ohio University, 2008).

5) Randomization and masking: Intervention assignment was determined using a computer-generated random sequence and permuted block sizes of eight and twelve. Envelopes containing the intervention were prepared and numbered between 1 and 1760, corresponding to the computer-generated sequence. These were stored in the pharmacy of the local research office and handed out in sequential order to RAs (by SAJ or LP). All research personnel involved in trial design, outcome measurements, and/or analysis, as well as parents/guardians of participants were blinded to intervention status.

6) Analyses: Development scores were calculated separately for each subtest. The raw score was calculated as the number of correct responses between the basal and the stopping point, added to the total number of unadministered items prior to the basal. Raw scores were converted to scaled scores between 1 and 19, derived from age-standardization tables (based on a developed country population).[17] Scaled scores were analyzed to make comparisons within the trial (i.e. among groups and different time points) and not as an indication of development delays or deficits compared to other populations.

The effect of deworming on development was examined for each subtest in unadjusted intention-to-treat analysis using one-way ANOVA. Developmental outcomes included absolute raw scores and scaled scores at the 24-month visit, and the change in raw and scaled scores from baseline to the 24-month visit. Multivariable linear regression analyses were also adjusted for baseline anthropometry, baseline development score (in the case of absolute score outcomes), age, sex, breastfeeding to 12 months of age and socioeconomic status (SES) (based on a proxy asset-based indicator)[15]. For children who were missing their 24-month visit, multiple imputation using a Markov Chain Monte Carlo model was used to impute development scores at follow-up (e.g. based on baseline values of age, sex, anthropometry and SES).

Additional multivariable linear regression analyses were conducted to examine the relationship between other baseline child, maternal and household factors and

development scores at the 24-month visit. Variables that were significant at $p<0.20$ in univariable analyses were included in further multivariable model building. The final model included all significant variables at $p<0.05$, as well as adjustment for age, intervention group, and the RA who performed the assessment.

All statistical analyses were performed using the Statistical Analysis Systems statistical software package version 9.3 (SAS Institute, Cary, NC, USA).

Role of the funding source

The funding agencies (Thrasher Research Fund; Canadian Institutes of Health Research; Fonds de Recherche du Québec – Santé, Research Institute of the McGill University Health Centre) had no role in study design, data collection, data analysis, data interpretation, manuscript writing, or the decision to submit the manuscript for publication. The corresponding author had full access to the data and final responsibility for the decision to submit the manuscript for publication.

RESULTS

Participant flow and baseline characteristics of the study population

Details on participant enrolment, follow-up, and baseline characteristics are described elsewhere[16]. Briefly, children were enrolled in the trial between September 2011 and June 2012 to reach the total required sample size of 1760. All children received their randomly assigned intervention at baseline. A total of 1606 children attended the 18-month visit: 1603 receiving the allocated intervention and three parents refused the receipt of the allocated intervention by their children. Eighty-eight percent (n=1563) of children attended their final follow-up visit between September 2012 and July 2013. Characteristics of children at baseline were similar by intervention group[16].

At baseline, only 17 children (1.0%) required two visits to complete the developmental assessment. At the 24-month visit, there were 38 children out of 1563 (2.4%) who required two visits to complete the test.

Development scores by intervention group

Raw and scaled scores on each of the four subtests were similar for all intervention groups at baseline (Table 1). Raw scores increased between the 12 and 24-month visits as is expected with increasing age, and were similar among intervention groups. Scaled scores increased over the 12-month period in receptive language and fine motor skills; however, over this same period of time, both cognitive and expressive language scaled scores decreased in all four groups, suggesting increasing developmental deficits.

Benefit, timing and frequency of deworming on development outcomes

When comparing the scaled score at the 24-month visit in each of the deworming intervention groups to the control group, no statistically significant benefit of deworming on any of the development subtests was detected in unadjusted or adjusted intention-to-treat analysis (Table 2). Results remained consistent when using the outcomes of absolute raw scores, and change in raw and scaled scores (results not shown). There was some evidence for improved cognitive outcomes in terms of timing, with greater scores in Group 2 compared to Group 1 at follow-up (Table 3). The effect size decreased with adjustment of baseline cognitive scores and nutritional status, and no effect was seen on any of the other subtests. There was no statistically significant effect of deworming frequency on any of the developmental outcomes (Table 4). No benefits of deworming on any cognitive outcomes were apparent in additional sensitivity analyses, including complete case analysis, per-protocol analysis, and subgroup analysis by malnutrition status (e.g. stunted and/or underweight) at baseline.

Adverse events

Eighteen serious adverse events were reported in the trial, none of which were deemed to be associated with the deworming intervention[16].

Predictors of development at 24 months

Variables that were examined but were not found to be statistically significantly related to developmental outcomes at the 24-month visit included continued breastfeeding to 12 months of age, up-to-date vaccinations at 12 months of age, marital status, iron supplementation received during the study, timing of introduction of liquids and foods, and place of delivery. Other child, maternal and household variables were found to be significantly related to development scores at the 24-month visit (Table 5). Predictors of cognitive and language score were similar in multivariable analyses and included baseline length (cm), female sex, maternal education, age at which child began to walk without support, and vitamin A supplementation. Predictors of fine motor score included baseline weight, sex, maternal education, and age at which child began to walk without support. SES was not found to be a significant predictor for any of the developmental outcomes.

DISCUSSION

This is the first randomized controlled trial to examine the potential effect of deworming on cognitive, language and fine motor skills in early preschool-age children using a rigorous developmental assessment instrument. Only one other trial has examined the potential benefit of deworming on development in early childhood, measured by cognitive and gross motor skills, but it was limited by a small sample size, the use of parental report for assessment of developmental outcomes, and a lack of a rigorous developmental test[14]. We chose to measure fine motor skills in the current trial, as the measure of gross motor skills is likely less variable and therefore less modifiable in children over one year of age. All other studies looking at the link between deworming and developmental outcomes have

been conducted in school-age populations, and are, therefore, not comparable to early preschool-age children in the critical window of development.

Overall, this trial was unable to demonstrate a benefit of any of the deworming schedules on development over the period of one year. There has been evidence from deworming in school-age populations that improvements in cognition may arise through a reduction in hookworm or *Trichuris* infections. The low prevalence of these two infections at baseline (less than 5%), may explain our findings. Other studies have demonstrated weak effects of nutrition interventions, and stronger effects of psychosocial interventions on short and long-term developmental outcomes. If there is a true benefit to deworming, it may be too early to detect this difference, or it may need to be combined with other cost-effective measures, such as micronutrient supplementation or improvements in child stimulation, the latter of which seems to have the greatest impact on development outcomes[7].

We were able to demonstrate improved developmental outcomes at the 24-month visit in children with greater height and weight measures at baseline, indicating the importance of targeting the high prevalence of malnutrition in this population. Not surprisingly, maternal education was found to be associated with development scores; however, SES was not. This population was specifically chosen to be homogeneous in terms of endemicity for STH, high prevalence of malnutrition, and lower SES; therefore, there is likely low variability in SES to detect differences in risk. The association of lower development scores with vitamin A supplementation is also somewhat surprising, but likely due to the fact that vitamin A is distributed strategically in the study area (i.e. to high risk individuals and by certain health centres) according to Ministry of Health guidelines[23].

The overall results are relevant to children in the second year of life in other STH-endemic areas. These results may not be generalizable to areas with much lower or higher STH infection prevalence and intensity or malnutrition, or to areas with high prevalence of co-infection with other tropical diseases (e.g. malaria).

Overall strengths of this study include the RCT design, a large sample size, a high follow-up rate and the inclusion of children in a very specific and narrow age range in a rapid phase of growth and development. This study also benefited from the use of the Bayley-III. We were able to demonstrate the feasibility of employing a rigorous assessment instrument in a research and LMIC context. The reaction from caregivers was positive, and few children required a second visit.

The trial is limited by the short follow-up time between assessments. In addition, the developmental instrument was adapted for our specific population in Peru, so there are limitations in generalizing the scores to other populations, even within Peru. The scaled scores are useful in making comparisons within the study, including detecting the increasing deficits over time; however, they should not be used to compare to other populations. We are also limited by the lack of detailed information on potential social confounders such as characteristics related to the home environment (e.g. mother-child interactions and child stimulation). Additional information on supplementations (e.g. iron, vitamin A), were assessed by self-report only, limiting the ability to detect differences between duration and timing of use.

Overall, the deworming interventions were not sufficient to improve development scores over one year, or to prevent the increasing developmental deficits in cognition and language skills. Due to the multifactorial nature of development, integrated interventions are likely necessary to combat developmental deficits in this vulnerable population[7]. Participants are currently being followed-up in an observational cohort with repeated yearly measurements on cognitive, language and motor functioning. This will allow us to detect any effect of the early deworming interventions on development in the longer-term.

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Table 1. Absolute raw and scaled development scores and change in scores at baseline (12-month visit) and follow-up (24-month visit) by intervention group (N=1760), Iquitos, Loreto, Peru (September 2011-July 2013).

	Group 1*[1] (n=440)		Group 2*[2] (n=440)		Group 3*[3] (n=440)		Group 4*[4] (n=440)	
	Raw scores	Scaled score**	Raw score	Scaled score	Raw score	Scaled score	Raw score	Scaled score
Baseline (12-month visit)								
Cognitive	42.5 (42.2, 42.7)	10.3 (10.0, 10.3)	42.5 (42.2, 42.8)	10.2 (10.0, 10.4)	42.4 (42.2, 42.7)	10.4 (10.3, 10.6)	42.4 (42.2, 42.7)	10.2 (10.0, 10.4)
Receptive language	12.9 (12.8, 13.1)	7.4 (7.2, 7.6)	12.8 (12.7, 13.0)	7.3 (7.1, 7.4)	13.0 (12.8, 13.1)	7.5 (7.3, 7.7)	13.0 (12.8, 13.1)	7.4 (7.2, 7.6)
Expressive language	13.4 (13.2, 13.6)	8.2 (8.1, 8.4)	13.5 (13.3, 13.6)	8.3 (8.1, 8.4)	13.5 (13.3, 13.7)	8.4 (8.2, 8.5)	13.5 (13.3, 13.7)	8.4 (8.2, 8.5)
Fine motor	29.2 (29.0, 29.3)	9.4 (9.2, 9.5)	29.2 (29.1, 29.4)	9.4 (9.3, 9.6)	29.2 (29.1, 29.3)	9.4 (9.3, 9.6)	29.2 (29.1, 29.3)	9.4 (9.3, 9.6)
Follow-up (24-month visit)								
Cognitive	58.8 (58.5, 59.1)	7.7 (7.6, 7.8)	59.3 (59.0, 59.6)	7.9 (7.8, 8.0)	59.1 (58.8, 59.4)	7.9 (7.7, 8.0)	59.1 (58.8, 59.4)	7.8 (7.6, 7.9)
Receptive language	23.9 (23.6, 24.1)	8.1 (8.0, 8.2)	23.9 (23.7, 24.2)	8.2 (8.0, 8.3)	23.9 (23.7, 24.1)	8.1 (8.0, 8.2)	23.9 (23.7, 24.1)	8.1 (8.0, 8.2)
Expressive language	24.6 (24.3, 24.8)	7.1 (7.0, 7.3)	24.5 (24.2, 24.9)	7.2 (7.0, 7.3)	24.6 (24.2, 24.9)	7.1 (7.0, 7.2)	24.6 (24.2, 24.9)	7.2 (7.0, 7.3)
Fine motor	39.4 (39.2, 39.6)	9.9 (9.7, 10.1)	39.5 (39.3, 39.7)	10.0 (9.9, 10.2)	39.4 (39.1, 39.6)	9.9 (9.7, 10.0)	39.4 (39.1, 39.6)	9.9 (9.7, 10.1)
Change from baseline to follow-up								
Cognitive	16.4 (16.0, 16.7)	-2.5 (-2.7, -2.3)	16.9 (16.5, 17.3)	-2.3 (-2.5, -2.1)	16.6 (16.2, 17.0)	-2.6 (-2.8, -2.4)	16.6 (16.2, 17.0)	-2.4 (-2.6, -2.2)
Receptive language	10.9 (10.7, 11.2)	0.7 (0.5, 0.9)	11.1 (10.9, 11.4)	0.9 (0.7, 1.1)	11.0 (10.7, 11.2)	0.6 (0.4, 0.8)	11.0 (10.7, 11.2)	0.7 (0.5, 0.9)
Expressive language	11.1 (10.8, 11.5)	-1.1 (-1.3, -0.9)	11.1 (10.7, 11.4)	-1.1 (-1.3, -0.9)	11.1 (10.7, 11.4)	-1.3 (-1.5, -1.1)	11.1 (10.7, 11.4)	-1.2 (-1.4, -1.0)
Fine motor	10.2 (9.9, 10.4)	0.5 (0.3, 0.7)	10.2 (10.0, 10.5)	0.6 (0.4, 0.8)	10.2 (9.9, 10.4)	0.5 (0.3, 0.7)	10.2 (9.9, 10.4)	0.5 (0.3, 0.7)

Results are expressed as mean (95% confidence interval)

*[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD = mebendazole at the 12 and 18-month visit; [4]Group 4 (PBO/PBO) = placebo at the 12 and 18-month visit

**Scaled scores are the raw scores scaled between 1 and 19 based on age of child in months and days and the specific subtest

Table 2. Overall benefit of deworming on absolute scaled development scores at the 24-month visit, using one-way ANOVA and multivariable linear regression analyses (N=1760*), Iquitos, Loreto, Peru (September 2011 – July 2013).

	Group 1 MBD/PBO**[1] (n=440)	Group 2 PBO/MBD**[2] (n=440)	Group 3 MBD/MBD**[3] (n=440)	Group 4 PBO/PBO**[4] (n=440)
Cognition				
Unadjusted difference	-0.07	0.14	0.10	*reference*
(95% CI)	(-0.24, 0.10)	(-0.04, 0.32)	(-0.08, 0.27)	
p value	0.431	0.122	0.291	
Adjusted‡ difference	-0.05	0.12	0.07	*reference*
(95% CI)	(-0.22, 0.12)	(-0.05, 0.29)	(-0.10, 0.24)	
p value	0.542	0.158	0.440	
Receptive language				
Unadjusted difference	-0.02	0.03	-0.05	*reference*
(95% CI)	(-0.19, 0.14)	(-0.14, 0.20)	(-0.23, 0.12)	
p value	0.780	0.730	0.543	
Adjusted difference	0.00	0.03	-0.06	*reference*
(95% CI)	(-0.16, 0.16)	(-0.13, 0.19)	(-0.23, 0.11)	
p value	0.990	0.710	0.502	
Expressive language				
Unadjusted difference	-0.01	0.00	-0.06	*reference*
(95% CI)	(-0.22, 0.19)	(-0.21, 0.21)	(-0.26, 0.15)	
p value	0.907	0.997	0.582	
Adjusted difference	0.03	-0.01	-0.05	*reference*
(95% CI)	(-0.16, 0.23)	(-0.21, 0.19)	(-0.24, 0.15)	
p value	0.755	0.914	0.640	
Fine motor skills				
Unadjusted difference	-0.02	0.12	-0.04	*reference*
(95% CI)	(-0.27, 0.24)	(-0.14, 0.37)	(-0.29, 0.21)	
p value	0.906	0.364	0.746	
Adjusted difference	0.00	0.10	-0.06	*reference*
(95% CI)	(-0.24, 0.24)	(-0.15, 0.35)	(-0.30, 0.19)	
p value	0.995	0.418	0.654	

Results are expressed as mean (95% Confidence Interval)

* Intention-to-treat analysis includes data from 1563 children for whom final outcome information was available, and 197 children who were lost to follow-up and whose outcome information was estimated using multiple imputation

**[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD = mebendazole at the 12 and 18-month visit; [4]Group 4 (PBO/PBO) = placebo at the 12 and 18-month visit

ǂ Adjusted models include age, sex, socioeconomic status, continued breastfeeding at 12 months of age, baseline height and weight, and baseline development score

Table 3. The effect of the timing of deworming on absolute scaled development scores at the 24-month visit, using one-way ANOVA and multivariable linear regression analyses (n=880*), Iquitos, Loreto, Peru (September 2011 – July 2013).

	Group 1 MBD/PBO**[1] (n=440)	Group 2 PBO/MBD**[2] (n=440)
Cognition		
Unadjusted difference	-0.21	*reference*
(95% CI)	(-0.38, -0.03)	
p value	0.019	
Adjusted‡ difference	-0.18	*reference*
(95% CI)	(-0.34, -0.01)	
p value	0.040	
Receptive language		
Unadjusted difference	-0.05	*reference*
(95% CI)	(-0.21, 0.11)	
p value	0.514	
Adjusted difference	-0.03	*reference*
(95% CI)	(-0.18, 0.12)	
p value	0.708	
Expressive language		
Unadjusted difference	-0.01	*reference*
(95% CI)	(-0.22, 0.19)	
p value	0.904	
Adjusted difference	0.04	*reference*
(95% CI)	(-0.15, 0.24)	
p value	0.672	
Fine motor skills		
Unadjusted difference	-0.13	*reference*
(95% CI)	(-0.36, 0.10)	
p value	0.262	
Adjusted difference	-0.10	*reference*
(95% CI)	(-0.33, 0.12)	
p value	0.382	

Results are expressed as mean (95% Confidence Interval)

* Intention-to-treat analysis includes data from 1563 children for whom final outcome information was available, and 197 children who were lost to follow-up and whose outcome information was estimated using multiple imputation

**[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit

ǂ Adjusted models include age, sex, socioeconomic status, continued breastfeeding at 12 months of age, baseline height and weight, and baseline development score

Table 4. The effect of the frequency of deworming on absolute scaled development scores at the 24-month visit, using one-way ANOVA and multivariable linear regression analyses (n=1320*), Iquitos, Loreto, Peru (September 2011 – July 2013).

	MBD/PBO**[1] (n=440)	PBO/MBD**[2] (n=440)	MBD/MBD**[3] (n=440)
Cognition			
Unadjusted difference	-0.17	0.04	*reference*
(95% CI)	(-0.34, 0.01)	(-0.13, 0.22)	
p value	0.063	0.630	
Adjusted‡ difference	-0.12	0.06	*reference*
(95% CI)	(-0.29, 0.05)	(-0.12, 0.23)	
p value	0.168	0.529	
Receptive language			
Unadjusted difference	0.03	0.08	*reference*
(95% CI)	(-0.14, 0.20)	(-0.09, 0.26)	
p value	0.713	0.343	
Adjusted difference	0.06	0.09	*reference*
(95% CI)	(-0.11, 0.23)	(-0.08, 0.26)	
p value	0.480	0.305	
Expressive language			
Unadjusted difference	0.05	0.06	*reference*
(95% CI)	(-0.16, 0.25)	(-0.15, 0.27)	
p value	0.657	0.587	
Adjusted difference	0.08	0.04	*reference*
(95% CI)	(-0.11, 0.27)	(-0.17, 0.24)	
p value	0.425	0.730	
Fine motor skills			
Unadjusted difference	0.03	0.16	*reference*
(95% CI)	(-0.20, 0.25)	(-0.07, 0.38)	
p value	0.827	0.173	
Adjusted difference	0.06	0.16	*reference*
(95% CI)	(-0.17, 0.28)	(-0.07, 0.38)	
p value	0.624	0.169	

Results are expressed as mean (95% Confidence Interval).

* Intention-to-treat analysis includes data from 1563 children for whom final outcome information was available, and 197 children who were lost to follow-up and whose outcome information was estimated using multiple imputation.

**[1]Group 1 (MBD/PBO) = mebendazole at the 12-month visit and placebo at the 18-month visit; [2]Group 2 (PBO/MBD) = placebo at the 12-month visit and mebendazole at the 18-month visit; [3]Group 3 (MBD/MBD) = mebendazole at the 12 and 18-month visit.

ǂ Adjusted models include age, sex, socioeconomic status, continued breastfeeding at 12 months of age, baseline height and weight, and baseline development score

Table 5. Child, maternal and household factors associated with raw development score at the 24-month visit in unadjusted and adjusted linear regression (n=1563*), Iquitos, Loreto, Peru (September 2011-July 2013).

