Maternal postpartum deworming: a novel strategy to reduce infant and maternal morbidity in low-and-middle-income countries

Layla S. Mofid, MSc
Department of Epidemiology, Biostatistics and Occupational Health
McGill University, Montréal, Canada
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ABSTRACT

Background: The World Health Organization identifies women of reproductive age as a high risk group for soil-transmitted helminth infections because these infections cause blood loss, iron deficiency anemia, and nutritional impairment. Deworming is a central component of control efforts aimed at reducing the burden of disease in endemic areas. The postpartum period offers a unique opportunity to reach women periodically throughout their reproductive lifespans. To date, no study has investigated the effect of maternal postpartum deworming on infant or maternal health outcomes.

Objectives: The objectives of this study were to determine the effectiveness of maternal postpartum deworming on the following infant and maternal health outcomes: 1) infant growth and morbidity; 2) maternal soil-transmitted helminth infection and intensity, anemia, and fatigue; and 3) breast milk quality indicators.

Methods: A double-blind, randomized, placebo-controlled trial was carried out in Iquitos, Peru. Mother-infant pairs were enrolled into the trial following delivery at Hospital Iquitos "Cesar Garayar Garcia". Prior to hospital discharge women were randomized to receive either a single-dose 400 mg albendazole tablet (i.e., deworming) or identical placebo, based on a pre-specified randomization schedule. Mother-infant pairs were visited in their homes at 1 and 6 months postpartum for outcome ascertainment. The primary outcome was mean infant weight gain between birth and 6 months of age. Continuous outcomes were compared between intervention groups using linear regression models and dichotomous outcomes were compared using log-binomial regression models. Analyses followed an intention-to-treat approach. Adjusted, complete-case, and per-protocol analyses were also performed.

Results: Between February and August 2014, 1010 mother-infant pairs were enrolled into the trial. A total of 999 (98.9%) and 970 (96.0%) mother-infant pairs completed their 1 and 6-month study visits, respectively. Infant weight gain between birth and 6 months of age was similar between intervention groups (4.3 kg ±0.04 *vs.* 4.4 kg ±0.04), as were secondary growth indices. The single-dose deworming intervention also did not have a differential effect on the occurrence

of hospitalizations, nor on the incidence of diarrhea, respiratory illness, fever, or ear infections in infants. Over the 6-month postpartum period, deworming significantly reduced the prevalence (RR: 0.5; 95% CI: 0.4, 0.6) and intensity of maternal helminth infections but had no demonstrable effect on maternal anemia (RR: 1.0; 95% CI: 0.9, 1.1) or fatigue score (mean difference: -0.4; 95% CI: -1.1, 0.3). Additionally, the mean concentration of vitamins and minerals in breast milk was similar between intervention groups.

Conclusions: Overall, while single-dose deworming did reduce maternal helminth infections, no statistically significant benefit was detected on infant growth or morbidity outcomes in the first 6 months of life. Maternal postpartum deworming also had no effect on the prevalence of maternal anemia, fatigue, and breast milk composition. The benefits of deworming may be more apparent in areas of higher prevalence and intensity of soil-transmitted helminth infection, over longer periods of follow-up, and with repeated administration. This first trial on maternal postpartum deworming contributes essential empirical evidence to inform World Health Organization policy and guidelines towards eliminating soil-transmitted helminthiasis as a public health problem.

RÉSUMÉ

Contexte*:* L'Organisation mondiale de la santé considère les femmes en âge de procréer comme un groupe à haut risque d'infection par les géohelminthes parce que ces infections causent des pertes sanguines, l'anémie ferriprive et une détérioration de l'état nutritionnel. Le déparasitage est un élément essentiel des efforts de réduction du fardeau de la maladie dans les régions où les géohelminthes sont endémiques. Toutefois, l'effet du déparasitage postpartum sur la santé maternelle et infantile n'a jamais été étudié à ce jour.

Objectifs*:* Les objectifs de cette étude étaient de mesurer l'efficacité du déparasitage maternel postpartum sur les issues suivantes: 1) la croissance et la morbidité infantiles: 2) la prévalence et l'intensité des géohelminthiases, l'anémie et la fatigue chez les mères; et 3) les indicateurs de la qualité du lait maternel.

Méthodes*:* Un essai à double insu contrôlé par placebo a été effectué à Iquitos, au Pérou. Des dyades mère-enfant ont été enrôlées dans l'étude après la naissance des enfants à l'Hôpital 'Cesar Garayar Garcia' d'Iquitos. Avant leur congé de l'hôpital, les femmes ont été désignées aléatoirement pour recevoir, soit une capsule unique de 400 mg d'albendazole (déparasitage), soit un placebo, selon un protocole préétabli. Mères et nourrissons ont été visités à domicile à la fin des premier et sixième mois de vie du nourrisson. Le principal résultat à l'étude était le gain pondéral des nourrissons durant leurs six premiers mois de vie. Les résultats continus ont été comparés entre les groupes d'intervention au moyen de régressions linéaires et les résultats dichotomiques ont été comparés au moyen de régressions log-binomiales. Les analyses suivaient l'approche de l'intention de traiter. Des analyses ajustées, des analyses complètes de cas et selon le protocole ont également été effectuées.

Résultats *:* De février à août 2014, 1010 dyades ont été enrôlées dans l'étude. En tout, 999 (98,9%) dyades ont complété la visite à un mois, et 970 (96,0%) ont complété celle à six mois. Le gain pondéral des nourrissons des deux groupes d'intervention durant leurs six premiers mois de vie étaient similaires (4,3 kg ±0.04 *vs.* 4,4 kg ±0.04), tout comme les indicateurs secondaires de la croissance. L'intervention de déparasitage à dose unique n'a eu aucun effet sur l'occurrence

des hospitalisations, ni sur l'incidence de la diarrhée, de la toux, de la fièvre ou de l'otite chez les nourrissons. Durant les six mois postpartum, le déparasitage a réduit significativement la prévalence (RR: 0,5; IC à 95% : 0,4; 0,6) et l'intensité des infections à géohelminthes chez les mères mais n'a eu aucun effet démontrable sur l'anémie (RR : 1,0; IC à 95% : 0,9; 1,1) ou le score de fatigue (différence des moyennes : -0,4; IC à 95% : -1,1; 0,3) chez les mères. De plus, les concentrations moyennes de vitamines et de minéraux dans le lait maternel des deux groupes d'intervention étaient similaires. .

Conclusions *:* Bien que l'intervention de déparasitage à dose unique ait réduit significativement les infections à géohelminthes chez les mères, aucun effet bénéfique statistiquement significatif n'a été détecté sur la croissance ou la morbidité des nourrissons durant leurs six premiers mois de vie. Le déparasitage postpartum n'a eu non plus aucun effet sur la prévalence de l'anémie et de la fatigue chez les mères, ni sur la composition du lait maternel. Les effets bénéfiques du déparasitage pourraient être plus apparents dans des zones où la prévalence et l'intensité des infections à géohelminthes sont plus élevées, après de plus longues périodes de suivi ou après des traitements répétés. Ce premier essai de déparasitage maternel postpartum apporte une preuve empirique essentielle qui informera les politiques et les lignes directrices de l'Organisation mondiale de la santé en vue de l'élimination des géohelminthiases comme problème de santé publique.

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DEDICATION

To Matthew

PREFACE AND CONTRIBUTION OF AUTHORS

Contribution of Authors

Layla S. Mofid, MSc (LSM) is the first author on all manuscripts. Under the guidance of her thesis supervisor, TWG, she was responsible for the formulation of the research question, and the development of the study design and research protocol. She helped write the grant application to the Bill and Melinda Gates Foundation, in which she is a co-investigator. She was responsible for preparing the applications to the research ethics board committees, in Canada (McGill University Health Centre), and in Peru (Asociación Civil Impacta Salud y Educación and the Instituto Nacional de Salud). She worked extensively with a software designer to develop a mobile data application and implemented its use in the trial. Ms. Mofid was present on-site in Iquitos, Peru from May 2013 to September 2014, where she assumed the role of Project Director of the trial. During this time, she was responsible for the hiring and training of a research team comprising a total of 22 people, developing and pilot-testing study instruments, preparing standard operating procedures, supervising study visits and laboratory activities, reviewing data collection and questionnaires daily, and carrying out quality control activities of all study procedures. Upon returning to Canada, Ms. Mofid was responsible for data cleaning, performing statistical analyses, drafting manuscripts, and disseminating results.

Theresa W. Gyorkos, PhD (TWG) is a Professor in the Department of Epidemiology, Biostatistics and Occupational Health at McGill University, and the senior author on all manuscripts. She is the Principal Investigator of the trial, and the thesis supervisor of LSM. She played a critical role in protocol development, including the conception and design of the study. In addition, she oversaw all academic, ethical, and logistic aspects of the trial. She provided substantive and methodological contributions during all stages of the trial, including interpretation of study results, and critical review of all manuscripts.

Martín Casapía, MD, MPH (MC) is the Director General of the Asociación Civil Selva Amazónica, a Professor in the Facultad de Medicina Humana at the Universidad Nacional de la Amazonía Peruana, and an Adjunct Professor in the Department of Epidemiology, Biostatistics and Occupational Health. He is a co-investigator on the trial and co-author on the manuscripts reporting trial results (i.e., Manuscripts 2-4). His role included providing local expertise on the

design and implementation of the trial, and presenting the trial to the ethics board committees in Peru, to Ministry of Health officials, and to the administration at Hospital Iquitos. He also aided in the interpretation and dissemination of study results.

Elham Rahme, PhD (ER) is a co-investigator on this project and co-author on the manuscripts reporting trial results (i.e., Manuscripts 2-4). She provided statistical and methodological expertise based on her involvement with numerous clinical research projects and trials. She contributed to the study design, analysis of data, interpretation of study results, and revision of manuscripts.

Grace S. Marquis, PhD (GSM) is a co-investigator on this project and co-author on Manuscripts 2 and 4. She provided expertise on nutrition, especially related to lactation performance and infant growth. Based on her extensive experience working in Peru and other low-and-middle income countries, she contributed to study methodology, results interpretation, and manuscript revision.

William D. Fraser, MD, MSC (WDF) is a co-investigator on this project and co-author on Manuscript 2. He provided expertise on obstetrics and randomized controlled trial methodology, and contributed to the research design, results interpretation, and manuscript revision.

Hugo Razuri, MD, MPH (HR) was the Canadian-based research coordinator during study implementation and co-author on Manuscripts 2-4. He provided input into the organization of fieldwork and communicated with collaborators on contractual aspects of the trial. He also contributed to interpretation of study findings and the revision of manuscripts.

Brittany Blouin, MSc (BB) was the Canadian-based research coordinator at the time of study conception and co-author on Manuscripts 2-4. She provided insight on the trial design and methodology, and also aided in grant writing. She provided input on the interpretation of study findings and contributed to the revision of manuscripts.

Lidsky Pezo, BSc (LP) was the local research coordinator in Iquitos, Peru and co-author on Manuscripts 2-4. She provided valuable input into the organization and logistics of study implementation, including preparing reports for research governing bodies in Peru; hiring, training and supervision of research assistants; and daily review of data collection. She also participated in the interpretation of study results and revision of manuscripts.

Antonio Montresor, MD (AM) is a co-investigator on this project and co-author on Manuscripts 2-4. He provided valuable substantive expertise on global health policy in his role as Medical Officer in the Department of Control of Neglected Tropical Diseases at the World Health Organization and as investigator on numerous research projects on deworming, worldwide. He provided input on study methodology, results interpretation, and manuscript revision.

Lindsay H Allen, PhD (LHA) is a co-investigator on this research project and co-author on Manuscripts 2 and 4. Along with **Setareh Shahab-Ferdows, PhD (SSF)** and **Daniela Hampel, PhD (DH)** (co-authors on Manuscript 4), they provided input on study methodology based on their experience in optimal methods for breast milk data collection. They also provided input into the interpretation of study results and revision of manuscripts.

Drs. Eder Aguilar, MD (EA) and **Hermánn Silva, MD (HS)** (co-authors on Manuscript 2) were the respective directors of the Departments of Obstetrics and Gynecology, and of Neonatology, at Hospital Iquitos during trial enrolment, and collaborators on this research project. They provided logistical support and input into study procedures taking place at the hospital, and as well as in the interpretation of study findings.

Statement of Originality

The work presented in this thesis is the result of an original research project formulated and conducted predominantly by LSM. To our knowledge, we are the first research group to propose maternal postpartum deworming as a viable and efficient strategy to target women of reproductive age in deworming programs. Though a well-developed framework exists for deworming in school-age children, the evidence base on deworming in other high risk groups, especially women of reproductive age, is lacking. In 1994, the World Health Organization held an Informal Consultation that concluded that the use of deworming in women of reproductive age is appropriate and safe, based on expert opinion and research conducted in other population groups. Additionally, the Consultation called for research on the effects of deworming on lactation performance. In 2006, the World Health Organization formulated their recommendations to include women of reproductive age (including pregnant women in their second and third trimester, and lactating women) as target populations for deworming activities.

The randomized controlled trial presented here represents the first study to evaluate the effects of integrating deworming into routine maternal postpartum care on a comprehensive range of infant and maternal health outcomes, while employing rigorous epidemiological methods. Despite the international recommendations of the World Health Organization, women of reproductive age continue to be excluded from national deworming programs and ministry of health guidelines in many endemic countries. Coverage estimates are also not available for this high risk group. Additionally, no previous clinical trials or observational studies have been specifically conducted in lactating women. For these reasons, there is unequivocal justification for a state of clinical equipoise. This study is also the first to assess the role of deworming on lactation performance, specifically on a broad panel of breast milk quality indicators. The overarching aim of this research is to inform clinical practice and global deworming guidelines. This research project contributes new evidence to the fields of parasite epidemiology, neglected tropical diseases, soil-transmitted helminths, and maternal and child health, and builds on the momentum generated by deworming efforts globally.

While LSM received active guidance throughout this study from her supervisor, thesis advisory committee members, and co-authors on substantive, methodological and statistical aspects, the conception, execution and drafting of the research reported in this thesis are her own.

Notation

For ease of reading, time points for the study are represented numerically (i.e., as 1 and 6 months) instead of written out in full within the text.

STATEMENT OF SUPPORT

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LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation	Definition
ACAZ	Mid-upper arm circumference-for-age
ACSA	Asociación Civil Selva Amazónica
AI	Adequate intake
aOR	Adjusted odds ratio
BCI	Bayesian Credible Interval
Ca	Calcium
CI	Confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CR	Cure rate
CV	Coefficient of variation
DALY	Disability-adjusted life year
DIRESA	Dirección Regional de Salud (Regional Ministry of Health) (in Peru)
DSMC	Data Safety and Monitoring Committee
epg	Eggs per gram (of feces)
ERR	Egg reduction rate
FAD	Flavin adenine dinucleotide
FAS	Fatigue assessment scale
Fe	Elemental iron
Hb	Hemoglobin
HC	Head circumference
HCAZ	Head circumference-for-age

HPLC-DAD	High performance liquid chromatography with diode array detection
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
IDA	Iron deficiency anemia
IFA	Iron-folic acid
IMCI	Integrated Management of Childhood Illnesses
INS	Instituto Nacional de Salud (National Institutes of Health) (in Peru)
ITT	Intention-to-treat
LAZ	Length-for-age z-score
LMIC	Low-and-middle-income country
MICE	Multiple Imputation by Chained Equations
MUAC	Mid-upper arm circumference
MUHC	McGill University Health Centre
NTD	Neglected tropical disease
OR	Odds ratio
PC	Preventive chemotherapy
pre-SAC	Preschool-aged children
RCT	Randomized controlled trial
REB	Research ethics board
RR	Risk ratio
SAC	School-aged children
SAE	Serious adverse event
SD	Standard deviation
SES	Socioeconomic status

SMD	Standardized mean difference
STH	Soil-transmitted helminthiasis or soil-transmitted helminths
UNICEF	United Nations Children's Fund
UPLC-MS/MS	Ultra-performance liquid-chromatography tandem mass spectrometry
WAZ	Weight-for-age z-score
WHO	World Health Organization
WRA	Women of reproductive age

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CHAPTER 1: INTRODUCTION

Soil transmitted helminths (STH) are a group of infections caused by four intestinal nematodes, namely, the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, and the hookworms *Ancylostoma duodenale* and *Necator americanus*. STHs are considered a disease of poverty in which the most vulnerable individuals living in areas where adverse health, social, and economic conditions predominate, carry the greatest disease burden. The World Health Organization (WHO) considers women of reproductive age (WRA) to be a high risk group for STH infections largely because of the high prevalence of anemia in this population that is caused, in part, by hookworm (de Benoist et al. 2008) and *T. trichiura* (Gyorkos et al. 2011a) infections. Iron deficiency is the most prevalent nutritional deficiency worldwide, and is an important cause of anemia, especially in pregnant and lactating women (Milman 2011). During lactation, anemia is thought to adversely affect mood, fatigue, maternal-infant bonding, and breastfeeding practices (Beard et al. 2005; Bozoky and Corwin 2002; Perez et al. 2005; Rioux et al. 2006), which can ultimately impact negatively on infant growth and development.

Deworming has been shown to be one of the safest and most cost-effective interventions for reducing STH burden in endemic countries, and it is the cornerstone of prevention and control measures against STH infections (WHO 2006a). In fact, in 2012, the Copenhagen Consensus ranked deworming among the top four solutions to address 'big issues facing the planet' in terms of cost and benefit (Copenhagen Consensus Centre: Expert Panel Findings 2012). The single-dose deworming drugs, albendazole and mebendazole, are recommended by WHO for efficient administration in large-scale deworming programs (WHO 2006a). In highly STH-endemic areas, WHO recommends annual or biannual mass deworming; that is, drug administration without prior diagnosis, to school-aged children, preschool-aged children, and WRA. Among the 112 countries considered endemic for STH infections (Knopp et al. 2012), there are no coverage estimates available for deworming in WRA, and few countries include this risk group among other deworming activities (Larocque and Gyorkos 2006). One major barrier to including WRA in deworming programs is likely the fear of inadvertently administering deworming drugs to women who may not be aware that they are in their first trimester of pregnancy (deworming being contraindicated in the first trimester of pregnancy (WHO 2002)). Consequently, this

vulnerable population has largely been excluded from deworming campaigns (Allen et al. 2002). Such exclusions negatively affect the health of these women, and may also indirectly affect the health of their children.

To date, no study has investigated the effect of providing deworming treatment to women during the early postpartum period on infant or maternal health outcomes. A 2010 systematic review on deworming in non-pregnant populations (Smith and Brooker 2010) included two studies in WRA: one trial (Gilgen et al. 2001) that specifically excluded lactating women, and one observational study (Casey et al. 2009) that included lactating women, but in which lactation status was not ascertained. These studies therefore do not provide useful data related to deworming in lactating women. Five trials to date (Elliott et al. 2005; Larocque et al. 2006; Ndibazza et al. 2010; Ndyomugyenyi et al. 2008; Torlesse and Hodges 2001) have been conducted in pregnant populations. They are summarized in three systematic reviews (Brooker et al. 2008; Imhoff-Kunsch and Briggs 2012; Salam et al. 2015). None of these trials used the same intervention (i.e., the same anthelmintic and micronutrient combination) or had the same follow-up time frame. While data from pregnant populations are of value, given the different nature of the interface between mother and child at the time of deworming, it is unclear whether their results are generalizable to lactating women.

There is a critical research gap in the published literature surrounding deworming in lactating women. In effect, the WHO recommendation to include lactating women in large-scale deworming programs is based on expert opinion, and not empirical evidence. Since, in many low-and-middle-income countries (LMICs) health centre-based delivery is being widely promoted to reduce maternal and infant mortality, the immediate postpartum period provides an ideal opportunity to reach this high risk group. As well, integrating deworming into routine postpartum care has the potential to benefit two vulnerable populations simultaneously (i.e., the mother and the newborn child), and can contribute to achieving the global targets aimed at improving maternal, infant, and young child nutrition outlined in the Global Nutrition Targets for 2025 (WHO 2014b). The aim of the present research is to contribute rigorous, empirical evidence that will inform global health policy regarding the benefits of maternal postpartum deworming to both mothers and infants.

2: LITERATURE REVIEW

2.1 Soil-transmitted helminthiasis

2.1.1 Epidemiology, burden of infection, and prevention and control interventions

Epidemiology and burden of infection

STHs are the most prevalent parasite infections of humans, with an estimated 1.45 billion people infected globally (Pullan et al. 2014). More than three-quarters of the world's population (5.3 billion) live in STH-endemic areas (Pullan and Brooker 2012) where high rates of poverty and malnutrition also exist. In 2010, the global prevalences of *A. lumbricoides, T. trichiura,* and hookworm infection were 14.5%, 8.3%, and 7.8%, respectively (Pullan et al. 2014). Infections are most common in the tropic and sub-tropic regions of sub-Saharan Africa, East Asia, China, India, and South America (Brooker et al. 2006).

STH infections cause the highest global burden of disease among all neglected tropical diseases (NTDs) (Murray et al. 2012). Morbidity associated with STH infection is a consequence of its worm burden (i.e., the intensity of infection, expressed either as the number of worms or number of eggs per gram (epg) of feces) (Bethony et al. 2006). Individuals harbouring more worms will commonly experience more worm-attributable morbidity (Bruschi 2014). For this reason, both the prevalence and the intensity of infection are used to accurately describe the impact of STH infections in human populations and to evaluate control measures (Hotez et al. 2008). Estimates of both prevalence and intensity of STH infection can be obtained from counting the number of epg in stool as analyzed by quantitative laboratory methods (WHO 2002). Thresholds for classifying light, moderate, and heavy infections have been established by WHO based on their association with morbidity, and are presented separately by species in Table 1. Worldwide, it has been estimated that more than 300 million people have heavy intensity infection, resulting in severe morbidity (Bruschi 2014; Hotez et al. 2006).

Mortality estimates resulting from STH infection vary, ranging from 9,000 to 135,000 deaths annually (Crompton 1999; Crompton and Nesheim 2002; Hotez et al. 2006; Hotez et al. 2009). However, these figures should be interpreted with caution because accurate estimation of

mortality attributable to STH is difficult (Crompton 1999; Crompton and Nesheim 2002). Since STH infections are rarely the principal cause of death, their disease burden on the population is often measured by disability-adjusted life years (DALYs) (Bethony et al. 2006). Pullman *et al*. (2014) estimated that DALYs attributable to STH infections were 5.18 million in 2010.

Table 1: Eggs per gram thresholds for classifying soil-transmitted helminth infections into light, moderate, and heavy intensity infections.

Helminth species	**Light intensity infection**	**Moderate intensity infection**	**Heavy intensity infection**
Ascaris lumbricoides	1- 4999	5000 - 49,999	≥ 50,000
Trichuris trichiura	1- 999	1000 - 9999	≥ 10,000
Hookworms*	1- 1999	2000 - 3999	≥ 4000

Source: WHO 2002

*The eggs of the two species of hookworm (*N. americanus* and *A. duodenale*) are indistinguishable from one another and are grouped together within the one category of 'hookworms'

In general, only moderate and heavy intensity infections cause significant clinical manifestations, and light intensity infections generally remain asymptomatic (Keiser and Utzinger 2008). However, even light intensity infections may contribute to growth retardation in the presence of malnutrition (Stephenson et al. 2000b). STH infections contribute to nutritional impairment, including malabsorption of nutrients, loss of appetite, chronic blood loss, and iron deficiency anemia (IDA) (Crompton and Nesheim 2002; Robertson et al. 1992; Stephenson et al. 2000a). The consequences of infection during childhood have been associated with growth faltering, and impaired memory and cognition, leading to reduced school attendance and lower educational performance (Crompton and Nesheim 2002). As a result, infections may have a profound impact on wage-earning potential, affecting future economic productivity (Bleakley 2007; Miguel and Kremer 2004).

Life cycle of parasites

The life cycles of STHs follow a common pattern, and for this reason, simultaneous infection with all three parasites is a frequent occurrence. Infection occurs, in *A. lumbricoides*, *T. trichiura* and some hookworm infections, by ingestion of soil that is contaminated with parasite eggs. For hookworm infection, a second mode of transmission can also occur, where skin is penetrated by infective larvae that are present in fecally contaminated soil. Inside the human host, eggs and larvae migrate and develop into adult worms inhabiting the large intestine (*T. trichiura*) or the small intestine (*A. lumbricoides* and hookworms). In the intestine, after mating, adult female worms produce large numbers of eggs which are passed in feces (Brooker et al. 2006). Heavily co-infected individuals are thought to excrete between two to five million eggs in their feces daily (Bruschi 2014). Since multiplication of worms cannot occur within the human host itself, the number of worms within the host can only increase through reinfection from parasite egg or larvae-contaminated environments (Ziegelbauer et al. 2012). Lack of sanitation, including the inadequate disposable of feces and poor hygiene, contribute to the contamination of the environment, and perpetuate the cycle of reinfection (Hotez et al. 2006).

Control measures

Prevention and control strategies aim to lower STH infection prevalence and reduce worm burden in heavily infected people. This can be achieved through improved sanitation, enhanced personal hygiene, health education, and periodic deworming. Two systematic reviews have demonstrated that improved sanitation and promotion of hygiene practices can have prolonged effects on the prevalence of STH infection (Strunz et al. 2014; Ziegelbauer et al. 2012). However, while improved sanitation to eliminate parasite-contaminated environments is the only definitive intervention to greatly reduce the risk of STH infections in vulnerable populations, it is costly, difficult to implement in resource-poor settings (Asaolu and Ofoezie 2003), and improvements are only observed after a long period of time (Brooker et al. 2004). Frequently, health education results in increased knowledge but may not always translate into behavioural change for a variety of reasons, the most important being poverty (Albonico et al. 2006).
Four anthelmintic drugs are currently recommended for the treatment and control of STH infections: albendazole, levamisole, mebendazole, and pyrantel pamoate (WHO 1996c). The benzimidazoles, albendazole and mebendazole, are among the most common deworming drugs

because of their high efficacy, safety, ease of administration, and availability in a single-dose format (WHO 2006a). Therapeutic action of this class of drug occurs in the gastrointestinal tract, where tubulin polymerization of the worms is inhibited, resulting in metabolic disruption, energy depletion, and eventual death (Keiser and Utzinger 2010; Venkatesan 1998). Due to their low aqueous solubility, benzimidazoles are poorly absorbed in the intestinal lumen, and have a relatively short half-life (2.5 – 12 hours) (Namwanje et al. 2011), making the frequency of adverse side effects very low (Montresor et al. 2003). If present, side effects are usually mild and self-limiting, ranging from nausea, headache, abdominal discomfort, and rash. Cure rates (CRs) (i.e., the percentage of STH-infected individuals who become negative following deworming treatment) and egg reduction rates (ERRs) (i.e., the percentage reduction in the number of epg of feces following deworming treatment) are the two most widely used indicators to evaluate the efficacy of chemotherapy against STH infections. A systematic review by Keiser and Utzinger found that single-dose oral administration of albendazole produced CRs of 88% (95% confidence interval (CI): 79%, 93%), 28% (95% CI: 13%, 39%) and 72% (95% CI: 59%, 81%) for *A. lumbricoides*, *T. trichiura*, and hookworm infections, respectively (Keiser and Utzinger 2008). Single-dose mebendazole produced CRs of 95% (95% CI: 91%, 97%), 36% (95% CI: 16%, 51%), and 15% (95% CI: 1%, 27%) for *A. lumbricoides*, *T. trichiura*, and hookworm infections, respectively. ERRs for single-dose albendazole against *A. lumbricoides*, *T. trichiura*, and hookworm infections have been reported as 87% – 100%, 0% – 90%, and 64% – 100%, respectively. Similarly, for mebendazole, the ERRs are 96% – 100%, 81% – 93% and 0% – 98%, respectively, for the three infections (Keiser and Utzinger 2008).