	Cognitive score		Language score		Fine motor score	
	Unadjusted	Adjusted**	Unadjusted	Adjusted	Unadjusted	Adjusted
Baseline weight (per kg increase)	0.3 (0.1, 0.4)	NS***	0.6 (0.4, 0.9)	NS	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)
Baseline length (per cm increase)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.1 (0.0, 0.1)	NS
Sex (female vs. male)	0.5 (0.2, 0.9)	0.7 (0.4, 1.0)	1.3 (0.8, 1.7)	1.5 (1.1, 2.0)	0.2 (0.0, 0.4)	0.3 (0.1, 0.5)
Maternal education (secondary incomplete vs. complete)	-0.7 (-1.0, -0.3)	-0.7 (-1.0, -0.4)	-1.7 (-2.2, -1.2)	-1.1 (-1.6, -0.7)	-0.3 (-0.5, -0.1)	-0.3 (-0.5, -0.1)
SES† (vs. first quartile)						
Second quartile	0.0 (-0.5, 0.4)	NS	0.3 (-0.3, 1.0)	NS	NS	NS
Third quartile	-0.2 (-0.6, 0.3)	NS	0.4 (-0.3, 1.0)	NS	NS	NS
Fourth quartile	0.3 (-0.1, 0.8)	NS	1.7 (1.0, 2.3)	NS	NS	NS
Periurban/rural residence vs. urban	-0.5 (-1.0, 0.0)	NS	-0.7 (-1.4, 0.0)	NS	-0.3 (-0.6, 0.1)	NS
Maternal employment outside home (yes vs. no)	0.5 (0.0, 1.0)	NS	NS	NS	NS	NS
Hospitalizations in first year of life (no vs. yes)	0.5 (0.0, 1.1)	NS	0.7 (-0.1, 1.5)	NS	NS	NS
Antenatal care attendance (no vs. yes)	-0.6 (-1.3, 0.1)	NS	-1.0 (-2.1, 0.0)	NS	NS	NS
Walking without support (per increasing year)	-0.2 (-0.3, -0.1)	-0.2 (-0.2, -0.1)	-0.4 (-0.5, -0.3)	-0.2 (-0.3, -0.1)	-0.1 (-0.2, -0.1)	-0.1 (-0.2, -0.1)
Number of children in home	NS	NS	-0.4 (-0.5, -0.2)	NS	NS	NS
Vitamin A received in past year (no vs. yes)	1.0 (0.6, 1.4)	0.5 (0.0, 0.9)	-0.9 (-1.5, -0.3)	-0.8 (-1.4, -0.1)	0.2 (0.0, 0.5)	NS

*Analyses restricted to children who attended the 24-month visit (i.e. n=197 excluded)

**Adjusted linear regression models control for age, intervention group, evaluator, and all other statistically significant variables in the multivariable model

***NS = not significant

†SES =socioeconomic status, where the first quartile corresponds to the poorest SES and the fourth quartile corresponds to the highest SES

8 GENERAL DISCUSSION AND CONCLUSION

Overall results and contributions

This is the first and largest double-blind, randomized, placebo-controlled trial of deworming to focus exclusively on 12 to 24 month old children. Prior to 2002, children in this age group were excluded from deworming interventions in STH-endemic countries, based on limited evidence of safety and benefit. A WHO Informal Consultation in 2002 recommended that deworming be provided as of 12 months of age; however, evidence remains limited and coverage continues to be suboptimal. Research from low- and middle-income countries stresses the importance of providing appropriate interventions in early childhood, particularly before two years of age, to prevent poor health, malnutrition, developmental deficits and other adverse effects into school-age and adulthood. There is only limited and contradictory evidence currently available on deworming benefits in early preschool-age children. This research was, therefore, both timely and essential to fill this important research gap.

The results demonstrate a high prevalence of malnutrition, especially in terms of stunting, which increases dramatically over the second year of life. Weight-for-age and length-for-age z scores also show a negative deviation compared to the international WHO growth standard over 12 months. This is consistent with the concept of the critical window before two years of age at which time growth faltering can increase in vulnerable child populations (Victora et al. 2010). The three-fold increase in STH infection prevalence over this same time period demonstrates that this is also a critical moment for rapid STH acquisition. This is not surprising as children start to become more mobile and exposure to pathogens in the environment increases. In a prior study in Iquitos, Peru, first STH infection was detected at eight months of age. Prevalence increased rapidly to almost 30% by 12 to 14 months of age (Gyorkos et al. 2011). A cohort study in Ecuador, following children from birth to three years of age, provides support to these findings (Menzies et al. 2014): first STH acquisition was demonstrated to occur around seven months of age and STH infection prevalence increased to 25% by three years of age. Children found to be STH positive over the course of the study

were treated, which may explain the lower increase in STH infection prevalence compared to the results from the current trial.

In examining the characteristics of the study population at baseline, a statistically significant independent association between stunting and STH infection was found. This is consistent with previous studies in a wider range of preschool-age children (Casapía et al. 2007, Gyorkos et al. 2011). No relationship between underweight and STH infection was apparent. The causal nature of the relationship between STH infection and malnutrition was explored by looking at the effect of deworming interventions provided at baseline on growth over 12 months. Overall, the greatest weight and length gains were seen in the group receiving deworming once at the 12-month visit. However, there was no statistically significant difference in weight or length gain when the different deworming interventions were compared to the control group. This is consistent with what has been reported in two previous trials (Kloetzel et al. 1982, Awasthi et al. 2013). An additional trial reported a benefit of mebendazole in improving growth in children under 30 months of age, but this was only found for mild wasting. No benefit on stunting was observed, and underweight, weight gain and length/height gain were not reported. Four additional trials demonstrated a statistically significant benefit of deworming on growth in terms of mean weight gain (Awasthi et al. 2000, Awasthi and Pande 2001, Alderman et al. 2006) and stunting (Awasthi et al. 2000). All four of these trials used albendazole as the deworming drug of choice, which is somewhat surprising as mebendazole is thought to have greater efficacy against the common STH parasites in early childhood (i.e. *Ascaris* and *Trichuris* infections) (Keiser and Utzinger 2008, Keiser and Utzinger 2010). One trial used a non-standard dose of 600 mg, higher than that recommended for use in any age group (Awasthi et al. 2000). The other three trials used the standard dose of 400 mg, which is still higher than the 200 mg recommended in 12 to 24 month old children (WHO 2002). Follow-up time was also longer in these trials, ranging from 18 months to three years. These differences, as well as the use of open-label designs, non-blinded outcome assessors, more frequent deworming schedules, different baseline STH prevalence

and intensity, study populations of much wider age ranges, and complete case analyses instead of intention-to-treat, may explain the variation in results.

The trial reported in this thesis provides important operational insight into deworming in early preschool-age children. It is the only trial that has looked specifically at the question of deworming timing and frequency. When deworming is provided, the results demonstrate a statistically significant improvement in weight and length gain from deworming just once at 12 months of age. No added benefit was observed from an additional dose provided at 18 months of age. This is insightful in light of the epidemiologic considerations of increasing STH infection prevalence over the second year of life. Although one round of deworming was not enough to reduce the STH infection burden at 24 months of age, it does appear that reducing even low prevalence and intensity of STH infection between 12 and 18 months of age can have an impact on growth. As deworming is contraindicated before 12 months of age, children should therefore be dewormed as soon as possible after their first birthday. This should be quite feasible using existing health infrastructure, as demonstrated in this trial. Deworming can be piggybacked onto interventions provided at 12 months, such as MMR vaccination and vitamin A supplementation, as recommended by WHO (WHO 2014).

In terms of the secondary outcome of development, there were increasing deficits in terms of cognition and expressive language over the one-year follow-up. Lower baseline weight and length were associated with lower development scores at the 24-month visit. Previous studies have demonstrated the important link between growth and development in both the short- and long-term (Grantham-McGregor et al. 2007, Martorell et al. 2010). However, the trial was not able to demonstrate a statistically significant benefit of deworming on development. This may be due to a lack of significant effect of the deworming interventions compared to the control group in terms of growth (which may be a mediating factor between STH infection and development), or due to the low prevalence of *Trichuris* and hookworm infections, which are thought to be related to cognitive functioning

(Stephenson 1987, Nokes et al. 1992, Ezeamama et al. 2012, Taylor-Robinson et al. 2012). Only one other deworming trial in preschool-age children included development outcomes (Stoltzfus et al. 2001), but it was also unable to demonstrate a statistically significant benefit of deworming on either cognition or gross motor skills.

Overall, there was no statistically significant benefit of deworming on growth and development in this population of preschool-age children. However, the results do indicate that, for children between 12 and 24 months of age, once-yearly deworming at 12 months of age provides the greatest growth benefits compared to later or more frequent deworming. A greater benefit may be apparent in areas of higher prevalence or intensity of infection. These results contribute to WHO policy and recommendations on deworming targeting preschool-age children in the over 100 STH-endemic areas of the world. They also contribute to providing practical guidance to governments in integrating deworming into early childhood health care.

Strengths and limitations

Strengths of this study include the randomized controlled trial design to determine the independent effect of deworming on growth and development outcomes, without the influence of confounding variables or external factors. The age group of children was also narrow and specific and provided information on children at a critical and homogeneous growth and development stage. The sample size was large, and there was a high follow-up rate over the 12 month time period, despite the fact that this is a highly mobile population. The high follow-up rate minimized the potential for bias from differential loss-to-follow-up. This was especially noteworthy as this area was affected by extreme seasonal flooding during the study, and many families were living in temporary shelters and difficult to locate. The outcome measurements were rigorous, both in terms of training and standardization, and in the use of appropriate instruments. The compliance to the treatment provided in the study was well-monitored as it was single-dose and provided by the RA during the study visit. In terms of analyses, the primary

results were expressed using an ITT approach, as recommended in the Consort guidelines. Multiple imputation, considered the gold standard, was used to impute outcome measurements for participants who were lost to follow-up. Other trials reviewed in preschool-age populations did not use an ITT approach, but rather expressed the final results using complete cases only, which could have led to biased results. In the current trial, the consistency of results from additional sensitivity analyses, including complete case and per-protocol analyses, suggest that the final population of children attending the visit was likely representative of the original study population.

Limitations of this study include the availability of deworming medications from other sources in the community. Although albendazole and mebendazole are contraindicated under two years of age in Peru, deworming is readily available from pharmacies, health centres and campaigns. The presence of community-based campaigns increased during the study period due to extreme flooding. Deworming is often provided without screening or a prescription to children with symptoms of gastrointestinal upset or diarrhea, which are common in early preschool-age children. The report of receiving deworming outside of the trial protocol was likely underestimated as it was by self-report at each follow-up visit (i.e. for the previous six months). The actual receipt of deworming outside of the trial differed among groups, with the highest non-compliance in the control group (i.e. Group 4). This non-compliance would likely have reduced the effect size between treated and untreated groups.

The study was also limited by the fact that stool specimens from those receiving placebo were analyzed by the lower sensitivity direct method. The storage of specimens to be read at the end of the trial may have contributed to an even further reduction in sensitivity. Therefore, misclassification of STH infection status would have affected Group 1 at the 18-month visit, Group 2 at the 12-month visit, and Group 4 at both the 12- and 18-month visits. This was considered a necessary trade-off to balance ethical, epidemiological and logistical issues. Some misclassification of specimens analyzed by the Kato-Katz method would

also have been present, as sensitivity and specificity are below 100% (Tarafder et al. 2010). The performance of this diagnostic method is thought to be further reduced when detecting light intensity infections, as was common in this study population (Booth et al. 2003, Knopp et al. 2008).

Due to the large sample size, and high morbidity of diarrheal illnesses, influenza, and other infections in this age group, active search of adverse events was not considered feasible. Thus, information on adverse events was collected in a passive manner and was dependent on report from caregivers or from notification from health centres (more common in the case of serious adverse events), between visits or at follow-up visits. Therefore, the true rate of adverse events in the study cannot be estimated.

The STH infection prevalence at baseline was approximately half of what had been found in a study conducted in the same study area in 2007. The number of children who could have potentially benefited from deworming in the trial was therefore reduced, resulting in a greater dilution of the effect size than had been anticipated.

Lastly, while the Bayley-III instrument was adapted for use in the study population and was administered in a stringent manner to ensure standardization among RAs, it was not additionally validated. As a result, the development results presented should be used for making comparisons within the same study population only, and not for comparison to other studies.

Generalizability

The overall results are relevant to children in the second year of life in other STH-endemic areas. These results may not be generalizable to areas with much lower or higher STH infection prevalence and intensity or malnutrition, or to areas with high prevalence of co-infection with other tropical diseases (e.g. malaria).

Results dissemination and future research

Three articles based on results from the trial have been submitted for publication in international peer-reviewed journals and included in this thesis. Presentations on various topics related to the trial have also been conducted in several national and international scientific conferences (see Appendix 9). The results will also be sent to newsletters such as Action Against Worms and to global initiatives such as Deworm the World, Children without Worms, the Mebendazole Advisory Committee, Partners for Parasite Control and Partnership for Child Development, among others. SAJ received a CIHR Planning and Dissemination Grant to support results dissemination activities, which are planned at the Pan American Health Organization (PAHO), WHO, and at various government, academic, and civil society institutions in Peru. Results will also be shared with study participants in Iquitos.

Additional publications that will be written by SAJ from data obtained from the project include:

1. Cost-effectiveness and policy implications of deworming in preschool-age children
2. Efficacy of mebendazole in 24-month old children
3. A systematic review of deworming in children under 5 years of age
4. An in-depth examination of the influence of compliance in the trial using Complier Average Causal Effect methodology
5. Ethical issues in the trial, including the use of open-label vs. placebo-controlled designs and collection and analysis of specimens from non-treated participants

Additional research which would benefit this and similar populations include a determination of the most appropriate combination of integrated nutrition, health and social interventions to combat the high prevalence of malnutrition and infection. This information is imperative to target the factors that prevent these vulnerable populations from escaping the vicious cycle of poverty.

The cohort of children recruited from the trial are currently being followed-up on a once-yearly basis up to five years of age to examine longer-term effects of the early deworming intervention on growth and development.

Implications for policy and practice

With the deadline to achieve the MDGs quickly approaching, and a shift in focus to the post-MDG agenda, it is imperative that attention remains on improving the health of children under two years of age. Poor health, malnutrition and developmental deficits in this critical window can have lasting detrimental effects into school-age and adulthood. Appropriate interventions are therefore needed to target the high infection and malnutrition burden in vulnerable child populations living in areas of extreme poverty. Currently, routine deworming of preschool-age children is not provided in Peru, and children under two years of age are specifically excluded from deworming programs. These practices are similar in other STH-endemic areas. This study contributes to providing practical guidance to governments and program managers in integrating deworming into health packages in early childhood, along with other routine interventions such as vaccinations and supplementations. Deworming can easily be provided using existing health infrastructure, or delivered in community-based campaigns for hard-to-reach populations. Overall, these results contribute to WHO policy and recommendations on deworming targeting preschool-age children in the over 100 STH-endemic areas of the world. They also contribute to providing practical guidance to governments in integrating deworming into early childhood health care.

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APPENDIX 1

Eligibility criteria checklist *(Criterios de Elegibilidad)*

CRITERIOS DE ELEGIBILIDAD

Nombre del participante: _____

Fecha de contacto: / /
(dd/mm/aaaa)

Marque según respuesta obtenida.

ITEM	CRITERIO	SI	NO
1	La visita de CRED de los 12 meses de edad de su niño(a) lo realiza en el establecimiento de salud del estudio.		
2	Vive usted con su niño en la zona de estudio (Iquitos o comunidades vecinas)		
3	Tiene usted programado llevar a su niño al EESS por una consulta médica por infección parasitaria.		
4	En los últimos 06 meses su niño(a) ha recibido tratamiento antiparasitario ó usó alguna de las siguientes plantas medicinales: Ojé ó Paico.		
5	Tiene planes de mudarse con su niño(a) fuera de la ciudad para los próximos 12 meses.		
6	Su niño(a) tiene menos de 12 meses ó más de 14 meses de edad.		
7	Presenta actualmente su niño(a) alguna condición médica crónica o congénita severa:		
	a) Labio leporino / paladar hendido		
	b) Congénitas de tubo neural: espina bífida		
	c) Congénitas de las extremidades		
	d) Congénitas de cabeza: microcefalia / macrocefalia		
	e) Prematuridad extrema ($\leq$ 28 ss)		
	f) Hipoxia al nacer (sufrimiento fetal severo, APGAR 5' $\leq$ de 3)		
	g) Desnutrición crónica severa		

Comentarios:_____

_____

_____

_____
Iniciales AI/ Fecha

PROTOCOLO: *Mejorar el crecimiento y desarrollo en la infancia temprana en los países de bajos recursos a través de un programa de desparasitación incorporado en la atención integrada de salud infantil*

APPENDIX 2

Informed Consent *(Consentimiento Informado)*

CONSENTIMIENTO INFORMADO

MEJORAR EL CRECIMIENTO Y DESARROLLO EN LA INFANCIA TEMPRANA EN LOS PAISES DE BAJOS RECURSOS A TRAVES DE UN PROGRAMA DE DESPARASITACION INCORPORADO EN LA ATENCION INTEGRADA DE SALUD INFANTIL

Asociación Civil SELVA AMAZONICA

McGill

Investigadores e Instituciones

Canadá: Dra. Theresa W. Gyorkos (Jefe de Protocolo), Instituto de Investigación del University Health Centre - McGill University); Lic. Serene A. Joseph (Co-investigadora, Universidad McGill); Dra. Grace Marquis (Co-investigadora, Universidad McGill); Dra. Elham Rahme (Co-investigadora, McGill University Health Centre); Dr. Brian Ward (Co-investigador, McGill University Health Centre)

Perú: Dr. Martín Casapia (Investigador Principal , Asociación Civil Selva Amazónica); Nut. Hilary M. Creed-Kanashiro (Consultora, Instituto de Investigación Nutricional).

Suiza : Dr. Antonio Montresor (Consultor, Organización Mundial de la Salud).

Introducción

Le invitamos a usted y a su hijo/a a participar en un programa de desparasitación que va a ser conducido por investigadores de Canadá, Suiza y Perú. Este tipo de programa de desparasitación donde un medicamento antiparasitario es proporcionado a los niños en edad preescolar en zonas altamente endémicas es recomendado por la Organización Mundial de la Salud. Este estudio está financiado por Fondos de Investigación Thrasher (Estados Unidos) y los Institutos Canadiense de Investigación en Salud (Canadá) y es

implementado a través de la Asociación Civil Selva Amazónica.

Si usted decide que su niño participe en el estudio, a usted y a su pareja se le solicitará que firme el CI antes de que usted decida la participación de su hijo/a en este estudio, es importante que usted entienda el contenido de este formato de consentimiento, los riesgos y beneficios para tomar una decisión informada, y que haga cualquier pregunta si hay algo que usted no entiende. Por favor lea completamente este formato de consentimiento y tómese su tiempo para decidir. Si usted decide que su hijo participe en este estudio, usted y su conyugue serán invitados a firmar este formato de consentimiento informado.

Propósito del estudio

El propósito de este estudio es determinar el beneficio de una intervención de desparasitación sobre el crecimiento y desarrollo de niños entre 12 y 24 meses de edad. El total de beneficio de la desparasitación en este grupo de edad, quienes están precisamente empezando a infectarse con parásitos intestinales, no es aun conocido. Este estudio nos permitirá determinar tres cosas: 1) si hay un beneficio de la desparasitación; 2) si hay un beneficio dependiendo de cuándo la desparasitación es proporcionada (por ejemplo, a los 12 meses de edad o 18 meses de edad); y 3) si hay un beneficio dependiendo de con qué frecuencia esto es proporcionado (por ejemplo, una o dos veces al año). Actualmente, no hay programas de desparasitación estándares ofrecidos para niños en edad preescolar en esta área y la desparasitación no está incluida como parte de los servicios de rutina proporcionadas por el Ministerio de Salud durante las visitas de CRED. Por lo tanto el régimen de desparasitación que su niño recibirá actualmente no está regularmente administrado en el Perú.

Número de participantes y* duración *de su participación.

Nosotros reclutaremos a 1760 niños durante sus visitas de rutina de CRED de los 12 meses de edad, en Iquitos y los alrededores. Cada niño participará en este estudio por 12 meses.

Este estudio tendrá una duración total de 20 meses

Tratamientos Del Estudio

Cada niño que participará en este estudio tomará una tableta a los 12, 18 y 24 meses de edad durante sus visitas de CRED, la tableta puede tener Mebendazol o Placebo. El placebo es una tableta que no tiene ningún medicamente activo pero parece idéntico al Mebendazol.

Cada niño será asignado al azar (como lanzar una moneda al aire) para estar en 1 de los 4 grupos de tratamiento y recibirá la tableta la cual debería ser tomada oralmente de acuerdo a la siguiente asignación:

Grupo 1: Administración de Mebendazol a los 12 meses y administración de placebo a los 18 meses.

Grupo 2: administración de placebo a los 12 meses y administración de Mebendazol a los 18 meses.

Grupo 3: administración de Mebendazol a los 12 meses y 18 meses.

Grupo 4: administración de placebo a los 12 meses y 18 meses.

El paquete de cuidado de salud estándar será dada junto con las tabletas en todas las visitas de CRED de acuerdo a las guías del Ministerio de Salud.

Todos los niños de este estudio recibirán Mebendazol a los 24 meses de edad.

Ni usted, ni el equipo de estudio sabrán qué tratamiento ha recibido su hijo(a).

Procedimientos del estudio

Si usted está de acuerdo que su niño participe en este estudio se le solicitará que nos proporcione su permiso para evaluar a su hijo(a) en tres oportunidades durante sus visitas de rutina de CRED: una vez a los 12 meses de edad, una vez a los 18 meses de edad y una vez a los 24 meses de edad al final del estudio.

1) en primer lugar, tomaremos el peso, talla y las medidas medio superior de la circunferencia del brazo de su hijo(a). Le haremos algunas preguntas a usted acerca de la salud de su hijo(a) y de información socio demográficas

general acerca de su familia. También le pediremos que nos proporcione una muestra de heces de su hijo(a).

2) En cada visita, todos los niños recibirán el servicio de rutina de CRED proporcionado por el centro de salud, incluyendo las vacunas programadas.

En las visitas de los 12 y 18 meses de edad, le pediremos a todos los niños muestras de heces . Algunas de estas muestras de heces serán seleccionadas para ser analizadas inmediatamente por un método de laboratorio. Sin embargo, todas las muestras de heces en ambas ocasiones se conservarán para ser examinadas al final del estudio por otro método de laboratorio. Al aceptar la participación de su hijo en este estudio, usted nos da permiso para examinar las muestras de heces de su hijo(a) por ambos métodos. Las muestras de heces serán utilizadas solamente en este estudio y serán destruidas inmediatamente después de que hayan sido analizadas en la visita final a los 24 meses. Se le entregará los resultados de todos los análisis de las muestras de heces al final del estudio.

Riesgos e incomodidades

La pastilla antiparasitaria Mebendazol, es muy segura y efectiva y está siendo administrada a los niños en edad preescolar en muchos países del mundo. La mayoría de los niños no presentan efectos secundarios después de la desparasitación. Si los síntomas ocurren, son leves y temporales y suelen desaparecer dentro de las 48 horas. Estos síntomas pueden incluir nauseas, vómitos y otros síntomas de malestar gastrointestinal, o dolor de cabeza y mareos.

Se molerá y se mezclará la pastilla con fruta o agua para que su hijo(a) no tenga ninguna dificultad en deglutir la tableta. La Organización Panamericana de la Salud y la Organización Mundial de la Salud consideran que el Mebendazol es muy seguro y de hecho, recomiendan el suministro de esta pastilla a los niños en edad preescolar donde los parásitos son comunes.

Beneficios Potenciales

Estos parásitos pueden causar alteración en el desarrollo y crecimiento físico e intelectual de su hijo(a). El beneficio directo que tendrá su hijo(a) al participar en este

estudio es que recibirá tratamiento para estos parásitos intestinales al final del estudio y también puede recibir tratamiento durante el estudio. Esto puede mejorar la salud de su hijo(a).

Alternativas de tratamiento

Alternativas de tratamiento disponibles en vez de Mebendazol incluyen Albendazol (con prescripción médica), que tiene menor eficacia para las lombrices más comunes en niños de 1 a 2 años. Ni el Mebendazol ni el Albendazol se suministran durante las visitas rutinarias de CRED.

Costos y compensación

No hay ningún costo asociado con su participación en este estudio. Todos los tratamientos y el análisis de las muestras de heces serán gratuitos durante la duración del estudio.

Usted recibirá un reembolso equivalente a S/.5.00 por transporte local cuando usted asista a la visita de estudio con su niño. Nosotros no daremos compensación por alimentación durante las visitas.

Confidencialidad

Toda la información obtenida durante este estudio será mantenida en estricta confidencialidad. Su nombre y el nombre de su hijo(a) quedarán anónimos para todas las personas que no son integrantes del equipo de investigación. La información que usted nos proporciona, incluyendo cuestionarios y formatos de consentimiento informado, se pondrá en un armario cerrado en la oficina del investigador y será mantenido por cinco años según procedimientos estándares de investigación. Los datos se mantendrán electrónicamente guardados en una computadora con contraseña para siempre; pero no se incluirán los nombres. Todas las muestras de heces serán guardadas en un refrigerador de investigación. Las muestras serán identificadas solamente con el número de identificación y sin nombre adjunto, y serán descartadas al final del estudio. Sólo el equipo de investigación podrá tener acceso a la información del estudio, y sólo después de primero haber recibido la aprobación del investigador principal. Los resultados de este estudio

09 Agos 2011
06 Agos 2012

serán publicados pero los nombres de los participantes no serán utilizados en ningún momento. Su identidad no será revelado en los resultados combinados. Para verificar los datos de la investigación, el Oficial de Garantía de Calidad de la Junta de Ética de Investigaciones del Centro Universitario de Salud McGill puede revisar estos registros. Además, habrá un Comité de Seguridad y Monitoreo de Datos (CSMD) que tendrá acceso a los datos en tres momentos durante el estudio para asegurar que la intervención de desparasitación sea segura. Al Firmar este formato de consentimiento, usted nos da permiso de proporcionar la información relacionada a su participación en este estudio a estos funcionarios.

Participación voluntaria y retiro de este estudio

La participación de su hijo(a) en este estudio es estrictamente voluntaria. Usted puede negar la participación de su hijo(a) en el estudio o puede descontinuar su participación en cualquier momento sin explicación, y sin penalidad o pérdida de beneficios a los que tiene derecho. Si usted descontinúa la participación de su hijo(a), su hijo no sufrirá ningún perjuicio sobre su atención medica o su participación en cualquier otro estudio de investigación.

Si hay algún nuevo descubrimiento acerca del medicamento durante la duración del estudio un miembro del equipo de investigación se comunicará directamente con usted.

Además, usaremos la información de este estudio para informar a los servicios de salud pública en el Perú y en otros paises en proporcionar programas de desparasitación para los niños en edad preescolar. Usted recibirá los resultados del estudio al final de la investigación es decir cuando el seguimiento haya terminado en todos los niños participantes en el estudio.

Tratamiento de daños o lesiones por su participación en este estudio

Si su hijo(a) sufre un daño o lesión relacionado a su participación en el estudio, el costo del tratamiento médico será cubierto por los patrocinadores, usted puede regresar al centro de salud si su niño presenta algún síntoma o puede solicitar la atención médica en la clínica Selva Amazónica donde un infectólogo o pediatra le proveerá atención médica y tratamiento sin costo.

Descontinuación total o parcial de la participación

Los niños deben descontinuar el producto de investigación por alguna de las siguientes razones

- Retiro del Consentimiento informado (decisión de los padres de retirarlo por alguna razón)
- Algún evento adverso severo

Asuntos Éticos

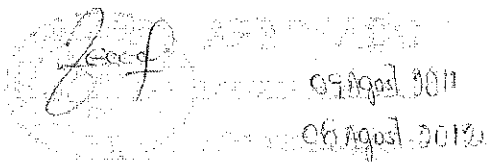
Nos gustaría asegurarle que este estudio fue revisado y aprobado por la Oficina de Revisión Ética del Centro Universitario de Salud McGill en Montreal, Canadá, el Comité de ética de la Universidad Peruana Cayetano Heredia, el Instituto Nacional de Salud en Lima, y la Dirección Regional de Salud de Loreto.

Persona de contacto

Si tiene alguna pregunta acerca del estudio, usted puede contactarse con el coordinador local del proyecto, Lic.Obst. Lidsky Pezo al 065-236277 anexo 151 (fijo) o 0800-13231 (línea gratuita) ó 965682004.

Si su hijo(a) sufre algún síntoma relacionado con este estudio, por favor comuníquese con el Dr. Martin Casapía al 065-236277 ó al 065-965621830. También puede presentarse a la oficina de la Asociación Civil Selva Amazónica, que está ubicada en Urb. Jardín N° 27, Fanning 4ta Cuadra, Iquitos.

Si tiene alguna pregunta sobre los derechos de su hijo(a) como participante en este estudio, o alguna duda o inquietud, comuníquese con el Dr. Leonid Lecca García Presidente del Comité Institucional de Ética de la Universidad Cayetano Heredia al teléfono 319-0000 anexo 2271, correo electrónico duict.cieh@oficinas-upch.pe Dirección del Comité de Ética de la Universidad Peruana Cayetano Heredia: Av. Honorio Delgado 430, Urb. Ingeniería, San Martín de Porras, Lima 31.



Declaración de consentimiento

He comprendido los contenidos de este formato de consentimiento informado, y estoy de acuerdo con la participación de mi hijo(a) en este estudio de investigación. He tenido la oportunidad de hacer preguntas y todas mis preguntas han sido resueltas a mi satisfacción. He tenido tiempo suficiente para considerar la información antes mencionada y pedir consejos si elijo hacerlo. Firmando este formato de consentimiento, no estoy renunciando a mis derechos legales. Estoy de acuerdo en guardar una copia del formato de consentimiento informado, que me darán en este momento.

Nombre de niño/a		
Nombre de la madre	Firma de la madre	Fecha (dd/mm/aaaa)
Nombre del padre/tutor legal	Firma de padre/tutor legal	Fecha (dd/mm/aaaa)
Nombre de testigo	Firma de testigo	Fecha (dd/mm/aaaa)
Nombre del entrevistador(a)	Firma del entrevistador(a)	Fecha (dd/mm/aaaa)

APPENDIX 3

Evaluation of Understanding (for consent form) *(Evaluación de Entendimiento)*

EVALUACION DE ENTENDIMIENTO (EDE)

Nombre del participante: _____ Fecha de contacto: / /

(dd/mm/aaaa)

ITEM	CRITERIO	SI	NO	REFORZADA	ACLARADA
1	Con este estudio sabremos si hay un beneficio |con la desparasitación en niños menores de 2 años.				
2	El Ministerio de Salud cuenta con un programa de desparasitación para niños de 1 año.				
3	El niño(a) participará por 1 año en el estudio.				
4	Traerá a su niño(a) al establecimiento de salud para su control de CRED cuando cumpla los 12, 18 y 24 meses de edad.				
5	En las visitas de control a su niño(a) le tomarán el peso, talla y pruebas para evaluar su desarrollo cognitivo y motor.				
6	Usted colectará una muestra de heces de su niño(a) en las 3 visitas del estudio y lo llevará al establecimiento de salud para entregar al asistente de investigación.				
7	Los resultados de los análisis de heces serán entregados al final del estudio				
8	Su niño(a) tomará una medicina o placebo en las 3 visitas del estudio.				
9	La medicina o placebo que se asigna a cada niño será decisión del asistente de investigación.				
10	La medicina de estudio puede causar síntomas como náuseas y dolor de estómago en los 02 primeros días de haberlo tomado.				
11	La participación de su niño(a) en el estudio es obligatorio.				
12	La atención que su niño(a) recibe en el estudio reemplaza a la atención brindada en el programa de CRED				

_____
Iniciales AI / Fecha

APPENDIX 4

Sworn declaration for single parent *(Declaración jurada)*

DECLARACION JURADA SIMPLE

Por la presente, yo_____,
Identificado/a con DNI Nº _____ en calidad de _____ del menor _____, declaro que después de haber recibido la información, absuelto las interrogantes y dudas del estudio "*Mejorar el crecimiento y desarrollo en la infancia temprana en los países de bajos recursos a través de un programa de desparasitación incorporado en la atención integrada de salud infantil*", Consiento Informado/a y Voluntariamente la participación de mi menor hijo/a en el estudio, así mismo que de acuerdo al Artículo 35 del Reglamento de Ensayos Clínicos del Instituto Nacional de Salud del MINSA, dispenso la no obtención del Consentimiento Informado por parte del padre/madre de mi menor hijo _____
por _____,
por lo que suscribo la presente Declaración Jurada Simple , estableciendo que lo manifestado está conforme a la realidad de los hechos.

_____ _____ _____

Firma **Fecha**

Nombre:
DNI:

_____
Iniciales AI / Fecha

APPENDIX 5

Baseline (12-month) household questionnaire and Bayley Record Forms

(Encuesta de visita domiciliaria a los 12 meses and Formato Bayley III)

Encuesta de visita domiciliaria a los 12 meses

Mejorar el crecimiento y desarrollo en la infancia temprana en países de bajos recursos a través de un programa de desparasitación incorporado en la atención integrada de salud infantil

1. IDENTIFICACIÓN DEL PARTICIPANTE, ENTREVISTADOR

INICIALES DE ENTREVISTADOR: _____

APELLIDO / NOMBRE DEL NIÑO/A: _____/_____

DIRECCIÓN: _____

DISTRITO: _____

FECHA DE ENTREVISTA: / / (dd/mm/aaaa)

CÓDIGO DE IDENTIFICACIÓN: ☐☐ - ☐☐☐ (EESS - PARTICIPANTE)

URBANO ☐ RURAL ☐ PERI-URBANO ☐

TELÉFONO: _____

2. CONSENTIMIENTO INFORMADO

A	¿Consentimiento obtenido?	☐ Sí *Realice la pregunta B*	☐ No *Excluir su participación*
B	¿Tiene consentimiento firmado por ambos padres o tutor?	☐ Sí *Realice la pregunta 1*	☐ No *Realice la pregunta C*
C	¿Razón que no tiene ambas firmas? ☐*No aplica (NA)*	☐ Padre/madre murió (1) ☐ Padre/madre soltero(a) (2)	☐ Padre/madre trabaja fuera (3) ☐ Otro_____ (4)

3. DATOS DE LA FAMILIA Y LA CASA

1	Señora, ¿Cuál es su nombre? (Apellido(s) / Nombre(s))	_____/_____	
2	¿Cuándo nació usted? (dd/mm/aaaa)	/ / *No sabe* ☐	Edad (años) _____
3	¿Estado civil de la madre?	☐ Casada/conviviente (1) ☐ Soltera, con pareja (2)	☐ Soltera, sin pareja (3) ☐ Separada/divorciada (4)
4	¿Cuál es el nivel de educación más alto que usted tiene? _____ ☐*No asistió (0)*	*Primaria:* ☐ Completa (1) ☐ Incompleta → # Grados completados: ____ *Secundario:* ☐ Completa (2) ☐ Incompleta → # Grados completados: ____ *Superior:* ☐ Completa (3) ☐ Incompleta → # Años completados: ____	
5	Señora, ¿A qué se dedica? *Especificar* _____	☐ Ama de casa (1) ☐ Trabajo fuera de la casa (2)	☐ Trabajo en casa (3)
6	Si tiene pareja, ¿A qué se dedica? *Especificar* _____ ☐*No tiene pareja* (6)	☐ Tareas de hogar (1) ☐ Trabajo fijo, ingreso fijo (2) ☐ Trabajo fijo, ingreso variable (3) No trabaja_____ (0)	☐ Eventual (4) ☐ Jubilado (5)
7	¿Cuántas personas en total viven en la casa (comparten olla familiar)? (incluir madre y niño participante)	_____ personas	
8	¿Cuántos hijos tiene usted?	¿0-4 años? _____ ¿5-14 años? _____ ¿15-17 años? _____ ¿≥18 años? _____	

Obs

POR CONFIRMAR DURANTE LA VISITA EN LA CASA

☐	**9**	¿De qué material está hecha la casa?	☐ Noble (1) ☐ Rústica (2)
☐	**10**	En su casa, ¿Qué usa usted para cocinar?	☐ Gas (1) ☐ Carbón (2) ☐ Leña (3) ☐ No cocina (4) ☐ Otro_____ (5)
☐	**11**	¿Tiene energía eléctrica pública en casa?	☐ Sí (1) ☐ No (0)
☐	**12**	¿Tiene radio en casa?	☐ Sí, funciona (1) ☐ Sí, no funciona (2) ☐ No (0)
☐	**13**	¿Tiene televisor en casa?	☐ Sí, funciona (1) ☐ Sí, no funciona (2) ☐ No (0)
☐	**14**	**A-** ¿Tiene agua potable en su casa (agua de grifo)?	☐ Sí (1) ☐ No (0)
☐		**B-** En caso que no, ¿de dónde obtiene el agua? _____ ☐*No aplica (NA)*	☐ Grifo vecino (1) ☐ Pozo artesiano (4) ☐ Camión cisterna (2) ☐ Pileta pública (5) ☐ Río (3) ☐ Agua de lluvia (6) ☐ Otro _____ (7)
	15	¿Qué hace con el agua antes de tomarlo?	☐ Toma directamente (1) ☐ Hierve (2) ☐ Trata (3)
☐	**16**	¿Dónde hace usted sus necesidades (describir cómo es su baño)?	☐ Directo al río (1) ☐ Taza con agua y desagüe público (5) ☐ Silo o letrina sin desagüe público (2) ☐ Silo o letrina conectada al desagüe público (6) ☐ Campo abierto (3) ☐ Pozo séptico (7) ☐ Letrina con drenaje al río (4) ☐ Otro_____ (8)
	17	¿Dónde hace su hijo sus necesidades?	☐ Pañal descartable (1) ☐ Bacín (4) ☐ Pañal de tela (2) ☐ Papel periódico (5) ☐ Baño (3) ☐ Otro _____ (6)
	18	¿Dónde bota las deposiciones de su hijo? →*si es basurero confirmar si es recogida por servicio público*	☐ Río (1) ☐ Basurero (3) ☐ Otro_____ (5) ☐ Campo/ Monte (2) ☐ Baño (4)

4. PREGUNTAS DEL EMBARAZO, PARTO, RECIEN NACIDO *(sobre el embarazo del hijo participante)*

Verificado en el carné de embarazo ☐ Sí ☐ No

1	**A-** ¿Usted asistió al Control Prenatal durante el embarazo con *(nombre)*?	☐ Sí (1) ☐ No (0)
	B- Si es que sí, ¿A cuántos controles? _____	☐*No aplica (NA)*
	C - Si es que sí, ¿Dónde? _____ ☐*No aplica (NA)*	☐ Hospital (1) ☐ Puesto de salud (3) ☐ Centro de salud (2) ☐ Particular (4) ☐ Otro_____ (5) Nombre del lugar: _____
2	¿Durante el embarazo, tomó usted…?	**Ai-** ¿Algún suplemento de hierro (ej. Sulfato ferroso)? ☐ Sí (1) ☐ No (0)
		Aii- Si es que sí, ¿por cuánto tiempo? _____ ☐meses ☐días ☐ *No aplica (NA)*
		Bi- ¿Algún antiparasitario (para los bichos)? (ej. Mebendazol/albendazol) ☐ Sí_____ (1) ☐ No (0)
3	¿Dónde dio a luz?	☐ Hospital (1) ☐ Puesto de salud (3) ☐ Casa (5) ☐ Centro de salud (2) ☐ Particular (4) ☐ Otro_____ (6) Nombre del lugar: _____

5. PREGUNTAS ACERCA DEL NIÑO (Verificado en el carné de CRED ☐ Sí ☐ No)

1 ¿Fecha de nacimiento del niño? (dd/mm/aaaa) ____/____/____ *No sabe* ☐ Edad (meses) ____

2 ¿Sexo del niño? ☐ Varón (1) ☐ Mujer (0)

3 A - ¿Cuál fue el peso/talla/puntaje de APGAR de su hijo al nacer?

A- Peso al nacer: _____.____ kg ☐*No sabe* ☐*Por referencia*

B- Talla al nacer: _____.____ cm ☐*No sabe* ☐*Por referencia*

C- APGAR a 5 min _____ ☐*No sabe* ☐*Por referencia*

B- ¿Al momento de nacer, demoró su hijo en llorar?