Large-scale deworming programs, also known as preventive chemotherapy (PC), with the benzimidazoles, are recommended by WHO as a medium-term measure to control STH infections because this approach is fast-acting, inexpensive, efficacious, and safe (Albonico et al. 1999; WHO 2006a). While PC does not prevent reinfection and must be repeated periodically, in school-age children it is considered to be the most cost-effective prevention and control strategy to combat STH burden of disease (World Bank 2003). PC has received considerable global support from numerous partner organizations and governments, including the donation of benzimidazoles by major pharmaceutical companies (i.e., Johnson & Johnson and GlaxoSmithKline (Addiss 2015)). WHO recommends PC in three high risk groups: school-age

children, preschool-age children, and WRA, at least once a year in areas where the prevalence of any STH infection exceeds 20%, and twice a year where the prevalence exceeds 50% (WHO 2006a). In areas where the prevalence of any STH infection is less than 20%, PC is not recommended, and individuals receive treatment on a case-by-case basis.

2.1.2 Soil-transmitted helminthiasis in women of reproductive age

WHO and other global health organizations consider WRA (15 to 49 years of age (WHO 2006b)), living in STH-endemic areas, to be a high risk group, due to their underlying poor iron status, increased iron demands, and blood loss due to menstruation, pregnancy, and lactation (Hotez et al. 2006; Stephenson et al. 2000a; WHO 2006a). This group includes: 1) adolescent girls; 2) non-pregnant and non-lactating adult women; 3) pregnant women; and 4) lactating women. More than a quarter of WRA in endemic areas are estimated to be infected with STHs (Brooker et al. 2008), causing significant disease burden in populations with underlying vulnerability to malnutrition. It has been postulated that changes in the immune response during pregnancy and lactation may moderate the susceptibility of pregnant and lactating women to hookworm infection, but this is poorly understood and needs to be investigated further (Roberts and Horsnell 2015; Stoltzfus et al. 1997). The extent of susceptibility to infection depends on depletion of iron stores in the host, which is particularly problematic for WRA in LMICs, since many of them are already iron deficient due to diets low in bioavailable iron (Albonico et al. 2006). In addition, increased susceptibility may extend over several years as WRA in LMICs commonly have multiple pregnancies throughout their reproductive years (WHO 1996b).

2.1.3 Deworming in women of reproductive age

2.1.3.1 Preamble to Manuscript 1

The following manuscript (Manuscript 1) is included within the literature review of the present thesis because it discusses the current knowledge base on deworming in WRA (i.e., adolescent girls, non-pregnant and non-lactating women, pregnant women, and lactating women), as well as the benefits and challenges of including different subgroups of WRA in deworming programs.

In 2012, the London Declaration united public and private partners with the goal to sustain, expand and extend drug access programs for NTDs worldwide (Addiss 2015). The donation of anthelmintic drugs by major pharmaceutical companies has led to increased discussion and political drive to eliminate the burden of STH in endemic areas through enhanced deworming efforts. While scaling-up deworming in school-aged children may be enough to meet the WHO targets for elimination (i.e., the prevalence of moderate-and-heavy intensity infection of < 1%), inclusion of WRA in deworming programs may enhance current efforts and help break transmission cycles earlier. Targeting WRA age can also benefit the women themselves since they are also considered a high risk group for STH infection and STH-attributable morbidity, but are rarely systematically included in deworming programs.

This manuscript is the first to propose the idea of maternal postpartum deworming as a strategy for targeting WRA. The manuscript has been submitted to the journal *Revista Panamericana de Salud Pública/Pan American Journal of Public Health*. Following this manuscript, the literature review continues with section 2.2 which discusses the impact of STH infection on WRA.

2.1.3.2 Manuscript 1: The case for maternal postpartum deworming

Authors and affiliations: Layla S. Mofid[1, 2], Theresa W. Gyorkos[1, 2*]

[1] Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montréal, Québec, Canada

[2] Division of Clinical Epidemiology, Research Institute of the McGill University Health Centre, Montréal, Québec, Canada

*Corresponding author: Dr. Theresa W. Gyorkos, Division of Clinical Epidemiology, Research Institute of the McGill University Health Centre, Royal Victoria Hospital, V Building, 687 Pine Avenue West, Montréal, QC, Canada, H3A 1A1; Telephone: 514-934-1934 x 44721 Email: theresa.gyorkos@mcgill.ca

ABSTRACT

Currently, prevention and control activities for soil-transmitted helminth infections have primarily focused on preventive chemotherapy in school-aged and preschool-aged children. Women of reproductive age have been largely excluded from national deworming programs despite their underlying risk of blood loss, anemia, and poor nutritional status. In this Current Topics article, we revisit the need for including women of reproductive age in deworming programs as both a means of improving their own health, and as a means of complementing other control strategies with the intent of achieving global targets for control. Lastly, we propose a novel strategy for providing deworming to lactating mothers and we underscore the need for empirical evidence to study the effectiveness and feasibility of this approach.

WORMS AND WOMEN

Infection with *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Necator americanus* and *Ancylostoma duodenale* (hookworms) – collectively referred to as the soil-transmitted helminths (STH) – are among the most prevalent infections of humankind, co-occurring on all inhabited continents (1). With over one billion people infected with STHs (2), and 5.3 billion people at risk of infection (3), the collective global burden of disease caused by these parasites exceeds that produced by any other neglected tropical disease (4). Infections are unequally distributed across socioeconomic strata, such that the most vulnerable individuals, who reside in areas where adverse health, social, and economic conditions predominate, carry the greatest disease burden (5). STHs contribute to malnutrition through blood loss, iron deficiency anemia, nutrient malabsorption, and impaired nutrient utilization (6). STHs have been linked to impaired growth, cognitive deficits, lower educational achievement, and reduced economic productivity in adulthood (7), thus perpetuating the cycle of poverty into future generations.

The disease burden of STHs in women of reproductive age (WRA) (i.e., women between the ages of 15 and 49 years) is largely unknown. While STH species-specific estimates are not available for WRA as a whole, Brooker *et al.* (2008) estimated that, in 2005, 37.7 million WRA in sub-Saharan Africa were infected with hookworm alone, including 6.9 million (26.7%) out of 25.9 million pregnant women (8). This figure is somewhat lower than the estimate of 44 million hookworm-infected women (35.5%) among 124 million pregnant women reported by Bundy *et al.* in 1995 (9), and may be reflective of differences in methodology and of improved conditions over time. Although STH prevalence estimates in WRA may vary, more than a quarter of WRA living in endemic areas are estimated to be infected, causing significant disease burden in a population with underlying vulnerability to malnutrition.

Periodic preventive chemotherapy (PC) with anthelmintic drugs (i.e., deworming) is the cornerstone of prevention and control measures against STH infections in highly endemic areas (10). The World Health Organization (WHO) has recommended that PC programs target the three groups at highest risk of infection: school-aged children (SAC), preschool-aged children (pre-SAC), and WRA (11). The WHO Preventive Chemotherapy Databank reports on deworming coverage for SAC and pre-SAC; however, no coverage estimates are available for

deworming in WRA, and few countries include this risk group among deworming activities (12). Integrating deworming into routine health services as a means of targeting WRA has been shown to be more economical than vertical deworming programs (13). This Current Topics article discusses the need for including WRA in large-scale national deworming programs as a means of improving their own health, the health of their children, and as a strategy to complement global strategic plans for STH control.

CURRENT DEWORMING STRATEGIES AND 2020 TARGETS

Deworming through PC is considered to be one of the most cost-effective means of reducing the burden of STHs (11). In fact, the Copenhagen Consensus ranked deworming among the top four solutions to address 'big issues facing the planet' in 2012 (14). Other strategies include improved sanitation, hygiene, and health education. While these approaches are arguably more comprehensive and long-term, they are also costly, require improved infrastructure and continued political commitment, and do not produce immediate measureable benefits (15). Therefore, PC has been promoted as a cost-effective, medium-term strategy to combat STHs because it is relatively inexpensive, easily implemented, and produces immediate impacts, even in resource-poor settings (16). Though reinfection will inevitably occur following treatment in highly endemic areas, interruption of transmission is thought possible through periodic PC at scheduled intervals (17). Increased momentum has been generated in global deworming efforts following the London Declaration, a public-private partnership dedicated to sustain and expand drug programs for neglected tropical diseases globally.

In response to the continued worldwide burden of disease due to STH infections, in 2012, WHO proposed a strategic plan to eliminate STH as a public health problem in children by 2020 (11). Specific targets include at least 75% coverage of PC in SAC and pre-SAC living in endemic areas. Operationally, this goal translates to a reduction in the prevalence of moderate-and-heavy infection to less than 1% in children. However, recent predictive modelling suggests that current PC efforts may not be sufficient to meet the 2020 goal without improving coverage and including WRA, particularly in regions where hookworm infection predominates (18).

WOMEN OF REPRODUCTIVE AGE: A HIGH RISK GROUP OFTEN FORGOTTEN

Women are the backbone of society due to their roles as caregivers and agents of change within their communities. Their health is intrinsically linked with that of their children, and their decisions regarding health, hygiene, and childrearing have a profound influence on the future of their children, their family, and their community. In many countries, WRA suffer from high levels of anemia, in part due to their underlying poor iron status, increased iron demands, and blood loss due to menstruation, pregnancy, and lactation (19). Such realities make WRA particularly vulnerable to the detrimental effects of STH infections caused by intestinal blood loss and malabsorption of micronutrients (11).

Of particular importance are infections with both hookworm and *T. trichiura*, owing to chronic blood loss from parasite feeding activity and lesions in the intestinal mucosa (19, 20). In fact, in 2010, hookworm infection was ranked as one of the top four causes of global anemia for both men and women (21), and accounted for up to 54% of moderate to severe anemia in pregnant women in Africa and Asia (7). Blood loss from hookworm infection varies according to species and infection intensity, with net daily losses estimated at 0.03 mL per worm in the case of *N. americanus* and 0.15 mL per worm for *A. duodenale* (20). Recent data have also shown that *T. trichiura* infections may play a greater role in anemia during pregnancy than previously thought (22). While daily fecal blood losses of 0.005 mL per worm caused by infection with *T. trichiura* (19) are significantly less than losses attributed to hookworm infection, when worm numbers are high, they may be sufficient to cause iron deficiency anemia, especially when co-occurring with hookworm infection (6). The benzimidazoles, albendazole and mebendazole, are the single-dose deworming drugs of choice in large-scale applications, with albendazole preferred in more hookworm-endemic areas due to its higher efficacy against hookworm infection (23). Costs associated with the implementation of PC vary primarily by the type of delivery strategy used. Much attention has been paid to SAC and pre-SAC through pharmaceutical drug donations, large-scale national deworming programs, and global reporting systems. While WRA may indirectly benefit from PC targeting children living in the same household, direct benefits of treatment are lost and additional indirect benefits accruing to the household are also lost. A major barrier to including WRA in deworming programs is likely the fear of inadvertently administering deworming drugs to women who may not be aware that they are in their first

trimester of pregnancy (at which time deworming in contraindicated). A comprehensive approach to PC programs targeting WRA is lacking.

SUBGROUPS OF WOMEN OF REPRODUCTIVE AGE

In 1994, WHO first discussed the inclusion of WRA (including pregnant and lactating women) in deworming recommendations, and called on the research community to address critical research gaps (24). The reproductive life course of women consists of several dynamic physiological stages, each presenting unique opportunities for large-scale deworming programs (Table 1).

Adolescent girls

Numerous experimental and observational studies of the effectiveness of deworming on a multitude of health outcomes have been conducted in children, and have included the subgroup of adolescent girls, defined as females between 10 and 19 years of age (25). However, few studies have been specifically conducted in this subgroup, have disaggregated data, or have reported results separately for adolescent girls. A recent Cochrane review included 45 randomized controlled trials in children aged ≤ 16 years (26). One selected subset of analyses was restricted to trials of children infected with STH (n=8), and another subset to trials where both infected and uninfected children were included within the same study population (n=37). For those trials of children known to be infected, single-dose deworming was found to have had positive effects on weight, height, and mid-upper arm circumference, but not on hemoglobin (Hb) concentration. For those trials treating both infected and uninfected children in endemic areas, single-dose deworming did not have an effect on anthropometry or Hb concentration. Many of the trials included in this review had short follow-up times that may have been insufficient to observe accrued benefits, and were insufficiently powered to detect differences between groups due to effect dilution (i.e., including both infected and uninfected children in the treated study population). This review is also limited by the wide age range, lack of stratification by sex, and a high degree of heterogeneity among the studies. Generalizability of the review's results to adolescent girls is therefore inappropriate.

Two studies on deworming have been specifically conducted in adolescent girls in India. Vir *et al.* conducted a community-based study in Uttar Pradesh in 150,700 girls aged 11 to 18 years (27). Over a four-year period, weekly iron-folic acid (IFA) supplementation, combined with monthly health education sessions and biannual deworming with single-dose albendazole, reduced the prevalence of anemia from 73.3% to 25.4%. The study did not evaluate the independent effects of deworming on anemia or Hb concentration, nor did it include a control group, so other reasons contributing to the decline in anemia, apart from the deworming intervention, cannot be ruled out. Lamba *et al.* conducted an intervention study in urban Agra among 300 adolescent girls divided into three intervention groups: a) IFA supplementation twice a week for three months; b) IFA supplementation twice a week for three months plus two doses of albendazole over a two-week period; and c) control (no supplementation or deworming) (28). Over three months of follow-up, the prevalence of anemia dropped from 80.7% to 35.5% in the group that received IFA plus deworming whereas the prevalence of anemia remained constant in the control group (84.3% vs. 81.2%). Because of the lack of information on randomization, treatment allocation, blinding, and statistical analysis, as well as a high rate of attrition (i.e., 35.3%), the risk of biases and confounding is high, and therefore no interpretation of these results can be made.

Adolescent girls are often included in school-based deworming programs which have been shown to be operationally feasible and highly cost-effective in a variety of settings. However, the extent to which they can benefit adolescent girls depends on school attendance and the ability to include non-enrolled children in PC activities. In many low-and-middle-income countries (LMICs), the rate of drop-out in girls during puberty spikes, making adolescent girls difficult to reach using school-based programs alone.

Non-pregnant and non-lactating adult women

Several studies have evaluated the effectiveness of deworming in adult populations, though these have frequently included children as well, and have not adequately separated results by age group or by sex. Two studies have specifically investigated deworming in non-pregnant and non-lactating adult women. Gilgen *et al.* conducted an RCT in Bangladesh among female tea pluckers, and included the following four intervention groups: 1) weekly iron supplementation

for 24 weeks; 2) single-dose 400 mg albendazole given at baseline and 12 weeks; 3) weekly iron supplementation for 24 weeks and single-dose 400 mg albendazole given at baseline and 12 weeks; and 4) placebos for both iron supplementation and albendazole (29). No statistically significant difference in labour productivity was found between the intervention groups over a 20-week period. Among women who were randomized to receive deworming only, there was no statistically significant change in Hb values pre and post-intervention. Important information about the trial methods is not mentioned (i.e., blinding, attrition rate, power, and sample size calculations), and as such, its quality cannot be determined. The second study was a population-based study conducted in Vietnam. Non-pregnant women were given weekly IFA supplementation plus single-dose albendazole at four-month intervals for one year and biannual deworming thereafter over 54 months (30). The prevalence of anemia declined from 38% to 18% and the prevalence of hookworm infection fell from 76% to 11%. The authors did not determine the effect of deworming alone on the prevalence of anemia, nor had they included a control group for comparison.

Non-pregnant and non-lactating women are not easily reachable through health facility-based platforms. Mass drug administration of whole communities, with its attendant costs, may be the only means to adequately reach this female subgroup.

Pregnant women

To date, five trials on deworming with single-dose deworming drugs during pregnancy have been conducted and are summarized in a recent Cochrane review (31) including a total of 4265 participants. Results indicated that deworming during pregnancy had no overall effect on maternal anemia, low birthweight, preterm birth, or perinatal mortality. This review, and the trials it includes, has several limitations. Foremost among these is that no single trial used the same intervention and variability, in terms of sample sizes, was high. Heterogeneity in baseline prevalences and intensities of STH infections (including species-specific prevalences and intensities), also limits the appropriateness of pooling individual trial results. Additionally, treatment effects were not stratified by baseline STH intensity categories, and thus effect dilution contributes to the null findings. Infant growth outcomes were only measured at birth, or shortly after birth, which also likely limited the potential to detect a benefit. Evidence to date suggests

that deworming after the first trimester of pregnancy is not associated with adverse birth outcomes (32, 33). While one study showed an association between maternal deworming and infant allergy (e.g., eczema) (34), current evidence on this topic is limited and requires further investigation.

Pregnant women can be reached in hospitals and health centres, where PC can piggyback on existing antenatal care services. However, ministries of health in endemic areas may be reluctant to integrate deworming into antenatal care following the first trimester of pregnancy due to unwarranted fears of harms to the growing fetus. This may contribute to the reason why pregnant women remain excluded from PC activities.

Lactating women

Despite the 1994 call from WHO for research on lactation performance following deworming in lactating women (24), to date, no study has assessed the effects of deworming following delivery (i.e., in the postpartum period) on maternal or infant outcomes. While data from other WRA subgroups provide important empirical evidence, given the unique interface between mother and child during the postpartum period, they may not be applicable to lactating women. A single pharmacokinetic study of albendazole in human breast milk estimated that breastfed infants of mothers administered a single 400 mg oral dose of albendazole were exposed to less than 0.1 mg/kg (of infant weight) of albendazole and its active metabolite, albendazole sulphoxide, over 36 hours (35). The authors concluded that these low concentrations would be unlikely to be considered harmful to breastfed infants.

Lactating women can be reached at multiple time points during early, mid, and late lactation. During the early postpartum period, deworming can be effectively integrated into routine services, provided that delivery services in hospitals or health centres are available, accessible, and used.

MATERNAL POSTPARTUM DEWORMING: A NEW STRATEGY

It is estimated that, every year, 468 million non-pregnant women worldwide are anemic (36). While the iron demands during the postpartum period are considerably less than those during pregnancy, pre-existing conditions, hemorrhage, and underlying poor nutritional status can make it difficult for women to fully replenish iron stores in the postpartum period (37, 38). Postpartum anemia is thought to affect up to 80% of women in LMICs (37). On average, a woman in sub-Saharan Africa spends as much as 28% of her lifespan pregnant, and 65% breastfeeding (24).

Over the last three decades, a large body of literature has developed on the interrelationship between gastrointestinal nematodes, deworming, and milk production in veterinary research. A narrative review of more than 80 trials found that anthelmintic treatment improved fat content in milk during lactation in 74% of the 35 studies which measured this outcome, though this difference was small (median increase of 2.1 kg) (39). The effect of deworming on milk yield is more striking. A meta-analysis of 75 trials found that the combined effect of treatment on milk production was 0.35 kg/cow per day, and that multiparous cows had higher responses to treatment than primiparous cows (40). Charlier *et al*. cautioned about comparing studies and meta-analyses, since the design, quality, and analysis of the studies varied substantially (41). Similar studies have not yet been conducted in human populations.

A critical research gap exists on the effects of maternal postpartum deworming on infant and maternal health outcomes, including on lactation performance. The integration of deworming into routine postpartum care may prove to be an operationally feasible strategy for the inclusion of women in deworming efforts, since women in the postpartum period are generally easily reachable; costs associated with drug distribution can be reduced; and government policies for hospital-based deliveries are promoted. In areas of STH endemicity, fertility rates are high, offering periodic opportunities to reduce the STH-attributable burden of disease in WRA during their most reproductive years (Figure 1).

FUTURE RESEARCH DIRECTIONS

As new plans are being discussed to include WRA in deworming activities, the best platform and target groups need to be considered. In particular, there is a need to study the effectiveness of maternal postpartum deworming as a means to improve maternal and child health. Deworming has the potential to improve maternal appetite, dietary intake, and nutrient absorption, and, in turn, may improve production of breast milk. Improvements to maternal nutritional status may have lasting consequences on maternal fatigue, mother-child interactions, and breastfeeding, and may, overall, enable mothers to better care for their newborn infants. Research on the benefits of maternal postpartum deworming is urgently needed to build on the deworming framework that has already been developed for children, and to take advantage of the momentum generated by deworming efforts globally.

Table 1: Summary of benefits and challenges of including subgroups of women of reproductive age in deworming programs.

Subgroup	Benefits of deworming	Challenges of deworming
Adolescent girls	- reduced partitioning of nutrients between STH and growth during puberty - easily reachable in school-based programs	- fear of inadvertent administration in first trimester of pregnancy - difficult to reach non-enrolled school children
Non-pregnant non-lactating women	- could improve work performance - could improve nutritional status prior to conception	- not easily reachable - fear of inadvertent administration in first trimester of pregnancy
Pregnant women	- could benefit mother and fetus simultaneously - could be easily integrated into antenatal care - women could be easily reachable in health centres/hospitals - no fear of inadvertent administration in first trimester of pregnancy	- some governments could be hesitant to administer drugs to pregnant women, despite evidence of lack of harm - antenatal care attendance is low in many countries
Lactating women	- could benefit mother and infant simultaneously - could be easily integrated into postpartum care - women could be easily reachable in health centres/hospitals - no fear of inadvertent administration in first trimester of pregnancy if given soon after delivery	- some governments could be hesitant to administer drugs to lactating women, despite evidence of lack of harm - the number of deliveries in health care settings may be suboptimal

STH = Soil-transmitted helminths (roundworms, whipworms and hookworms)

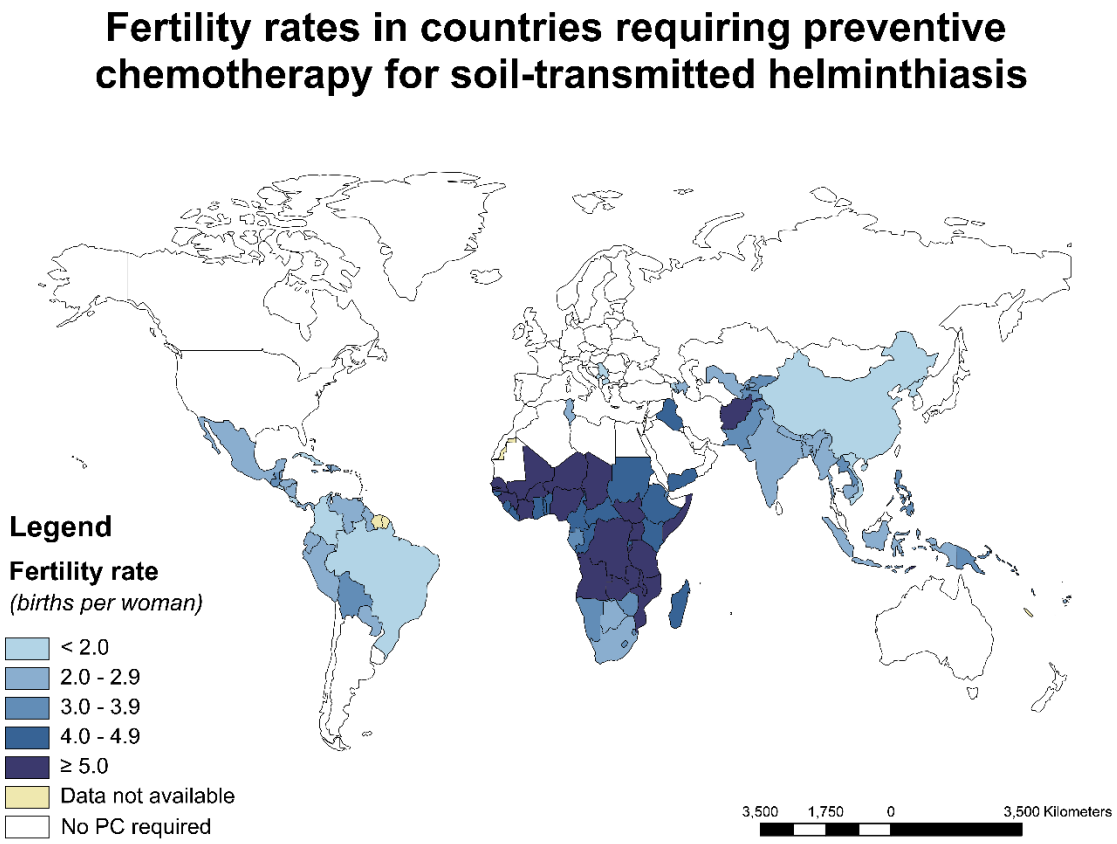

Figure 1: World map showing fertility rates (blue shading) among countries determined to be in need of preventive chemotherapy (PC) for soil-transmitted helminthiasis according to WHO guidelines.*

*Fertility data are taken from the World Development Indicators Databank (World Bank, 2013) and the World Statistics Pocketbook (United Nations Statistics Division, 2010 - 2015). Data on the need for preventive chemotherapy in children for soil-transmitted helminth infection are taken from the WHO Preventive Chemotherapy Databank (World Health Organization, 2013). Fertility rates (in brackets) for endemic regions that could not be displayed are: Cape Verde (2.3), Comoros (4.6), French Polynesia (2.1), Kiribati (3.8), Marshall Islands (4.1), Mauritius (1.4), Micronesia (3.3), Nauru (4.3), Sao Tome and Principe (4.6), Tonga (3.8), and Tuvalu (3.2).

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2.2 Impact of soil-transmitted helminthiasis in women of reproductive age

2.2.1 Overview

STHs are an important cause of intestinal bleeding, anemia, malnutrition, and competition for micronutrients. STH infections can cause nutritional disturbances due to inefficient nutrient absorption, lower dietary intake, and changes in nutrient metabolism (Crompton and Nesheim 2002). For example, STH infections can affect appetite and reduce food intake, lowering consumption of energy, iron, and micronutrients, especially zinc, folate, and vitamin B_{12} (Crompton and Nesheim 2002). Worm infections can also minimize the absorption of protein, and fat, thus reducing nutrient availability to the host. Infections with helminths can reduce vitamin A absorption by as much as 70%, and also interfere with vitamin A utilization, which is required for hematopoiesis (Nalubola and Nestel 1999; WHO 2003).