☐ Sí (1) → ¿Por cuánto tiempo? _____ ☐min ☐ seg

→ ¿Necesitó reanimación u otro tipo de asistencia? ☐Si (1) ☐No (0) ☐*No sabe (99)*

☐ No (0)

☐ *No sabe (99)*

4 ¿A cuántos controles de Crecimiento y Desarrollo (CRED) ha asistido su hijo desde que nació?

☐*No asistió antes*

A- Entre 0-5 meses _____ **B-** Entre 6 a 11 meses _____

C- ¿Fecha de la última visita (dd/mm/aaaa)? ____/____/____

D- Establecimiento de salud (EESS) _____

5 **A-** ¿Su hijo recibió la vacuna de Sarampión, Rubeola y Paperas? ☐ Sí *(→ B)* (1) ☐ No *(→ C)* (0)

B-Si es que sí, ¿cuándo? (dd/mm/aaaa) ____/____/____

☐*No aplica (NA)*

C- Si es que no, ¿está programada la vacunación? ☐ Sí (1) → ¿Para cuándo? (dd/mm/aaaa) ____/____/____

☐*No aplica (NA)* ☐ No (0) → ¿Por qué?_____

6 ¿Qué otras inmunizaciones recibió su hijo en el EESS desde que nació? *(Confirmar en carné)*

Marcar Si o No o No Sabe

	1ra dosis	2da dosis	3ra dosis
Tuberculosis (BCG)			
Antihepatitis			
Antipolio			
Pentavalente			
Rotavirus			
Antineumococica			
Otro_____			

7 ¿Su hijo recibió vitamina A...?

A- ¿A los 6 meses de edad? ☐ Sí (1) ☐ No (0)

B - ¿En algún otro momento? ☐ Sí (1) ¿Cuándo?_____ ☐ No (0) *No sabe* (99)

8 **A-** ¿Su hijo toma algún suplemento de hierro? (ej. Sulfato ferroso) *(ver si tiene muestra)* ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B- Si es que sí, ¿desde hace cuánto tiempo lo está tomando? _____ ☐días ☐semanas ☐meses

☐*No aplica (NA)*

9 **A-** ¿Recibió su hijo un antiparasitario en los primeros 6 meses de vida? ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B- Si es que sí, ¿cuál fue el medicamento? ☐ Mebendazol (1) ☐ Albendazol (2)

☐*No aplica (NA)* ☐ Otro_____ (3) ☐ *No sabe* (99)

9

C- Si es que sí, ¿en qué forma? ☐ Jarabe (1) ☐ Pastilla (2) ☐ *No sabe* (99)

☐*No aplica (NA)*

D –Si es que sí, ¿cuál fue la dosis? ☐ Único (1) ☐ Otro:_____(# veces por día) × _____ (# días)

☐*No aplica (NA)*

E –Si es que sí, ¿dónde lo recibió? ☐ EESS (1) ☐ Campaña en iglesia (4) ☐ Farmacia (2) ☐ Otro_____ (5) ☐ Campaña en escuela (3) ☐ *No sabe* (99)

☐*No aplica (NA)*

10

A- ¿Su hijo toma algún otro suplemento, vitamina o medicamento actualmente? ☐ Sí (1) ☐ No (0)

B- Si es que sí, especificar: *(Preguntar si tiene una muestra)* **1-**_____ **3-**_____ **5-**_____

☐*No aplica (NA)* **2-**_____ **4-**_____ **6-**_____

11

A- ¿En algún momento su hijo ha tenido dengue (confirmado en EESS)? ☐ Sí (1) ☐ No (0)

B - Si es que sí, ¿cuántos meses tenía? _____ meses

☐*No aplica (NA)*

12

A- ¿En algún momento su hijo ha tenido malaria (confirmado en EESS)? ☐ Sí (1) ☐ No (0)

B - Si es que sí, ¿cuántos meses tenía? _____ meses

☐*No aplica (NA)*

13

A- ¿Su hijo ha sufrido de otro problema de salud que requirió hospitalización? ☐ Sí (1) ☐ No (0)

B- Si es que sí, especificar: _____

☐*No aplica (NA)*

14

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido tos o alguna dificultad respiratoria? ☐ Sí (1) ☐ No (0)

B- Si es que sí… **i-** ¿Por cuánto tiempo? _____ días

☐*No aplica (NA)* **ii-** ¿Qué medidas tomaron? _____

15

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido diarrea (**4 o más** deposiciones líquidas)? ☐ Sí (1) ☐ No (0)

B- Si es que sí… **i-** ¿Por cuánto tiempo? _____ días

☐*No aplica (NA)* **ii-** ¿Con sangre visible en las heces? ☐ Sí (1) ☐ No (0)

ii- ¿Qué medidas tomaron? _____

16

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido fiebre? ☐ Sí (1) ☐ No (0)

B- Si es que sí… **i-** ¿Por cuánto tiempo? _____ días

☐*No aplica (NA)* **ii-** ¿Medido con termómetro? ☐ Sí (1) → _____ ° C ☐ No (0)

ii- ¿Qué medidas tomaron? _____

17

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido algún problema de oído? ☐ Sí (1) ☐ No (0)

B- Si es que sí… **i-** ¿Dolor de oído? ☐ Sí (1) ☐ No (0)

☐*No aplica (NA)* **ii-** ¿Descarga ó secreción de los oidos? ☐ Sí (1) ☐ No (0)

iii- ¿Qué medidas tomaron? _____

iv- ¿Diagnóstico médico? ☐ Sí (1) ☐ No (0)

6. PREGUNTAS SOBRE EL DESARROLLO DEL NINO

1	*Observar si el niño cumple los siguientes logros de desarrollo motor grueso:*							
	A- pararse con apoyo	☐	Sí	(1)	☐ No	(0)	☐ *No sabe*	(99)
	B- gatear	☐	Sí	(1)	☐ No	(0)	☐ *No sabe*	(99)
	C- caminar con apoyo	☐	Sí	(1)	☐ No	(0)	☐ *No sabe*	(99)
	D- pararse solo	☐	Sí	(1)	☐ No	(0)	☐ *No sabe*	(99)
	E- caminar solo	☐	Sí	(1)	☐ No	(0)	☐ *No sabe*	(99)
2	**A-** Señora, ¿cuántos meses tenía su hijo cuando empezó a caminar solo?	_____ meses			☐ *No sabe*	(99)	☐ *No aplica (NA)*	

7. PREGUNTAS DE LACTANCIA Y ALIMENTACIÓN COMPLEMENTARIA

1	**A-** ¿Usted (*la madre*) le dio de lactar a (*nombre*)? ☐ Sí (1) ☐ No (0) **B-** Si es que sí, ¿sigue dando de lactar a su hijo? ☐ Sí (1) → ¿Cuántas veces? mañana_____ tarde_____ noche____ ☐ No (0) → ¿Hasta cuántos meses le dio de lactar? _____ meses ☐ *No aplica (NA)*	
2	**A-**¿Le ha dado algún té/infusión/agüita a su hijo? ☐ Sí (1) ☐ No (0) **B-** Si es que sí.. ☐ *No aplica (NA)* **i-** ¿Desde qué edad le dio? _____ ☐meses ☐días **ii-** ¿Sigue dándole? ☐ Sí (1) ☐ No (0) → ¿Hasta qué edad le dio? _____ ☐meses ☐días	
3	**A-** ¿Cuál fue lo primero que le dio a (*nombre*) de tomar que no fue leche materna ni fue té/infusión/agüita? *Especificar*_____	☐ Leche no materna (1) ☐ Jugo (2) ☐ Masato (3) ☐ Caldo (4) ☐ Gaseosa (5) ☐ Avena/quaker (6) ☐ Yogur (7) ☐ Otro_____ (8)
	B- ¿Cuántos días o meses tenía cuando le dio de tomar por primera vez? _____ ☐meses ☐días	
4	**A-** ¿Cuál fue la primera comidita que le dio a (*nombre)* para comer o probar?	☐ Sopa (1) ☐ Puré (2) ☐ Maduro (3) ☐ Galleta (4) ☐ Galleta (5) ☐ Alimento sancochado, aplastado (6) ☐ Otro _____ (7)
	B- ¿Cuántos días o meses tenía cuando le dio esta comidita por primera vez? _____ ☐meses ☐días	

5	¿Cuántos meses tenía su hijo cuando empezó a comer..?	**A-** Purés, mazamorras o papillas	_____	☐meses ☐días	☐*No sabe*	☐*No aplica (NA)*
		B- Maduro	_____	☐meses ☐días	☐*No sabe*	☐*No aplica (NA)*
		C- Frutas	_____	☐meses ☐días	☐*No sabe*	☐*No aplica (NA)*
		D-Verduras	_____	☐meses ☐días	☐*No sabe*	☐*No aplica (NA)*
		E- Alimentación de origen animal (Ej. carnes, ave, hígado, pescado, vísceras, sangrecita, huevo) *Especificar*_____	_____	☐meses ☐días	☐*No sabe*	☐*No aplica (NA)*

8. RECORDATORIO DE CONSUMO DE 24 HORAS: APRENDER QUE COME EL NIÑO

1	¿Cómo fue el apetito del niño ayer? *(Leer opciones)*	☐ Usual (normal) (1)	☐ Menos que usual (2)	☐ Más que usual (3)		
	→*Si consumió menos que lo usual (e.j. debido a enfermedad) preguntar cuando comió normal y pasar ese día*					
2	Señora: Vamos a hablar ahora sobre la alimentación que recibió (todito que comió) ayer su hijo. Solo la de ayer, desde que se despertó hasta que se durmió en la noche.					

Preguntas de Comida	**Hora Aprox.**	**Todos los alimentos/bebidas/lactadas. Si el alimento es una preparación listar ingredientes**	**Consistencia** Espeso=1; Sólido=2; Aguado=3
¿Ayer cuál fue la primera cosa que comió (nombre del niño) desde que se despertó? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
Después de su última comida ¿comió algo más antes de dormirse?			
Y durante la noche ¿le dio algo más?			

PREGUNTAS A LLENAR DESPUES DE COMPLETAR EL RECORDATORIO:

3	¿Comió un producto animal (carne/pescado/vísceras/ave/huevos) ayer?	☐ Sí (1)	☐ No (0)	
4	Número de preparaciones con consistencia: espesa ____	sólido____	aguado____	

COMENTARIOS: _____

_____

_____

_____

Asociación Civil SELVA AMAZONICA

McGill UNIVERSITY

L'Institut de recherche du Centre universitaire de santé McGill
The Research Institute of the McGill University Health Centre

Formato Bayley III: Cognitivo

ID: ___ ___ - ___ ___ ___ Edad del niño (meses y días): _____ Fecha de prueba ____/_____/____ Entrevistador _____

INSTRUCCIONES: Un niño de 12 meses puede empezar en el ítem 31. Si el niño pasa los primeros 3 ítems (31, 32, 33), avanza hacia adelante. Si el niño no pasa los 3 primeros ítems, va al ítem 28 y seguir adelante. En caso el niño no pasa los ítems 28, 29 y 30 empezar por el ítem 25. Detener la prueba cuando el niño obtiene un puntaje de 0 (cero) en 5 ítems consecutivos. Marcar 1 si el niño recibe crédito y 0 si el niño no recibe crédito.

	ITEM	MATERIALES	CRITERIO DE EVALUACION Y COMENTARIOS	INSTRUCCIONES VERBALES	CREDITO
	25. Busca el objeto caído	*el oso o patito de goma*	**Crédito:** si el niño mira hacia el suelo para buscar el juguete	Ninguno	
26 31	**26. Serie Campana: Manipula**	*campana*	**Crédito:** si el niño manipula la campana mientras la mira con evidente interés a los detalles	Ninguno	
27 33 37	**27. Serie Recoger Cubos: alcanza para el segundo cubo**	*3 cubos*	**Crédito:** si el niño alcanza el segundo cubo o intenta hacerlo mientras mantiene el primer cubo	Ninguno	
	28. Tira toallita para obtener objeto	*sonaja* *2 toallitas*	**Crédito:** si el niño jala la toallita y alcanza la sonaja	Ninguno	
	29. Jala cuerda adaptadamente **→ 41**	*aro con cuerda*	**Crédito:** si el niño coge la cuerda, la jala para asegurar el aro, y luego agarra el aro.	Ninguno	
	30. Conserva 2 cubos **→33, 37**	*2 cubos* ***3 segundos***	**Crédito:** Si el niño recoge/agarra los dos cubos al mismo tiempo durante 3 segundos	Ninguno	
26 31	**31. Serie Campana: Suena a propósito**	*campana*	**Crédito:** si el niño mantiene la campana por el mango y la toca a propósito	Ninguno	

INICIO 12 meses

	32. Mira dibujos en libro de cuentos	*Libro de cuentos*	**Crédito**: si el niño mira uno o más imágenes específicas con interés	**"Mira, ve"** (Max 2 veces)	
27 33 37	**33. Serie Recoger Cubos: Conserva 2 de los 3 cubos ofrecidos**	*3 cubos* ***3 segundos***	**Crédito**: si el niño mantiene los 2 primeros cubos por al menos 3 segundos después de atender visualmente al 3er cubo.	Ninguno	
	34. Busca los objetos perdidos	*3 cubos* *taza con asa*	**Intentos: 2 Crédito**: si el niño se percata que la taza esta vacía (mira dentro de la taza) Intento 1 ☐ Intento 2 ☐	Ninguno	
	35. Saca el cubo de la taza	*3 cubos* *taza con asa* ***cronómetro*** ***2 minutos***	**Crédito**: si el niño saca los tres cubos (con intención) dentro de 2 minutos	**"Sacalos"** (Max 2 veces)	
36 54	**36. Serie Cubos: Pone 1 cubo en la taza**	*9 cubos* *taza con asa*	**Crédito**: si el niño coloca un cubo dentro de la taza o intenta hacerlo, aunque no lo suelte (no importa si se queda dentro después que esté colocado). Número de cubos en taza: _____	1er cubo: **"Pon el cubo en la taza. Ponlo en la taza"** (Max 3 veces) 8 cubos: **"Pon los cubos en la taza. Ponlos todos"** (Max 3 veces)	
27 33 37	**37. Serie Recoger Cubos: 3 cubos**	*3 cubos*	**Crédito**: si el niño mantiene los dos primeros cubos y recoge o intenta recoger (golpea, alcanza, etc.) el tercer cubo	Ninguno	
	38. Explora agujeros en portapalos **→ 47, 55**	*portapalos*	**Crédito**: si el niño mete un dedo dentro de un o más agujeros	**"¿Ves?"** (Max 2 veces)	
	39. Empujar el carro	*Carro*	**Crédito**: si el niño empuja el carro (con intención) con las cuatro ruedas sobre la mesa	**"Empújalo"** (Max 3 veces)	
	40. Encuentra objeto escondido **→45, 50**	*2 toallitas* *pulsera*	**Intentos: 2 Crédito**: si en el primero intento el niño encuentra la pulsera debajo de la toallita correcta, una vez por toallita. Intento 1: ☐ Izquierda ☐ Derecha Intento 2: ☐ Izquierda ☐ Derecha	**"Mira, ve la pulsera. La voy a esconder".** **"Lo escondí debajo de aquí"** **"Encuentra la pulsera. ¿Dónde está la pulsera?"** (Max 1 vez por intento)	

41. Suspende el aro por la cuerda	*Aro con cuerda*	**Crédito**: si el niño obtiene el aro y lo suspende por la cuerda	**"¿Puedes hacerlo?".** (Max 2 veces)	
42. Saca el cereal del frasco **→ 46**	*Frasco sin tapa* *cereales*	**Intentos: 3 Crédito**: si el niño saca intencionalmente el cereal del frasco Intento 1 ☐ Intento 2 ☐ Intento 3 ☐	**"Ahora sácalo"** (Max 2 veces)	
43. Caja transparente: recupera el juguete (frente) **→52**	*caja transparente,* *juguete (ej, patito),* ***cronómetro*** **20 segundos**	**Crédito**: si hasta 20 segundos el niño coge el juguete metiendo la mano a través del lado abierto	**"Coge el juguete. Adelante. Ve por el".** (Max 1 vez)	
44. Aprieta objeto	*Patito de goma*	**Crédito**: si el niño intenta apretar el patito	**"Apriétalo"** (Max 2 veces)	
45. Encuentra objeto escondido (intercambiar) **→ 50**	*2 toallitas* *pulsera*	**Intentos: 2 Crédito**: si en el primero intento el niño encuentra la pulsera debajo de la toallita correcta, una vez por toallita. Intento 1: ☐ Izquierda ☐ Derecha Intento 2: ☐ Izquierda ☐ Derecha	**"Mira, ve la pulsera. La voy a esconder".** **"Lo escondí debajo de aquí"** **"Encuentra la pulsera. ¿Dónde está la pulsera?"** (Max 1 vez por intento)	
46. Destapar el frasco	*Frasco con tapa*	**Crédito**: si el niño destapa el frasco	**"Destapa el frasco".** (Max 2 veces)	
47 55 **47. Serie Portapalos 2 agujeros**	*Portapalos* *6 palitos amarillos* ***cronómetro*** ***70 segundos***	**Intentos: 3 Crédito**: si el niño pone el mismo palito dos veces o más en el portapalos, o pone dos o más palitos que se quedan en el portapalos Intento 1: # palitos ___ Tiempo (6 palitos)_____ Intento 2: # palitos ___ Tiempo (6 palitos)_____ Intento 3: # palitos ___ Tiempo (6 palitos)_____	**"Pon los palitos en los huequitos. Ponlos todos"** (Max 2 veces)	
48 53 **48. Serie Juego Relacional: independiente**	*muñeca, oso* *vasito, cuchara* *pelota chica* *toallitas, cubos*	**Crédito**: si el niño demuestra juego relacional independiente o hacia otros, usando objetos con un fin adecuado, sin imitar sus acciones	**"Tengo sed. Necesito agüita"** **"Tu, qué vas a hacer?"** (Max 2 veces)	

49 56	**49. Serie Tablero Rosado: 1 pieza**	*tablero rosado, piezas rojas,* ***cronómetro*** **180 segundos**	**Crédito**: si el niño coloca correctamente por lo menos una pieza dentro de 180 segundos # piezas colocadas correctamente:	**"Pon todas las piezas".** (Max 2 veces)	
	50. Encuentra objeto escondido (cambio de sitio visible)	*2 toallitas pulsera*	**Intentos: 2 Crédito**: si en el primero intento el niño encuentra la pulsera debajo de la toallita correcta, una vez por toallita. Intento 1: ☐ Izquierda ☐ Derecha Intento 2: ☐ Izquierda ☐ Derecha	**"Mira, ve la pulsera. La voy a esconder".** **"Lo escondí debajo de aquí"** **"Lo estoy escondiendo de nuevo"** **"Encuentra la pulsera. ¿Dónde está la pulsera?"** (Max 1 vez por intento)	
	51. Serie Tablero Azul: 1 pieza	*tablero azul piezas azules* ***cronómetro 150 segundos***	**Crédito**: si el niño coloca correctamente por lo menos una pieza dentro de 150 segundos, aunque luego la saque.	**"Ponlo en su lugar"** (Max 2 veces)	
	52. Caja transparente: recupera el juguete (lados)	*caja transparente, juguete,* ***cronómetro 20 segundos/lado***	**Crédito**: si hasta 20 segundos el niño coge el juguete metiendo la mano a través del lado abierto, una vez por lado	**"Coge el juguete. Adelante. Ve por el".** (Max 1 vez)	
48 53	**53. Serie Juego Relacional: otros**	*muñeca, oso vasito, cuchara pelota chica toallitas, cubos*	**Crédito**: si el niño demuestra juego relacional hacia otros, usando los objetos correctamente	**"Tengo sed. Necesito agüita".** **"Tu, qué vas a hacer?"** (Max 1 vez)	
	54. Serie Cubos: Pone 9 cubos en la taza	*9 cubos taza con asa*	**Crédito**: si el niño coloca los 9 cubos dentro de la taza Número de cubos en taza: _____	**"Pon los cubos en la taza. Ponlos todos"** (Max 3 veces)	
47 55	**55. Serie Portapalos: 6 palitos**	*portapalos 6 palitos amarillos* ***cronómetro 70 segundos***	**Intentos: 3 Crédito**: si el niño pone los 6 palitos dentro de 70 segundos (todos los 6 palitos puestos en el mismo intento) Intento 1: # palitos ___ Tiempo (6 palitos)_____ Intento 2: # palitos ___ Tiempo (6 palitos)_____ Intento 3: # palitos ___ Tiempo (6 palitos)_____	**"Pon los palitos en los huequitos. Ponlos todos"** (Max 2 veces)	
49 56	**56. Serie Tablero Rosado: completo**	*tablero rosado, piezas rojas* ***cronómetro 180 segundos***	**Crédito**: si el niño correctamente coloca las tres piezas dentro de 180 segundos # piezas colocadas correctamente:	**"Pon todas las piezas".** (Max 2 veces)	

Formato Bayley III: Lenguaje Receptivo

ID: ___ ___ - ___ ___ ___ Edad del niño (meses y días): _____ Fecha de prueba ____/____/____ Entrevistador _____

INSTRUCCIONES: Un niño de 12 meses puede empezar en el ítem 8. Si el niño pasa los primeros 3 ítems (8, 9, 10), avanza hacia adelante. Si el niño no pasa los 3 primeros ítems, va al ítem 5 y seguir adelante. Detener la prueba cuando el niño obtiene un puntaje de cero en 5 ítems consecutivos. Marcar 1 si el niño recibe crédito y 0 si el niño no recibe crédito.