2.2.2 Anemia and iron deficiency

Definition, consequences and determinants

Anemia is defined as a condition in which the number of circulating red blood cells and their capacity to carry oxygen to the body's tissues is insufficient to meet the physiologic needs of the host (WHO 2011b). Iron deficiency is considered the most common and widespread nutritional disorder in the world (Krafft 2013), and the most frequent cause of anemia (de Benoist et al. 2008). Other causes of anemia include parasitic infections, such as malaria, hookworm, and schistosomiasis; nutritional deficiencies in vitamins A, B_{12}, C, and folic acid; hemorrhage; and congenital hereditary defects (WHO 2001). When the Hb concentration is below the optimal level in an individual, iron deficiency is said to be present. It is defined as the absence of iron stores when the signs of inadequate iron supply to the tissues is apparent (WHO 2004b). IDA is considered a subset of iron deficiency, at the extreme lower end of the distribution (below the 95^{th} percentile) in a population. IDA accounts for half of the burden of anemia worldwide (WHO 2001), and was ranked among the top 15 causes of global DALYs in 2010 (Murray and Lopez 2013).

In otherwise healthy individuals, symptoms of anemia do not begin until Hb levels fall to 80 g/L or lower (Yip et al. 1996). Clinical consequences of poor iron status include poor immune

function, reduced memory and concentration, lower aerobic capacity, and decreased work performance (Beard 2000; Bruner et al. 1996; WHO 2004b). Socioeconomic status (SES) is considered an upstream determinant of IDA due to its association with food insecurity, lack of access to appropriate health care services, and inadequate sanitation and hygiene (WHO 2001). More proximal determinants of IDA include the intake and absorption of iron and other micronutrients; presence of blood loss due to trauma or infections with hookworms, schistosomes, and *Helicobacter pylori*; recurrent inflammation caused by malaria, diarrhea, and pneumonia; and life stages where increased iron requirements are present, such as during infancy, adolescence, pregnancy, and lactation (Pasricha et al. 2013).

Measurement of anemia

The most common and reliable population-based measure to assess the prevalence of anemia is the concentration of Hb in the blood (de Benoist et al. 2008; WHO 2001). The measurement of Hb is rapid, inexpensive, and appropriate in field settings. Normal Hb values can vary with age, sex, ethnicity, pregnancy status, smoking, and altitude (WHO 2001). Table 3 displays the WHO cut-offs for anemia based on blood Hb concentrations. Hb concentration defines anemia most often in individuals who already have severe iron deficiency (WHO 2001). This indicator has low specificity because there are a variety of causes of anemia, and IDA represents only 50% of anemias (Lynch 2010). Despite these issues, Hb concentration remains a widely used proxy for iron deficiency due to the availability of Hb assays and the ease of use of point-of-care hemoglobinometers, such as HemoCue®, using venous or capillary blood (Pasricha et al. 2013).

Anemia in WRA

WRA is considered to be the group at highest risk for anemia (de Benoist et al. 2008). It is estimated that 468 million non-pregnant women and 56 million of pregnant women worldwide are anemic on an annual basis (de Benoist et al. 2008). Susceptibility of IDA in WRA is a result of poor iron status, blood loss during menstruation, and high iron demands during pregnancy and lactation (Tolentino and Friedman 2007). WRA lose approximately 35 mL of blood containing 15.5 mg of iron during each period of menstruation (WHO 1994). During pregnancy and birth, around 790 mg of iron is needed for optimal maternal and infant health (Crompton and Nesheim 2002), and each day of lactation 0.75 mg of iron is transferred from mother to baby (WHO

1996b). An adult woman in Africa spends as much as 28% of her lifespan pregnant, and 65% breastfeeding (WHO 1995). Consequently, WRA in the developing world have high iron requirements that are often not met through diet, and further diminished through additive interactions from infections, such as STH and malaria (Mwangi et al. 2006).

Table 2: Thresholds of hemoglobin blood concentrations for defining anemia, by subpopulation at sea level.

Subpopulation	**Hemoglobin concentration (g/L)**
Children (0.5 – 4.9 years)	<110
Children (5– 11.9 years)	<115
Children (12–14.9 years)	<120
Men (≥ 15 years)	<130
Non-pregnant women (≥ 15 years)	<120
Pregnant women	<110
Lactating women	Not specified

Source: WHO 2001

Adolescence

During adolescence, caloric, protein, and mineral requirements significantly increase to meet the demands of rapid somatic growth and increased erythrocyte mass at the beginning of menses (Soares et al. 2010). The requirements for iron increase by 70% after the age of 10, making adolescence a critical time when iron deficiency can have both immediate and long-lasting effects in later life (Nelson 1996). In fact, it has been estimated that a 14-year-old girl has iron requirements 30% higher than her mother (Hallberg 2001). Available data on dietary intake in adolescents suggest that girls are unlikely to obtain sufficient iron to replenish iron stores during this period, due to suboptimal iron intakes, especially in LMICs (Tapiero et al. 2001). Studies in adolescent girls have demonstrated that iron supplementation can enhance cognition and improve attention and mood (Ballin et al. 1992; Bruner et al. 1996; Groner et al. 1986; Rowland et al.

1988). Of particular concern is pregnancy during adolescence, since increased iron requirements are needed to provide for the growth of both the mother and of the growing fetus, where there is simultaneous competition for nutrients and minerals (Beard 2000; Hallberg 2001; Soares et al. 2010). Soares and colleagues found reduced iron stores in nulliparous adolescent girls in late pregnancy and prior to delivery compared with nulliparous adults (Soares et al. 2010). This may be the result of the increased iron demands of pregnancy in an already iron-depleted adolescent population.

Pregnancy

IDA is by far the most common nutritional problem during pregnancy, and can cause a variety of adverse maternal and fetal health outcomes, especially for women living in LMICs (Soares et al. 2010). It is estimated that, annually, 30% of non-pregnant women, and 42% of pregnant women are anemic, worldwide (de Benoist et al. 2008). As a result, many women are anemic prior to conception, and are unable to recuperate iron stores during pregnancy (Allen 2000). During the second trimester of pregnancy, iron absorption increases by 50%, and by the third trimester, it increases by four times the normal rate to deal with the exponential growth of the fetus and provide > 80% of fetal iron needs (WHO 2004b). The iron demands in pregnancy are three times higher than during menstruation (Tapiero et al. 2001). Iron deficiency in the first trimester of pregnancy has been associated with poor pregnancy outcomes, including low birthweight, preterm delivery, intrauterine growth retardation, and lower Apgar scores (Allen 2000; Kalaivani 2009). A meta-analysis of observational studies on anemia during pregnancy and birth outcomes found that maternal anemia in early pregnancy was associated with increased risk of preterm birth (pooled adjusted odds ratio [aOR]: 1.3; 95% CI: 1.0, 1.7), but had no statistically significant relationship with low birthweight or fetal growth restriction (Xiong et al. 2000). Although some retrospective studies have suggested an association between maternal anemia and maternal mortality (Chi et al. 1981; Llewellyn-Jones 1965), prospective, intervention studies have not been conducted, since large sample sizes would be required, and it would be unethical to leave anemic pregnant women untreated (Allen 2000). It is likely that studies on the association between anemia and maternal mortality are confounded by concurrent infection, hemorrhage, poor nutritional status and pregnancy hemodilution (Brabin et al. 2001).

Postpartum period and lactation

The prevalence of postpartum anemia in LMICs is thought to be as high as 80% (Milman 2011), constituting a serious public health problem (de Benoist et al. 2008). At delivery, blood losses can exceed 1290 mg of iron (Beard 2000), leading to deficiencies in the postpartum period. Infants born to iron-deficient mothers are more likely to be anemic in the first 6 months of life (Tapiero et al. 2001). Improvements to postpartum iron stores are important when interpregnancy intervals are short, thus providing protection for the mother in her subsequent pregnancy (Allen 2000). More importantly, intergenerational transfer of poor iron status from mother to child increases an infant's vulnerability to iron deficiency and has been associated with poor growth, lagged cognitive and behavioural development, and increased exposure to infections (Balarajan et al. 2011; Beard et al. 2005). Maternal anemia during lactation has been associated with negative impacts on perceived milk supply (Henly et al. 1995), duration of exclusive breastfeeding (Henly et al. 1995; Rioux et al. 2006), emotional stability (Beard et al. 2005), fatigue (Beard et al. 2005), and maternal-infant bonding (Perez et al. 2005).

Anemia and STH infection

Anemia is considered one of the most detrimental effects of STH infection (Hotez et al. 2006). Inadequately treated infection with hookworm can lead to chronic blood loss, depletion of host iron stores, and the development of IDA (Pawlowski et al. 1991). As much as 54% of moderate to severe anemia in pregnant women is thought to be attributable to hookworm infection, in Africa and Asia (Navitsky et al. 1998; Stoltzfus et al. 1997). A hookworm intensity of 2500 to 8000 epg, corresponding to a presence of 40 to 160 adult worms in the small intestine, has been associated with Hb levels below 110 g/L (Bundy et al. 1995; Lwambo et al. 1992), which is indicative of anemia. However, studies have shown that anemia may occur even with light intensity hookworm infection (i.e., less than 40 adult worms), depending on the iron status of the host (Olsen et al. 1998). A 2008 meta-analysis in pregnant women found that even light intensity hookworm infection (i.e., 1-1999 epg) is associated with a significant decrease in Hb levels (standardized mean difference (SMD) -0.24; 95% CI: -0.36, -0.13), and that this effect is magnified when comparing those with heavy and light intensity infections (SMD -0.57; 95% CI: -0.87, -0.26) (Brooker et al. 2008). The degree of iron deficiency caused by hookworm infection depends on the species, with *A. duodenale* causing greater blood loss (i.e., mean of 0.15 mL per

worm per day) than *N. americanus* (i.e., 0.03 mL per worm per day) (Crompton 2000). In 2010, infection with hookworm was ranked as one of the top four causes of global anemia for both men and women (Kassebaum et al. 2014). The synergistic relationship between concurrent infection with hookworm and malaria has been shown to be additive and to increase the risk of severe anemia (Brooker et al. 2007).

The association between infection with *T. trichiura* and anemia is less understood. The majority of studies that have assessed the prevalence of anemia among infected and uninfected individuals have not shown a significant association (Gyorkos and Gilbert 2014). However, studies that have categorized *T. trichiura* infection by WHO-recognized categories of intensity have demonstrated that moderate-and-heavy infection with *T. trichiura* is statistically significantly associated with lower Hb levels and/or anemia, in pregnant women (Gyorkos et al. 2011a) and in children (Quihui-Cota et al. 2010; Ramdath et al. 1995; Robertson et al. 1992). Furthermore, concomitant infection with *T. trichiura* and hookworm has been shown to be associated with lower Hb levels, compared to single infection (Robertson et al. 1992), with the risk of anemia steadily increasing with higher intensities of either infection (Gyorkos et al. 2011a).

2.2.3 Fatigue

Fatigue is a multidimensional symptom that is highly prevalent in individuals suffering from chronic diseases, like cancer, multiple sclerosis, and infections (Michielsen et al. 2003; Michielsen et al. 2004). The postpartum period is a time when women are particularly susceptible to fatigue because of the physical and emotional demands of childbirth, breastfeeding, and adapting to new parenting roles (Bozoky and Corwin 2002; Gardner and Campbell 1991). Elevated fatigue in lactating mothers has the potential to alter children's developmental trajectory (Beard et al. 2005) by contributing to early breastfeeding cessation (Rioux et al. 2006), lack of responsiveness, irritability, and depression (Beard et al. 2005; Perez et al. 2005). Research to date has not adequately assessed how anemia and fatigue affect maternal quality of life, and a mother's ability to cope and care for her newborn child (Allen 2000).

Fatigue and soil-transmitted helminth infections

The burden of STH infections in children and adults includes listlessness, weakness, lethargy, lack of energy, difficulty in completing daily activities, and shortness of breath, and are likely to be a result of iron deficiency (Crompton et al. 2003; Crompton and Nesheim 2002; Pawlowski et al. 1991). Fatigue is an important consequence of STH infection because it can affect mood (Bruner et al. 1996), work productivity (Crompton and Nesheim 2002), and mother-child interactions (Beard et al. 2005). Only one cross-sectional study in Malaysia has assessed the association between STH and fatigue, and found that STH-infected children were more likely to complain of tiredness compared to uninfected children (36% vs. 12%, $p = 0.019$) (Huat et al. 2012). However, the authors make no mention of the type of scale they used to measure fatigue. A gap in the research literature currently exists on the effects of STH infection on fatigue, and the short or long-term consequences of maternal fatigue on both maternal and infant health outcomes.

2.3 Milk production during infections

2.3.1 Soil-transmitted helminth infections and milk production in veterinary research

In the last three decades, a large body of literature has developed on the effect of gastrointestinal nematodes, deworming, and milk production in veterinary research. Poor nutrition, stress, acute illness, gestation, and lactation may reduce host immunity, thereby decreasing the animal's defense mechanisms against parasites (Armour 1989). Around parturition, expression of immunity is reduced, specifically to nematode infections (Barger 1993). Functions regulating immunity are greatly influenced by the nutritional status of the host, since they are given lower priority compared to functions of maintenance, growth, and reproduction (Coop and Kyriazakis 2001).

Milk quality

Nematode infections are known to suppress appetite in both dairy and beef cattle, leading to lower feed intake and poor growth (Gross et al. 1999). Additionally, the digestibility and absorption of feed is suspected to decrease in the presence of gastrointestinal parasites, due to

physical damage in the gut (Villalba et al. 2014). As such, poor nutritional status of the host may ensue, and would likely have an impact on milk production, since nutrients are prioritized for maintenance (Coop and Kyriazakis 2001). A common characteristic of gastrointestinal parasite infections in ruminants is the loss of protein in the gut, with effects exacerbated in situations where nutrient availability is further reduced by low food intake (Coop and Kyriazakis 2001). A study in goats found that milk produced from untreated animals had 29.9% lower fat content, 23.3% lower protein content, and 19.6% lower lactose content, compared to their anthelmintic-treated counterparts (Rinaldi et al. 2007). A narrative review by Gross et al. (1999) found that treatment with anthelmintics improved yield of milk fat over the course of lactation in 74% of included studies, though this difference was small (median increase of 2.1 kg) (Gross et al. 1999). Other studies in ruminants, however, have not observed any effect of anthelmintic treatment on the nutrient composition of milk (Alberti et al. 2012; Forbes et al. 2004; Hoste and Chartier 1993).

2.3.2 Breast milk studies in human populations

Maternal nutritional status and breast milk

Lactating women in LMICs are at risk of suffering from a shortage of dietary energy and protein, and micronutrient deficiencies, due to a suboptimal diet, low nutrient stores, and changes in nutrient utilization. Variation in milk composition occurs within the same feeding, diurnally, daily, and with progression of lactation. Strong variation is also observed from woman to woman, and between milk samples from the same woman (Picciano 2001). Maternal nutritional status is not thought to affect milk composition unless malnutrition is severe (Black et al. 2008). Fat and energy content in breast milk are related and appear to be influenced by maternal nutrition (Emmett and Rogers 1997). The percentage of maternal body fat and adipose stores may affect the fat concentrations in milk by buffering daily dietary fluctuations, so that fat content in milk is consistent (Institute of Medicine 1991; Picciano 2001). Dietary protein intake may alter total protein and nitrogen content in breast milk (Picciano 2001), although studies on the relationship between protein intake, supplementation, and milk protein content have reported contradictory results (Emmett and Rogers 1997). On the other hand, carbohydrate concentration

in breast milk, mainly lactose and oligosaccharides, are thought to be insensitive to changes in maternal diet and nutritional status (Emmett and Rogers 1997; McVeagh and Miller 1997).

While evidence that milk concentrations of macronutrients are affected by maternal nutritional status is largely inconclusive, maternal nutritional status is regarded as a major factor influencing some micronutrient content in human milk (Dewey and Cohen 2007; Zavaleta et al. 1995). In general, milk concentrations of water-soluble vitamins are more dependent on maternal nutritional status compared to fat-soluble vitamins, and increases in vitamin intake commonly result in higher milk levels (Institute of Medicine 1991). Vitamin A, iodine, selenium, thiamin, riboflavin, pyridoxine, and cobalamin are responsive to maternal intake and deficiency (Black et al. 2008; Institute of Medicine 1991). Maternal vitamin A deficiency is especially important, since infant stores are low at birth, and breast milk is the main determinant of infant vitamin A status deficiency (Black et al. 2008). There is no evidence, however, that iron, copper, and zinc are affected by maternal nutritional status (Domellof et al. 2004). Some studies have shown that malnutrition can affect immunological factors in colostrum (Miranda et al. 1983) and mature milk (Chang 1990); however, other studies have failed to observe such an association (Hennart et al. 1991; Lönnerdal et al. 1996).

Although there is little evidence to indicate that nutritional status of the mother directly affects milk production (i.e., quantity produced), her perception of malnourishment may influence her feeding behaviours, such as duration of exclusive breastfeeding and introduction of complementary foods (Dewey and Cohen 2007). Such choices may influence infant nutrition, with impacts on growth, development, and morbidity. Infant characteristics, such as birthweight and breastfeeding time, are thought to be more related to milk output, compared to maternal factors (e.g., age, parity, anthropometric indices, nutritional status) (Dewey et al. 1991; Pérez-Escamilla et al. 1995). Some studies have reported seasonal changes in milk output (Brown et al. 1986; Prentice et al. 1986), which may be a result of differences in dietary intake (i.e., related to harvest period), changes in breastfeeding patterns, or infant illness. Though some studies have found an association between maternal energy intake and infant milk intake (Butte et al. 1984; Prentice et al. 1986), associations may reflect reverse causation, where mothers who have high milk output also have high food intake due to increased appetite (Institute of Medicine 1991).

Maternal infection and breast milk composition

Maternal infections, including STHs, can cause nutritional disturbances in lactating mothers due to reduced food intake, impaired absorption, and competition for nutrients (Crompton and Nesheim 2002). Consequently, co-occurring malnutrition and infection in mothers may impact breast milk production and affect the nutritional status of infants. However, few studies have assessed the effect of maternal infection on breast milk quality. Acute infections during lactation that change trace element (e.g., copper, iron, zinc) concentrations in the serum of mothers, do not appear to influence content in breast milk (Dorea 2000a; Dorea 2000b). Zavaleta *et al.* (1995) conducted a study among low-income Peruvian women who were exclusively breastfeeding and found that those with acute febrile infection were more likely to have lower serum zinc concentrations and higher serum copper concentrations than healthy women (Zavaleta et al. 1995). However, infection was not related to milk volume, milk protein, or trace element concentrations. The authors made no mention of adjustment for important confounders, such as maternal age and parity. Another cross-sectional study by Dhonukshe-Rutten *et al.* (2005) found that mothers infected with intestinal parasites had significantly higher zinc concentrations in their breast milk than non-parasitized women (Dhonukshe-Rutten et al. 2005). However, results of this study should be interpreted with caution because of the small sample size (n=68), high rate of attrition (31%) over a two-week period, and lack of consideration of important confounders in analyses. Most importantly, women infected with STH at baseline were given either 400 mg of albendazole or placebo, yet group assignment was not taken into account in subsequent analyses, nor was blinding or randomization mentioned. More research is needed on this topic to corroborate results and better understand the interaction between intestinal parasites and milk composition.

2.4 Infant Growth and Development

2.4.1 Overview

Malnutrition is highly prevalent in LMICs, and is the principal cause of mortality in children worldwide (Bryce et al. 2005; Murray and Lopez 1997). Undernutrition is one form of malnutrition, and includes stunting, wasting, and deficiencies of crucial vitamins and minerals

(Black et al. 2008). In LMICs, it has been estimated that 178 million children under five years of age are stunted, 112 million are underweight, and 55 million are wasted (Black et al. 2008). Worldwide, approximately 45% of child deaths (3.1 million) are considered to be a direct or indirect result of undernutrition (Black et al. 2013). Poor nutrition predisposes children to infection, leading to increased risk of mortality (Barros et al. 2010), higher risk of cognitive deficits, lower educational achievement, and lower productivity as adults (Victora et al. 2008).

2.4.2 Breastfeeding and infant growth

It is well established that human milk is the optimal source of nutrients for infant growth and development (Nakamori et al. 2009), due to its nutritional content, and immunologic and antimicrobial properties that change according to the needs of the growing infant (Field 2005). Breastfeeding is considered one of the most important means to reduce neonatal morbidity and mortality by preventing infant infection and by promoting physical growth and intellectual and motor development (Saugstad 2011). Suboptimal breastfeeding, including non-exclusive breastfeeding in the first six months of life, is estimated to be responsible for 10% of disease burden in children under the age of five years, and 1.4 million deaths (Black et al. 2008). Breastfeeding offers protection against both infections and chronic conditions, reducing the risk of upper respiratory conditions, gastrointestinal infections, and onset of obesity (Pérez-Escamilla 2003). A 2012 Cochrane Review found that infants who were exclusively breastfed until six months of age had reduced morbidity from gastrointestinal infections and had no observable deficits in growth compared to infants who were introduced to complementary foods at earlier ages (Kramer and Kakuma 2012). In response to evidence on the benefits of exclusive breastfeeding until six months of age, WHO now recommends: 1) initiation of breastfeeding within the first hour of life; 2) exclusive breastfeeding until six months of age; and 3) continued breastfeeding with introduction of complementary foods for two years or more (UNICEF/WHO 2015). Infant weight gain is considered an important anthropometric indicator of lactation performance (i.e., breastfeeding adequacy and sufficient milk transfer from mother to breastfeeding infant) (Henly et al. 1995) and adequate energy intake (Lucas et al. 2012).

2.4.3 Maternal nutritional status and infant growth

Maternal nutrient depletion is a serious concern in many LMICs and can contribute to an increased risk of birth defects, preterm birth, fetal growth restriction, and maternal anemia and mortality (King 2003). Short interpregnancy intervals are thought to contribute to poor outcomes through a variety of mechanisms, including depletion of micronutrients and macronutrients, hormonal dysregulation, presence of inflammatory response markers, maternal anemia, and incomplete recovery from physiological stresses (Conde-Agudelo and Belizan 2000; DeFranco et al. 2015; Smits and Essed 2001). During pregnancy, maternal undernutrition may reduce energy and protein availability, resulting in inadequate nutrient exchange between mother and fetus (Neufeld 2004). Such deficiencies may play a role in fetal growth retardation, leading to low birthweight, stunting, and inadequate catch-up growth during infancy. Adolescent pregnancies are of particular concern due to the superimposition of increased nutritional requirements of pregnancy and an intense growing period of the mother, where competition for micronutrients between mother and fetus can ensue (King 2003; Scholl et al. 1994). It has also been hypothesized that maternal parasitic infections during pregnancy may affect fetal immune responses and also decrease vaccine efficacy in children (Malhotra et al. 2015).

Lactation is a period of higher nutritional burden than during pregnancy, when energy requirements increase by 25%, protein needs by 54%, and micronutrient demands by 0-93%, depending on the specific vitamin or mineral (Dewey and Cohen 2007). Factors such as maternal dietary intake, nutritional status, and exposure to infections may have an effect on the concentrations of macronutrients and immunological factors in breast milk (Marquis et al. 2003). Physiologic stressors, such as the continuation of breastfeeding during a new pregnancy, have been shown to have an effect of milk composition (Marquis et al. 2003), yet no study has evaluated the effect of maternal helminth infections on milk quality, and subsequent potential effect on infant health.

2.4.4 Potential for maternal postpartum deworming

The health of mothers and their children are intimately intertwined and the 1,000-day period from conception through to two years of age is a critical window of opportunity in shaping the health and development of children (Bryce et al. 2008; Victora et al. 2008). Since growth

faltering generally occurs before two years of age (Victora et al. 2010), interventions targeting this crucial time of development can have the greatest impact on future health throughout the entire lifespan. Maternal postpartum deworming is an intervention that has not yet been rigorously evaluated in terms of improving maternal morbidity and supporting the healthy growth of children.

Maternal postpartum deworming may improve capacity for breastfeeding and quality of milk by improving appetite, dietary intake, and nutrient absorption. Mothers with improved nutritional status and enhanced energy levels may be more likely to initiate and continue exclusive breastfeeding to the six-month recommended time point, and beyond. Additionally, improved maternal nutritional status may influence the passage of certain nutrients (e.g., thiamin, riboflavin, vitamin B_6 and vitamin B_{12}, among others (Allen 2012)) to breast milk during breastfeeding. This may, in turn, improve the nutritional status of infants and enhance growth and development (Figure 1).

Deworming is recommended by WHO because it is safe, cost-effective, and holds promise in reducing anemia and improving the nutritional status of new mothers. Maternal postpartum deworming could be an ideal means of reaching WRA because: a) it can be easily administered through routine postpartum care; b) it supports pro-hospital policies for delivery; c) it is single-dose and well tolerated with little to no side effects; d) it is well-known and culturally acceptable in many STH-endemic communities; and e) it could tackle a multitude of nutritional disturbances, including both micronutrient (e.g., iron, vitamin A), and macronutrient (e.g., fat, protein) deficiencies. Providing empirical evidence on the benefits of deworming in lactating women is imperative since this high risk population has been largely excluded from deworming programs, despite vulnerability to anemia and nutrient depletion. The lack of information on deworming in the postpartum period, combined with the potential to benefit two vulnerable populations simultaneously, provides clear justification for the need for methodologically sound research on maternal postpartum deworming. This research will build on increasing global deworming initiatives, and maintain momentum to tackle NTDs among the world's most vulnerable populations.

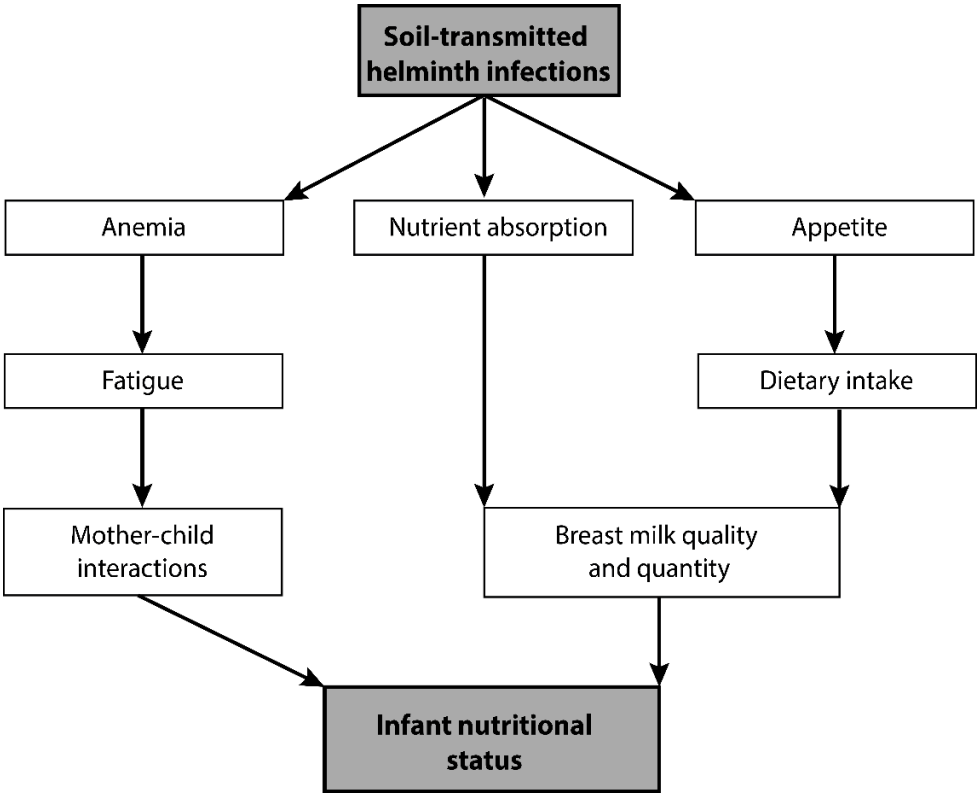

Figure 1**:** Proposed mechanism for the effect of STH infections (and deworming) on maternal and infant health.