ITEM	MATERIALES	CRITERIO DE EVALUACION Y COMENTARIOS	INSTRUCCIONES VERBALES	CREDITO
5. Responde a la voz de una persona → 9	*ninguno*	**Crédito:** si el niño responde claramente a la voz de la persona	Llamar nombre del niño en voz normal (Max 3 veces)	
6. Busca con giro de la cabeza	*campana* *sonaja*	**Intentos:** 2 sonidos **Crédito:** si el niño gira la cabeza a propósito en la dirección del sonido por lo menos una vez Intento 1: ☐ Campana oreja derecha ☐ Campana oreja izquierda Intento 2: ☐ Sonaja oreja derecha ☐ Sonaja oreja izquierda	NA	
7. Distinguir sonidos	*hoja de papel, sonaja*	**Crédito:** si el niño responde claramente a los dos sonidos	NA	
8. Mantiene juego con objetos →10 INICIO 12 meses	*objetos de interes* *Ej. muñeca* *sonaja* ***cronómetro*** ***60 segundos***	**Crédito:** si generalmente el niño está interesado en los objetos durante por lo menos 60 segundos Objeto 1: _____ Objeto 2: _____ Objeto 3: _____ Objeto 4: _____	**"Mira, cuantos juguetes"** (Max 2 veces por juguete)	
9. Responde a su nombre	*ninguno*	**Crédito:** si el niño responde las dos veces que llama su nombre, pero no para el nombre desconocido	Si necesita: Llamarle de su nombre. Espera. Llamarle con un nombre desconocido. Espera Llamarle de su nombre. (Max 1 vez)	

	10. Interrumpe actividad **→ 12**		**Crédito**: si el niño mira hacia arriba y para brevemente el juego cuando lo llaman por su nombre.	Llamarle por su nombre (2 veces)	
	11. Reconoce 2 palabras familiares	*Ninguno*	**Crédito**: si el niño responde a por lo menos dos palabras familiares Palabra 1: _____ Palabra 2: _____	Decir palabras desconocidos. (4 palabras) Decir palabras conocidos. (4 palabras)	
	12. Responde a una instrucción de no-no	*Objeto de interes*	**Crédito**: si ante la orden del no-no, el niño frena su intención de coger el juguete aunque luego quiera de nuevo agarrarlo	Decir: "**No-No**" en voz firme	
	13. Se interesa en la rutina de juego de otra persona **→ LE11**	***cronómetro*** ***60 segundos***	**Crédito**: si el niño mantiene atención y disfruta del juego durante por lo menos 60 segundos	**NA**	
	14. Responde al pedido de rutinas sociales	*Ninguno*	**Intentos: 3 Crédito:** si el niño responde de una manera apropiada en el caso de por lo menos 1 pedido verbal Rutina #1: ☐ Rutina #2: ☐ Rutina #3: ☐	Pedir que juegue contigo (sin usar gestos) Max 2 por juego	
15 19	**15. Serie identifica objetos: 1 correcto**	*libro de cuentos* *vasito* *cuchara* *pelota chica* *muñeca*	**Crédito**: si el niño identifica (apunta) correctamente por lo menos 1 de los objetos libro ☐ vasito ☐ cuchara ☐ pelota chica ☐ muñeca ☐	**Dónde está el __(ej vaso)?** o **Muéstrame el vaso** o **Coge el vaso** o **Vaso?** (Max 2 veces por objeto)	
	16. Identifica objetos en el ambiente	*Objetos en el ambiente*	**Intentos: 2 Crédito**: si el niño identifica correctamente un objeto que nombra Intento 1: ☐ Intento 2: ☐	**Dónde están los zapatos?** **Muéstrame tus zapatos?** **Zapatos?** (Max 2 por objeto)	
17 21	**17. Serie identifica fotos: 1 correcto**	*Libro de Fotos (versión Perú)*	**Crédito**: si el niño apunta correctamente por lo menos 1 de las fotos de prueba aguaje ☐ árbol☐ sandalias☐ pollo ☐ pelota ☐ hamaca ☐ motocarro ☐ cuchara ☐ plátanos ☐ taza ☐ gorro ☐ gato ☐	**Muéstrame** (foto) O **Apunta** (foto) (Max 2 por foto)	

	18. Entiende palabras inhibitorias	*pelota* *cubos*	**Crédito**: si el niño para brevemente el juego en respuesta a las palabras inhibitorias	**"¡Espera!"** o **"¡Mi turno!"** o **"¡Para!".** (Max 1)	
15 19	**19. Serie identifica objetos: 3 correcto**	*libro de cuentos* *vasito cuchara* *pelota chica muñeca*	**Crédito**: si el niño identifica (apunta) correctamente por lo menos 3 de los objetos libro ☐ vasito ☐ cuchara ☐ pelota chica ☐ muñeca ☐	**Dónde está ___(ej vaso)?** o **Muéstrame ___** o **Coge __** o **Vaso?** (2 por objeto)	
	20. Sigue instrucciones de una parte	*muñeca* *cuchara* *oso* *peine* *pañuelo*	**Intentos: 3** **Crédito**: si el niño responde correctamente en al menos 2 pruebas. Prueba 1: ☐ Prueba 2: ☐ Prueba 3: ☐	1. **"El bebe tiene hambre. Dale de comer al bebe"** (2 veces) 2. **"Esto es un peine. Peina al bebé"** (2 veces) 3. **"Esto es una toallita. Limpia la cara del bebé".**(2 veces)	
17 21	**21. Serie identifica fotos: 3 correcto**	*Libro de Fotos (versión Perú)*	**Crédito**: si el niño identifica (apunta) correctamente por lo menos 3 de las fotos de prueba aguaje ☐ árbol☐ sandalias☐ pollo ☐ pelota ☐ hamaca ☐ motocarro ☐ cuchara ☐ plátanos ☐ taza ☐ gorro ☐ gato ☐	**Muéstrame** (foto) O **Señala con el dedo** (foto) (Max 2 por foto)	
	22. Identifica 3 tipos de ropa	*Ropa del niño, mama o entrevistador (3 total)*	**Crédito**: si el niño identifica (apunta, toca, mira claramente) por lo menos 3 tipos de ropa	**"Señala con el dedo _____ de tu mama/ti/mi"** o **"Muéstrame _____ de tu mama/ti/mi".** (Max 2 por ropa)	
	23. Serie identificar fotos de acción: 1 correcto	*Libro de Fotos (versión Perú) página 6-9*	**Crédito**: si el niño apunta correctamente por lo menos 1 de las fotos saludar ☐ nadar ☐ dormir ☐ comer ☐ beber ☐ jugar ☐ lavar ☐ correr ☐	**"Muéstrame" o "Señálame con tu dedo la persona que esta…"** (Max 2 por foto)	
	24. Identifica 5 partes del cuerpo	*muñeca*	**Crédito**: si el niño apunta correctamente por lo menos 5 partes del cuerpo barriga ☐ boca ☐ cabeza ☐ manos ☐ nariz ☐ ojos ☐ orejas ☐	**"Muéstrame __ del bebé"** o **"Dónde están __ del bebé?"**, o **"Ojos?"** o **"Señálame con tu dedo"** o **"Toca"** (Max 2 por parte de cuerpo)	
	25. Sigue instrucciones de 2 partes	*3 Objetos de interés*	**Crédito**: si el niño sigue correctamente por lo menos uno de las instrucciones de dos partes en su totalidad Instrucción 1: ☐ Instrucción 2: ☐	**"Ve por (objeto) y ponlo sobre la mesa, por favor"** (2 veces) **"Recoge (objeto) y dámelo"** (Max 2 veces)	

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Formato Bayley III: Lenguaje Expresivo

ID: ___ ___ - ___ ___ ___ Edad del niño (meses y días): _____ Fecha de prueba ____/_____/____ Entrevistador _____

INSTRUCCIONES: Un niño de 12 meses puede empezar en el ítem 7. Si el niño pasa los primeros 3 ítems (7, 8, 9), avanza hacia adelante. Si el niño no pasa los 3 primeros ítems, va al ítem 5 y seguir adelante. Detener la prueba cuando el niño obtiene un puntaje de 0 (cero) en 5 ítems consecutivos. ***La mayoría de estos ítems se puede observar a lo largo de la prueba.*** **Marcar 1 si el niño recibe crédito y 0 si el niño no recibe crédito.**

	ITEM	MATERIALES	CRITERIO DE EVALUACION Y COMENTARIOS	INSTRUCCIONES VERBALES	CREDITO
	5. Vocalizando o riéndose socialmente	*ninguno*	**Crédito:** si el niño vocaliza (se ríe, arrulla, chilla, carcajea) o se ríe frente a la atención.	NA	
	6. 2 Sonidos vocales	*ninguno*	**Crédito:** si el niño vocaliza por lo menos 2 sonidos vocales diferentes y distintos	NA	
INICIO 12 meses	**7. Llama la atención**	*ninguno*	**Crédito:** Si el niño intenta llamar la atención de usted u otros	NA	
	8. 2 sonidos consonantes	*objetos de interes* *Ej. oso* *sonaja*	**Crédito:** si el niño produce al menos dos sonidos consonantes diferentes y distintos	Si necesita, **"pa-pa-pa"** (tocando la sonaja) (max 3 veces) o **"da-da-da"** (hacer soltar el oso) (max 3 veces)	
	9. Usa gestos	*ninguno*	**Crédito:** si el niño usa por lo menos un gesto para expresar sus necesidades/deseos	NA	
10 **13**	**10. Serie combinación consonante-vocal: 1 combinación**	*ninguno*	**Crédito:** si el niño imita o hace espontáneamente por lo menos una combinación repetitiva de consonantes-vocales (Ej. baba o dada)	Si necesita, repetir sonidos **"papapa", "bebebe"** **"mamama"** (max 3 sonidos)	
	11. Participa en rutina de juego **→17, LR13**	*objetos para el juego* *Ej. toallita*	**Crédito:** si el niño participa activamente en por lo menos un juego	Pedir que juegue contigo Max 3 juegos (2 pedidos por juego)	

	12. Balbucea expresivamente	*ninguno*	**Crédito**: si el niño produce por lo menos una vocalización que contiene inflexiones y que es expresiva. O dice una palabra distinguible independientemente de inflexión.	NA	
	13. Serie combinación de consonantes-vocales: 4 combinaciones	*objetos de interés* *Ej. oso. muñeca*	**Crédito**: si el niño imita o hace espontáneamente por lo menos cuatro combinaciones consonante-vocal repetitivas (p.e. gaga, baba, papa y mama).	Si se necesita: **Ej. gagaga, bababa, papapapa, mamama** (Max 4 sonidos)	
	14. Use aproximaciones de una palabra	*ninguno*	**Crédito:** Si el niño produce por lo menos 1 aproximación de una palabra	**NA**	
	15. Dirige la atención de otro persona	*objetos de interés*	**Crédito**: si el niño apunta o muestra por lo menos un objeto a usted o a la madre	**NA**	
	16. Imita palabra	*Ninguno*	**Crédito**: si el niño imita por lo menos una palabra incluso si consiste solamente en vocales	Si se necesita: **Ej. Mamá, Bebé, Gracias, Pelota** (Max 4 palabras)	
	17. Inicia interacción de juego **→ 24**	*objetos de interés,* *Ej. toallita* *pelotita*	**Crédito**: si el niño inicia por lo menos una interacción para jugar	**NA**	
18 **23**	**18. Serie use aproximaciones de palabras: 2 palabras**	*objetos de interés,* *Ej. toallita* *taza*	**Crédito**: si el niño dice 2 palabras diferentes de manera apropiado. Son aceptables aproximaciones siempre y cuando la meta del niño esté claro Palabra 1: _____ Palabra 2: _____ Palabra 3: _____ Palabra 4: _____ Palabra 5: _____ Palabra 6: _____	**NA**	
	19. Utiliza la palabra para expresar sus necesidades	*Ninguno*	**Crédito**: si el niño usa por lo menos una palabra en un esfuerzo de expresar sus necesidades	**NA**	
20 **27**	**20. Serie Nombrar Objeto: 1 objeto**	*taza, pelota* *muñeca, cuchara* *oso, u otros*	**Crédito**: si el niño identifica correctamente por lo menos un objeto, aunque no lo pronuncie bien taza ☐ pelota ☐ muñeca ☐ cuchara ☐ oso ☐ otro ☐ _____ otro ☐ _____ otro ☐ _____	*Si toca objeto:* **"Qué es eso? Qué tienes?"** (2 veces/obj) *Si no toca objeto*: **"Qué es esto? Qué tengo en la mano?"** (2 veces por objeto)	

	21. Combina palabra y gesto	*Ninguno*	**Crédito**: si el niño combina una palabra y un gesto	**NA**	
22 28	**22. Serie nombra fotos: 1 foto**	*Libro de Fotos (versión Perú) pagina 10-15*	**Crédito**: si el niño nombra correctamente por lo menos 1 foto cuchara ☐ gorro ☐ pelota ☐ aguaje ☐ taza ☐ pollo ☐ árbol ☐ botas ☐ motocarro ☐ plátano ☐ hamaca ☐ gato ☐	**"Dime qué es esto"** o **"Qué es esto?"** (2 veces cada foto)	
18 23	**23. Serie use aproximaciones de palabras: 8 palabras**	*objetos de interés* *Ej. taza* *pelota*	**Crédito**: si el niño dice 8 palabras diferentes de manera apropiado (o aproximación de la palabra). Palabra 1: _____ Palabra 2: _____ Palabra 3: _____ Palabra 4: _____ Palabra 5: _____ Palabra 6: _____	**NA**	
	24. Responde sí o no verbalmente a preguntas	*Ninguno*	**Crédito**: si el niño usa sí o no (o variaciones) apropiadamente en por lo menos dos respuestas	**Te gusta este(a) _ (objeto)?** **Quieres _____ (objeto)?** **Puedes darme ___ (objeto)?** (2 veces cada objeto x 4 obj)	
	25. Imita expresiones de dos palabras	*Ninguno*	**Crédito**: si el niño imita una frase de dos o más palabras (o aproximación de la palabra) Frase 1: _____ Frase 2: _____	Si se necesita: Ej **"Mama, va", "Papa come" "Bebe toma" "Mi pelota" "Perro grande"** (Max 3 frases)	
	26. Usa expresiones de dos palabras	*Ninguno*	**Crédito**: si el niño usa por lo menos una frase de dos o más palabras espontáneamente Frase 1: _____ Frase 2: _____	NA	
20 27	**27. Serie nombrar objeto: 3 objetos**	*taza* *pelota* *muñeca* *cuchara* *oso* *u otros objetos*	**Crédito**: si el niño identifica correctamente por lo menos tres objetos taza ☐ pelota ☐ muñeca ☐ cuchara ☐ oso ☐ otro ☐ _____ otro ☐ _____ otro ☐ _____	si toca el objeto: **Qué es eso? Qué tienes?** (2 veces/objeto) si no toca el objeto: **Qué es esto? Qué tengo en la mano?** (2 veces por objeto)	
22 28	**28. Serie nombra fotos: 5 fotos**	*Libro de Fotos (versión Perú) pagina 10-15*	**Crédito**: si el niño nombra correctamente por lo menos 5 fotos cuchara ☐ gorro ☐ pelota ☐ aguaje ☐ taza ☐ pollo ☐ árbol ☐ botas ☐ motocarro ☐ plátano ☐ hamaca ☐ gato ☐	**"Dime qué es esto"** o **"Qué es esto?"** (2 veces por foto)	

Formato Bayley III: Motor Fino

ID: ___ ___ - ___ ___ ___ Edad del niño (meses y días): _____ Fecha de prueba ____/_____/____ Entrevistador _____

INSTRUCCIONES: Un niño de 12 meses puede empezar en el ítem 22. Si el niño pasa los primeros 3 ítems (22, 23, 24), avanza hacia adelante. Si el niño no pasa los 3 primeros ítems, va a ítem 19 y seguir adelante. En caso el niño no pasa los ítems 19, 20 y 21 empezar por el ítem 16. Detener la prueba cuando el niño obtiene un puntaje de cero en 5 ítems consecutivos. Marcar 1 si el niño recibe crédito y 0 si el niño no recibe crédito.

	ITEM	MATERIALES	CRITERIO DE EVALUACION Y COMENTARIOS	INSTRUCCIONES VERBALES	CREDITO
	16. Alcanza de manera unilateral	*objetos pequeños*	**Crédito:** si el niño tiende a alcanzar con una sola mano más a menudo que con las dos manos	NA	
17 20 24 26	**17. Serie Bolitas: agarrar con un rastrillo**	*pequeñas bolitas de cereales*	**Crédito:** si el niño recoge los cereales con la mano como rastrillo, en un intento de agarrar el cereal, aunque no llegue a agarrarlo. O si el niño usa un agarrón más avanzado	NA	
	18. Serie Cubos: oposición parcial del pulgar	*3 cubos*	**Crédito**: si el niño agarra el cubo de manera que el pulgar se opone en parte a los dedos. O si el niño usa un agarrón más avanzado	NA	
	19. Transfiere aro	*aro*	**Crédito:** si el niño transfiere el aro de mano a mano	"**Agárralo**". (max 2 veces)	
17 20 24 26	**20. Serie Bolitas: agarra con la mano entera**	*pequeñas bolitas de cereales*	**Crédito**: si el niño utiliza toda la mano para agarrar el cereal. O si el niño usa un agarrón más avanzado	NA	
	21. Transfiere el cubo	*1 cubo*	**Crédito**: el niño transfiere el cubo de mano a mano.	**NA**	
18 22 INICIO 12 meses	**22. Serie Cubos: agarra con el pulgar-yema del dedo**	*1 cubo*	**Crédito**: si el niño utiliza el pulgar y la yema de cualquier de los dedos para agarrar el cubo	**NA**	

	23. Juntar cubos a la línea media	*2 cubos*	**Crédito**: si el niño junta los cubos en su línea media	**"Ahora tu, golpea los cubos" (max 1 vez)**	
17 20 24 26	**24. Serie Bolitas: oposición parcial del pulgar**	*pequeñas bolitas de cereales*	**Crédito**: si el niño agarra el cereal para que el pulgar se opone al menos en parte a los dedos. O si usa un agarrón más avanzado.	**NA**	
	25. Levanta la taza por el asa	*taza con asa*	**Crédito:** si el niño levanta la taza por el asa con una sola mano.	**NA**	
17 20 24 26	**26. Serie bolitas: agarra pulgar-dedos**	*pequeñas bolitas de cereal*	**Crédito**: si el niño utiliza la yema del pulgar y de cualquier dedo para agarrar el cereal	**NA**	
	27. Hojea las páginas del libro	*Libro de Fotos (original)*	**Crédito**: si el niño intenta hojear una página o varias páginas a la vez,	**"Coge el libro, mira las fotos"** (1 vez)	
28 34 37 48	**28. Serie Agarrar: agarra de puño**	*papel* *2 crayolas*	**Crédito**: si el niño agarra la crayola con un agarrón del puño, mientras marca en el papel. O si el niño usa un agarrón mas avanzado.	**"Ahora tu, dibuja"** (max 2 veces)	
	29. Aísla el dedo índice extendido	*portapalos*	**Crédito**: si el niño extiende su dedo índice, manteniendo curvados los otros dedos.	**NA**	
	30. Hace garabatos espontáneamente	*1 crayola* *papel*	**Crédito**: si el niño hace garabatos en el papel de forma espontánea e intencionalmente	**"Ahora tu, dibuja" (max 2 veces)**	
31 38	**31. Serie Apilar Cubos: 2 cubos**	*9 cubos*	**Intentos: 3 Crédito**: si el niño apila por lo menos dos cubos en uno de los intentos. Intento 1: ☐ Intento 2: ☐ Intento 3: ☐	**"Mira mi torre".** **"Usa estos cubos para hacer una gran torre"** (con 3 cubos) **"Haz una torre lo mas grande que puedas. Usa todos los cubos"** (con 9 cubos)	