CHAPTER 3: RESEARCH OBJECTIVES

3.1 Primary objective

The principal research objective is to determine the effectiveness of maternal postpartum deworming on mean weight gain in infants between birth and 6 months of age.

3.2 Secondary objectives

The secondary research objectives are to determine the effectiveness of maternal postpartum deworming on:

1. Infant health outcomes up to 6 months of age:
   a) growth (i.e., weight and height indices, head circumference indices, and mid-upper arm circumference indices); and
   b) infant morbidity (i.e., occurrence of hospitalizations since birth, and incidence of diarrhea, respiratory illness, fever, and ear infection in the previous two weeks).

2. Maternal health outcomes at 6 months postpartum:
   a) STH infection and intensity;
   b) anemia;
   c) self-reported fatigue; and

3. Indicators of breast milk quality at 1 and 6 months postpartum

CHAPTER 4: METHODS

4.1 Research design

The effectiveness of maternal postpartum deworming on infant and maternal health outcomes was assessed using a parallel, double-blind, randomized, placebo-controlled trial design. The intervention, deworming with single-dose albendazole, was integrated into routine maternal postpartum care at Hospital Iquitos "Cesar Garayar Garcia" in Iquitos, Peru. For a detailed description of the trial protocol see the published manuscript (Mofid et al. 2016) in Appendix A.

4.1.1 Study population

Study setting

The study was conducted in Iquitos, the capital of the Region of Loreto in the Amazon Basin of northeastern Peru. Iquitos is the most populated city of the Peruvian Amazon, with more than 400,000 inhabitants (INEI 2008). The city is situated along the Amazon, Nanay, and Itaya rivers (3.75° S, 73.25° W, 120 m above sea level), and only accessible by air and river, making it geographically isolated from the rest of the country (Morrison et al. 2010). Iquitos is comprised of four districts: Iquitos, Punchana, Belén, and San Juan Bautista. The climate is considered tropical, with an average daily temperature of 25.8°C (22.0–32.2°C) and an average annual precipitation of 3.6 m (Forshey et al. 2010). The main industries in the area include fishing, oil, logging, agriculture, and tourism. A large proportion of the adult population is employed informally, often working outside the city for long periods at a time. Diets in the area are primarily based on carbohydrates, with low consumption of proteins from animal origin (Apaza and Varas 2008; Paima and Zevallos 2012). The areas along the rivers are densely populated, with the majority of individuals living in houses built on raised platforms to combat annual flooding during the rainy season (October to April). Flooding, inadequate sanitation and waste management, and poor access to potable water, are factors that contribute to the endemicity of STH infections, and a state of permanent fecal contamination of the environment. While malaria is more of a problem in the rural areas surrounding the city (Maheu-Giroux et al. 2010), the incidence of dengue within the city of Iquitos is high and varies seasonally (Stoddard et al. 2014).

Study population

Recruitment, baseline assessment, and treatment allocation of participants took place at one hospital site, the Hospital Iquitos "Cesar Garayar Garcia". This hospital has a catchment area that includes the poor and highly STH-endemic district of Belén. In 2004, prevalences of 60% for *A. lumbricoides,* 80% for *T. trichiura, and* 45% for hookworm were reported in over 1,000 pregnant women enrolled in a previous randomized controlled trial (RCT) (Larocque et al. 2006). This hospital can be considered to be representative of health facilities globally that serve STH-endemic populations of low SES. At the time of the study, there was no routine deworming for WRA in Peru.

4.1.2 Eligibility Criteria

Inclusion criteria

Women were eligible to participate in the trial if they met all of the following inclusion criteria:

1) they delivered at Hospital Iquitos (to include participants at high risk for STH infections);
2) they planned to reside in Iquitos or a neighbouring area for the next 24 months (to facilitate follow-up of mother-infant pairs);

Exclusion criteria

Mothers and their newborn babies (mother-infant pairs) were ineligible to participate in the trial based on one or more of the following exclusion criteria:

1) they delivered a stillborn child (to exclude children who could not be followed up);
2) they delivered a child with a serious congenital abnormality or serious medical condition (to exclude children who would require exceptional medical care and follow-up);
3) they delivered a baby with a gestational age less than 32 weeks (to exclude children who would require exceptional medical care and follow-up)
4) they delivered a baby with an Apgar score less than 4 at five minutes (to exclude children who would require exceptional medical care and follow-up);
5) they had a multiple birth (to exclude children having special weight considerations, and additional care needs);

6) mother or baby were transferred to another hospital or hospitalized for a period of more than three days after delivery (to exclude participants with serious medical conditions who would require additional specialized care).
7) they were unable to communicate in Spanish (to ensure high-quality data collection).

Exposure of infants to HIV infection (as indicated through a positive HIV test of the mother) was considered a serious medical condition that required hospitalization. As such, the trial did not include mother-infant pairs in which HIV status in the mother was confirmed to be positive. Peruvian Ministry of Health guidelines do not recommend breastfeeding in HIV-positive mothers (MINSA 2013b).

4.1.3 Interventions

Following delivery at Hospital Iquitos, eligible consenting mothers were randomized to one of two intervention groups:

Experimental group: Standard postpartum care plus deworming
Control group: Standard postpartum care plus placebo

Deworming consisted of a single-dose 400 mg tablet of albendazole (manufactured by GlaxoSmithKline Inc. and donated by WHO). The placebo was identical to the albendazole tablet in terms of size, taste, colour, and markings (manufactured and purchased from Laboratorios Hersil in Lima, Peru). All mother infant-pairs received routine postpartum care as per Peruvian Ministry of Health guidelines (MINSA 2013a; 2013b).

4.1.4 Randomization

A statistician not otherwise involved in the trial prepared a randomization schedule using computer-generated simple randomization with a 1:1 allocation ratio. Prior to the onset of recruitment, two external pharmacists not directly involved in the trial packaged small, opaque, sequentially-numbered envelopes containing the randomly assigned intervention (albendazole or placebo) according to the randomization schedule. All envelopes were stuffed with cotton, sealed, and packed in styrofoam boxes that were clearly labeled with the project name, date, and

range of treatment assignment numbers. Boxes were stored in a temperature-regulated pharmacy in the research facility (Asociación Civil Selva Amazónica) under lock-and-key. Envelopes were only accessible by the project director (LSM) and research coordinator (LP) for daily distribution to the project supervisors (ARB and NPV) to meet the estimated sample size. The randomization list was stored in a secure cabinet under lock-and-key in Canada, and was not accessible to any research personnel (until after the primary data analysis was completed), with the exception being on specific request of the Data Safety and Monitoring Committee (DSMC).

4.2 Recruitment Strategy

4.2.1 Census of study area

Enumeration of pregnant women in the catchment area of Hospital Iquitos (i.e., the target population) was carried out between December 2013 and January 2014, in conjunction with the local ministry of health (Dirección Regional de Salud (DIRESA)). During door-to-door canvassing, households where pregnant women in their second and third trimester resided were identified, and information was collected on the name, age, marital status, expected due date, history of antenatal care, anticipated place of birth, and details of the location of the house (e.g., points of reference). This information was cross-referenced with a list of pregnant women from each health centre within the hospital's catchment area (i.e., 6 de Octubre, 9 de Octubre, América, Belen, Cardozo, Moronacocha, Progreso, San Juan, Santo Tomas, Tupac Amaru, Vargas Guerra). The complete roster of potentially eligible participants was used by research assistants for pre-recruitment of participants.

4.2.2 Pre-recruitment

A two-stage approach was used for recruitment into the trial. In stage one, trained research assistants, including nurses, nurse-midwives, and nurse-technicians, visited the homes of women in their third trimester of pregnancy based on a roster of pregnant women residing in the hospital catchment area. At this visit, research assistants explained the research study to women and their partners and assessed pre-delivery eligibility. In the case that the eligibility criteria were met and both parties wished to participate in the trial, research assistants read the informed consent

document aloud, answered any questions about study procedures, and asked both women and their partners to sign the document. The document was written in Spanish at a basic literary level and described the trial with respect to its aims and expected contributions, the extent of participant involvement, potential benefits and inconveniences of participation, and the voluntary nature of initial and continued participation. In the case that a woman or her partner were under the age of 18 years, written assent was obtained, and informed consent was requested from their parent, guardian, or spouse/partner over the age of 18 years. Women who did not have a partner or whose partner was absent for an indefinite period of time (e.g., death, separation, etc.) were asked to sign a sworn statement to declare the father's absence, in accordance with Peruvian ethics guidelines. Following informed consent, evaluation of understanding the consent form was assessed by asking the participants a series of questions. Research assistants reinforced the themes addressed by each question, and clarified any misunderstanding.

4.2.3 Recruitment

In stage two of trial recruitment, women presenting for delivery in the labour room of Hospital Iquitos were approached by research assistants, reminded of the study, asked whether they were still interested in participating in the trial (i.e., reaffirmation of consent), and assessed for post-delivery eligibility. In the case that a woman presented for delivery at the hospital without previously consenting to participate, she was assessed for eligibility, invited to participate in the trial, and she and her partner were asked to provide informed consent within 10 hours of delivery.

Baseline Assessment

Following informed consent procedures, a baseline questionnaire was administered to women to collect information on sociodemographic characteristics (e.g., age, education), obstetric and medical history (e.g., parity, use of antenatal services, deworming during pregnancy), and environmental exposures (e.g., water source, housing structure). Information about delivery, including Apgar score, gestational age, type of delivery, and infant complications, was extracted from the hospital registry. Information on maternal morbidity (e.g., hemorrhage, hypertension, pre-eclampsia) and medication and vitamin supplementation during pregnancy was obtained from medical charts. Following delivery, baseline anthropometry of infants was performed by

research personnel, as detailed in section 4.3.1. Women were asked to provide a stool specimen upon arrival in the hospital and prior to treatment allocation.

4.2.4 Treatment allocation

Sequentially-numbered treatment envelopes, containing the single tablet of the randomly allocated intervention, were brought to the maternity ward of the hospital daily for treatment allocation. Mother-infant pairs were visited bedside once they had their final medical examination and received their discharge documentation from hospital staff. Hospital discharge usually occurs within 24 hours of a vaginal delivery and within 72 hours of a caesarean section. At this time, the research assistant administered the tablet contained in the next sequentially-numbered envelope, and recorded the treatment assignment code. The participant's identification number and the date and time of treatment allocation were written on each envelope, and stapled to the informed consent document of the participant. Ingestion of the tablet with water was directly observed by the research assistant, and women were monitored for a 30-minute period to observe any immediate adverse effects. Before mother-infant pairs left the hospital, research assistants informed the mothers of the date of the scheduled 1-month study visit (at their home), and recorded this information, along with the contact details of the study coordinator, in the infant's Growth and Development Booklet.

4.2.5 Sample size

The sample size calculation was based on detection of the smallest clinically meaningful difference between intervention groups in the primary outcome, mean infant weight gain between birth and 6 months of age. An estimate of mean weight gain was obtained from recent data on children aged between 5 and 7 months residing in the study area (i.e., 4.24 kg with a standard deviation [SD] of 1.014 kg) (Gyorkos et al. 2011b). Previous authors have claimed that an mean infant weight gain difference of approximately 500 g would be clinically meaningful based on trials of enriched formula feeding in infants (Chouraqui et al. 2008; Trabulsi et al. 2011; Ziegler et al. 2007). However, since the intervention for this trial would be given to women rather than to infants, and since there was the possibility for effect dilution from treating both STH-infected and uninfected mothers, a minimum mean weight gain difference of 200 g in infants was anticipated between the two intervention groups.

The sample size calculation is, therefore, based on an expected effect size of 0.20 kg, an SD of 1.014 kg, a significance level (α) of 0.05, and a power (1-β) of 0.80. Based on the above specifications and a two-sided independent *t*-test, the estimated sample size per group was 404 mother-infant pairs. As informed by a previous study in the same hospital population (Gyorkos et al. 2012), the required sample size was increased to 1010 mother-infant pairs to account for a 20% attrition rate. Sample size and power calculations were conducted using PS Power and Sample Size Calculations Version 3.0 (Copyright © 1997 by Dupont and Plummet).

4.3 Follow-up

4.3.1 Outcome ascertainment

All mother-infant pairs were visited in their homes by research assistants at 1 and 6 months following delivery to ascertain primary and secondary outcomes.

Anthropometric measurements

Weight was measured in duplicate at birth, and at 1 and 6 months following delivery using a portable electronic scale, accurate to the nearest 0.01 kg (Seca 354, Seca Corp., Baltimore, USA), and calibrated routinely with standard weights to ensure high accuracy and precision. Length was measured in duplicate at birth, and at 1 and 6 months following delivery as recumbent crown-heel length on a flat surface using a stadiometer (Seca 417, Seca Corp., Baltimore, USA), accurate to the nearest millimetre. Both weight and length were measured in unclothed infants. Head circumference (HC) was measured in duplicate at birth, and at 1 and 6 months following delivery using a non-stretch Teflon measuring tape (Seca 212, Seca Corp., Baltimore, USA), accurate to the nearest millimetre. Mid-upper arm circumference (MUAC) was measured in duplicate at the 6-month time point only using a non-stretch measuring tape (UNICEF S0145620), accurate to the nearest millimetre. The mean of the first and second anthropometric measurement was used in analyses.

Breastfeeding practice

Research assistants administered a questionnaire to the mothers at the 1 and 6-month study visits. The first component of the questionnaire was designed to assess current breastfeeding practices (e.g., duration of exclusive breastfeeding, timing of first introduction of complementary foods). Questions on breastfeeding practice were adapted from the WHO indicators for assessing infant and young child feeding practices (WHO 2008a).

Infant morbidity

The second section of the questionnaire was comprised of morbidity indicators modified from the Integrated Management of Childhood Illnesses (IMCI) Chart (WHO 2014b). Mothers were asked about their infant's hospitalizations since enrollment and episodes of diarrhea, respiratory illness, fever, and ear infection experienced by their infant in the previous two weeks, including duration and treatment. These indicators have been previously used for research purposes in LMICs (Blouin et al. 2011; Humphrey et al. 1996; Larocque et al. 2006).

Maternal fatigue

The third part of the questionnaire contained questions on self-reported fatigue using a standardized scale to quantify the degree of physical and cognitive fatigue experienced by mothers. At 6 months postpartum, fatigue was assessed using the Fatigue Assessment Scale (FAS) (Michielsen et al. 2003). This scale has been shown to have good psychometric properties (Michielsen et al. 2003; Michielsen et al. 2004) and has been used to measure fatigue in women during the postpartum period (Cooklin et al. 2012b; Giallo et al. 2014).

Maternal STH infection

At the 6-month study visit, women were also given instructions on how to collect a specimen of stool, along with a small plastic container labeled with their unique study identification number, a sealable bag, gloves, and a wooden stick. Research assistants returned the following day to collect the specimens and to transport them to the local research facility. Following collection of the stool specimen, all mothers were offered deworming (i.e., single-dose 400 mg albendazole) if they were not newly pregnant (i.e., in their first trimester of pregnancy).

4.3.2 Adverse event reporting

Serious adverse events (SAE) in mothers and infants were passively reported to research personnel by participants during study visits or between study visits. SAEs were defined as: death; life-threatening condition; in-patient hospitalization or prolongation of an existing hospitalization; persistent or significant disability/incapacity; cancer; and accidental or intentional overdose, in accordance with WHO criteria (WHO 2006a). Ethics committees in Peru (Asociación Civil Impacta Salud y Educación and the Instituto Nacional de Salud (INS)) and Canada (McGill University Health Centre (MUHC)) were notified of SAEs occurring in either the mother or the child within a period of 24 hours from when the event was first reported to research personnel. The DSMC received a summary of adverse events by intervention group at pre-specified intervals (see 4.6.2).

4.4 Substudy

A separate substudy was carried out to evaluate the effect of deworming on breast milk composition in a subsample of trial participants at 1 and 6 months following delivery.

4.4.1 Assembly of substudy

During initial informed consent procedures, women were asked if they would also like to take part in a substudy to assess breast milk production. Of those who agreed to participate, and who were available during the assessment period, a random sample of 200 mother-infant pairs were selected, stratified by sex of the infant. In the event that a mother-infant pair who participated in the 1-month assessment was unavailable at the scheduled assessment at 6 months postpartum, another pair was randomly selected from the trial population for assessment at the 6-month substudy visit only.

4.4.2 Breast milk quality assessments

Collection

Breast milk samples were collected from mothers who were selected to participate in the substudy. To standardize breast milk collection methods, every effort was made to collect

samples between 6:00 am and 11:00 am to avoid extremes in diurnal variations (Allen 2012). Women were assisted in providing a casual milk sample of 50 mL using a Medela Symphony electric breast pump (Medela AG, Switzerland). Collection occurred by pumping milk into a pre-sterilized container with a leak-proof seal from the breast from which the infant had not last fed. All milk samples were transferred to the local laboratory on ice and stored in a refrigerator at 4°C prior to processing.

Laboratory analysis of breast milk

Each sample was homogenized, aliquoted into pre-sterilized falcon tubes, and stored at −80°C. Frozen samples were transported on dry ice to the Western Human Nutrition Research Center (WHNRC) in California for storage at −70°C and subsequent micronutrient assessment. The following indicators of breast milk quality were analyzed: retinol, β-carotene, α-tocopherol, γ-tocopherol, thiamin, riboflavin, flavin adenine dinucleotide (FAD), nicotinamide, pyridoxal, cobalamin, copper, iron, and zinc. Total riboflavin (vitamin B_2) was calculated as riboflavin + FAD × 0.479 (Allen et al. 2015).

Milk samples were analyzed in dim light due to the photosensitivity of some vitamins. High performance liquid chromatography with diode array detection (HPLC-DAD) was used for the measurement of retinol, β-carotene, α-tocopherol, and γ-tocopherol. Riboflavin, FAD, nicotinamide, and pyridoxal were measured by ultra-performance liquid-chromatography tandem mass spectrometry (UPLC-MS/MS), using previously described and validated methods (Hampel et al. 2012). Thiamin was measured using the thiochrome method by high performance liquid chromatography with fluorescence detection (HPLC-FL) (Hampel et al. 2016). The IMMULITE 1000 solid-phase, competitive chemiluminescent enzyme immunoassay (Siemens) was used to measure cobalamin concentrations (Hampel et al. 2014). Copper, iron, and zinc were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using the Varian VISTA AX CCD. The creamatocrit method was used to estimate the concentration of fat (g/L) in breast milk samples. All laboratory analyses were conducted blinded to intervention group status.

4.4 Laboratory analysis of stool specimens

Due to the ethical constraints preventing the detection of STH infection and subsequent randomization to receive placebo, baseline STH infection status could not be determined for all trial participants. Thus, only specimens collected in the hospital from women randomized to the experimental group were analyzed immediately using the Kato-Katz technique (i.e., because these participants received deworming as part of the trial protocol and thus ethical practices were followed). To maintain blinding of research personnel, laboratory technologists, and research coordinators, a code-switching method was implemented. All stool specimens were labeled with a unique study identification code that was given to each participant at recruitment. Once specimens arrived at the local research facility and before they were transferred to the laboratory, the study coordinator (LP) replaced the study identification code of the specimen with a laboratory code, based on a list containing treatment allocation codes and laboratory codes. Laboratory technologists were provided with the list of laboratory codes, with instructions on which specimens were to be analyzed immediately. Each list was stored on separate password-protected computers, one in the coordinator's office, and the other in the laboratory. A master list containing linked information was stored under lock-and-key in the research office in Canada. This method of preserving blinding has been successfully implemented in a previous trial (Joseph et al. 2014).

Fresh stool specimens collected in the hospital and at the 6-month follow-up visit were analyzed by a trained microscopist using the Kato-Katz technique within 24 hours of initial collection (WHO 2004a). This technique is recommended for the assessment of STH prevalence and also to quantify the intensity of infection (i.e., epg of stool) (Montresor et al. 1998). Sensitivity estimates for a single specimen analyzed using the Kato-Katz technique have been estimated at 96.9% (95% Bayesian Credible Interval [BCI]: 96.1%, 97.6%) for *A. lumbricoides*, 91.4% (95% BCI: 90.5%, 92.3%) for *T. trichiura*, and 65.2% (95% BCI: 60.0%, 69.8%) for hookworm (Tarafder et al. 2010). In order to improve sensitivity, slides were examined within one hour of preparation since, after this time, hookworm eggs collapse and become less visible (Montresor et al. 2002). STH intensity, based on parasite-specific egg counts, was categorized according to WHO cut-offs (WHO 2002) (see Table 1 in section 2.1.1).

4.5 Quality assurance

4.5.1 Blinding

Blinding of investigators, research assistants, laboratory technologists, and data analysts was ensured from recruitment until the end of the 6-month study visit (i.e., ascertainment of the primary outcome). Only one member of the research team, the Canadian-based research coordinator (HR), remained unblinded during the trial, to ensure integrity of the study procedures (e.g., randomization list generation, packaging of study interventions) and to communicate with the DSMC.

4.5.2 Mobile application

A mobile data collection application, called eEPI, was developed by members of the research team (LSM, BB, and TWG) in conjunction with a software developer (ReFacta Inc.) for use with Android tablets. Electronic data collection enhanced data integrity by automating question jump patterns, validating responses, ensuring data completeness, and providing prompts to research assistants. It also eliminated the need for manual data transcription and entry. Standard operating procedures were incorporated into questionnaires to ensure that the technique and order of study procedures were standardized. Data were uploaded daily to the on-site server and saved on multiple password-protected platforms (i.e., hard-drive, external disc drive, cloud).

4.5.3. Maximization of follow-up rate

In the case that participants were not available during their scheduled follow-up home visit, research assistants attempted to contact them by phone, and returned to their place of residence until they could be located. Every effort was made to visit participants as close to the target date of their study visit as possible. When logistically feasible, research assistants traveled to participants' homes for follow-up visits when they had relocated outside of the study site area. Participants were only considered lost to follow-up if they could not be located or contacted, if the distance to the new residence was too far away from the study site, if either mother or child had died, or in cases of voluntary withdrawal from the trial.

4.5.4 Training and Standardization

Between July and December 2013, a team of 22 research personnel (one research coordinator, two supervisors, three laboratory technologists and 16 research assistants) were hired and trained in bioethics practice guidelines, research design, electronic data entry, informed consent procedures, and all field aspects of the study protocol. During this time, research personnel received theoretical and practical training on study procedures, including informed consent, questionnaire administration, infant anthropometry, and breast milk collection. Standardization of anthropometric measurements among research assistants was conducted according to WHO guidelines (WHO 2008b), in order to achieve inter and intra-rater reliability of over 95% (de Onis et al. 2004; Ulijaszek and Kerr 1999). Between November and December 2013, all study instruments were pilot-tested, and necessary adjustments to procedures and instruments were made. Prior to commencement of the trial, all study procedures were pre-tested in the field and in the hospital.

4.5.5 Supervision

Daily review of data collection occurred at the end of each work day with the study director (LSM), research coordinator (LP), and each research assistant. At this time, electronic data were checked for completeness, accuracy, and coherence with each research assistant. Inconsistencies were discussed until an agreement was reached, and changes were made accordingly. All modifications to questionnaire data were recorded using a built-in audit table. On-site observation of study visits occurred routinely during each phase of the trial, in both the hospital and field to ensure that study procedures were being executed correctly. Following supervision of study visits, points of clarifications and suggestions for improvement were discussed with each research assistant, as appropriate, to ensure consistency and to maintain integrity of the data.

4.5.6 Laboratory quality control

Asociación Civil Selva Amazónica

In accordance with WHO guidelines, a subsample of 10% of the microscope slides were re-examined by a second laboratory technologist, without knowledge of the previous result, in order

to maintain inter-rater reliability of STH intensity measurements. In the case of an egg count discrepancy larger than 10%, results were discussed by the two technologists and further slides were co-examined until consistency was achieved, to prevent subsequent errors.

Western Human Nutrition Research Center

Assessment of vitamin and mineral content (retinol, β-carotene, α-tocopherol, γ-tocopherol, thiamin, riboflavin, FAD, nicotinamide, pyridoxal, cobalamin, copper, iron, and zinc) in breast milk was internally and externally validated at the beginning, middle (every 14-20 samples), and at the end of each run. Internal validation occurred by using internal standards made of pooled human milk samples from healthy women. Commercially available external standards with known analyte concentrations were also used to validate the equipment. Depending on the assay, the acceptable coefficient of variation (CV) ranged from 1%-23%.

4.6 Trial Oversight

4.6.1 Ethics approval

Research ethics board (REB) approval for the trial was granted by the Asociación Civil Impacta Salud y Educación of Lima, Peru. Since all RCTs in Peru require a second REB approval from the Peruvian National Institute of Health, ethics approval was also obtained from the INS of Lima, Peru. In Canada, the trial received REB approval from the Research Institute of the MUHC in Montreal, Québec. Additional approvals were sought from the Dirección Regional de Salud (DIRESA) (Regional Ministry of Health, Peru) and the study hospital (i.e., Hospital Iquitos "Cesar Garayar Garcia"). All approvals were renewed on time throughout the trial, according to their renewal schedule. In accordance with INS requirements, clinical trial liability insurance was purchased.

4.6.2 Trial monitoring

A Trial Steering Committee, consisting of the principal investigator and all co-investigators, was formed to oversee trial procedures. This committee was responsible for reviewing the study protocol, ensuring the trial was being conducted in accordance with the principles of good

clinical practice, and appointing three international experts with expertise in deworming, biostatistics, and clinical trial methodology to comprise the DSMC. The DSMC, consisting of three members from the USA and Peru, was responsible for reviewing the protocol prior to study initiation, and evaluating study conduct and the occurrence of SAEs at four time points throughout the trial: at the halfway point of recruitment; following completion of recruitment and following each follow-up visit. After each review, the DSMC provided approval for trial continuation. The trial is registered with clinicaltrials.gov (NCT01748929).

4.7 Statistical analyses

4.7.1 Variable classification

Infant growth indices

Mean weight, length, and HC gains were calculated by subtracting the values at each study visit by the values at birth. Daily weight and length gains were calculated by dividing the respective gains by the age of the child (in days) between study visits. Sex-specific z-scores for weight-for-age (WAZ), length-for-age (LAZ), HC-for-age (HCAZ), and MUAC-for-age (ACAZ) were calculated using WHO Anthro software (version 3.2.2, 2011) and macros (WHO 2011a). Prevalence of underweight and stunting were defined as z-scores for WAZ and LAZ, respectively of < - 2 SD from the median of the WHO reference population, according to the WHO Child Growth Standards (WHO 2009).