32 40 41 43	**32. Serie imita trazos: al azar**	*papel* *2 crayolas*	**Crédito:** si el niño produce un trazo en cualquier dirección Intento 1: ☐ Intento 2: ☐	**"Mira como va…zip! Hazlo tu!".**	
	33. Mete 10 cereales en el frasco (en 60 segundos).	*10 cereales frasco sin tapa* *cronometro* ***60 segundos***	**Intentos: 2 Crédito:** si el niño coloca 10 cereales dentro del frasco en 60 segundos o menos, un cereal a la vez Intento 1 ☐ Intento 2 ☐	**"Quiero saber cuántos cereales puedes poner en el frasco, tan rápido que puedas. Te voy a decir cuando puedes parar. Listo….ya!".**	
28 34 37 48	**34. Serie Agarrar: agarra transicional**	*papel* *2 crayolas*	**Crédito:** si el niño agarra la crayola usando sus dedos en oposición parcial con el pulgar y hace un trago sobre la hoja de papel.	**NA**	
	35. Monedas en ranura	*el chanchito* *5 monedas*	**Intentos: 2 Crédito:** si el niño coloca por lo menos 3 monedas en la ranura Intento 1 ☐ Intento 2 ☐	**"Pon la moneda en el chanchito"**	
	36. Lego: desarmar	*Cubos Lego*	**Crédito:** si el niño remueve todos los cubos de la unidad.	**"Ahora tu, desarme todo".**	
28 34 37	**37. Serie Agarrar: agarra intermedia**	*papel* *2 crayolas*	**Crédito:** si el niño agarra la crayola usando un agarrón intermedio y hace un trago sobre la hoja de papel.	**NA**	
31 38	**38. Serie apilar cubos: 6 cubos**	*9 cubos*	**Intentos: 3 Crédito:** si el niño apila por lo menos 6 cubos en uno de los tres intentos. Intento 1: ☐ Intento 2: ☐ Intento 3: ☐	**"Mira mi torre".** **"Usa estos cubos para hacer una gran torre" (con 3 cubos)** **"Haz una torre lo mas grande que puedas. Usa todos los cubos"** (9 cubos)	

APPENDIX 6

Baseline (12-month) health centre questionnaire

(Cuestionario de visita CRED a los 12 meses)

Cuestionario de visita CRED a los 12 meses

Mejorar el crecimiento y desarrollo en la infancia temprana en países de bajos recursos a través de un programa de desparasitación incorporado en la atención integrada de salud infantil

IDENTIFICACIÓN DEL PARTICIPANTE, ENTREVISTADOR

INICIALES DE ENTREVISTADOR: _____

CÓDIGO DE IDENTIFICACIÓN: [ ][ ] - [ ][ ][ ]
(EESS - PARTICIPANTE)

FECHA DE ENTREVISTA: [/ /]
(dd/mm/aaaa)

1. MEDIDAS DEL NIÑO

1

A- Confirmación de la muestra de heces: ☐ Entregado ☐ Rotulado ☐ En buen estado

B- ¿Dónde entregó la muestra? ☐ ESSS (1) ☐ Casa (2)

C- ¿Fecha/hora de colección? Fecha (dd/mm/aaaa): / /
Hora: _____ : _____ ☐am ☐pm

D- ¿Fecha/hora de entrega? Fecha (dd/mm/aaaa): / /
Hora: _____ : _____ ☐am ☐pm

2 Escala Bayley Cognitivo: ____ Lenguaje Receptivo: ____ Lenguaje Expresivo: ____ Motor Fino: ____

➔ *Si aplicaron la prueba en dos días, indicar la fecha de la segunda visita:* Fecha 2 (dd/mm/aaaa): / / ☐ *No aplica (NA)*

3 Peso del niño

A- Peso: _____ kg

B- ¿Balanza? ☐ Balanza SECA (1) ☐ Balanza EESS (2)

C- ¿Pañal? ☐ Con pañal (1) ☐ Sin pañal (2)

D- ¿Ropa? ☐ Con ropa (1) ☐ Sin ropa (2)

E- ¿Sentado? ☐ Sentado (1) ☐ Echado (2) ☐ Parado (3)

4 Talla del niño

A- Talla: _____ cm

B- ¿Tallimetro? ☐ Tallimentro SECA (1) ☐ Tallimetro EESS (2)

C- ¿Pañal? ☐ Con pañal (1) ☐ Sin pañal (2)

D- ¿Ropa? ☐ Con ropa (1) ☐ Sin ropa (2)

E- Comentarios: _____

➔ANTES DE SEGUIR A LA ADMINISTRACION DEL TRATAMIENTO, ASEGURAR QUE TODOS LOS FORMATOS ESTÉN COMPLETOS Y VERIFICAR QUE SE HA ENTREGADO LA MUESTRA DE HECES.

SI ES QUE NO,

NO PASAR AL SIGUIENTE PASO HASTA QUE LA MUESTRA HAYA SIDO ENTREGADA.

2. ADMINISTRACIÓN DEL TRATAMIENTO

FECHA DE ADMINISTRACIÓN: / / (dd/mm/aaaa)

Código del sobre: | | | | |

→*Anotar este código en el frasco de muestra de heces*

1 ¿El niño tomó la pastilla triturada con..?

☐ Jugo (1) ☐ Yogur (2)
☐ Otro_____ (3)

2

A- ¿El niño tragó toda la pastilla triturada? ☐ Si (1) ☐ No (0)

B- Si es que no, explicar:

☐ *No aplica (NA)* _____

3

A- ¿Después de la administración del tratamiento, tuvo el niño algún efecto secundario **menor** mientras estaba en el EESS? ☐ Si (1) ☐ No (0)

B- ¿Si es que si, cual(es)?

☐ Dolor de estómago (1) ☐ Vómitos (2)
☐ Nausea (3) ☐ Otro_____ (4)

Cita de seguimiento a los 18 Meses:

Visita domiciliaria: Fecha: _____ _____/_____/_____ Hora: _____ AM ☐ PM ☐
día de la semana dd mm aaaa

Visita EESS: Fecha: _____ _____/_____/_____ Hora: _____ AM ☐ PM ☐
día de la semana dd mm aaaa

Otros Comentarios: _____

_____

_____

_____

_____

APPENDIX 7

Follow-up 1 (18-month) questionnaire

(Encuesta de visita EESS a los 18 meses)

Asociación Civil SELVA AMAZONICA

McGill UNIVERSITY

L'Institut de recherche du Centre universitaire de santé McGill
The Research Institute of the McGill University Health Centre

Encuesta de visita EESS a los 18 meses

Mejorar el crecimiento y desarrollo en la infancia temprana en países de bajos recursos a través de un programa de desparasitación incorporado en la atención integrada de salud infantil

1. IDENTIFICACIÓN DEL PARTICIPANTE, ENTREVISTADOR

INICIALES DE ENTREVISTADOR: _____

CÓDIGO DE IDENTIFICACIÓN: [ ][ ] - [ ][ ][ ]
(EESS - PARTICIPANTE)

DIRECCIÓN: _____

DISTRITO: _____

URBANO ☐ RURAL ☐
PERI-URBANO ☐

TELÉFONO: _____

FECHA DE ENROLAMIENTO: / / (dd/mm/aaaa)

FECHA DE ENTREVISTA: / / (dd/mm/aaaa)

2. PREGUNTAS ACERCA DEL NIÑO (Verificado en el carné de CRED ☐ Sí ☐ No)

1

A- ¿Cual fue la edad gestacional de su hijo? _____ semanas ☐ *No sabe* (99)
(verificar en el carné de embarazo) *¿Verificado?* ☐ Si (1) ☐ No (0)

B- ¿Cuál fue su fecha de la ultima menstruacion antes del embarazo de…? (dd/mm/aaaa) / / ☐ *No sabe* (99)
(verificar en el carné de embarazo) *¿Verificado?* ☐ Si (1) ☐ No (0)

2

¿A cuántos controles de CRED ha asistido su hijo en los últimos 6 meses? _____ ☐ *No asistió desde enrolamiento*
(desde fecha de enrolamiento hasta fecha actual)

B- ¿Fecha de la última visita (dd/mm/aaaa)? / /

C- Establecimiento de salud (EESS) _____

3

A- ¿Su hijo/a recibió la vacuna de SPR? ☐ Si *(→ B)* (1) ☐ No (0)

B- ¿Si es que sí, cuándo? (dd/mm/aaaa) / /
☐ *No aplica (NA)*

4

A- ¿Su hijo/a recibió la vacuna de DPT? ☐ Si *(→ B)* (1) ☐ No *(→ C)* (0)

B- ¿Si es que sí, cuándo? (dd/mm/aaaa) / /
☐ *No aplica (NA)*

C- ¿Si es que no, está programada la vacunación? ☐ Si (1) → ¿Para cuándo? (dd/mm/aaaa) / /
☐ *No aplica (NA)* ☐ No (0) → ¿Por qué? _____

5

¿Su hijo recibió vitamina A en los últimos seis meses (desde enrolamiento en el estudio)? ☐ Sí (1) → ¿Cuándo? _____ ☐ No (0) ☐ *No sabe* (99)

6

A- ¿Su hijo toma algún suplemento de hierro? (ej. Sulfato ferroso) *(ver si tiene muestra)* ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B- Si es que sí, ¿desde hace cuánto tiempo lo está tomando? _____ ☐días ☐semanas ☐meses
☐ *No aplica (NA)*

7

A- ¿Recibió su hijo un antiparasitario en los últimos seis meses (desde enrolamiento en el estudio) (aparte de la pastilla que recibió en el estudio)? ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B- Si es que sí, ¿cuál fue el medicamento? ☐ Mebendazol (1) ☐ Albendazol (2)
☐ *No aplica (NA)* ☐ Otro _____ (3) ☐ *No sabe* (99)

C- Si es que sí, ¿en qué forma? ☐ Jarabe (1) ☐ Pastilla (2) ☐ *No sabe* (99)
☐ *No aplica (NA)*

D- Si es que sí, ¿hace cuánto tiempo que lo tomó? _____ ☐días ☐semanas ☐meses
☐ *No aplica (NA)*

7	**E** –Si es que sí, ¿cuál fue la dosis? ☐ *No aplica (NA)*	☐ Único (1) ☐ Otro:____(# veces al día) × ____ (# días)
	F –Si es que sí, ¿dónde lo recibió? ☐ *No aplica (NA)*	☐ EESS (1) ☐ Campaña en iglesia (4) ☐ Farmacia (2) ☐ Otro_____ (5) ☐ Campaña en escuela (3) ☐ *No sabe* (99)
8	**A-** ¿Su hijo toma algún otro suplemento, vitamina o medicamento actualmente? **B-** Si es que sí, especificar: *(Preguntar si tiene una muestra)* ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) **1-**_____ **3-**_____ **2-**_____ **4-**_____
9	**A-** ¿Su hijo ha tenido dengue en los últimos seis meses (desde enrolamiento en el estudio) (confirmado en EESS)? **B -** Si es que sí, ¿cuántos meses tenía? ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) _____ meses
10	**A-** ¿Su hijo ha tenido malaria en los últimos seis meses (desde enrolamiento en el estudio) (confirmado en EESS)? **B -** Si es que sí, ¿cuántos meses tenía? ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) _____ meses
11	**A-** ¿En las últimas dos semanas (15 días) su hijo ha tenido tos o alguna dificultad respiratoria? **B-** Si es que sí… ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) **i-** ¿Por cuánto tiempo? _____ días **ii-** ¿Qué medidas tomaron? _____
12	**A-** ¿En las últimas dos semanas (15 días) su hijo ha tenido diarrea (**4 o más** deposiciones líquidas por día)? **B-** Si es que sí… ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) **i-** ¿Por cuánto tiempo? _____ días **ii-** ¿Con sangre visible en las heces? ☐ Sí (1) ☐ No (0) **iii-** ¿Qué medidas tomaron? _____
13	**A-** ¿En las últimas dos semanas (15 días) su hijo ha tenido fiebre? **B-** Si es que sí… ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) **i-** ¿Por cuánto tiempo? _____ días **ii-** ¿Medido con termómetro? ☐ Sí (1) → ____ °C ☐ No (0) **iii-** ¿Qué medidas tomaron? _____
14	**A-** ¿En las últimas dos semanas (15 días) su hijo ha tenido algún problema de oído? **B-** Si es que sí… ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) **i-** ¿Dolor de oído? ☐ Sí (1) ☐ No (0) **ii-** ¿Descarga ó secreción de los oidos? ☐ Sí (1) ☐ No (0) **iii-** ¿Qué medidas tomaron? _____ **iv-** ¿Diagnóstico médico? ☐ Sí (1) ☐ No (0)
15	**A-** *Observar si el niño camina solo:* **B-** Señora, ¿cuántos meses tenía su hijo cuando empezó a caminar solo?	☐ Sí (1) ☐ No (0) ☐ *No sabe* (99) _____ meses ☐ *No sabe* (99) ☐ *No aplica (NA)*
16	**A-** ¿Usted (*la madre*) le dio de lactar a (*nombre*)? **B-** Si es que sí, ¿sigue dando de lactar a su hijo? ☐ *No aplica (NA)*	☐ Sí (1) ☐ No (0) ☐ Sí (1) → ¿Cuántas veces? mañana____ tarde____ noche____ ☐ No (0) → ¿Hasta cuántos meses le dio de lactar? _____ meses

3. RECORDATORIO DE CONSUMO DE 24 HORAS: APRENDER QUE COME EL NIÑO

1 ¿Cómo fue el apetito del niño ayer? *(Leer opciones)* ☐ Usual (normal) (1) ☐ Menos que usual (2) ☐ Más que usual (3)

→*Si consumió menos que lo usual (e.j. debido a enfermedad) preguntar cuando comió normal y pasar ese día y anotar la fecha del recordatorio: Fecha (dd/mm/aaaa)* ____/____/_____

2 Señora: Vamos a hablar ahora sobre la alimentación que recibió (todito que comió) ayer su hijo. Solo la de ayer, desde que se despertó hasta que se durmió en la noche.

Preguntas de Comida	Hora Aprox.	Todos los alimentos/bebidas/lactadas. Si el alimento es una preparación listar ingredientes	Consistencia Espeso=1; Sólido=2; Aguado=3
¿Ayer cuál fue la primera cosa que comió (nombre del niño) desde que se despertó? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
Después de su última comida ¿comió algo más antes de dormirse?			
Y durante la noche ¿le dio algo más?			

PREGUNTAS A LLENAR DESPUES DE COMPLETAR EL RECORDATORIO:

3 ¿Comió un producto animal (carne/pescado/vísceras/ave/huevos) ayer? ☐ Sí (1) ☐ No (0)

4 Número de preparaciones con consistencia: espeso ____ sólido____ aguado____ LM _____

4. MEDIDAS DEL NIÑO

	A- Confirmación de la muestra de heces:	☐ Entregado		☐ Rotulado		☐ En buen estado		
	B- ¿Dónde entregó la muestra?	☐ EESS (1)		☐ Casa (2)				
1	**C-** ¿Fecha/hora de colección?	Fecha (dd/mm/aaaa): / /						
		Hora: ____ : ____	☐am	☐pm				
	D- ¿Fecha/hora de entrega?	Fecha (dd/mm/aaaa): / /						
		Hora: ____ : ____	☐am	☐pm				
2	Peso del niño	**A-** Peso: _____ kg						
		B- ¿Balanza?	☐ Balanza SECA (1)		☐ Balanza EESS (2)			
		C- ¿Pañal?	☐ Con pañal (1)		☐ Sin pañal (2)			
		D- ¿Ropa?	☐ Con ropa (1)		☐ Sin ropa (2)			
		E- ¿Sentado?	☐ Sentado (1)		☐ Echado (2)	☐ Parado (3)		
3	Talla del niño	**A-** Talla: _____ cm						
		B- ¿Tallimetro?	☐ Tallimentro SECA (1)		☐ Tallimetro EESS (2)			
		C- ¿Pañal?	☐ Con pañal (1)		☐ Sin pañal (2)			
		D- ¿Ropa?	☐ Con ropa (1)		☐ Sin ropa (2)			
		E- Comentarios: _____						

→ NO PASAR A LA ADMINISTRACION DEL TRATAMIENTO HASTA QUE HAYA:

1. **ASEGURADO QUE TODOS LOS FORMATOS ESTÉN COMPLETOS**
2. **VERIFICADO QUE SE HA ENTREGADO LA MUESTRA DE HECES**
3. **CONFIRMADO EL CODIGO DE TRATAMIENTO**

5. ADMINISTRACIÓN DEL TRATAMIENTO *(CONFIRMAR EL CODIGO DE IDENTIFICACION EN EL SOBRE)*

	FECHA DE ADMINISTRACIÓN: / / (dd/mm/aaaa)	Código del sobre: ☐ ¿Reemplazo? → ***Anotar este código en el frasco de muestra de heces***
1	¿El niño tomó la pastilla triturada con..?	☐ Jugo (1) ☐ Yogur (2) ☐ Otro _____ (3)
2	**A-** ¿El niño tragó toda la pastilla triturada?	☐ Si (1) ☐ No (0)
	B- Si es que no, explicar: _____ ☐ *No aplica (NA)*	
3	**A-** ¿Después de la administración del tratamiento, tuvo el niño algún efecto secundario **menor** mientras estaba en el EESS?	☐ Si (1) ☐ No (0)
	B- ¿Si es que sí, cual(es)?	☐ Dolor de estómago (1) ☐ Vómitos (2) ☐ Náusea (3) ☐ Otro _____ (4)

CITA DE SEGUIMIENTO A LOS 24 MESES:

Visita domiciliaria: Fecha - _____ ____/____/_____; Hora - _____:_____ ☐AM ☐PM

Visita EESS: Fecha - _____ ____/____/_____; Hora - _____:_____ ☐AM ☐PM

Comentarios: _____

APPENDIX 8

Follow-up 2 (24-month) questionnaire

(Encuesta de visita EESS a los 24 meses)

Encuesta de visita EESS a los 24 meses

Mejorar el crecimiento y desarrollo en la infancia temprana en países de bajos recursos a través de un programa de desparasitación incorporado en la atención integrada de salud infantil

1. IDENTIFICACIÓN DEL PARTICIPANTE, ENTREVISTADOR

INICIALES DE ENTREVISTADOR: _____

CÓDIGO DE IDENTIFICACIÓN: ☐☐ - ☐☐☐ (EESS - PARTICIPANTE)

DIRECCIÓN: _____

DISTRITO: _____

FECHA DE ENROLAMIENTO: / / (dd/mm/aaaa)

FECHA DE ENTREVISTA: / / (dd/mm/aaaa)

URBANO ☐ RURAL ☐
PERI-URBANO ☐

TELÉFONO: _____

2. CARACTERÍSTICAS SOCIODEMOGRÁFICAS

1

A- ¿En algún momento desde enrolamiento, usted ha cambiado de domicilio (aunque luego haya vuelto)? (*verificar datos de cuestionario domiciliario 12 meses*) ☐ Si (1) → 2.1B ☐ No (0) → 2.2

B- Si es que sí, ¿fue a causa de la inundación? ☐ Si (1) →2.1C ☐ No (0) → 2.1E

_____ ☐*No aplica (NA)*

C- Si es que sí, ¿dónde estuvo usted hospedado? ☐ Familiar ciudad (1) ☐ Carpa (3) ☐ Otro (5)

☐ Familiar fuera ciudad (2) ☐ Albergue (4) Especificar: _____

_____ ☐*No aplica (NA)*

D- Si es que sí, ¿cuánto tiempo estuvo usted fuera de su casa? _____ ☐días ☐semanas ☐meses ☐ Sigue desplazado

_____ ☐*No aplica (NA)*

E- Actualmente, ¿usted vive en una casa diferente a donde vivía cuando nos reunimos la primera vez (hace un año)? ☐ Sí (1) ☐ No (0)

_____ ☐*No aplica (NA)*

2 ¿De qué material está hecha la casa? ☐ Noble (1) ☐ Rústica (2)

3

A- ¿Tiene agua potable en su casa (agua de grifo)? ☐ Sí (1) ☐ No (0)

B- En caso que no, ¿de dónde obtiene el agua?

_____ ☐*No aplica (NA)*

☐ Grifo vecino (1) ☐ Pozo artesiano (4)
☐ Camión cisterna (2) ☐ Pileta pública (5)
☐ Río (3) ☐ Agua de lluvia (6)
☐ Otro _____ (7)

4 ¿Qué hace usted con el agua antes de tomarlo? ☐ Toma directamente (1) ☐ Hierve (2) ☐ Trata (3)

5	¿Dónde hace usted sus necesidades (describir cómo es su baño)?	☐ Directo al río	(1)	☐ Taza con agua y desagüe público	(5)		
		☐ Silo o letrina sin desagüe público	(2)	☐ Silo o letrina conectada al desagüe público	(6)		
		☐ Campo abierto	(3)	☐ Pozo séptico	(7)		
		☐ Letrina con drenaje al río	(4)	☐ Otro_____	(8)		
6	¿Dónde hace su hijo sus necesidades?	☐ Pañal descartable	(1)	☐ Bacín	(4)		
		☐ Pañal de tela	(2)	☐ Papel periódico	(5)		
		☐ Baño	(3)	☐ Otro _____	(6)		
7	¿Dónde bota las deposiciones de su hijo? → *si es basurero confirmar si es recogida por servicio publico*	☐ Río	(1)	☐ Basurero	(3)	☐ Otro_____	(5)
		☐ Campo/ Monte	(2)	☐ Baño	(4)		

3. PREGUNTAS ACERCA DE LA MADRE

1	¿Cuál es el estado civil actual de la madre?	☐ Casada/conviviente	(1)	☐ Soltera, sin pareja	(3)		
		☐ Soltera, con pareja	(2)	☐ Separada/divorciada	(4)		
2	**A-** Señora, ¿usted ha dado a luz en el último año?	☐ Sí	(1)	☐ No	(0)		
	B- Si es que sí, ¿cuántos meses tiene su(s) bebe(s)? *(Confirmar en carné de embarazo)* ☐*No aplica (NA)*	1) _____ meses		2) _____ meses			
3	**A-** Señora, ¿usted está actualmente embarazada?	☐ Sí	(1)	☐ No	(0)	☐ *No sabe*	(99)
	B- Si es que sí, ¿cuántos meses de embarazo tiene? *(Confirmar en carné de embarazo)* ☐*No aplica (NA)*	_____ meses					

4. PREGUNTAS ACERCA DEL NIÑO (Verificado en el carné de CRED ☐Sí ☐No)

1 ¿A cuántos controles de Crecimiento y Desarrollo (CRED) ha asistido su hijo?:

A- Entre 12 a 17 meses ____ **B-** Entre 18 a 23 meses ____ **C-** Desde 24 meses ____

C- ¿Fecha de la última visita (dd/mm/aaaa)? ____/____/_____

D- Establecimiento de salud (EESS) _____

2 ¿Qué inmunizaciones recibió su hijo en el EESS desde que nació? *(Confirmar en carné)*

Marcar Si o No o No Sabe

	1ra dosis	2da dosis	3ra dosis
Tuberculosis (BCG)			
Antihepatitis			
Antipolio			
Pentavalente			
Antiamarílica			
Sarampion, Rubeola, Paperas (SPR)			
DPT			
Rotavirus			
Antineumococica			
Otro_____			

3 ¿Su hijo recibió vitamina A en los últimos seis meses (desde la visita 2)? ☐ Sí (1) → ¿Cuándo?_____ meses ☐ No (0) ☐ *No sabe* (99)

4

A- ¿Su hijo toma algún suplemento de hierro? (ej. Sulfato ferroso) *(ver si tiene muestra)* ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B- Si es que sí, ¿desde hace cuánto tiempo lo está tomando? ☐*No aplica (NA)* _____ ☐días ☐semanas ☐meses

5

A- ¿Su hijo toma algún otro suplemento, vitamina o medicamento actualmente? ☐ Sí (1) ☐ No (0)

B- Si es que sí, especificar: *(Preguntar si tiene una muestra)* ☐*No aplica (NA)*

1-_____ **3-**_____

2-_____ **4-**_____

6

A- ¿Su hijo ha tenido dengue en los últimos seis meses (desde la visita 2) (confirmado en EESS)? ☐ Sí (1) ☐ No (0)

B - Si es que sí, ¿cuántos meses tenía? ☐*No aplica (NA)* _____ meses

7

A- ¿Su hijo ha tenido malaria en los últimos seis meses (desde la visita 2) (confirmado en EESS)? ☐ Sí (1) ☐ No (0)

B - Si es que sí, ¿cuántos meses tenía? ☐*No aplica (NA)* _____ meses

8

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido tos o alguna dificultad respiratoria? ☐ Sí (1) ☐ No (0)

B- Si es que sí… ☐*No aplica (NA)*

i- ¿Por cuánto tiempo? _____ días

ii- ¿Qué medidas tomaron? _____

9

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido diarrea (**4 o más** deposiciones líquidas por día)? ☐ Sí (1) ☐ No (0)

B- Si es que sí… ☐*No aplica (NA)*

i- ¿Por cuánto tiempo? _____ días

ii- ¿Con sangre visible en las heces? ☐ Sí (1) ☐ No (0)

iii- ¿Qué medidas tomaron? _____

10

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido fiebre? ☐ Sí (1) ☐ No (0)

B- Si es que sí… ☐*No aplica (NA)*

i- ¿Por cuánto tiempo? _____ días

ii- ¿Medido con termómetro? ☐ Sí (1) → _____ ° C ☐ No (0)

iii- ¿Qué medidas tomaron? _____

11

A- ¿En las últimas dos semanas (15 días) su hijo ha tenido algún problema de oído? ☐ Sí (1) ☐ No (0)

B- Si es que sí… ☐*No aplica (NA)*

i- ¿Dolor de oído? ☐ Sí (1) ☐ No (0)

ii- ¿Descarga ó secreción de los oidos? ☐ Sí (1) ☐ No (0)

iii- ¿Qué medidas tomaron? _____

iv- ¿Diagnóstico médico? ☐ Sí (1) ☐ No (0)

12

¿El niño caminaba en la visita 2 (verificar en cuestionario)? ☐ *Si → 4.13* ☐ *No → 4.12*

Señora, ¿cuántos meses tenía su hijo cuando empezó a caminar solo? _____ meses ☐ *No sabe* (99) ☐ *No aplica (NA)*

13

¿El niño seguía lactando en la visita 2 (verificar en cuestionario)? ☐ *Si → 4.13* ☐ *No → Sección 5*

Señora, ¿sigue dando de lactar a su hijo? ☐*No aplica (NA)*

☐ Sí (1) → ¿Cuántas veces? mañana_____ tarde_____ noche_____

☐ No (0) → ¿Hasta cuántos meses le dio de lactar? _____ meses

5. PREGUNTAS ACERCA DE DESPARASITACION

1

A- ¿Recibió su hijo un antiparasitario en los últimos 6 meses (desde la visita 2) (aparte de la pastilla que recibió en el estudio) ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B- Si es que sí, ¿cuál fue el medicamento? ☐ Mebendazol (1) ☐ Albendazol (2) ☐ Otro_____ (3) ☐ *No sabe* (99)

☐*No aplica (NA)*

C- Si es que sí, ¿en qué forma? ☐ Jarabe (1) ☐ Pastilla (2) ☐ *No sabe* (99)

☐*No aplica (NA)*

D- Si es que sí, ¿hace cuánto tiempo lo tomó? _____ ☐días ☐semanas ☐meses

☐*No aplica (NA)*

E –Si es que sí, ¿cuál fue la dosis? ☐ Único (1) ☐ Otro:_____(# veces por día) × _____ (# días)

☐*No aplica (NA)*

F –Si es que sí, ¿dónde lo recibió? ☐ EESS (1) ☐ Campaña en iglesia (4) ☐ Farmacia (2) ☐ Otro_____ (5) ☐ Campaña en escuela (3) ☐ *No sabe* (99)

☐*No aplica (NA)*

G – Si es que sí, ¿sacaron un análisis de heces? ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

☐*No aplica (NA)*

2

A – ¿Usted cree que su hijo recibió la pastilla activa (mebendazol) durante el estudio? ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

B –Si es que sí, ¿después de qué visita(s)? ☐ Visita 1 (1) ☐ Visita 2 (2) ☐ Ambas (3) ☐ *No sabe* (99)

☐*No aplica (NA)*

C –Si es que sí, ¿por qué? *(marcar todo que responde)* ☐ mejor apetito (1) ☐ gusanos en heces (3) ☐ no se enferma (5) ☐ mas energía (2) ☐ ha crecido (4) ☐ Otro (6) Especificar: _____ ☐ *No sabe* (99)

☐*No aplica (NA)*

D- Si es que no, ¿por qué? *(marcar todo que responde)* ☐ no mejoró apetito (1) ☐ no gusanos en heces (3) ☐ se enferma (5) ☐ no mejoró energía (2) ☐ no ha crecido (4) ☐ Otro (6) Especificar: _____ ☐ *No sabe* (99)

☐*No aplica (NA)*

Si respondió que vio gusanos → 5.2F Si respondió que no vio gusanos → 5.3 En todo otro caso → 5.2E

E – Señora, ¿usted vio lombrices (gusanos) en las heces de su hijo(a) después de haber recibido la pastilla de estudio? ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

☐*No aplica (NA)*

F –¿Después de que visita(s) vio lombrices? ☐ Visita 1 (1) ☐ Visita 2 (2) ☐ Ambas (3) ☐ *No sabe* (99)

☐*No aplica (NA)*

G –¿Que cree usted que significa ver lombrices en las heces? ***(marcar todo que responde)*** ☐ niño ha sido tratado (1) ☐ Otro (3) ☐ niño tenía parásitos (2) ☐ *No sabe* (99) Especificar: _____

☐*No aplica (NA)*

3 Si el antiparasitario NO fuera gratuito (es decir, tendría un costo), ¿usted participaría en un programa de desparasitación para sus hijos? ☐ Sí (1) ☐ No (0) ☐ *No sabe* (99)

6. RECORDATORIO DE CONSUMO DE 24 HORAS: APRENDER QUE COME EL NIÑO

1	¿Cómo fue el apetito del niño ayer? *(Leer opciones)*	☐ Usual (normal) (1) ☐ Menos que usual (2) ☐ Más que usual (3)
	→Si consumió menos que lo usual (e.j. debido a enfermedad) preguntar cuándo comió normal y pasar ese día y anotar la fecha del recordatorio: Fecha (dd/mm/aaaa) ____/____/________	
2	Señora: Vamos a hablar ahora sobre la alimentación que recibió (todito que comió) ayer su hijo. Solo la de ayer, desde que se despertó hasta que se durmió en la noche.	

Preguntas de Comida	Hora Aprox.	Todos los alimentos/bebidas/lactadas. Si el alimento es una preparación listar ingredientes	Consistencia Espeso=1; Sólido=2; Aguado=3
¿Ayer cuál fue la primera cosa que comió (nombre del niño) desde que se despertó? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
¿Luego de esto, cuál fue la siguiente cosa que comió ayer? ¿Comió algo más?			
Después de su última comida ¿comió algo más antes de dormirse?			
Y durante la noche ¿le dio algo más?			

PREGUNTAS A LLENAR DESPUES DE COMPLETAR EL RECORDATORIO:

3	¿Comió un producto animal (carne/pescado/vísceras/ave/huevos) ayer?	☐ Sí (1) ☐ No (0)
4	Número de preparaciones con consistencia: espeso ____ sólido____ aguado____ LM _____	

7. MEDIDAS DEL NIÑO

1	**A-** Confirmación de la muestra de heces:	☐ Entregado	☐ Rotulado	☐ En buen estado		
	B- ¿Fecha/hora de colección?	Fecha (dd/mm/aaaa): / /				
		Hora: ____ : ____	☐am	☐pm		
	C- ¿Fecha/hora de entrega?	Fecha (dd/mm/aaaa): / /				
		Hora: ____ : ____	☐am	☐pm		
2	Escala Bayley Cognitivo: ___ Lenguaje Receptivo: ___ Lenguaje Expresivo: ____ Motor Fino: ___					
	➔ *Si aplicaron la prueba en dos días, indicar la fecha de la segunda visita:*	Fecha 2 (dd/mm/aaaa): / /	☐ *No aplica (NA)*			
3	Peso del niño	**A-** Peso: ____ kg				
		B- ¿Balanza?	☐ Balanza SECA (1)	☐ Balanza EESS (2)		
		C- ¿Pañal?	☐ Con pañal (1)	☐ Sin pañal (2)		
		D- ¿Ropa?	☐ Con ropa (1)	☐ Sin ropa (2)		
		E- ¿Sentado?	☐ Sentado (1)	☐ Parado (2)		
4	Talla del niño	**A-** Talla: ____ cm				
		B- ¿Tallímetro?	☐ Tallímetro SECA (1)	☐ Tallímetro EESS (2)		
		C- ¿Pañal?	☐ Con pañal (1)	☐ Sin pañal (2)		
		D- ¿Ropa?	☐ Con ropa (1)	☐ Sin ropa (2)		
		E- ¿Posición?	☐ Echado (1)	☐ Parado (2)		
5	Comentarios:					

➔NO PASAR A LA ADMINISTRACION DEL MEBENDAZOL HASTA QUE HAYA:

1. **ASEGURADO QUE TODOS LOS FORMATOS ESTÉN COMPLETOS**
2. **VERIFICADO QUE SE HA ENTREGADO LA MUESTRA DE HECES**
3. **CONFIRMADO EL CODIGO DE LA MUESTRA**

8. ADMINISTRACIÓN DE MEBENDAZOL

FECHA DE ADMINISTRACIÓN: / / (dd/mm/aaaa)

CÓDIGO DE IDENTIFICACIÓN: | | | - | | | |

➔***Anotar este código en el frasco de muestra de heces***

1	**A-** ¿Después de la administración del tratamiento, tuvo el niño algún efecto secundario **menor** mientras estaba en el EESS?	☐ Si (1)	☐ No (0)		
	B- ¿Si es que sí, cual(es)?	☐ Dolor de estómago (1)	☐ Vómitos (2)		
		☐ Náusea (3)	☐ Otro_____ (4)		

Comentarios: _____

APPENDIX 9

Abstracts from related presentations

Global Health Conference, Montreal, Canada, November 2011

Ensuring equitable access to participation in an RCT of deworming of preschool-age children: use of a community-based pre-recruitment census.

Serene A. Joseph, Martín Casapía, Theresa W. Gyorkos

1. McGill University, Montreal, QC, Canada
2. Asociación Civil Selva Amazónica, Iquitos, Peru

Background: Ensuring equitable access to participation in research studies can be challenging, particularly in developing countries. Those who may benefit the most from health interventions are often more difficult to identify due to lower use of health services. A pre-recruitment strategy was designed to overcome these challenges for a randomized-controlled trial of deworming, incorporated into routine health-centre based growth and development ('Crecimiento y Desarrollo' or 'CRED') visits, in 12-month old children living in the Peruvian Amazon.

Methods: A pre-recruitment census was conducted between April and July 2011 in 10 participating health centre jurisdictions in and around Iquitos, the capital of the Loreto region. Each house was visited to determine the age of children living in the home. In houses where there was a child < 12 months of age, the following information was obtained: name of child, date of birth, name of the health centre that the child regularly attended, number of 'CRED' visits since birth, and date of last 'CRED' visit.

Results: Information was obtained on a total of 3208 children from birth to 11 months of age. Of these children, 62.1% (n=1991) attended one of the participating study health centres, 32.5% (n=1044) attended non-study health centres, hospitals or private clinics, and 5.4% (n=173) did not attend any health centre. Only 29.4% (n=943) of children attended the recommended number of one CRED visit per month from birth, ranging from a high of 49.2% at 1 month of age to a low of 14.6% at 11 months of age.

Conclusion: The pre-recruitment census provided accurate and up-to-date information on potential study participants. These results will be used to inform additional strategies for recruitment, such as targeting higher-risk children with no or lower than recommended CRED attendance who may have been missed by recruitment in the health centre alone. The census also allowed an additional contact between the research team and participants, which will help to increase awareness of and interest in the study. Similar community-based strategies will be used to maintain high follow-up throughout the duration of the trial.

European Congress of Tropical Medicine and International Health, Copenhagen, Denmark, September 2013

Malnutrition and the critical growth window in an STH-endemic area of Peru: worm infections a barrier to achieving child-related MDGs?

Serene A. Joseph[1,2], Martin Casapia[3], Brittany Blouin[2], Theresa W. Gyorkos[1,2]

1. McGill University; 2. McGill University Health Centre; 3. Asociacion Civil Selva Amazonica;

Introduction: Children under 2 years of age are in the most critical window for growth and development. As mobility increases, this time period also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical environments. The association between malnutrition and worm infection, however, has been understudied in this vulnerable age group.

Materials and Methods: A double-blind, randomized, placebo-controlled trial of a deworming intervention is currently being conducted in 12-month old children in Iquitos, an STH-endemic area of the Peruvian Amazon. Baseline enrolment data was collected between September 2011 and June 2012. Anthropometric measurements were taken, including length and weight. Stool specimens were collected from all participants, with half of the specimens (i.e. from those receiving active mebendazole treatment) being analyzed immediately with the Kato-Katz technique. The association between malnutrition and STH infection was analyzed using logistic regression.

Results: A total of 1760 children were enrolled into the trial. Baseline data of all participants showed a prevalence of stunting, underweight and wasting of 24.2%, 8.7% and 2.3%, respectively. Of the 880 participants with analyzed stool specimens, the prevalence of any STH infection was 14.6%. The distribution by parasite species was 11.5% for Ascaris, 4.5% for Trichuris and 0.6% for hookworm. A significant association was observed between malnutrition (stunting) and infection with at least one STH species (aOR = 1.68; 95% CI: 1.06, 2.66).

Conclusions: Although previously thought to be negligible, the high STH prevalence by 12 months of age and the associated link with malnutrition is of concern. Follow-up of these children at 18 and 24 months of age is nearing completion. This data will provide increased insight into the longer-term effects and causality between STH infection and malnutrition, such that appropriate interventions can be targeted to this vulnerable age group and improvements in child-related MDGs can be achieved.

Canadian Conference on Global Health, Ottawa, Canada, October 2013

Vaccinations and routine growth and development visits in child health care in Peru: Opportunities for piggybacking deworming and achieving the MDGs

Serene A. Joseph, Martín Casapía, Brittany Blouin, Theresa W. Gyorkos

1. McGill University, Montreal, QC, Canada
2. Asociación Civil Selva Amazónica, Iquitos, Peru

Objective: With the deadline for the MDGs approaching, emphasis needs to be placed on cost-effective, easily integrated solutions for targeting multiple conditions simultaneously. Deworming is one such intervention that has been shown to target multiple MDGs. Despite reasonable deworming coverage rates in school-age children (through school-based campaigns), coverag e remains low in preschool-age children. In Peru, routine growth and development clinics (CRED) are scheduled in early childhood (once monthly from birth to 12 months), at which time interventions such as vaccinations are provided. These CRED visits may be an ideal avenue to piggyback deworming, thereby targeting the youngest age group.

Methods: A deworming trial is currently being conducted in Iquitos, an STH-endemic area of the Peruvian Amazon. Enrolment took place between September 2011 and June 2012 in children attending their routine 12-month CRED visits. Eligibility criteria included attendance of the 12-month CRED visit, but previous attendance was not required. A socio-demo-epi questionnaire was administered at baseline. Previous CRED attendance, along with vaccinations to date, were requested from the mother, and verified from medical records.

Outcome: A total of 1760 children were enrolled in the trial. The mean number of CRED visits before enrolment was 7.6 (SD ± 3.5) (range 0 to 13). Only 3.6% had no previous visits. Baseline data indicated that 34.6% of participants had received their Measles, Mumps and Rubella (MMR) vaccine (scheduled at 12 months of age), although an additional 43.8% had an appointment for this scheduled. Seventy-eight percent of participants had all other vaccinations according to schedule; this percentage was over 85% for all vaccines except the pneumococcal vaccine.