Maternal anemia and fatigue

Anemia was defined as a Hb value of less than 120 g/L (WHO 2001). Cut-offs for mild, moderate, and severe anemia were defined as Hb concentrations 110-119 g/L, 80-109 g/L, and <80 g/L, respectively (WHO 2011b). The total FAS score was calculated by reverse scoring items 4 and 10 and then summing all 10 items (Michielsen et al. 2004), to give a range of possible scores between 10 and 50. The prevalence of elevated fatigue was categorized as FAS scores ≥ 22 (De Vries et al. 2004b). For comparison with other studies conducted in the postpartum period, a modified 5-item version of the FAS score was also calculated using items 1, 2, 3, 5, and 9 (Chau and Giallo 2015; Cooklin et al. 2012a; Giallo et al. 2014).

Maternal STH infection and intensity

The prevalence of STH was reported separately for each STH species, and also as the prevalence of at least one of the three species (i.e., any STH infection). STH intensity was expressed as both an arithmetic mean epg, and was also categorized into light, moderate, and heavy infections according to WHO cut-offs (Montresor et al. 1998).

Breast milk quality

Important micronutrients in breast milk were analyzed and expressed as the concentration per volume of breast milk. Fat-soluble vitamins (i.e., retinol, β-carotene, α-tocopherol, and γ-tocopherol) were also analyzed by the concentration per lipid content in breast milk. Low breast milk vitamin A was defined as milk retinol below the established cut-off of ≤ 1.05 μmol/L (WHO 1996a).

Socioeconomic status

A proxy for SES was calculated using an asset-based index including the following six variables: presence of a gas stove, television, radio, electricity in the house, tap water connection in the house, and housing material of cement or bricks. Principal Components Analysis was used to derive weights for the construction of the index (Filmer and Pritchett 2001). The first principal component explained 39.2% of the total variance of the underlying construct of SES. The SES index was categorized into quartiles for subsequent analyses. This method for estimating SES has been used previously in studies conducted in the same geographic area (Gyorkos et al. 2013; Joseph et al. 2014).

4.7.2 Descriptive analyses

Study flow, including participant recruitment, eligibility, and losses to follow-up were depicted in a Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Continuous and categorical variables at baseline were expressed as means (with SDs) and proportions, as appropriate, for description of the study population and to allow for comparisons in prognostic variables between intervention groups. In addition, baseline characteristics were compared between those who remained in the study at 6 months and those who were lost to follow-up to assess the possibility of differential losses.

4.7.3 Estimation of baseline prevalence of soil-transmitted helminth infections

While the Kato-Katz technique is recommended for the quantitative assessment of STH infection and intensity, it has less than perfect sensitivity and specificity (Tarafder et al. 2010). Thus, the baseline species-specific prevalences of *A. lumbricoides*, *T. trichiura*, and hookworm were corrected using Bayesian methods described by Joseph *et al.* (1995). Bayesian analyses were performed using the BayesDiagnosticTests Version 3.10.2 Software Package using prior information on the sensitivities and specificities for a single specimen using the Kato-Katz technique for each helminth species provided by Tarafder *et al.* (2010).

4.7.4 Multiple imputation

The effect of maternal postpartum deworming on primary and secondary outcomes was first examined using an intention-to-treat (ITT) approach, in which participants were analyzed according to their assigned intervention group, irrespective of supplemental treatment (i.e., contamination) or loss to follow-up. Missing outcome values were imputed using Multiple Imputation by Chained Equations (MICE) with 20 imputations. Variables that were related to the outcome, and/or related to loss-to-follow-up, were included in the imputation model. For imputation of infant growth and morbidity outcomes, baseline variables used to impute missing outcome data included: weight, length and HC at birth, infant sex, gestational age, maternal age, marital status, education level, number of people residing in the household, SES index, and intervention group. Initially, interaction terms for intervention group and infant sex, birthweight, and birth length, were included in the imputation model, but since no evidence of interaction was observed, interaction terms were dropped from the final imputation model. For imputation of maternal outcomes (i.e., STH infection status, Hb, and fatigue score), the following baseline variables were included in the imputation model: district of residence, maternal age, marital status, education level, number of individuals residing in the household, SES index, and intervention group. The imputation of breast milk quality outcomes included only mother-infant pairs who had participated in either the 1 or 6-month substudy visit. Baseline variables used to impute missing outcome data from the substudy were infant sex, gestational age, maternal age, marital status, education level, number of people residing in the household, SES index, and intervention group. Initially an interaction term for intervention group and infant sex was

included in the imputation model for the substudy outcomes but was later dropped because analysis models did not show evidence of effect measure modification by infant sex.

4.7.5 Effect of deworming on infant growth and morbidity

The primary outcome was mean infant weight gain between birth and 6 months of age. Continuous growth indices were compared between intervention groups using univariable linear regression. The prevalences of underweight and stunting, as well as the risk of infant morbidity (i.e., hospitalization, diarrhea, cough or difficulty breathing, fever and ear infections) were compared between intervention groups using univariable log-binomial regression. The estimation of the risk ratio (RR) is preferable to standard logistic regression since the odds ratio (OR) overestimates associations when the outcomes of interest are not rare (Knol et al. 2012). In some cases, estimation of the RR through log-binomial regression may fail to converge (Cummings 2009). In the presence of convergence difficulty, the RR was estimated using a Poisson regression model with a robust variance estimator. While the log-binomial and Poisson regression models should produce similar point estimates for the RR, Poisson methods for binomial data typically produce standard errors that are too large (Knol et al. 2012). In order to relax the assumption that the data are from a Poisson distribution, a robust variance estimator was used to obtain standard errors and CI that are approximately correct under a misspecified model distribution (Cummings 2009; Zou 2004).

In addition to the estimation of crude intervention effects, multivariable linear regression and multivariable log-binomial regression were used to evaluate the effectiveness of deworming on infant growth and morbidity while adjusting for baseline covariates that were *a priori* determined to be important confounders in the published literature (Senn 1994). These included maternal age, education, SES, infant sex, and gestational age. To evaluate the presence of potential effect measure modification due to infant sex, birthweight, and birth length on deworming, interaction terms were included in separate multivariable linear regression models with the primary outcome, mean infant weight gain.

4.7.6 Effect of deworming on maternal health outcomes

The prevalence of STH infection, anemia, and elevated fatigue were compared between intervention groups using univariable log-binomial regression. The proportion of women with moderate-and-heavy infection was estimated by combining WHO epg thresholds, and compared between groups using log-binomial regression. Poisson regression with a robust variance estimator was used if problems with convergence were experienced.

The effect of deworming on maternal Hb concentrations and continuous fatigue scores was examined separately using univariable linear regression models. Fatigue scores included total FAS score and modified FAS score at 6 months postpartum. Multivariable linear and log-binomial regression models were performed to adjust for baseline values of maternal age, education, district of residence, number of living people residing in household, and SES.

4.7.7 Effect of deworming on breast milk composition

Since concentrations of biochemical indicators are known to have a skewed distribution, the median and interquartile range of indicators were compared between intervention groups, and unadjusted differences were examined using the Wilcoxon rank-sum test.

Separate linear regression models were used to assess the effect of deworming on breast milk concentrations of each biochemical indicator. Skewed dependent variables were log-transformed for inclusion in regression analyses to satisfy normality assumptions. The presence of effect measure modification by infant sex was assessed by including interaction terms between intervention group and sex in models with each biochemical indicator. The effect of deworming on the prevalence of low breast milk vitamin A was assessed using log-binomial regression. Multivariable models were adjusted for maternal age, education, SES, and infant sex. In instances where outliers were present in the distribution of biochemical indicators, extreme values were deleted and analyses were repeated to evaluate robustness of results.

4.7.8 Sensitivity analyses

In addition to ITT analyses, the following sensitivity analyses were performed : 1) a complete-case approach which included all participants who had outcome data for the 6-month follow-up visit; 2) a per-protocol approach which included those who had outcome data for the 1 and 6-month follow-up visits and did not report taking deworming outside of the trial protocol at either time point; 3) an approach restricted to those who had a 6-month follow-up visit within 3 days of the target date (for infant growth outcomes only). All analyses were conducted using STATA/SE for Windows (version 4.0, Stata Corp., College Station, TX, USA).

CHAPTER 5: RESULTS – INFANT HEALTH OUTCOMES

5.1 Preamble to Manuscript 2

As increasing worldwide attention is being focused on achieving global targets for reducing the prevalence and disease burden of STH infections, previously missed opportunities for intervention are being investigated. In this light, interventions during lactation have the potential to benefit both mother and infant. Because deworming in the early postpartum period can be easily integrated within routine postpartum care, endemic countries may find it a feasible and cost-effective intervention to complement other deworming activities, especially in those areas where the disease burden is highest. To date, no study has assessed the effectiveness of maternal postpartum deworming on maternal or infant health outcomes.

The pre-requisite of equipoise in this trial has been established from a review of the current literature in addition to consideration of expert opinion provided by parasite epidemiologists, field researchers, and global policy experts. This trial fills a research gap which has been identified by WHO. Specifically, the trial results will contribute to the evidence base on the effectiveness of a deworming intervention targeted to an important subgroup of WRA (i.e., lactating women). The overarching aim of the trial is to provide original scientific evidence to inform global health policy on deworming.

The following manuscript (Manuscript 2) presents the first chapter of results. It summarizes the rationale of the trial and includes a brief description of the methods. A more detailed description of the research procedures can be found in the published trial protocol in Appendix A (Mofid et al. 2016). This manuscript reports on the effectiveness of maternal postpartum deworming on the primary outcome of mean infant weight gain between birth and 6 months of age. It also presents the results of other infant growth indices and indicators of infant morbidity. For clarity and to avoid redundancy, this manuscript focuses on infant outcomes and Manuscript 3 focuses on maternal outcomes. The manuscript conforms to the CONSORT 2010 guidelines for the reporting of parallel RCTs. It has been prepared according to the specifications of the journal *PLOS Neglected Tropical Diseases*.

5.2 Manuscript 2: A double-blind randomized controlled trial of maternal postpartum deworming to improve infant weight gain in the Peruvian Amazon

Short title: Maternal postpartum deworming and infant growth

Authors and Affiliations: Layla S. Mofid[1,2], Martín Casapía[3], Eder Aguilar[4], Hermánn Silva[4], Antonio Montresor[5], Elham Rahme[2,6], William D. Fraser[7], Grace S. Marquis[8], Jozef Vercruysse[9], Lindsay H. Allen[10], Brittany Blouin[1,2], Hugo Razuri[2], Lidsky Pezo[3], Theresa W. Gyorkos[1,2]*

[1] McGill University, Department of Epidemiology, Biostatistics and Occupational Health, Montréal, Québec, Canada

[2] Research Institute of the McGill University Health Centre, Division of Clinical Epidemiology, Montréal, Québec, Canada

[3] Asociación Civil Selva Amazónica, Iquitos, Peru

[4] Hospital Iquitos "César Garayar García," Iquitos, Peru

[5] Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

[6] Department of Medicine, McGill University, Montréal, Québec, Canada

[7] Centre de recherche et Département d'obstétrique et de gynécologie, Université de Sherbrooke, Sherbrooke, Québec, Canada

[8] McGill University, School of Dietetics and Human Nutrition, Ste. Anne-de-Bellevue, Québec, Canada

[9] Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[10] USDA, ARS Western Human Nutrition Research Center, University of California, Davis, California, USA

*Corresponding author: Email: theresa.gyorkos@mcgill.ca (TWG)

ABSTRACT

Background: Nutritional interventions targeting the critical growth and development period before two years of age can have the greatest impact on health trajectories over the life course. Compelling evidence has demonstrated that interventions investing in maternal health in the first 1000 days of life are beneficial for both mothers and their children, and can contribute to improving equity and alleviating poverty. One such potential intervention is deworming, integrated into maternal postpartum care in areas where soil-transmitted helminth infections are endemic.

Methodology/Principal Findings: 1010 mother-infant pairs were recruited into a trial aimed at assessing the effectiveness of maternal postpartum deworming on infant and maternal health outcomes. Following delivery, mothers were randomly assigned to receive either single-dose 400 mg albendazole, or matching placebo. Mother-infants pairs were followed-up at 1 and 6 months postpartum. There was no statistically significant difference in mean weight gain between infants in the experimental and control groups (4.3 kg ±0.04 *vs.* 4.4 kg ±0.04) at 6 months postpartum. Further, maternal postpartum deworming did not have a differential effect on the occurrence of infant hospitalizations, nor on the incidence of diarrhea, cough, fever, or ear infections.

Conclusions/Significance: In a study population composed of both infected and uninfected mothers, maternal postpartum deworming with single-dose albendazole was insufficient to impact infant growth and morbidity indicators at 1 or 6 months of age. The benefits of postpartum deworming should be further investigated in study populations having higher overall prevalences and intensities of soil-transmitted helminth infections and, in particular, where the prevalences and intensities of whipworm and hookworm infections are of public health concern.

Trial registration: ClinicalTrials.gov (NCT01748929)

AUTHOR SUMMARY

Worldwide, over one billion people are infected with intestinal worms (roundworms, whipworms, and hookworms). In worm-endemic areas, women of reproductive age are a high risk group for infection because of their poor nutritional status and increased physiological needs during pre-pregnancy, pregnancy, and lactation. To measure the effect of providing mothers with deworming treatment soon after delivery, we conducted a trial in 1010 mother-infant pairs. Mothers were randomly assigned to receive either a single-dose deworming tablet or a placebo tablet. Mothers and their infants were visited in their homes at 1 and 6 months following delivery. At the 6-month time point, among all mother-infant pairs, we could not detect an effect of deworming on infant growth or morbidity. The potential benefit of maternal postpartum deworming in populations with higher prevalences and intensities of intestinal worms, particularly where infections with whipworm and hookworm predominate, warrants further investigation.

INTRODUCTION

Worldwide, more than 200 million children under the age of five years fail to reach their full developmental potential [1]. Suboptimal nutrition and growth during fetal and early childhood development is widely regarded as having adverse long-term consequences, including higher susceptibility to infections, decreased learning potential, lower economic productivity, and greater risk of mortality [2]. The most critical period of pre and postnatal development (coined the first 1000 days of life) is a time when interventions can have the greatest impact on preventing negative health and economic outcomes over the life course [3, 4]. Given the intrinsic relationship between maternal and child health, the nutritional status of mothers is considered to be a pivotal driver of infant growth, development, and survival [5, 6]. To date, most studies have focused almost exclusively on maternal micronutrient deficiencies [7], and have not adequately addressed the multi-factorial causes of maternal malnutrition, including the role of maternal infection on future infant growth and morbidity.

The soil-transmitted helminth (STH) infections (*Ascaris lumbricoides* (the roundworm), *Trichuris trichiura* (the whipworm) and the hookworms, *Necator americanus* and *Ancylostoma duodenale*) are considered the most prevalent parasitic infections of humans, affecting more than 1.45 billion people worldwide [8]. The global significance of STHs lies in their high burden of disease, attributed to blood loss, anemia, and malabsorption of nutrients, that can cause or further exacerbate nutritional deficiencies [9]. Sequelae of chronic infection with STHs include fatigue, loss of appetite, growth faltering, and impaired cognition [9-11]. While much of the current research and focus of large-scale control programs have targeted preschool and school-aged children, women of reproductive age (WRA) remain a largely neglected group [12] despite their underlying poor iron status due to inadequate diet and high risk of blood loss owing to menstruation, pregnancy, and childbirth [11, 13, 14]. While species-specific estimates are not available for WRA as a whole, it is estimated that, at any one time, as many as 37.7 million WRA are infected with hookworms alone [15].

WHO recommends that single-dose deworming (i.e., single tablets of 400 mg albendazole or 500 mg mebendazole) be given to WRA, including pregnant women (after the first trimester) and lactating women in endemic areas, where the prevalence of infection exceeds 20% [14]. Among

the 112 countries considered endemic for STH infections [16], no coverage estimates are available for deworming in WRA (i.e., the proportion of WRA reached by the deworming intervention), and few countries include this risk group among other deworming activities [17]. The evidence base on deworming in WRA is limited, and largely focuses on deworming during pregnancy. Randomized controlled trials (RCTs) and observational studies conducted in pregnant women have been largely inconclusive, providing mixed evidence on the benefits of deworming on maternal anemia, low birthweight, and perinatal mortality [18, 19]. The uptake and health benefits of deworming during pregnancy may not be optimal because in many low-and-middle-income countries (LMICs) antenatal care attendance is low and reinfection rates can be high. The early postpartum period presents an innovative opportunity to reach WRA because deworming can be easily integrated into routine care, and many LMICs have, or are currently adopting and actively promoting, pro-hospital and health centre delivery policies.

While maternal deworming will be of direct benefit to the infected woman (by curing or reducing her burden of STH infection), the unique interface between mother and child during lactation suggests that benefits may also accrue to the newborn infant. To address this research gap, we designed a double-blind RCT aimed at comparing the effectiveness of single-dose albendazole *vs*. placebo administered to women following in-hospital delivery on infant growth and morbidity in an area of Peru known to be highly endemic for STH infection.

METHODS

Research design and setting

This study is a single-centre, double-blind, randomized, placebo-controlled trial conducted in the Amazon region of Iquitos, Peru. A recent meta-analysis estimated that a quarter of Peru's population was infected with STHs [20]. In Iquitos, the prevalence of infection was much higher than the country average with prevalences > 80% in school-aged children [21], > 40% in preschool-aged children [22], and > 90% in pregnant women [23]. Iquitos was chosen as the study site because of its high STH endemicity, a high proportion of women delivering in hospital, and because, at the time of the trial, there were no routine deworming programs targeting WRA.

Recruitment

A detailed description of study procedures can be found in the published trial protocol [24]. Briefly, mother-infant pairs were recruited into the trial using a two-stage approach. First, from pregnancy registries accessed in health centre records, women who were in their third trimester of pregnancy were visited in their homes by research personnel in order to assess eligibility using the following inclusion criteria: planned delivery at Hospital Iquitos "César Garayar García"; and intention to remain in the study area for a 24-month period. Following a detailed explanation of the trial, women and their partners were asked to provide informed consent and, if this was obtained, a baseline questionnaire was administered.

Upon arrival at the Hospital Iquitos "César Garayar García" for delivery, women were approached by research assistants and asked to re-confirm their consent to participate in the trial. Following delivery, mothers and newborns were assessed for the following exclusion criteria: stillborn; serious congenital abnormality or serious medical condition; gestational age < 32 weeks; Apgar score < 4 at 5 minutes; multiple birth; mother or baby transferred to another hospital, or hospitalization of mother or baby for > 3 days; and inability to communicate in Spanish. In the case that women presented at the hospital for delivery prior to being visited in their home by a research assistant, every effort was made to recruit mothers into the trial as soon as possible before or after delivery (i.e., within 10 hours of birth).

Randomization and treatment allocation

A computer-generated randomization schedule was prepared prior to recruitment by a statistician, not otherwise involved in the trial, using a random number sequence according to simple randomization with a 1:1 allocation ratio. Group assignments were concealed in opaque, sequentially-numbered envelopes according to the randomization sequence. Envelopes were stored in a secure temperature-regulated pharmacy. A print copy of the randomization schedule was stored in a sealed envelope under lock-and-key at the research office in Canada (Research Institute of the McGill University Health Centre).

Eligible, consenting women were visited at bedside prior to hospital discharge and were administered the tablet in the next sequentially-numbered envelope. Participants, research personnel, and data analysts were blinded to group assignment.

Mothers randomized to the experimental group received single-dose 400 mg albendazole and mothers randomized to the control group received single-dose placebo. The albendazole tablets were manufactured by GlaxoSmithKline Inc. and donated for this trial by WHO. The placebo tablets were manufactured to be identical to the albendazole tablets in all aspects, including size, taste, color, and markings and were manufactured by Laboratorios Hersil in Lima, Peru. Both groups received the current standard of routine postpartum care from hospital personnel. At 6 months postpartum, all mothers were offered deworming as part of the trial protocol, if they were not newly pregnant (i.e., in the first trimester of pregnancy).

Sample size

The required sample size was estimated for the primary outcome of mean infant weight gain between birth and 6 months of age. An estimate of mean weight gain was obtained from recent data on children between 5 and 7 months of age residing in the study area (i.e.,4.24 kg with a standard deviation of 1.01 kg) [25]. The trial sample size was computed using a two-sided independent *t*-test, with a significance level of 0.05, a power of 0.80, a standard deviation of 1.01 kg, a minimum detectable difference in weight gain of 0.20 kg, and taking into account an attrition rate of 20%. Based on the above specifications, a total of 1010 participants was calculated to be the sample size needed to declare that intervention groups would be different if, in fact, there would be a true between-group difference of 0.2 kg (or more) in weight gain between birth and 6 months of age. Sample size calculations were carried out using PS Power and Sample Size Calculations Version 3.0 (Copyright © 1997 by Dupont and Plummer). Based on the number of deliveries at the study hospital in 2011, recruitment was expected at the rate of 300 mother-infant pairs per month, suggesting a total recruitment period of approximately 3.5 months.

Data collection

Epidemiologic and clinical data

A baseline questionnaire was administered to women following receipt of informed consent to obtain information on sociodemographic characteristics. Pregnancy and delivery information about mothers (e.g., pregnancy complications) and their infants (e.g., gestational age, Apgar score, and birth time) was obtained from medical charts prior to randomization.

Baseline soil-transmitted helminth infection

In order to estimate baseline prevalence and intensity of STH infection, women were asked to also provide a stool specimen in the hospital when they presented for delivery. Specimen collection from participants randomized to the experimental group were analyzed immediately using the Kato-Katz technique. This is because they were allocated to the experimental group and would receive deworming treatment as part of the trial protocol (therefore respecting ethical guidelines). The analysis of fresh stool specimens using the Kato-Katz technique is recommended for the assessment of STH prevalence and also to quantify the intensity of infection [27]. While specificity for a single stool specimen using this technique had been estimated at over 93%, sensitivity has been reported at 96.9% (95% Bayesian Credible Interval [BCI]: 96.1, 97.6) for *A. lumbricoides*, 91.4% (95% BCI: 90.5, 92.3) for *T. trichiura*, and 65.2% (95% BCI: 60.0, 69.8) for hookworm [28]. In order to preserve blinding of laboratory technologists, research personnel, and study coordinators, a code-switching method was implemented that had successfully been used in another trial [29]. Briefly, the local study coordinator replaced participant identification codes on specimen containers with a laboratory code prior to transfer to the laboratory, according to a pre-defined list. In the laboratory, technologists were provided with another list containing laboratory codes and indications of which specimens were to be analyzed immediately. Each list was kept on a password-protected computer, one in the administration office of the trial in Peru (Asociación Civil Selva Amazónica), and the other in the laboratory. A master list linking information was kept under lock-and-key at the research office in Canada (Research Institute of the McGill University Health Centre).

Anthropometry

Anthropometry in infants was measured immediately after delivery in the hospital, and during follow-up at participants' homes at 1 and 6 months following delivery. Weight was measured in the unclothed infant using a digital balance (Seca 354, Seca Corp., Baltimore, USA), accurate to 0.01 kg. Recumbent length was measured using a stadiometer (Seca 417, Seca Corp., Baltimore, USA), accurate to 0.1 cm. Head circumference (HC) was measured using non-stretchable tape (Seca 212, Seca Corp., Baltimore, USA), accurate to 0.1 cm. Mid-upper arm circumference (MUAC) was measured at the 6-month study visit only using non-stretchable tape (UNICEF S0145620), accurate to 0.1 cm. All anthropometric measurements were performed in duplicate and the mean value was used for analyses. All research assistants were trained on anthropometry assessment according to WHO guidelines [26] in order to ensure accuracy, precision, and standardization of measurements.

Infant morbidity

At each follow-up visit, information about infant morbidity and hospitalization since birth was obtained by self-report from the mothers. Infant morbidity indicators were adapted from the Integrated Management of Childhood Illness (IMCI) [30] and included episodes of diarrhea, cough or difficulty breathing, fever, and ear infections in the previous two weeks. In the case of reported hospitalizations since birth, hospital medical charts were reviewed to confirm admission, diagnosis, and treatment. All serious adverse events (SAEs) were categorized according to WHO guidelines [14] and reported to the relevant research ethics boards and the trial's Data Safety and Monitoring Committee.

Every attempt was made to visit trial participants as close to the target date of their scheduled study visit as possible in order to ascertain the most accurate anthropometric data. To minimize loss to follow-up, research assistants made numerous attempts to locate and/or communicate with participants. Participants were considered lost to follow-up only in circumstances where they could not be located, the area of relocation was too far away, either the mother or child had died, or due to voluntary withdrawal from the study. All data collected during home visits were recorded electronically using a data application on a mobile tablet and reviewed daily for accuracy and completeness by the research coordinator (LP) and project director (LSM).

Statistical analyses

Weight, length and HC gains were calculated by subtracting the values at each study visit by the values at the previous visit. Daily weight and length gains were calculated by dividing the respective gains by the age of the child (in days) between study visits. Z-scores for weight-for-age (WAZ), length-for-age (LAZ), HC-for-age (HCAZ), and MUAC-for-age (ACAZ) were calculated using WHO Anthro software (version 3.2.2, 2011) and macros for Stata. The prevalences of underweight and stunting were defined as z-scores for WAZ and LAZ respectively, of < -2 SD from the median of the WHO reference population.

Bayesian models for each helminth species were used to correct prevalence estimates by adjusting for imperfect sensitivity and specificity in the absence of a gold standard. A non-informative prior was used for the baseline prevalence and prior information on sensitivity and specificity for each helminth species were taken from a recent publication by Tarafder *et al.* (2010) [28]. Bayesian analyses were performed using the BayesDiagnosticTests Version 3.10.2 Software Package, using the methods of Joseph *et al.* (1995) [31].

The effectiveness of deworming on infant growth and morbidity outcomes was first assessed according to the intention-to-treat (ITT) principle, such that participants were analyzed according to their assigned intervention group. Multiple Imputation by Chained Equations (MICE) with 20 imputations was used to impute anthropometric and morbidity outcome data for those infants who missed their 1 and/or 6 month follow-up visits. Variables that were related to the outcome, and/or related to loss-to-follow-up were included in the imputation model, including weight, length and HC at birth, infant sex, gestational age, maternal age, marital status, education level, number of people residing in the household, socioeconomic status, and intervention group.

As one objective of the trial was to assess the possibility of effect measure modification by infant sex, birthweight and birth length on infant growth indices, the initial imputation model also included interaction terms for intervention group and infant sex, birthweight, and birth length. However, since no evidence of interaction was observed in the analysis models, interaction terms were dropped from the final imputation model.

Unadjusted ITT analyses for continuous outcomes were performed using univariable linear regression. Unadjusted ITT analyses for dichotomous outcomes were performed using univariable log-binomial regression modeling. Participants were categorized into quartiles of socioeconomic status using an asset-based index derived from the following six variables: presence of a gas stove, television, radio, electricity in the house, tap water connection in the house, and housing material of cement or bricks [29, 32, 33]. The index was constructed using weights derived from Principal Components Analysis. Adjusted analyses used multivariable linear regression modeling for continuous outcomes and multivariable log-binomial regression for dichotomous outcomes, with the following covariables: maternal age, education, socioeconomic index, infant sex, and gestational age. In the case where log-binomial regression models could not converge, the risk ratio was estimated using a Poisson regression model with a robust variance estimator.