Discussion: The majority of children in the study population attend CRED visits, and vaccination coverage is high. Although less than half of children were currently vaccinated against MMR, this is likely due to the fact that enrolment was done at the same age that MMR is scheduled. Those who do not attend formal clinic visits regularly can also be targeted at home through MMR campaigns, which are periodically conducted to increase coverage. Conclusion: Piggybacking deworming activities with vaccinations, both in health clinics and in community campaigns, may be successful in targeting the youngest age groups who are not yet exposed to school-based deworming campaigns. This can ultimately increase coverage of multiple interventions in the most vulnerable populations and help to extend heath and social benefits beyond the 2015 MDG deadline.

3rd North American Congress of Epidemiology, Montreal, Canada, June 2011

Weighing the ethical and epidemiological rigour in RCTs of early childhood deworming: a fine but necessary balance to support evidence-based policy.

S.A. Joseph* and T.W. Gyorkos

International organizations such as the World Health Organization and the Pan American Health Organization recommend mass deworming of children in endemic areas, including preschool-age children as of 1 year of age. Still, there remain important knowledge gaps regarding the most appropriate treatment regimes, delivery strategies and outcomes of interest, particularly for preschool-age children. The unique nutritional demands and growth pattern in early childhood, especially in children under two years of age, warrant the need for new trials rather than merely extrapolating results from older children. But how then do we ensure that we provide the most rigorous epidemiologic evidence while balancing the ethical concerns inherent in a study in which some children receive deworming treatment while others do not? A deworming RCT that is being conducted in 12 to 24 month old children in Iquitos, Peru will be used as a case study to discuss these important considerations, including: deciding on the most appropriate comparison group(s), including the use of a placebo control and provision of standard of care services; conducting a blinded or open-label study; obtaining high participation rates and maintaining low loss to follow-up and high compliance of children when not all will receive active treatment; determining if and when to collect, analyze and store stool samples; among other issues. Taking into account the unique ethical and epidemiological characteristics of RCTs of deworming in young children will ensure that the results obtained will properly inform and support global evidence-based policy recommendations, reduce the burden of intestinal parasite infection and ultimately improve early childhood growth and development.

European Congress of Tropical Medicine and International Health, Barcelona, Spain, October 2011

Deworming benefits during the critical window of growth and development: reviewing the literature and identifying the research gaps for children 12 to 24 months of age

SA Joseph, TW Gyorkos

Introduction: As of 2002, WHO has recommended the inclusion of children between 12 and 24 months of age in large-scale deworming activities. These children are in the most critical window of growth and development, at which time deworming interventions may have important implications for short and long-term health, nutrition and social outcomes. Therefore, the objective of this study was to review the literature on deworming in early preschool-age children.

Methods: A search of relevant databases (e.g. Medline, Embase, Lilacs, Cochrane) was undertaken. Inclusion criteria of studies were: 1) randomized controlled trials (RCTs) with a placebo or usual care control group; 2) growth and/or development outcomes; 3) age < 24 months at recruitment; 4) use of WHO recommended drugs, albendazole or mebendazole; 5) healthy study populations.

Results: A total of 5 RCTs fit the inclusion criteria. Frequency of treatment ranged from once yearly to every 3 months. Two trials included school-age children and 2 trials included children < 12 months in their study populations. No trials focused on or reported age-disaggregated results for children < 2 years of age. Anthropometric outcomes were reported as continuous (e.g. weight gain, Z scores) and categorical (e.g. underweight) measurements. Only one trial measured development, which was by parents' self-report. No adverse events were reported in any of the studies.

Conclusions: A thorough understanding of the benefits of deworming in children 12 to 24 months of age has been limited by the: the inclusion of children under 1 year of age, and/or a lack of age-disaggregated data in studies including children > 2 years of age; the variable frequency of treatment; and the heterogeneity in outcome measurement and reporting. An RCT on the benefits of deworming, including appropriate timing and frequency, on growth and development in children 12 to 24 months of age is currently being undertaken in Iquitos, Peru to help fill this important research gap.

International Congress of Parasitology, Mexico City, Mexico, August 2014

A randomized-controlled trial to determine the benefit of deworming on growth in early preschool-age children in Iquitos, Peru

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BACKGROUND: WHO recommends deworming of young children as of 12 months of age in soil-transmitted helminth (STH) endemic areas; however, the optimal timing and frequency have been understudied in early preschool-age children. These children may be particularly vulnerable to adverse effects from STH infection as they are in the most critical window for growth.

METHODS: We conducted a randomized controlled trial of deworming (500mg single-dose mebendazole) in 12 month-old children in Iquitos, an STH-endemic area of the Peruvian Amazon. A total of 1760 children were enrolled between September 2011 and July 2013 and followed for one year. Children were randomly allocated to one of four groups: 1) deworming at 12 months of age and placebo at 18 months of age; 2) placebo at 12 months of age and deworming at 18 months of age; 3) deworming at 12 and 18 months of age; or 4) placebo at 12 and 18 months of age. Height, weight and STH infection were assessed at each visit.

RESULTS: A total of 1563 children (88.8%) attended their 24-month visit. At baseline, the prevalence of STH infection was 12.4%. The species distribution was 11.5% for *Ascaris*, 4.5% for *Trichuris* and 0.6% for hookworm. STH prevalence rose to over 40% at 24 months. There was a statistically significant improvement in weight gain in those receiving deworming once at 12 months, compared to those receiving deworming once at 18 months ($p=0.028$). No additional benefit was detected for twice-yearly deworming.

CONCLUSIONS: Our results indicate that once-yearly deworming at 12 months of age has important benefits on growth. These results contribute to the evidence-base on deworming policy in over 100 STH-endemic countries worldwide. Emphasis should be placed on providing children in this vulnerable age group with cost-effective, integrated interventions to reduce health and nutritional burdens.

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Title: The effect of deworming timing and frequency on growth in early preschool-age children: results of a randomized-controlled trial of mebendazole in one to two-year old children in the Peruvian Amazon.

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Children under two years of age are in the most critical window for growth and development. As mobility increases, this time also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical environments. WHO recommends deworming as of 12 months in endemic areas; however, the optimal timing and frequency have been understudied in this age group. Many countries still exclude children 12-23 months in deworming programs. We conducted a randomized-controlled trial of deworming (500mg single-dose mebendazole) in 12 and 13 month-old children in Iquitos, an STH-endemic area of the Peruvian Amazon. A total of 1760 children were enrolled from September 2011 to June 2012 at 12 participating health centres. Children were randomly allocated to one of four groups: 1) deworming at 12 months of age and placebo at 18 months of age; 2) placebo at 12 months of age and deworming at 18 months of age; 3) deworming at 12 and 18 months of age; or 4) placebo at 12 and 18 months of age (i.e. control group). Participants were followed up to 24 months of age to assess the benefit of deworming on the main outcome of weight gain. Results were analyzed with an intention-to-treat approach. A total of 1563 children (88.8%) attended their 24 month visit. STH prevalence rose from 12.2% at 12 months to over 40% at 24 months. Mean weight gain (kg) between 12 and 24 months was: Group 1): 2.05 (±0.7); Group 2): 1.94 (±0.8); Group 3): 2.04 (±0.7); and Group 4): 2.00 (±0.7). There was a statistically significant improvement in weight gain in those receiving deworming once at 12 months, compared to those receiving deworming once at 18 months ($p=0.028$). No difference was detected between those receiving deworming once at 12 months vs. twice at 12 and 18 months ($p=0.88$). Results remained significant when adjusting for baseline characteristics. Additional analyses were performed to take into account clustering, multiple testing, missing data and compliance. Overall, our results indicate that deworming, provided once-yearly at 12 months of age, has important benefits on growth in early preschool-age children. These results contribute to the evidence-base on deworming policy in over 120 STH-endemic countries worldwide. Emphasis should be placed on translating results into practice, such that children in this vulnerable age group are targeted with the most cost-effective, integrated interventions to reduce health and nutritional burdens.

APPENDIX 10

Supplementary analyses

MANUSCRIPT A

1. Risk factors for stunting and underweight in 12 and 13-month old children in Iquitos, Peru, September 2011 to June 2012 **(n=1760).**
   ****Includes children with specimens analyzed by both the Kato-Katz and the direct method**

Child, maternal and household characteristics	Stunting		Underweight	
	Crude RR (95% CI)	Adjusted RR (95% CI)	Crude RR (95% CI)	Adjusted R (95% CI)
Any STH infection (yes vs. no)	1.30 (1.03, 1.64)	1.30 (1.02, 1.65)	1.45 (0.97, 2.18)	1.46 (0.96, 2.22)
Sex (male vs. female)	1.56 (1.30, 1.87)	1.60 (1.32, 1.93)	1.65 (1.19, 2.29)	1.66 (1.18, 2.33)
Age (13 vs. 12 months)	1.52 (1.20, 1.92)	1.51 (1.19, 1.92)	NS	NS
Birth weight (per kg increase)	0.89 (0.86, 0.92)	0.49 (0.41, 0.56)	0.32 (0.25, 0.43)	0.35 (0.27, 0.46)
Continued breastfeeding at 12 months (no vs. yes)	1.30 (1.02, 1.67)	NS	1.35 (0.86, 2.11)	NS
Vaccinations up-to-date (no vs. yes)	1.17 (0.95, 1.44)	NS	1.50 (1.06, 2.12)	NS
Vitamin A supplementation in previous year (no vs. yes)	1.18 (0.99, 1.40)	NS	1.53 (1.12, 2.10)	NS
Any hospitalization since birth (yes vs. no)	1.57 (1.23, 1.99)	NS	NS	NS
Mean development score (per 1 point increase)	0.95 (0.94, 0.97)	0.96 (0.95, 0.98)	0.92 (0.90, 0.94)	0.93 (0.91, 0.95)
First vs. fourth SES quartile	1.72 (1.31, 2.25)	1.48 (1.12, 1.96)	2.69 (1.61, 4.51)	2.14 (1.24, 3.68)
Second vs. fourth SES quartile	1.56 (1.18, 2.05)	1.43 (1.08, 1.89)	2.54 (1.52, 4.25)	1.92 (1.12, 3.29)
Third vs. fourth SES quartile	1.70 (1.30, 2.23)	1.44 (1.10, 1.89)	1.85 (1.06, 3.21)	1.53 (0.86, 2.70)
Maternal iron supplementation during pregnancy (no vs. yes)	1.19 (0.96, 1.49)	NS	1.36 (0.93, 2.00)	NS

MANUSCRIPT B

1a. Overall benefit of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=1103).

**** Per-protocol analyses including only those with all three visits and who did not report taking deworming outside of the trial protocol**

	Group 1	Group 2	Group 3	Group 4
Unadjusted weight difference	0.055407	-0.12618	0.038382	reference
p value	0.3715	0.0418	0.5376	reference
Adjusted weight difference	0.055161	-0.11395	0.042142	reference
p value	0.3705	0.0651	0.4966	reference
Unadjusted height difference	0.216854	-0.13688	0.071763	reference
p value	0.1923	0.41	0.6674	reference
Adjusted height difference	0.262321	-0.06511	0.124989	reference
p value	0.107	0.6897	0.4452	reference
Unadjusted WAZ difference	0.060546	-0.13122	0.032896	reference
p value	0.2921	0.0224	0.5687	reference
Adjusted WAZ difference	0.058447	-0.11582	0.036569	reference
p value	0.3009	0.041	0.5201	reference
Unadjusted HAZ difference	0.075558	-0.07542	0.019732	reference
p value	0.2031	0.2036	0.7406	reference
Adjusted HAZ difference	0.08779	-0.04713	0.038238	reference
p value	0.132	0.4198	0.5143	reference

1b. Overall benefit of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=1563).

**** Complete case analyses including only those who attended the 24-month visit**

	Group 1 (n=388)	Group 2 (n=398)	Group 3 (n=381)	Group 4 (n=396)
Outcome				
Weight change, kg	2.05 (0.7)	1.94 (0.8)	2.04 (0.7)	2.00 (0.7)
p value	0.336	0.214	0.418	*reference*
Length change, cm	9.84 (2.1)	9.57 (1.9)	9.69 (1.9)	9.64 (2.0)
p value	0.151	0.589	0.753	*reference*
WAZ change	-0.22 (0.7)	-0.35 (0.7)	-0.23 (0.7)	-0.27(0.6)
p value	0.319	0.106	0.433	*reference*
HAZ change	-0.49 (0.7)	-0.60 (0.7)	-0.54 (0.7)	-0.55 (0.7)
p value	0.201	0.345	0.846	*reference*

1c. Overall benefit of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=185).

**** Analyses restricted to STH-infected children at baseline**

	Group 1	Group 2	Group 3	Group 4
Unadjusted weight difference	-0.15556	-0.02535	-0.04003	reference
p value	0.3132	0.8703	0.7907	reference
Adjusted weight difference	-0.2043	-0.05899	-0.08763	reference
p value	0.1985	0.7078	0.5709	reference
Unadjusted height difference	-0.68213	-0.32813	-0.52098	reference
p value	0.0681	0.3824	0.1539	reference
Adjusted height difference	-0.67514	-0.33407	-0.48388	reference
p value	0.0769	0.3764	0.1926	reference
Unadjusted WAZ difference	-0.11881	0.015729	0.027545	reference
p value	0.4652	0.9235	0.8626	reference
Adjusted WAZ difference	-0.17931	-0.02629	-0.03579	reference
p value	0.2831	0.8739	0.8258	reference
Unadjusted HAZ difference	-0.153	-0.02917	-0.07018	reference
p value	0.3957	0.8723	0.6904	reference
Adjusted HAZ difference	-0.14503	-0.03369	-0.06403	reference
p value	0.4363	0.8554	0.7243	reference

2a. The effect of timing of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=1103).

**** Per-protocol analyses including only those with all three visits and who did not report taking deworming outside of the trial protocol**

	Group 1	Group 2
Unadjusted weight difference	0.181584	reference
p value	0.0031	reference
Adjusted weight difference	0.169107	reference
p value	0.0055	reference
Unadjusted height difference	0.353737	reference
p value	0.0313	reference
Adjusted height difference	0.327426	reference
p value	0.0419	reference
Unadjusted WAZ difference	0.19177	reference
p value	0.0007	reference
Adjusted WAZ difference	0.17427	reference
p value	0.0018	reference
Unadjusted HAZ difference	0.150973	reference
p value	0.0101	reference
Adjusted HAZ difference	0.13492	reference
p value	0.0193	reference

2b. The effect of timing of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=1563).

**** Complete case analyses including only those who attended the 24-month visit**

	Group 1 (n=388)	Group 2 (n=398)
Outcome		
Weight change, kg	2.05 (0.7)	1.94 (0.8)
p value	0.028	*reference*
Length change, cm	9.84 (2.1)	9.57 (1.9)
p value	0.048	*reference*
WAZ change	-0.22 (0.7)	-0.35 (0.7)
p value	0.009	*reference*
HAZ change	-0.49 (0.7)	-0.60 (0.7)
p value	0.027	*reference*

2c. The effect of timing of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=185).

**** Analyses restricted to STH-infected children at baseline**

	Group 1	Group 2
Unadjusted weight difference	-0.13021	reference
p value	0.3442	reference
Adjusted weight difference	-0.14531	reference
p value	0.2981	reference
Unadjusted height difference	-0.354	reference
p value	0.2874	reference
Adjusted height difference	-0.34107	reference
p value	0.3083	reference
Unadjusted WAZ difference	-0.13454	reference
p value	0.3545	reference
Adjusted WAZ difference	-0.15302	reference
p value	0.2977	reference
Unadjusted HAZ difference	-0.12383	reference
p value	0.4411	reference
Adjusted HAZ difference	-0.11134	reference
p value	0.4968	reference

3a. The effect of frequency of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=1103).

**** Per-protocol analyses including only those with all three visits and who did not report taking deworming outside of the trial protocol**

	Group 1	Group 2	Group 3
Unadjusted weight difference	0.017025	-0.16456	reference
p value	0.782	0.0075	reference
Adjusted weight difference	0.013019	-0.15609	reference
p value	0.8315	0.0108	reference
Unadjusted height difference	0.145091	-0.20865	reference
p value	0.3794	0.2059	reference
Adjusted height difference	0.137332	-0.19009	reference
p value	0.3955	0.2391	reference
Unadjusted WAZ difference	0.02765	-0.16412	reference
p value	0.6278	0.004	reference
Adjusted WAZ difference	0.021879	-0.15239	reference
p value	0.6966	0.0067	reference
Unadjusted HAZ difference	0.055827	-0.09515	reference
p value	0.3433	0.1061	reference
Adjusted HAZ difference	0.049552	-0.08537	reference
p value	0.3919	0.14	reference

3b. The effect of frequency of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=1563).

**** Complete case analyses including only those who attended the 24-month visit**

	Group 1 (n=388)	Group 2 (n=398)	Group 3 (n=381)
Outcome			
Weight change, kg	2.05 (0.7)	1.94 (0.8)	2.04 (0.7)
p value	0.8829	0.0413	*reference*
Length change, cm	9.84 (2.1)	9.57 (1.9)	9.69 (1.9)
p value	0.2673	0.3952	*reference*
WAZ change	-0.22 (0.7)	-0.35 (0.7)	-0.23 (0.7)
p value	0.836	0.017	*reference*
HAZ change	-0.49 (0.7)	-0.60 (0.7)	-0.54 (0.7)
p value	0.2834	0.2588	*reference*

3c. The effect of frequency of deworming on anthropometric outcomes over 12 months, using one-way ANOVA and multivariable linear regression analysis (n=185).

**** Analyses restricted to STH-infected children at baseline**

	Group 1	Group 2	Group 3
Unadjusted weight difference	-0.11554	0.014673	reference
p value	0.3833	0.9127	reference
Adjusted weight difference	-0.11667	0.028643	reference
p value	0.3811	0.8323	reference
Unadjusted height difference	-0.16114	0.192857	reference
p value	0.6146	0.5512	reference
Adjusted height difference	-0.19126	0.149814	reference
p value	0.549	0.6442	reference
Unadjusted WAZ difference	-0.14636	-0.01182	reference
p value	0.2956	0.9333	reference
Adjusted WAZ difference	-0.14352	0.0095	reference
p value	0.3061	0.9468	reference
Unadjusted HAZ difference	-0.08282	0.041012	reference
p value	0.5924	0.7931	reference
Adjusted HAZ difference	-0.081	0.03034	reference
p value	0.6043	0.8485	reference

4. Baseline characteristics of children who attended the 24-month visit (n=1563) compared to those who did not attend the 24-month visit (n=197).

	Attended last visit (n=1563)	Did not attend last visit (n=197)
Child characteristics		
Weight, kg	8.71(1.0)	8.75 (1.0)
Length, cm	72.1 (2.4)	72.4 (2.6)
Age, months	12.1 (0.3)	12.2 (0.4)
Birth weight, kg	3.1 (0.5)	3.1 (0.5)
Birth length, cm	49.4 (2.5)	49.3 (2.4)
Development score	98.1 (6.0)	98.2 (6.1)
Sex, female	749 (47.9)	91 (46.2)
Continued breastfeeding at 12 months	1397 (89.4)	178 (90.4)
Up-to-date vaccinations	1255 (80.5)	155 (78.7)
Received vitamin A in previous year	821 (52.5)	100 (50.8)
Hospitalizations since birth	148 (9.5)	15 (7.6)
Walking without support	387 (24.8)	46 (23.5)
Maternal and household characteristics		
Married or common-law	1258 (80.5)	165 (83.8)
Maternal secondary education completed	501 (32.1)	53 (26.9)
Maternal employment outside the home	161 (10.3)	18 (9.1)
Periurban/rural residence	1385 (88.6)	175 (88.8)
Potable water in home	801 (51.3)	97 (49.2)
Earth/wood house material	1205 (77.1)	149 (75.6)
SES - Lowest quartile	403 (25.8)	55 (27.9)
SES - 2nd lowest quartile	371 (23.7)	48 (24.4)
SES - 2nd highest quartile	420 (26.9)	40 (20.3)
SES - Highest quartile	369 (23.6)	54 (27.4)

Results are expressed as means (SD) or frequency (%)