The following sensitivity analyses were also planned, *a priori*, to be undertaken: 1) a complete-case approach restricted to those participants who had complete baseline and 6-month data; 2) a per-protocol approach including those who had complete outcome data and did not report taking deworming outside of the trial protocol at either time point, and 3) an approach restricted to those who had a 6-month follow-up visit within 3 days of the target date (for infant growth outcome models).

Ethical considerations

Ethics approval for this trial was obtained from the research ethics committees of the Asociación Civil Impacta Salud y Educación (Peru), the Instituto Nacional de Salud (INS) (Peru) and the McGill University Health Centre (MUHC) (Canada). As per INS guidelines, all women and their partners provided written informed consent for participation in the trial. An independent Data Safety and Monitoring Committee comprised of three international experts was put in place to monitor trial progression, review all SAEs, and approve continuation of the trial at pre-specified time points.

RESULTS

Trial Recruitment

Recruitment into the trial took place between February and August 2014, over a period of nearly 6 months. Of the 2134 women and their partners who were approached to participate in the trial, 1010 were enrolled and randomized (Figure 1). The remaining 1124 mother-infant pairs did not participate because: the mother did not meet the pre-delivery eligibility criteria (n=752), either mother or infant did not meet the post-delivery eligibility criteria (n=134), either mother or her partner declined to participate (n=175), or the sample size was reached before enrolment could take place (n=63). Those who declined to participate were more likely to live in urban areas (OR: 2.8; 95% CI: 1.4, 5.5), and be primigravida (OR: 1.6; 95% CI: 1.1, 2.4). All 1010 mother-infant pairs recruited into the trial were randomized to the experimental (n=510) or control (n=500) groups.

Description of study population

Baseline maternal and infant characteristics for the two intervention groups are summarized in Table 1. Groups were similar in terms of maternal and infant characteristics. However, some differences between groups were found in household characteristics (i.e., access to potable water in the home and the type of housing structure).

In the hospital, 64 participants (12.5%) in the experimental group provided a stool specimen that was analyzed immediately using the Kato-Katz technique. Those who provided a stool specimen for analysis in the hospital where similar in terms of baseline characteristics to those who did not provide a specimen. Due to the RCT design, prevalences and intensities of infection are expected to be similar between intervention groups. Uncorrected and corrected prevalences of STH infection, along with intensities are presented in Table 2. The proportion of women testing positive for any STH infection was 48.4%, of which 29.7% tested positive for *A. lumbricoides*, 26.6% tested positive for *T. trichiura*, and 6.3% tested positive for hookworm. Bayesian methods that adjust for imperfect sensitivity and specificity produced corrected prevalences of 31.4% (95% BCI: 13.7%, 49.4%) for *A. lumbricoides*, 18.5% (95% BCI: 1.0%, 43.5%) for *T. trichiura*, and 28.7% (95% BCI: 1.9%, 88.6%) for hookworm.

Follow-up

A total of 968 (95.8%) infants completed both their 1 and 6 month study visits. Of the 42 infants who were lost-to-follow-up, 7 (0.7%) were visited only at baseline, 31 (3.1%) were visited at 1 month only, and 4 (0.4%) were visited at 6 months only. The causes of attrition were: emigration from study area (n=35), infant death (n=5), maternal death (n=1), and temporary withdrawal (n=1). The mean number of days between the baseline and first follow-up visit was 32.0 days (±3.6) and the mean number of days between the baseline and second follow-up visit was 186.7 days (±13.3). Follow-up rates were similar in the intervention groups at both follow-up time points. Overall, baseline characteristics were similar between participants who remained in the trial at 6 months and those who were lost to follow-up. However, infants who missed their 6-month study visit (n=38) had mothers who were more likely to be single (RR: 2.6; 95% CI: 1.3, 5.3). The proportion of women who reported having received deworming outside of the trial protocol was 3.3% (16/490) in the experimental group and 2.7% (13/478) in the control group.

In the infants, the prevalence of underweight increased from 3.8% (95% CI: 2.6%, 4.9%) at birth to 7.4% (95% CI: 5.8%, 9.0%) at 1 month, and then decreased to 3.6% (95% CI: 2.5%, 4.8%) at 6 months. The prevalence of stunting steadily increased from 6.0% (95% CI: 4.6%, 7.5%) at birth, to 12.4% (95% CI: 10.3%, 14.4%) at 1 month to 13.5% (95% CI: 11.3%, 15.6%) at 6 months.

Effect of deworming on primary and secondary anthropometric outcomes

Weight, length, and HC gains, as well as z-scores for WAZ, LAZ, HCAZ, and ACAZ between intervention groups are compared in Table 3. The primary outcome, mean infant weight gain between birth and 6 months of age, was similar between intervention groups (4.3 kg ±0.04 *vs.* 4.4 kg ±0.04). There were no differences between groups in terms of the primary or secondary anthropometric outcomes in unadjusted or adjusted ITT analyses at 1 (data not shown) or 6 months postpartum. Deworming did not have a statistically significant effect on the prevalence of underweight or stunting at 1 (data not shown) or 6 months postpartum (Table 4), nor on the daily weight and length gains (Table 5). Results were consistent in sensitivity analyses. There was no statistical evidence that the effect of deworming on anthropometric outcomes differed between boys and girls, or varied according to birthweight or birth length.

Effect of deworming on infant morbidity

The occurrence of hospitalization, and the incidence of diarrhea, respiratory problems, and fever, as assessed at 6 months postpartum are shown in Table 6. The incidence of ear infection did not differ between intervention groups but was too low (< 1%) to perform statistical modelling. No significant difference was observed in indicators of infant morbidity between intervention groups at 1 (data not shown) or 6 months postpartum. Results were consistent in adjusted analyses as well as further sensitivity analyses.

Serious adverse events

Between baseline and the 6-month visit, 65 SAEs were reported in infants. The frequency of SAEs was similar between intervention groups, with 34/510 (6.7%) occurring in the experimental group, and 31/500 (6.2%) occurring in the control group. Of the 5 infant deaths that were reported over the 6 months of follow-up, 2 occurred in the experimental group, and 3 occurred in the control group. No SAE was found to have been related to the administration of albendazole (to the mothers).

DISCUSSION

This is the first trial assessing the effect of maternal postpartum deworming on infant and maternal health outcomes. We were unable to demonstrate an overall effect of maternal postpartum deworming on infant growth or morbidity indicators up to 6 months of age within the total study population. The low prevalence of STH infection in this population may have reduced the ability of detecting a benefit due to effect dilution (i.e., the trial population comprising both STH-infected and uninfected mothers).

Although no previous studies of deworming have been conducted in lactating women, trials have been carried out in pregnant populations. There are inconsistencies in study findings among published reports of trials of deworming in second and third trimester pregnant women where infant growth was an outcome measure. In a trial conducted in Uganda between 2003 and 2005, treatment with albendazole in the second and third trimester of pregnancy showed no benefit in terms of mean birthweight or the proportion of infants born with low birthweight [34]. In

contrast, in a trial of single-dose mebendazole and daily elemental iron conducted in Peru, a beneficial effect on the proportion of infants born with very low birthweight (<1500 g) was observed [17]. In the present study, the prevalence and intensity of STH infection was low, and therefore closer to the baseline STH infection profile in the previous Uganda trial (68% prevalence) compared to the previous Peru trial (91% prevalence). Low STH prevalence overall may account for the fact that an effect was not observed in the total study population (which included both infected and uninfected mothers).

Our study has several strengths. First is the minimization of measured and unmeasured confounding by external factors achieved through the RCT design. The sample size was large and recruitment of the study population was completed within a period of 6 months. A high follow-up rate was maintained throughout the trial, minimizing the potential for bias caused by differential loss to follow-up. In-depth training and standardization of anthropometric measurements ensured a high degree of accuracy and precision of outcome ascertainment, thus reducing the potential for measurement error. While deworming medication is readily available for purchase over-the-counter in the study area, compliance with the study protocol was high and non-differential between intervention groups. The consistency of our results between ITT, complete-case, and per-protocol analyses demonstrates the robustness of our findings. Results from the present study may be generalizable to other populations of lactating women in STH-endemic areas with similar prevalence and intensity profiles, and where deliveries in health facilities are actively promoted.

The limitations encountered in the conduct of this trial include a lower than anticipated baseline prevalence of STH (considerably lower than the 91% reported in Larocque *et al* (2006) from the same study area) [23]. This could be the reason why a benefit of deworming in the total study population was not detected. Collecting stool specimens from women prior to enrolment was challenging and resulted in a less than optimal yield. WHO recommends use of the Kato-Katz technique on fresh stool specimens for the assessment of STH prevalence and intensity. However, due to ethical constraints, specimens collected from those participants randomized to the control group could not be immediately analyzed by this technique. The less than perfect sensitivity and specificity of the Kato-Katz technique likely resulted in some misclassification of

baseline STH infection status, and likely underestimated the true prevalence of infection in the trial population. However, this was minimized by correcting the prevalences using Bayesian methods (Joseph et al. 1995). Lastly, infant morbidity indicators were based on maternal reporting. We have no reason to believe that reporting differed by intervention group, although misclassification in morbidity status may have reduced the observed differences in morbidity outcomes between the two intervention groups.

It has been well established that only individuals infected with STHs will benefit from deworming and, as a result, WHO only recommends the implementation of deworming programs in areas where the prevalence of infection exceeds 20% [14]. This recommendation is based on the observation that moderate-and-heavy STH intensity is not normally found at prevalences lower than 20% [35]. Many argue that, in order to evaluate the impact of deworming interventions, analyses must be restricted to those who were infected at baseline, and thus could benefit from treatment [36]. However, new trends in ethics guidelines prevent the detection of STH infection at baseline and subsequent randomization of infected individuals to control groups, thereby withholding treatment from those in need. Without accurate individual participant data on baseline infection status, subgroup analyses restricted to infected populations are limited. For these reasons, the standard parallel RCT design may not be adequate to evaluate the effectiveness of deworming. There is a need for developing more novel research designs, such as stepped-wedge RCTs (i.e., cluster RCTs in which cross-over from control to intervention is randomized until all clusters receive the intervention [37]) with careful consideration of secular trends.

Overall, this is the first trial to provide rigorous empirical evidence on the benefits of maternal postpartum deworming. Future research is needed, not only on the biological mechanisms underpinning the potential link between maternal postpartum deworming and benefits to the infant, but also in study populations having higher prevalences and intensities of STH infections, especially *T. trichiura* and hookworm infections which have a direct effect on anemia. The postpartum period is an ideal time to reach women periodically during their reproductive years because they are easily accessible, especially in areas where hospital-based deliveries are promoted, and where deworming can be easily integrated into standard postpartum care.

Targeting treatment to the groups at highest risk of STH infection may produce the greatest health impacts.

CONTRIBUTORS

LSM, BB, and TWG were responsible for trial conception and protocol development, with critical input from MC, AM, ER, WDF, GSM, JV, and LHA. LSM, LP, and HR were responsible for on-site trial management including training, logistics, development of study tools, and overseeing of data collection. LSM, TWG, and ER were involved in data analysis. LSM and TWG were responsible for manuscript preparation. All authors contributed to the interpretation of data and the preparation and revision of the manuscript.

DECLARATION OF INTERESTS

We declare that we have no conflicts of interest.

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Table 1: Baseline characteristics of mothers and infants (N=1010) by intervention group, Iquitos, Peru for the recruitment period of February to August 2014.

	Albendazole n = 510	**Placebo n = 500**
Maternal characteristics		
Age (years) mean ±SD	25.2 ±6.8	25.6 ±7.0
Married or cohabiting *n* (%)	447 (87.7)	443 (88.6)
Primigravida *n* (%)	134 (26.3)	127 (25.4)
Less than secondary education *n* (%)	291 (57.1)	299 (59.8)
Employment outside home *n* (%)	60 (11.8)	58 (11.6)
Deworming during pregnancy *n* (%)	11 (2.2)	8 (1.6)
Iron supplementation during pregnancy *n* (%)	478 (93.7)	477 (95.4)
Vaginal delivery *n* (%)	393 (77.1)	378 (75.6)
Infant characteristics		
Male *n* (%)	248 (48.6)	251 (50.2)
Birthweight (kg) mean ±SD	3.2 ±0.4	3.2 ±0.4
Birth length (cm) mean ±SD	48.6 ±1.8	48.6 ±1.9
Birth head circumference (cm) mean ±SD	33.6 ±1.3	33.6 ±1.2
Gestational age (weeks) mean ±SD	38.7±0.8	38.6 ±0.9
Apgar score at 5 minutes mean ±SD	9.8±0.5	9.8 ±0.6
Household characteristics		
Peri-urban or rural residence *n* (%)	490 (96.1)	485 (97.0)
Access to potable water in home *n* (%)	396 (77.7)	367 (73.4)
Home with dirt or wooden floor *n* (%)	321 (62.9)	352 (70.4)
Number of people residing in household mean ±SD	5.9 ±2.8	5.8 ±2.6

SD = standard deviation

Table 2: Baseline prevalence and intensity of soil-transmitted helminth infections as assessed by single stool specimens collected in the hospital (n=64) and analyzed using the Kato-Katz technique.

STH species	Prevalence
A. lumbricoides	
Prevalence – uncorrected (95% CI)	29.7 (19.6, 42.3)
Prevalence – corrected (95% BCI)*	31.4 (13.7, 49.4)
Intensity – median epg (range)	336 (48 – 11376)
T. trichiura	
Prevalence – uncorrected (95% CI)	26.6 (17.0, 39.0)
Prevalence – corrected (95% BCI)*	18.5 (1.0, 43.5)
Intensity – median epg (range)	96 (24 – 1704)
Hookworm	
Prevalence – uncorrected (95% CI)	6.3 (2.3, 15.9)
Prevalence – corrected (95% BCI)*	28.7 (1.9, 88.6)
Intensity – median epg (range)	120 (48 – 168)
Any STH	
Prevalence – uncorrected (95% CI)	48.4 (36.2, 60.9)

STH = soil-transmitted helminth; epg = eggs per gram (of feces);
CI = confidence interval; BCI = Bayesian credible interval
*Corrected prevalences performed using Bayesian estimation of disease prevalence in the absence of a gold standard. A uniform density over the range [0,1] (α=1, β=1) was used for the prior distribution for the prevalence of soil-transmitted helminthiasis; Informative information on test parameters, including α and β values for prior distributions, are taken from Tarafder et al. 2010, and are based on published literature and expert opinion.

Table 3: Effect of maternal postpartum deworming on infant anthropometric outcomes over their first 6 months of life (N=1010*), Iquitos, Peru, starting from the first day of recruitment in February 2014 to the last day of the 6-month follow-up in February 2015.

Outcome	**Albendazole**	**Placebo**
Mean weight gain (kg), 0 – 6 mo	4.3 ±0.04	4.4 ±0.04
Unadjusted difference (95% CI)	-0.02 (-0.1, 0.08)	*reference*
p value	0.675	
Adjusted** difference (95 % CI)	-0.01 (-0.1, 0.09)	*reference*
p value	0.809	
Mean length gain (cm), 0 – 6 mo	16.1 ±0.08	16.0 ±0.09
Unadjusted difference (95% CI)	0.08 (-0.2, 0.3)	*reference*
p value	0.539	
Adjusted** difference (95 % CI)	0.1 (-0.1, 0.3)	*reference*
p value	0.418	
Mean head circumference gain (cm), 0 – 6 mo	8.5 ±0.05	8.5 ±0.05
Unadjusted difference (95% CI)	0.04 (-0.1, 0.2)	*reference*
p value	0.612	
Adjusted** difference (95 % CI)	0.04 (-0.09, 0.2)	*reference*
p value	0.520	
WAZ, 6 mo	-0.2 ±0.05	-0.2 ±0.04
Unadjusted difference (95% CI)	-0.04 (-0.2, 0.09)	*reference*
p value	0.551	
Adjusted** difference (95 % CI)	-0.05 (-0.2, 0.08)	*reference*
p value	0.457	

LAZ, 6 mo	-1.0 ±0.04	-1.0 ±0.04
Unadjusted difference (95% CI)	0.03 (-0.08, 0.2)	*reference*
p value	0.555	
Adjusted** difference (95 % CI)	0.01 (-0.1, 0.1)	*reference*
p value	0.827	
HCAZ, 6 mo	-0.6 ±0.04	-0.6 ±0.04
Unadjusted difference (95% CI)	-0.02 (-0.1, 0.09)	*reference*
p value	0.731	
Adjusted** difference (95 % CI)	-0.05 (-0.2, 0.06)	*reference*
p value	0.393	
ACAZ, 6 mo	0.1 ±0.04	0.1 ±0.04
Unadjusted difference (95% CI)	0.004 (-0.1, 0.1)	*reference*
p value	0.945	
Adjusted** difference (95 % CI)	-0.006 (-0.1, 0.09)	*reference*
p value	0.910	

WAZ = weight-for-age; LAZ = length-for-age; HCAZ = head circumference-for-age; ACAZ = mid-upper arm circumference-for-age; CI = confidence interval

*Intention-to-treat analysis includes data from 972 infants for whom anthropometric outcomes were available, and 38 infants who were lost to follow-up and whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, socioeconomic index, infant sex, and gestational age

Table 4: Effect of maternal postpartum deworming on prevalence of infant underweight and stunting at 6 months of age (N=1010*), Iquitos, Peru, starting from the first day of the 6-month visit in August 2014 to the last day of the 6-month visit in February 2015.

Outcome	**Albendazole**	**Placebo**
Prevalence underweight (95% CI)	3.9 (2.2, 5.6)	3.4 (1.8, 5.0)
Unadjusted RR (95% CI)	1.1 (0.6, 2.2)	*reference*
p value	0.691	
Adjusted** RR (95 % CI)	1.2 (0.6, 2.3)	*reference*
p value	0.574	
Prevalence stunted (95% CI)	12.8 (9.8, 15.7)	14.2 (11.1, 17.3)
Unadjusted RR (95% CI)	0.9 (0.7, 1.2)	*reference*
p value	0.496	
Adjusted** RR (95 % CI)	0.9 (0.7, 1.3)	*reference*
p value	0.668	

RR = risk ratio; CI = confidence interval

*Intention-to-treat analysis includes data from 972 infants for whom anthropometric outcomes were available, and 38 infants who were lost to follow-up and whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, socioeconomic index, infant sex, and gestational age

Table 5: Comparison of daily of infant weight and length gains between intervention groups (N=1010*), Iquitos, Peru, starting from the first day of recruitment in February 2014 to the last day of the 6-month follow-up in February 2015.

	Albendazole Mean ±SD	Placebo Mean ±SD	Unadjusted difference (95% CI)	Adjusted** difference (95% CI)
Weight gain (g/d)				
0 – 1 mo	30.1 ±0.6	30.7 ±0.5	-0.6 (-2.1, 0.9)	-0.7 (-2.1, 0.8)
>1 – 6 mo	21.8 ±0.2	21.8 ±0.2	-0.08 (-0.7, 0.5)	0.02 (-0.6, 0.6)
Length gain (cm/d)				
0 – 1 mo	0.1 ±0.002	0.1 ±0.002	-0.00006 (-0.005, 0.005)	0.0005 (-0.005, 0.006)
>1 – 6 mo	0.08 ±0.0005	0.08 ±0.0005	0.0003 (-0.001, 0.002)	0.0004 (-0.0009, 0.002)

SD = standard deviation; CI = confidence interval

*Intention-to-treat analysis includes data from 999 and 972 infants at 1 and 6 months, respectively for whom anthropometric outcomes were available, and 11 and 38 infants at 1 and 6 months, respectively who were lost to follow-up and whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, socioeconomic index, infant sex, and gestational age

Table 6: Effect of deworming on infant morbidity indicators at 6 months of age (N=1010*), Iquitos, Peru, starting from the first day of recruitment in February 2014 to the last day of the 6-month follow-up in February 2015.

Outcome	**Albendazole**	**Placebo**
Hospitalizations % (95% CI)	6.8 (4.6, 9.0)	5.5 (3.5, 7.6)
Unadjusted RR (95% CI)	1.2 (0.7, 2.0)	*reference*
p value	0.423	
Adjusted** RR (95 % CI)	1.3 (0.8, 2.2)	*reference*
p value	0.291	
Diarrhea % (95% CI)	8.8 (6.3, 11.4)	9.1 (6.5, 11.7)
Unadjusted RR (95% CI)	1.0 (0.6, 1.4)	*reference*
p value	0.859	
Adjusted** RR (95 % CI)	1.0 (0.6, 1.4)	*reference*
p value	0.824	
Cough % (95% CI)	14.1 (11.1, 17.2)	13.3 (10.2, 16.4)
Unadjusted RR (95% CI)	1.1 (0.8, 1.5)	*reference*
p value	0.687	
Adjusted** RR (95 % CI)	1.0 (0.8, 1.4)	*reference*
p value	0.783	
Fever % (95% CI)	28.9 (24.9, 32.9)	25.8 (21.8, 29.7)
Unadjusted RR (95% CI)	1.1 (0.9, 1.4)	*reference*
p value	0.270	
Adjusted** RR (95 % CI)	1.1 (0.9, 1.4)	*reference*
p value	0.197	

RR = risk ratio; CI = confidence interval

*Intention-to-treat analysis includes data from 972 infants for whom anthropometric outcomes were available, and 38 infants who were lost to follow-up and whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, socioeconomic index, infant sex, and gestational age

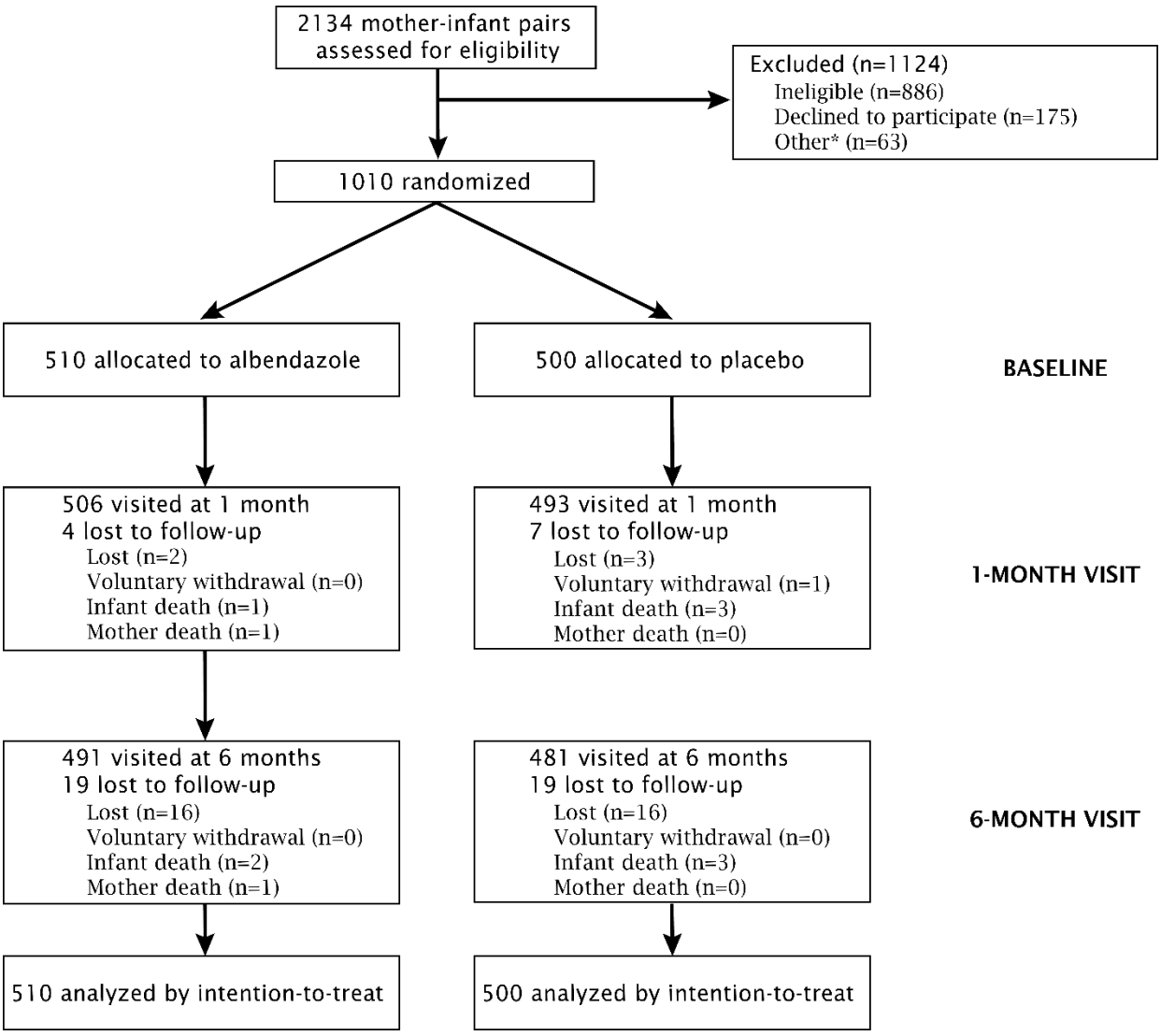

Figure 1: Trial flow diagram

*63 women were screened but were not enrolled because the sample size of the trial was already met.

CHAPTER 6: RESULTS – MATERNAL HEALTH OUTCOMES

6.1 Preamble to Manuscript 3

In the literature, anemia and fatigue are described as important consequences of STH infection, particularly in areas where the prevalence of hookworm infection is high. In WRA, and especially after childbirth, these consequences can have profound effects on maternal and child well-being. Anemia and fatigue in the postpartum period may also affect breastfeeding practices, maternal-infant bonding and child developmental trajectories in the long-term. For these reasons, the effectiveness of maternal postpartum deworming on the prevalence and intensity of STH infections, anemia, and self-reported fatigue was evaluated over the first 6 months of lactation, when exclusive breastfeeding is recommended.

The previous manuscript (Manuscript 2) provided a detailed description of trial procedures, including recruitment, randomization, treatment allocation, and outcome ascertainment. It also provided results for the primary trial outcome of mean infant weight gain between birth and 6 months of age, along with other infant growth indices and indicators of infant morbidity. The manuscript described in this chapter (Manuscript 3) comprises the second results chapter of the thesis. It focuses on the effect of maternal postpartum deworming on maternal health outcomes, including intestinal worm infections, anemia, and fatigue.

Manuscript 3 follows the CONSORT 2010 guidelines for the reporting of parallel RCTs. It has been prepared according to the specifications of the *American Journal of Tropical Medicine and Hygiene*. Preliminary results were presented at the Maternal and Neonatal Health Beyond 2015 Colloquium (Rabat, Morocco, November 2015). In addition, select aspects of this manuscript, including the selection of the validated instrument to measure fatigue, were also presented at the Canadian Conference on Global Health (Montreal, Quebec, Canada, November 2015).

6.2 Manuscript 3: The effect of maternal postpartum deworming on maternal health outcomes in Iquitos, Peru: a randomized-controlled trial

Running title: Postpartum deworming and maternal health

Authors and affiliations: Layla S. Mofid[1,2], Martín Casapía[3], Antonio Montresor[4], Elham Rahme[2,5], Brittany Blouin[1,2], Hugo Razuri[2], Lidsky Pezo[3], Theresa W. Gyorkos[1,2]*

[1] McGill University, Department of Epidemiology, Biostatistics and Occupational Health, Montréal, Québec, Canada

[2] Research Institute of the McGill University Health Centre, Division of Clinical Epidemiology, Montréal, Québec, Canada

[3] Asociación Civil Selva Amazónica, Iquitos, Peru

[4] Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

[5] Department of Medicine, McGill University, Montréal, Québec, Canada

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*Corresponding author: Dr. Theresa W. Gyorkos, Division of Clinical Epidemiology, Research Institute of the McGill University Health Centre, Royal Victoria Hospital, V Building, 687 Pine Avenue West, Montréal, QC, Canada, H3A 1A1; Telephone: 514-934-1934 x 44721
Email: theresa.gyorkos@mcgill.ca

ABSTRACT

Anemia and fatigue are common consequences of infection caused by intestinal parasites in women of reproductive age living in parasite-endemic areas. No previous studies have determined the role of intestinal parasite infections on symptoms of fatigue using standardized scales. This study aims to determine the effectiveness of deworming on the prevalence and intensity of intestinal parasites, anemia, and fatigue among women up to 6 months postpartum. A randomized controlled trial was conducted in Iquitos, Peru in 1010 mother-infant pairs. Following delivery, women were randomly allocated to receive single-dose deworming (albendazole) or matching placebo. At 6 months postpartum, mothers provided stool specimens for detection of intestinal parasite infection, and finger-prick blood samples for assessment of blood hemoglobin concentration. The Fatigue Assessment Scale was used to ascertain the self-reported presence of physical and cognitive symptoms of fatigue. A total of 970 (96.0%) participants attended their 6-month follow-up visit. The risk of parasite infection at 6 months postpartum was significantly lower in the group who received albendazole compared to placebo (RR: 0.5; 95% CI: 0.4, 0.6). There was no statistically significant benefit of deworming on maternal anemia (48.1% *vs.* 48.6%) or elevated fatigue (61.3% *vs.* 64.1%). In the present study population where baseline intestinal parasite infection and intensity were low, deworming was highly effective at reducing the burden of infection but no overall benefit on maternal anemia or fatigue was detected. Additional nutritional interventions, such as postpartum micronutrient supplementation, should be considered to improve maternal health and support mothers' capacity for infant caregiving.

INTRODUCTION

Soil-transmitted helminth infections (STHs; i.e., infections of *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms, *Necator americanus* and *Ancylostoma duodenale*) are considered the most prevalent infections among all neglected tropical diseases, affecting more than 1 billion people globally.[1] Infections are most common in the tropics and subtropics in conditions where poverty, malnutrition, and co-infections with other microorganisms proliferate. STHs cause immense disease burden, surpassing that produced by all other neglected tropical diseases.[2] Serious consequences of infection include nutritional impairment, chronic blood loss, and iron deficiency anemia.[3] Lethargy, listlessness, and lack of energy have also been reported in individuals infected with STHs and are likely sequelae to anemia.[4]

Postpartum anemia affects as many as 80% of women in low-and-middle income countries (LMICs)[5] because of excessive bleeding during childbirth, pre-existing conditions present during pregnancy, and underlying poor nutritional status.[6] The presence of anemia in women following delivery has been linked to depression, stress, cognitive impairment, and lack of energy.[7] Fatigue is considered one of the principal symptoms of anemia.[8] New mothers are at increased risk of fatigue due to the physical and emotional demands of childbirth, breastfeeding, and adapting to new parenting roles.[9, 10] Reduced productivity, well-being, and overall quality-of-life are serious consequences of postpartum fatigue.[11] In lactating women, fatigue has the potential to negatively impact children's course of development though altered maternal mood,[10] irritability, depression,[7, 12] and early termination of breastfeeding.[13] Maternal nutritional status, including infection, may exacerbate fatigue levels during this transitional period.

Fatigue is defined as a persistent feeling of physical and mental exhaustion that decreases capacity for daily activities.[14] It is commonly reported following infection with viral, bacterial, and parasitic pathogens.[15] The occurrence of chronic fatigue syndrome has been characterized in individuals with Epstein Barr virus infection, Ross River virus infection, viral meningitis, Dengue fever, brucellosis, Lyme disease, Q-fever, and giardiasis.[16] While fatigue is often described as a symptom of STH infection, little research has been carried out on this topic. Only one cross-sectional study in Malaysia has been conducted and it found that STH-infected children were more likely to complain of tiredness compared to uninfected children (36% *vs.*

12%, $p = 0.019$).[17] No intervention studies have evaluated the impact of deworming on the reduction of fatigue in any population, including that of lactating women.

Given the importance of maternal well-being on child health and development trajectories, it is important to quantify the extent to which STH infections and deworming affect anemia and fatigue, especially in the postpartum period. The aim of this study was to investigate the benefit of maternal postpartum deworming on STH infection, anemia, and self-reported fatigue over the first 6 months of lactation, when exclusive breastfeeding is recommended.

MATERIALS AND METHODS

Study population and setting

A detailed description of the trial methods is available elsewhere.[18] Briefly, a single-centre, double-blind, randomized controlled trial (RCT) was conducted in Iquitos, Peru. Participants were mother-infant pairs who were enrolled following delivery at Hospital Iquitos "César Garayar García". This hospital site was chosen due to its high rate of births, and because its catchment area includes individuals living in areas of high reported STH endemicity.[19]

Recruitment

Recruitment took place using a two-stage process. In the first stage, women were visited in their homes by research assistants during their third trimester of pregnancy and screened for eligibility. Women were considered eligible to participate if they planned to deliver at Hospital Iquitos "César Garayar García"; and intended to remain in the study area for a period of 24 months. Women meeting eligibility criteria were invited to participate in the trial, and informed consent was sought from both women and their partners.

In stage two, women presenting for delivery at Hospital Iquitos "César Garayar García" were re-screened for eligibility. Mother-infant pairs were considered eligible based on the following inclusion criteria: a) delivery at Hospital Iquitos "César Garayar García"; and b) planned residence in the study area for a period of 24-months. Mother-infant pairs were considered ineligible based on the following exclusion criteria: a) stillborn; b) infant with serious congenital

abnormalities or serious medical condition; c) infant with gestational age < 32 weeks; d) infant with Apgar score at 5 minutes < 4; e) multiple birth; f) mother or baby transferred to another hospital or hospitalized for > 3 days following delivery; g) mother unable to communicate in Spanish. A baseline questionnaire was administered to participants to collect information on sociodemographic characteristics and obstetric history. Birth information, including gestational age, Apgar score, and birth time, was obtained from medical charts.

Intervention groups and treatment allocation

After delivery and prior to hospital discharge, participating women were visited at bedside by research assistants and randomly allocated to one of two intervention groups, experimental or control, based on the randomization schedule. The experimental group received a single-dose 400 mg albendazole (i.e., deworming) tablet. The control group received a matching placebo tablet. The placebo tablet was identical to the albendazole tablet in terms of shape, colour, markings and taste, and was manufactured by Laboratorios Hersil (Lima, Peru). Tablets were directly observed to be swallowed by research personnel. Routine postpartum care for mothers and infants was provided by hospital personnel according to Peruvian Ministry of Health standards.[20, 21]

Sample size and power

This trial was designed with adequate power to determine whether maternal postpartum deworming could improve mean infant weight gain over the first 6 months of life (i.e., the primary outcome).[18] It was calculated that 1010 mother-infant pairs were needed to detect a minimum clinically significant difference of 0.20 kg between intervention groups in mean infant weight gain between birth and 6 months of age. The estimated sample size took into account a power of 80%, a significance level of 5%, an attrition rate of 20%, and assumed a standard deviation of 1.01 kg. With this sample size, the trial had 80% power to detect a minimum difference of 1.50 g/L of hemoglobin (Hb) between groups (assuming a standard deviation of 12.0 g/L)[22] and a minimum difference in the fatigue score of 1.10 points (assuming a standard deviation of 8.4 points).[23] These differences are smaller than those considered clinically relevant from the literature.[22, 23] Sample size and power calculations were conducted using PS Power and Sample Size Calculations Version 3.0 (Copyright © 1997 by Dupont and Plummet).

Randomization and blinding

A biostatistician not otherwise involved in the trial generated a computer-based randomization schedule using simple randomization and a 1:1 allocation ratio. Tablets were prepared in identical opaque envelopes and sequentially-numbered by two independent pharmacists. The sealed envelopes were stored in a temperature-regulated pharmacy in the local research facility. All research personnel responsible for the design, implementation and data analysis of the trial, as well as study participants, were blinded to group assignment.

Measurement of outcomes

Mother-infant pairs were visited by research assistants in their homes at 1 and 6 months following delivery. Infant outcomes, including the trial's primary outcome of infant weight gain between birth and 6 months of follow-up, are reported elsewhere.[24] Maternal outcomes, including STH infection, Hb concentration, anemia, and fatigue, are reported here.

Stool examination

Stool specimens were collected from mothers at 6 months postpartum for the determination of STH prevalence and intensity. Specimens were transferred to the laboratory of the local research facility and processed within 24 hours of collection using the Kato-Katz technique. Microscope slides were quantitatively examined for helminth ova by trained technologists. A random subsample of 10% of slides were re-examined by a second trained technologist for quality assurance.

Hemoglobin

At 6 months postpartum, capillary blood samples were obtained from a finger prick using sterile lancets. The concentration of Hb (g/L) was determined using a portable HemoCue® Hb 201 + System (Hemocue, Inc, Ängelholm, Sweden).

Fatigue

Fatigue was measured at the 6-month follow-up visit using the validated 10-item Fatigue Assessment Scale.[25] The questionnaire was translated into Spanish and pilot-tested prior to implementation, at which time adjustments to language were made as necessary. Trained

research assistants administered the questionnaire to participants and a visual representation of the scale was displayed to help participants in responding. To each of the 10 items of the FAS, participants answered how they generally felt using a 5-point Likert-type scale (i.e., 1 = "never," 2 = "sometimes," 3 = "regularly", 4 = "often", 5 = "always"). Responses to items 4 and 10 were reverse coded. A total score was calculated by summing the responses of all items. High scores are indicative of higher levels of fatigue.

The FAS is a rigorous instrument that was originally developed to assess mental and physical fatigue symptoms in the Dutch general population.[26] The scale has been shown to have robust psychometric properties and sufficient ease of use,[27] including high internal consistency (i.e., 0.90), test-retest reliability (i.e., 0.89), discriminant validity with depression and emotional stability, convergent validity (i.e., 0.60 to 0.78) with other established measures of fatigue, and strong evidence of unidimensionality.[25, 28] It has been used to measure chronic fatigue in individuals with a variety of conditions, including sarcoidosis,[28] cancer,[29] arthritis,[30] fibromyalgia,[31] and inflammation.[32] Its use has also been extended for identifying parental fatigue in both men and women.[33]

Data management and analyses

All data collected during study visits were recorded in real-time on mobile tablets using a data collection application. The application was implemented to improve data quality by minimizing missing data and incoherent responses. The effect of maternal postpartum deworming on fatigue was first examined using an intention-to-treat (ITT) approach, such that participants were analyzed according to their assigned intervention group. Missing outcome data were imputed using Multiple Imputation by Chained Equations (MICE) with 20 imputations. Variables related to the outcome, and/or related to loss to follow-up were used to impute missing outcome variables, and included baseline values of district of residence, maternal age, marital status, education, number of individuals residing in the household, socioeconomic status, and intervention group.

The prevalence of STH infection was reported separately by STH species, as well as in terms of infection with any one of the STH species, and as co-infection with *T. trichiura* and hookworm.

Intensity of infection, based on parasite-specific egg counts, was categorized according to the World Health Organization (WHO) cut-offs,[34] and reported as the prevalence of moderate-and-heavy infection for each STH species. Anemia was defined as a Hb concentration below 120 g/L.[35] Cut-offs for mild, moderate, and severe anemia were defined as Hb concentrations 110-119 g/L, 80-109 g/L, and < 80 g/L, respectively. Since studies examining postpartum fatigue have implemented a modified 5-item version of the FAS,[11, 36, 37] the current trial reports both the total score and the modified score for comprehensiveness and comparability. The total FAS score was also dichotomized into those without fatigue (FAS score < 22) and those with elevated fatigue (FAS score ≥ 22)[38] for further analyses.

Continuous variables (i.e., mean Hb concentration and FAS score) were compared between intervention groups using separate linear regression models. Dichotomous variables (i.e., the risk of STH infection and moderate-and-heavy infection, anemia, and elevated fatigue) were compared between intervention groups using separate log-binomial regression models. In the case where log-binomial regression models could not converge, Poisson regression with a robust variance estimator was performed. Covariables in adjusted analyses were determined by a review of the literature and expert opinion, and included maternal age, education, district of residence, number of living people residing in household, and socioeconomic status (i.e., based on a composite asset-based index[24, 39, 40]).

The following sensitivity analyses were also carried out: a) a complete-case approach, restricted to those participants who had complete baseline and 6-month data; and b) a per-protocol approach including those who had complete outcome data and did not report taking deworming outside of the trial protocol at any time.

Ethics approval and trial monitoring

Written informed consent was obtained from all women and their spouses/partners for the participation of the mother and infant in the study. The trial protocol was approved by regulatory bodies in Peru (Asociación Civil Impacta Salud y Educación and the Instituto Nacional de Salud) and Canada (McGill University Health Centre). Additional approval was granted by the Regional Ministry of Health (Dirección Regional de Salud (DIRESA)) of Loreto, and the Hospital Iquitos

"César Garayar García". An external Data Safety and Monitoring Committee was formed to monitor adverse events and the progress of the trial.

RESULTS

Trial profile and baseline characteristics

The trial profile outlining participant flow throughout the study is shown in Figure 1. Details on participant enrolment are described elsewhere.[24] Briefly, a total of 2134 mother-infant pairs were screened for eligibility, of which 886 were deemed ineligible, 175 declined to participate and 63 were screened but did not give birth before the sample size was reached. Enrolment of the required sample size of 1010 mother-infant pairs took place between February and August 2014. All mothers received their randomly assigned intervention at baseline according to the randomization schedule. Overall, baseline characteristics were similar between intervention groups.[24]

Follow-up

A total of 970 mothers (96.0%) were visited in their homes at the 6-months postpartum. Among those visited at 6 months, there were no missing data for maternal Hb or FAS items.

Soil-transmitted helminthiasis

At the 6-month follow-up visit, 962 participants (95.2%) provided a stool specimen that was analyzed immediately using the Kato-Katz technique. In ITT analysis, the risk of any STH infection was significantly lower in the experimental group (RR: 0.5; 95% CI: 0.4, 0.6), as was the risk of species-specific infection with *A. lumbricoides* (RR: 0.4; 95% CI: 0.3, 0.5), *T. trichiura* (RR: 0.5; 95% CI: 0.4, 0.6), and hookworm (RR: 0.1; 95% CI: 0.06, 0.3) (Table 1). Similarly, the risk of co-infection with *T. trichiura* and hookworm was significantly lower in the experimental group (RR: 0.2; 95% CI: 0.05, 0.4) compared to the control group. The deworming intervention was also effective in reducing the proportion of participants with moderate-and-heavy infection with *A. lumbricoides* (RR: 0.2; 95% CI: 0.08, 0.3), *T. trichiura* (RR: 0.3; 95% CI: 0.2, 0.5), and hookworm (RR: 0.2; 95% CI: 0.1, 0.4) (Table 2).

Hemoglobin and anemia

At 6 months postpartum, the mean concentration of Hb did not differ between intervention groups (mean difference: 1.1; 95% CI: -0.4, 2.7) (Table 3). Nearly half of participants (i.e., 48%) were classified as anemic, with mild-to-moderate anemia being the most prevalent. The single deworming intervention had no effect on the prevalence of overall anemia (RR: 1.0; 95% CI: 0.9, 1.1), nor the prevalences of mild, moderate, or severe anemia.

Fatigue

The mean total fatigue score was similar between intervention groups (23.5 ±0.2 *vs.* 23.9 ±0.3), as was the proportion of mothers considered fatigued (61.3% *vs.* 64.1%) (Table 4) at 6 months postpartum. Additionally, the mean modified FAS score did not differ between intervention groups (11.8 ±0.2 *vs.* 12.0 ±0.2).

Results were consistent in adjusted analyses, as well as complete-case and per-protocol analyses.

Serious adverse events

Between enrolment and 6 months of follow-up, a total of 13 serious adverse events (SAEs) were reported in mothers. The SAE profile was similar between intervention groups (6 in experimental group *vs.* 7 in control group). One maternal death was reported and it occurred in the experimental group. None of the SAEs were deemed to be associated with the deworming intervention.

DISCUSSION

This is the first RCT to examine the effectiveness of maternal postpartum deworming on anemia and symptoms of fatigue. WHO recommends that, in areas endemic for STH, women of reproductive age, including pregnant (after the first trimester) and lactating women, be included in routine deworming programs because of their increased vulnerability to the morbidity associated with STH infection, especially blood loss and iron deficiency anemia.[41] Albendazole was chosen as the anthelmintic for the deworming intervention in the current trial because of its higher efficacy against hookworm infection, which is often more prevalent in adult populations.

As expected, the deworming intervention was highly effective at reducing the prevalence and intensity of STH infection at 6 months postpartum. These results are consistent with a meta-analysis on reinfection with STHs which found that the risk of *A. lumbricoides* (RR: 0.7; 95% CI: 0.6, 0.8), *T. trichiura* (RR: 0.5; 95% CI: 0.4, 0.7), and hookworm (RR: 0.6; 95% CI: 0.3, 0.9) was lower in those who were administered anthelmintics 6 months prior.[42] Findings in the present study suggest that while reinfection may have occurred within 6 months of parasite clearance, groups were still significantly different in terms of the burden of STHs.

Overall, this trial was unable to demonstrate a benefit of one-time deworming on mean maternal Hb levels, or the prevalence of anemia at 6 months postpartum. These findings are in agreement with a recent Cochrane review that found that administration of single-dose deworming during the second trimester of pregnancy had no effect on the risk of anemia in the third trimester (RR: 0.9; 95% CI: 0.8, 1.1).[43] A meta-analysis in non-pregnant populations demonstrated that, among RCTs, treatment with albendazole corresponded to a 1.89 g/L increase (95% CI: 0.13, 3.63) in mean Hb.[44] The same review found that deworming treatment with any benzimidazole in isolation had little to no impact on the risk of mild (RR: 0.98; 95% CI: 0.89, 1.06) and moderate anemia (RR 0.87; 95% CI: 0.59, 1.15). In LMICs where STH infections are endemic, the causes of anemia are multi-factorial, ranging from diet and infection to genetic disorders.[45] In areas of concurrent poverty where diets are low in bioavailable iron, deworming alone may not be enough to replenish iron stores without micronutrient supplementation, including iron. While over 90% of women in our study population reported taking iron supplementation (i.e., iron folic-acid) during pregnancy, this proportion dropped to 55% at 1 month following delivery and 3% at 6 months postpartum. Therefore, deworming may need to be coupled with other cost-effective interventions, such as iron supplementation with high tolerability, to observe improvements in maternal anemia.

In the current trial, mean fatigue scores and the risk of elevated fatigue were found to be similar between intervention groups. The prevalence of elevated fatigue in our trial population (i.e., 62.6%) was higher than that reported in Europe among breast cancer or colorectal cancer patients (i.e., 32-41%),[46, 47] but lower than patients with sarcoidosis (i.e., 73-79%).[38, 48, 49] Studies in postpartum women in Australia have reported mean modified FAS scores of 12-14 points[11, 35, 36]

which are similar to those reported in the current trial (i.e., 11.9 ±0.1). Results of the current study indicate that while fatigue is a common complaint in women during the postpartum period, single-dose deworming may not be sufficient to improve symptoms, especially because the prevalence of anemia is also high.

Overall strengths of the trial include its RCT study design, large sample size, and low rate of attrition. The implementation of a validated instrument to measure fatigue with good psychometric properties has benefited the study. All study instruments were rigorously pilot-tested for understandability and appropriateness within the LMIC context, and study visits were continually monitored to ensure a high degree of quality in outcome ascertainment. Results of this study are generalizable to other populations endemic for STH infection where antenatal and health-centre based delivery services are readily available.

The trial is limited by the short follow-up time. It may be that improvements to Hb levels and symptoms of fatigue take time to accrue, especially during the transitional time of early parenthood. Additionally, there was a lower than expected baseline prevalence of STH infection (i.e., 48.4%)[24] that may have contributed to the inability to detect a difference in anemia and fatigue outcomes between intervention groups. The study is also limited by the lack of baseline data for Hb concentrations and fatigue scores which did not allow for an assessment of changes within individual participants over time. Use of the FAS has previously been limited to populations in high-income countries where the social and demographic profile of respondents is vastly different from those in the current trial. Therefore, fatigue scores from the current study are likely not generalizable to those reported to date in other postpartum women.

Overall, maternal postpartum deworming, with a one-time administration of single-dose albendazole, was not sufficient to improve maternal Hb levels, the risk of anemia, and reported fatigue over an initial postpartum period of 6 months. However, the prevalence of STH infection was significantly reduced among those who received the deworming intervention. Improvements to maternal nutritional status may require coupling deworming with micronutrient supplementation that is culturally acceptable and well tolerated by the target population. As

follow-up for this trial is still ongoing up to 24 months of age, future analyses will determine the long-term effects of deworming on maternal health outcomes.

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DISCLOSURES

None.

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Table 1: Effect of maternal postpartum deworming on soil-transmitted helminth prevalence at 6 months postpartum (N=1010*), Iquitos, Peru, starting from the first day of the 6-month visit in August 2014 to the last day of the 6-month visit in February 2015.

Outcome	**Albendazole**	**Placebo**
Prevalence		
A. lumbricoides % (95% CI)	9.9 (7.2, 12.5)	27.5 (23.5, 31.5)
Unadjusted RR (95% CI)	0.4 (0.3, 0.5)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.4 (0.3, 0.5)	*reference*
p value	<0.0001	
T. trichiura % (95% CI)	18.7 (15.3, 22.2)	36.2 (32.0, 40.5)
Unadjusted RR (95% CI)	0.5 (0.4, 0.6)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.5 (0.4, 0.7)	*reference*
p value	<0.0001	
Hookworm % (95% CI)	1.2 (0.2, 2.2)	9.1 (6.5, 11.7)
Unadjusted RR (95% CI)	0.1 (0.06, 0.3)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.1 (0.06, 0.3)	*reference*
p value	<0.0001	
Co-infection *T. trichiura*/hookworm % (95% CI)	0.8 (0.02, 1.6)	5.5 (3.5, 7.5)
Unadjusted RR (95% CI)	0.2 (0.05, 0.4)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.2 (0.06, 0.5)	*reference*
p value	0.001	

Any STH % (95% CI)	24.8 (21.0, 28.7)	50.2 (45.7, 54.7)
Unadjusted RR (95% CI)	0.5 (0.4, 0.6)	*reference*
p value	<0.0001	
Adjusted[†] RR (95 % CI)	0.5 (0.4, 0.6)	*reference*
p value	<0.0001	

STH = soil-transmitted helminth; RR = risk ratio; CI = confidence interval

*Intention-to-treat analysis includes data from 962 women for whom stool specimens were available, and 48 women whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, district of residence, number of people residing in the household, and socioeconomic index.

Table 2: Effect of maternal postpartum deworming on the proportion of participants having moderate-and-heavy intensity of soil-transmitted helminth infection at 6 months postpartum (N=1010*), Iquitos, Peru, starting from the first day of the 6-month visit in August 2014 to the last day of the 6-month visit in February 2015.

Outcome	**Albendazole**	**Placebo**
Proportion of trial population		
Moderate/Heavy *A. lumbricoides* % (95% CI)	1.9 (0.7, 3.2)	12.0 (9.1, 14.9)
Unadjusted RR (95% CI)	0.2 (0.08, 0.3)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.2 (0.09, 0.3)	*reference*
p value	<0.0001	
Moderate/Heavy *T. trichiura* % (95% CI)	7.1 (4.9, 9.4)	21.7 (18.0, 25.4)
Unadjusted RR (95% CI)	0.3 (0.2, 0.5)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.3 (0.2, 0.5)	*reference*
p value	<0.0001	
Moderate/Heavy Hookworm % (95% CI)	4.0 (2.3, 5.8)	17.7 (14.3, 21.1)
Unadjusted RR (95% CI)	0.2 (0.1, 0.4)	*reference*
p value	<0.0001	
Adjusted** RR (95 % CI)	0.2 (0.1, 0.4)	*reference*
p value	<0.0001	

RR = risk ratio; CI = confidence interval

*Intention-to-treat analysis includes data from 962 women for whom stool specimens were available, and 48 women whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, district of residence, number of people residing in the household, and socioeconomic index.

Table 3: Effect of maternal postpartum deworming on hemoglobin and anemia at 6 months postpartum (N=1010*), Iquitos, Peru, starting from the first day of the 6-month visit in August 2014 to the last day of the 6-month visit in February 2015.

Outcome	**Albendazole**	**Placebo**
Mean hemoglobin g/L ±SD	119.9 ±0.6	118.8 ±0.6
Unadjusted difference (95% CI)	1.1 (-0.4, 2.7)	*reference*
p value	0.192	
Adjusted** difference (95 % CI)	1.1 (-0.6, 2.6)	*reference*
p value	0.221	
Anemia % (95% CI)	48.1 (43.6, 52.5)	48.6 (44.1, 53.1)
Unadjusted RR (95% CI)	1.0 (0.9, 1.1)	*reference*
p value	0.874	
Adjusted** RR (95 % CI)	1.0 (0.9, 1.1)	*reference*
p value	0.851	
Mild anemia % (95% CI)	27.2 (23.2, 31.1)	27.6 (23.6, 31.6)
Unadjusted RR (95% CI)	1.0 (0.8, 1.2)	*reference*
p value	0.887	
Adjusted** RR (95 % CI)	1.0 (0.8, 1.2)	*reference*
p value	0.911	
Moderate anemia % (95% CI)	20.2 (16.7, 23.8)	19.8 (16.2, 23.4)
Unadjusted RR (95% CI)	1.0 (0.8, 1.3)	*reference*
p value	0.859	
Adjusted** RR (95 % CI)	1.0 (0.8, 1.3)	*reference*
p value	0.857	
Severe anemia % (95% CI)	0.4 (0, 0.9)	1.0 (0.1, 1.9)
Unadjusted RR (95% CI)	0.4 (0.08, 2.0)	*reference*
p value	0.262	
Adjusted** RR (95 % CI)	0.4 (0.08, 2.1)	*reference*
p value	0.275	

SD = standard deviation; RR=risk ratio; CI = confidence interval

*Intention-to-treat analysis includes data from 970 women for whom hemoglobin outcomes were available, and 40 women who were lost to follow-up and whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, district of residence, number of people residing in the household, and socioeconomic index.

Table 4: Effect of maternal postpartum deworming on total fatigue score and the prevalence of fatigue at 6 months postpartum (N=1010*), Iquitos, Peru, starting from the first day of the 6-month visit in August 2014 to the last day of the 6-month visit in February 2015.

Outcome	**Albendazole**	**Placebo**
Mean fatigue score ±SD	23.5 ±0.2	23.9 ±0.3
Unadjusted difference (95% CI)	-0.4 (-1.1, 0.3)	*reference*
p value	0.277	
Adjusted** difference (95 % CI)	-0.3 (-1.0, 0.4)	*reference*
p value	0.415	
Fatigue % (95% CI)	61.3 (57.0, 65.6)	64.1 (59.8, 68.3)
Unadjusted RR (95% CI)	1.0 (0.9, 1.1)	*reference*
p value	0.370	
Adjusted** RR (95 % CI)	1.0 (0.9, 1.0)	*reference*
p value	0.332	

SD = standard deviation; RR=risk ratio; CI = confidence interval

*Intention-to-treat analysis includes data from 970 women for whom fatigue outcomes were available, and 40 women who were lost to follow-up and whose outcome data were imputed using multiple imputation.

**Adjusted for maternal age, education, district of residence, number of people residing in the household, and socioeconomic index.

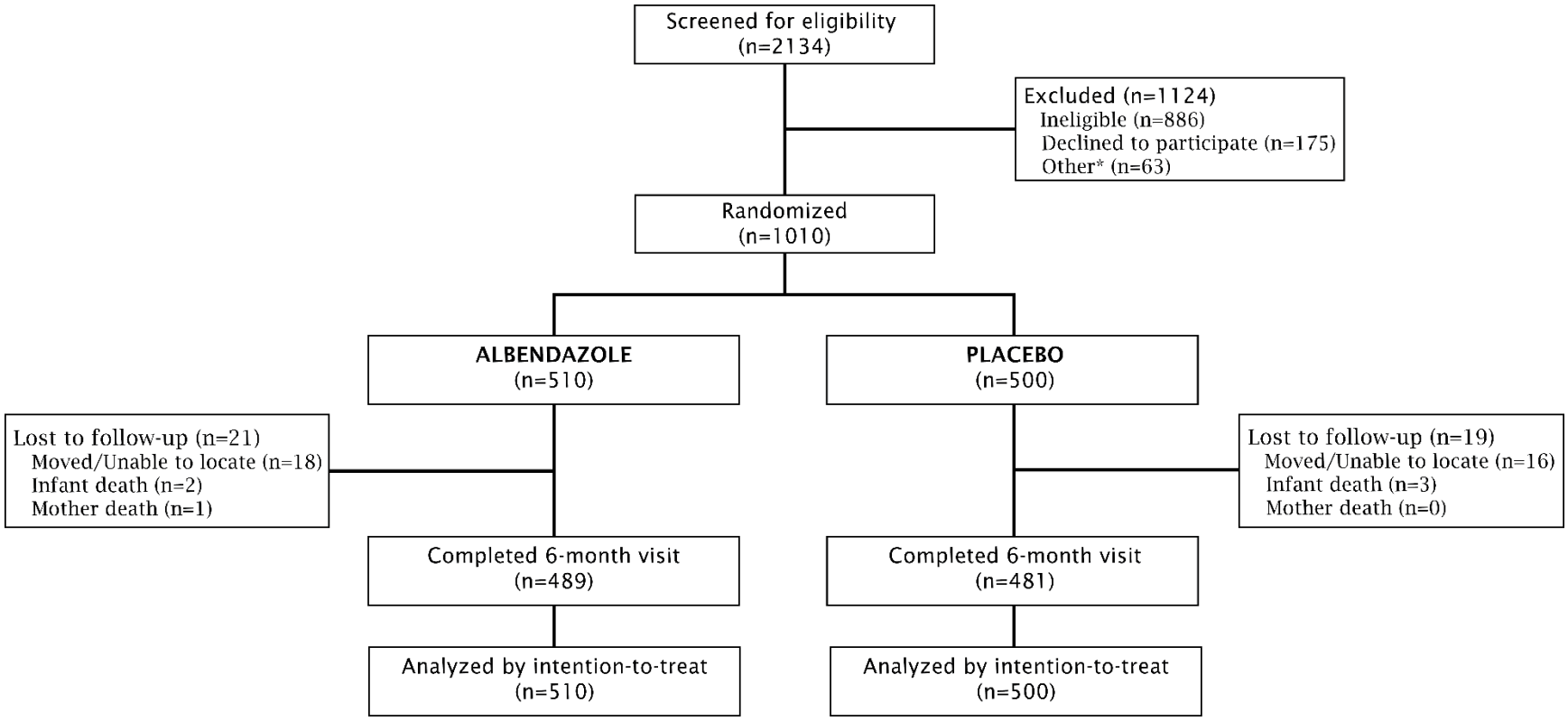

Figure 1: Flow of participants in the trial, from recruitment, randomization, follow-up through to data analysis.

*63 women were screened but were not enrolled because the sample size of the trial was already met

CHAPTER 7: RESULTS – BREAST MILK COMPOSITION

7.1 Preamble to Manuscript 4

The following manuscript (Manuscript 4) comprises the third and final chapter of results of this thesis. The manuscript presents the results of the effectiveness of maternal postpartum deworming on the secondary outcomes of breast milk concentrations of micronutrients.

The RCT on maternal postpartum deworming provided the source population for the substudy focusing on breast milk quality. The methods section of this manuscript is divided into two sections; procedures of the main trial and the substudy on the breast milk quality assessment. From among the 1010 trial mother-infant pairs enrolled into the trial, a random subsample of 200 mother-infant pairs, stratified by infant sex, were selected to participate in the substudy. Mature breast milk samples were collected from mothers at 1 and 6 months following delivery. As no study has previously assessed the effect of deworming on breast milk quality, a broad panel of micronutrients was measured. Due to the exploratory nature of this study, the type 1 error rate was not adjusted for multiple comparisons to avoid dismissal of potentially relevant findings (Perneger 1998; Rothman 1990).

Manuscript 4 conforms to the CONSORT 2010 guidelines for the reporting of parallel RCTs. The manuscript has been prepared according to the specifications of the *Journal of Nutrition*.

7.2 Manuscript 4: Effectiveness of maternal postpartum deworming on nutrient composition of breast milk

Running title: Deworming and breast milk composition

Authors and Affiliations: Layla S. Mofid, MSc[1,2], Martín Casapía, MD, MPH[3], Antonio Montresor, MD[4], Elham Rahme, PhD[2,5], Grace S. Marquis, PhD[6], Lindsay H. Allen, PhD[7], Setareh Shahab-Ferdows, PhD[7], Daniela Hampel, PhD[7], Brittany Blouin, MSc[1,2], Hugo Razuri, MD, MPH[2], Lidsky Pezo, MPH[3], Theresa W. Gyorkos, PhD[1,2]*

[1] McGill University, Department of Epidemiology, Biostatistics and Occupational Health, Montréal, Québec, Canada

[2] Research Institute of the McGill University Health Centre, Division of Clinical Epidemiology, Montréal, Québec, Canada

[3] Asociación Civil Selva Amazónica, Iquitos, Peru

[4] Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

[5] Department of Medicine, McGill University, Montréal, Québec, Canada

[6] McGill University, School of Dietetics and Human Nutrition, Sainte Anne-de-Bellevue, Québec, Canada

[7] USDA Western Human Nutrition Research Center, University of California, Davis, California, USA

*Corresponding author: Dr. Theresa W. Gyorkos, Division of Clinical Epidemiology, Research Institute of the McGill University Health Centre, Royal Victoria Hospital, V Building, 687 Pine Avenue West, Montréal, QC, Canada, H3A 1A1; Telephone: 514-934-1934 x 44721; Fax: 514-934-8293; Email: theresa.gyorkos@mcgill.ca

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Authors' last names: Mofid, Casapia, Montresor, Rahme, Marquis, Allen, Shahab-Ferdows, Hampel, Blouin, Razuri, Pezo, Gyorkos

ABSTRACT

Background. Soil-transmitted helminth infections are thought to contribute to poor nutritional status through a variety of mechanisms, including reduced food intake, and nutrient malabsorption, loss, and altered metabolism. Lactating women are considered a high risk group for infection-related morbidity because of their underlying poor nutritional status, among other risk factors. The inter-relationships among parasite infection, deworming, and breast milk quality has received little previous research attention.

Objective. The present study was conducted to determine the effectiveness of single-dose treatment with albendazole on indicators of breast milk quality, including vitamins and minerals.

Methods. A random sample of 200 mother-infant pairs participating in a randomized controlled trial on maternal postpartum deworming (single-dose albendazole *vs.* single-dose placebo) were selected to participate in the breast milk substudy at 1 and 6 mo postpartum. Casual breast milk samples were collected from women and analyzed for vitamins (i.e., A, B_1, B_2, B_3, B_6, B_{12}, E) and minerals (i.e., copper, iron, zinc).

Results. The concentrations of vitamins and minerals in breast milk were similar in the two intervention groups at both time points. Over 87% of the women had low breast milk vitamin A ($\leq$ 1.05 μmol/L), with no meaningful differences between groups. Median micronutrient values in breast milk in our study population were lower than those recommended for adequate intake in healthy infants.

Conclusion. Further research, ideally in areas of higher parasite endemicity, is needed to fully understand the effect of soil-transmitted helminth infection and the role of deworming on breast milk composition.

Trial registration. Clinicaltrials.gov NCT01748929

Keywords: lactation; vitamins; minerals; deworming; soil-transmitted helminth; Peru

INTRODUCTION

Breast milk is considered the optimal source of nutrients for promoting infant growth and development over the first 6 mo of life. The nutritional, immunologic, and antimicrobial properties of breast milk change according to the needs of the growing infant and offer protection against infections and chronic conditions during infancy and childhood (1). Exclusive breastfeeding until 6 mo of age has been associated with improved infant outcomes; namely, a lower risk of upper respiratory conditions (2), a lower risk of gastrointestinal infections (3), delayed onset of obesity (4), and even a lower risk of infant mortality (5). However, the degree of benefit conferred by breast milk depends on the nutritional status of the mother, the composition of her milk, and sufficient intake of breast milk by the infant (6).

The lactation period is a time of nutritional vulnerability for women, due to high physiological demands, depleted nutrient stores, and metabolic changes (7). The complex interplay among maternal diet, infection, and physiological stresses on maternal nutrition may influence the nutrient composition of breast milk. Human studies have evaluated the effect of maternal nutritional status on the quality of breast milk, but the role of infection on the nutritional content of human milk has been underexplored. The effect of parasitic infections, including the soil-transmitted helminths (STH), on breast milk quality requires investigation, since there is well-established evidence of STH-attributable effects on nutrient malabsorption, chronic blood loss, and iron deficiency anemia (8-10).

STH infections are endemic in at least 112 countries (11) affecting populations which are often burdened by poverty and concurrent malnutrition. Owing to the underlying vulnerability of lactating women, the World Health Organization (WHO) recommends their inclusion in large-scale deworming programs in areas where the prevalence of STH infection exceeds 20% (12). A critical research gap currently exists on the effects of maternal postpartum deworming on infant and maternal health outcomes, including lactation performance. It was hypothesized that deworming could have an effect on maternal appetite, nutrient absorption, and fat digestion, with subsequent effects on enhanced micronutrient content in breast milk. The objective of this study was to evaluate the effectiveness of maternal postpartum deworming on breast milk composition

over the first 6 mo postpartum in an area of the Peruvian Amazon that is known to be highly endemic for STH infection.

METHODS

Main trial

Study setting and population

The study design and procedures have been previously described in detail (13). Briefly, a double-blind, placebo-controlled randomized trial was carried out to assess the effectiveness of maternal postpartum deworming with single-dose (400 mg) albendazole on infant and maternal health outcomes. The trial took place in Iquitos, Peru. This city is situated in the Amazon Basin of northeastern Peru at the confluence of the Amazon, Nanay, and Itaya rivers. Among residents, carbohydrates are the main source of caloric intake, and diets are generally low in proteins from animal origin (14, 15). During pregnancy, iron-folic acid supplements (i.e., 60 mg Fe + 400 mcg folic acid) and calcium supplements (i.e., 2 g Ca) are provided to women by the Peruvian Ministry of Health (16).

Recruitment

Women in their third trimester of pregnancy were identified using rosters of antenatal care clinics in surrounding health centres. Pregnant women meeting the following inclusion criteria were invited to participate in the trial: a) presenting for delivery at the Hospital Iquitos "Cesar Garayar Garcia"; and b) planning to reside in the study area for the next 24 mo. Mother-infant pairs were excluded from participation if they met one or more of the following exclusion criteria: a) delivery of a stillborn infant; b) delivery of an infant with a serious congenital abnormality or serious medical condition; c) infant having a gestational age < 32 wk; d) infant having an Apgar score < 4 at 5 minutes; e) delivery of twins or multiples; f) mother or baby requiring transfer to another hospital prior to discharge, or mother or infant requiring hospitalization for > 3 d; and g) inability to communicate in Spanish.

Ethics approval and registration

The trial was approved by the Research Ethics Boards of the Asociación Civil Impacta Salud y Educación (Peru), the Instituto Nacional de Salud (Peru) and the McGill University Health Centre (Canada). Written informed consent was obtained from all women and from their spouses/partners prior to enrollment. The trial was monitored regularly by an external Data Safety and Monitoring Committee and is registered at Clinicaltrials.gov (NCT01748929).

Randomization and blinding

Assignment to intervention group was determined by a statistician not otherwise involved in the trial prior to trial onset using a computer-generated schedule according to simple randomization with a 1:1 allocation ratio. Sequentially-numbered opaque envelopes were packaged with the corresponding intervention tablet (i.e., a 400 mg single-dose albendazole tablet or identical placebo tablet) according to the randomization schedule by two external pharmacists. All envelopes were stored in a temperature and humidity-controlled pharmacy under lock-and-key. All research assistants, laboratory technologists, and local study coordinators who were responsible for trial design, recruitment, outcome assessment, and data analyses, as well as participants, were blinded to intervention group assignment.

Enrolment

Between February and August 2014, a total of 1010 pregnant women were enrolled into the trial at Hospital Iquitos "Cesar Garayar Garcia". Following delivery, and prior to hospital discharge, mothers were randomly assigned to receive the albendazole tablet or the placebo tablet, according to the randomization schedule. Research assistants visited mothers at bedside to administer the tablet in the next sequentially-numbered envelope, and directly observed its ingestion with water. All mothers and infants received standard postpartum care as per Peruvian Ministry of Health guidelines by hospital personnel. A baseline questionnaire was administered to mothers by research assistants in order to collect information on sociodemographic characteristics, use of deworming and iron supplementation during pregnancy, and obstetric history. Information on gestational age, Apgar score, and clinical history was obtained from hospital records.

Outcome ascertainment

Infant feeding

Breastfeeding practices were ascertained by administering a questionnaire to mothers at 1 and 6 mo postpartum. Questions regarding the duration of exclusive breastfeeding, timing of the first introduction of complementary foods, and frequency of food intake were adapted from the WHO indicators for assessing infant and young child feeding practices (17). Infants were considered exclusively breastfed if they ingested only breast milk, with the exception of vitamins and medications. Infants were considered predominantly breastfed if their main source of nourishment was breast milk, but they had also consumed nonfood–based liquids (i.e., water, juice, tea) (18).

Substudy

One of the secondary objectives of the trial was to determine the effectiveness of deworming on breast milk composition at 1 and 6 mo postpartum. During informed consent procedures, women and their partners were asked if they wished to participate in an additional assessment of breast milk quality. Of those who agreed to participate in the substudy, a random subsample of mother-infant pairs (n=200), stratified by infant sex, was selected to participate at two time points: 1 and 6 mo postpartum. The same mother-infant pairs who were included in the 1-mo assessment were expected to also participate in the 6-mo assessment. However, in the case that a mother-infant pair who had been included in the 1-mo assessment was lost-to-follow-up or could not provide a milk sample at 6 mo, another pair from the trial was randomly selected to participate in the 6-mo assessment only. Women with mastitis were not eligible to be included in the substudy. An equal distribution of participants between intervention groups was expected, and therefore stratification by intervention group was not conducted.

Breast milk sample collection

Casual breast milk samples were collected using a Medela Symphony electric breast pump (Medela AG, Baar, Switzerland). Every effort was made to collect samples between 6h00 and 11h00 am to minimize diurnal variations. Research assistants aided women in collecting approximately 50 mL of milk into presterilized leak-proof containers from the breast from which the infant had not last fed, irrespective of the time elapsed since the last breastfeeding episode.

Milk samples were immediately wrapped in aluminum foil and stored in a cooler on ice until being transferred to the laboratory. Samples were refrigerated at 4°C until processing.

Biochemical indicators

A wide array of micronutrients in breast milk were analyzed based on their relationship with nutritional status, their function in immunomodulating infections (19), and their hypothesized relationship with STH infections (8). Specifically, 13 micronutrients (retinol, β-carotene, α-tocopherol, γ -tocopherol, thiamin, riboflavin, flavin adenine dinucleotide (FAD), nicotinamide, pyridoxal, cobalamin, copper, zinc, iron) were analyzed in this substudy. Total vitamin B_2 was calculated as riboflavin + FAD × 0.479 (20).

Laboratory analyses

Upon arrival at the laboratory, all milk samples were homogenized and aliquoted into sterile cryogenic falcon tubes and stored at -80°C. Falcon tubes were wrapped in foil to ensure protection from light. Samples were shipped on dry ice to the Western Human Nutrition Research Center, California (USA) for storage at -70°C and subsequent micronutrient assessment. The methods of analysis used to measure micronutrients in breast milk are summarized in Table 1. Samples were analyzed in subdued lighting to prevent photo-oxidative damage and degradation. Retinol, β-carotene, α-tocopherol, and γ-tocopherol were analyzed using high performance liquid chromatography with diode array detection (HPLC-DAD). The concentrations of riboflavin, FAD, nicotinamide, and pyridoxal were measured by ultra-performance liquid-chromatography tandem mass spectrometry (UPLC-MS/MS), using methods and validation described elsewhere (21). Thiamin was measured using the thiochrome method by high performance liquid chromatography with fluorescence detection (HPLC-FL), as described previously (22). Analysis of vitamin B_{12} was carried out using the IMMULITE 1000 solid-phase, competitive chemiluminescent enzyme immunoassay (Siemens), using previously described and validated methods (23). The concentrations of copper, iron, and zinc were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using the Varian VISTA AX CCD. The concentration of milk fat (g/L) was estimated using the creamatocrit method (24). All samples were assayed blind to intervention group status.

Sample size and power

The sample size selected for the substudy (n=200) was based on the number of independent milk samples required to detect a difference of at least 0.3 µmol/L in breast milk retinol because it is the micronutrient in breast milk thought to be most likely affected by the deworming intervention (25). Using a two-sided *t*-test, an alpha value of 0.05, a power of 0.80, and assuming a common standard deviation of 1.00 µmol/L (26), a sample size of 175 was required. In order to account for attrition and the possibility that some mothers would experience insufficient milk supply at the 6-mo time point, another 25 samples were added for a total sample size of 200 participants. Sample size and power calculations were conducted using PS Power and Sample Size Calculations Version 3.0 (Copyright © 1997 by Dupont and Plummet).

Data management and statistical analysis

All data collected during home visits were recorded electronically using a data application on a mobile tablet, and reviewed daily for accuracy and completeness. Laboratory management of samples, including labeling, tracking, and storage was organized using the Laboratory Data Management System (Version 11.1.0.30, Frontier Science, Amherst, NY, USA).

Since the concentrations of vitamins and minerals are known to be positively skewed, the medians and interquartile ranges were compared between intervention groups among participants with complete outcome data for the substudy. Crude differences in medians were assessed using the non-parametric Wilcoxon rank-sum test. Skewed dependent variables were log-transformed to satisfy normality assumptions of regression models. Linear regression models were performed to compare the concentrations of micronutrients in breast milk between intervention groups according to the intention-to-treat (ITT) principle, such that participants were analyzed according to their assigned intervention group. Multiple Imputation by Chained Equations (MICE) with 20 imputations was used to impute breast milk micronutrient concentrations for mother-infant pairs who were selected to participate in the substudy but who did not have outcome data for either the 1 or the 6 mo visit. The MICE model included infant sex, gestational age, maternal age, marital status, education, number of people residing in the household, socioeconomic status, and intervention group. As one objective of the substudy was to assess the possibility of effect measure modification by infant sex, the initial MICE model also included an interaction term for

intervention group and infant sex. However, the interaction term was dropped from the final MICE model because there was no statistical evidence of interaction in analysis models.

Separate linear models were used to evaluate the effectiveness of maternal postpartum deworming on continuous concentrations of micronutrients in breast milk at 1 and 6 mo postpartum. For fat soluble vitamins (i.e., retinol, β-carotene, α-tocopherol, and γ –tocopherol) the effect of deworming was assessed on the concentrations per volume, and also the concentrations standardized by the lipid content (g/L) in each breast milk sample. According to established criteria by WHO, low breast milk vitamin A was defined as ≤ 1.05 μmol/L (27). The prevalence of low breast milk vitamin A concentrations (i.e., ≤ 1.05 μmol/L) was compared between intervention groups using log-binomial regression models. In the case where log-binomial regression models did not converge, a Poisson regression model with a robust variance estimator was used to estimate the risk ratio (RR). An asset-based proxy of socioeconomic status was calculated from the following baseline variables: ownership of a radio and television, type of cooking fuel, type of flooring, presence of water connection to the house, and presence of wired electricity in the house. The composite index was combined using weights generated by Principal Components Analysis and divided into quartiles for subsequent analyses (28-30). Models were adjusted for baseline confounders deemed important from the published literature and from expert opinion. Covariables included in models were: maternal age, education, socioeconomic index, and infant sex. The presence of effect measure modification by infant sex was assessed by including an interaction term for intervention group and infant sex in models with each micronutrient. Sensitivity analyses were employed to assess the robustness of study findings, and included: a) a complete-case analysis; b) a per-protocol analysis; and c) an analysis in which extreme outliers were deleted from models for the vitamin or mineral in question. Results are presented with 95% confidence intervals (CIs) and differences were considered statistically significant at $P < 0.05$.

Concentrations of micronutrients in breast milk samples were compared to daily adequate intakes (AI) of micronutrients for infants aged 0 to 6 mo (31). Daily micronutrient concentrations were estimated by multiplying the median concentration of each micronutrient by an average daily breast milk intake assumed for a healthy infant of 0.78 L/day (32). The proportion of median

concentrations meeting AIs was calculated by dividing the calculated median daily intake of each micronutrient by the concentration used to set the AI. All analyses were conducted using STATA/SE for Windows (version 4.0, Stata Corp., College Station, TX, USA).

RESULTS

Participant flow and baseline characteristics

Data collection for the substudy took place between April and December 2014. At enrolment, groups were similar in terms of baseline characteristics (Table 2). Some differences between groups were noted in the type of housing structure (i.e., dirt or wooden *vs.* concrete floor). Overall, mother-infant pairs selected to participate in the substudy were similar to those in the total trial population. However, those who participated in the substudy had more people residing in the household (mean difference: 0.4; 95% CI: 0.03, 0.8). The mean maternal age of substudy participants was 25.2 y ±7.2 y; 51.2% of the infants were male; and the mean gestational age was 38.7 wk ±0.9 wk.

Follow-up

Of the 215 mother-infant pairs who were selected to participate in the substudy, 185 (86.0%) participated in both the first and second assessments, 15 (7.0%) participated in the first assessment only, and 15 (7.0%) participated in the second assessment only (Figure 1). The main reasons why those who completed the first assessment could not complete the second assessment were: relocation to another city or temporarily away from the study area at the time of assessment (n=11), withdrawal for unknown reasons (n=3), and insufficient milk supply (n=1). On average, mothers who were lost-to-follow up after the 1-mo visit were more likely to be younger (RR: 0.8; 95% CI: 0.7, 0.9), and primiparous (RR: 3.2; 95% CI: 1.2, 8.3), compared to those who remained in the substudy. The mean period of time between baseline and the first milk quality assessment was 38.3 d (±1.6 d) and the mean period of time between baseline and the second assessment was 181.7 d (± 3.1 d). The median time of day for milk collection at the 1-mo assessment was 8h13 (range: 6h02, 10h59), and the median time of day for collection at the 6-mo assessment was 7h53 (range: 5h35, 14h22). At both time points, the majority of participants (i.e., > 85%) had last consumed food more than three hours prior to milk collection.

Infant feeding

Among those who participated in the substudy at 1 mo postpartum, 49.0% (95% CI: 42.1%, 56.0%) of mothers were exclusively breastfeeding, and 44.0% (95% CI: 37.2%, 51.0%) were predominantly breastfeeding their infants. By 6 mo postpartum, the proportion of mothers exclusively breastfeeding fell to 1.0% (95% CI: 0.2%, 4.0%), and the proportion predominantly breastfeeding fell to 6.5% (95% CI: 3.8%, 10.9%). The prevalence of exclusive and predominant breastfeeding did not differ between intervention groups (data not shown).

Breast milk composition

The distribution of micronutrient concentrations in breast milk is displayed in Table 3. The concentrations of micronutrients in breast milk were similar between intervention groups in crude and adjusted analyses (Tables 3 and 4). Similar results were observed when the concentration of fat-soluble vitamins was standardized by the lipid content in breast milk (data not shown). Results were consistent in complete-case and per-protocol sensitivity analyses, as well as analyses where extreme outliers of the micronutrients were removed from models (data not shown). There was no statistical evidence that the effect of deworming on the concentration of breast milk indicators differed by infant sex.

The prevalence of low breast milk vitamin A off was high (Table 5), with over 87% of women being classified below the cut-off (i.e., ≤ 1.05 μmol/L) at 1 and 6 mo postpartum. The proportion of mothers with low breast milk vitamin A was similar between intervention groups at both time points, in unadjusted and adjusted analyses, as well as further sensitivity analyses.

Estimation of adequate intakes by infants

The concentrations of vitamins and minerals by intervention group at both 1 and 6 mo postpartum were compared to AI values determined for infants from 0 to 6 mo of age (31), and are displayed in Table 6. At 1 mo postpartum, the median concentration of copper and zinc present in breast milk samples exceeded those used to set AI values; however, by 6 mo postpartum, concentrations fell to approximately 70% and 50% of AI values, respectively. Less than one-third of median concentrations met AI values for retinol, less than a quarter met AI values for riboflavin, and less than half met AI values for iron, at either time point.

DISCUSSION

A WHO Informal Consultation in 1994 called for research on lactation performance following deworming in lactating women. The results presented here, where deworming consisted of a single-dose of albendazole, provide a starting point for research on this topic. Overall, an effect of maternal postpartum deworming on the mean concentration of vitamins and minerals in breast milk at 1 or 6 mo postpartum could not be detected. The majority of participants (i.e., > 87%) had low breast milk vitamin A, according to WHO criteria.

Infection with STHs is thought to affect host nutritional status through a variety of mechanisms, including lower food intake and inadequate nutrient uptake, caused by nutrient loss, malabsorption, and altered metabolism (8). The presence of worms in the gut, and consequent damage to the intestinal mucosa, are thought to reduce fat absorption, and can lead to inefficient absorption of fat-soluble vitamins, such as vitamin A and its precursors (8). Only one study has attempted to investigate the effect of infections with *Ascaris lumbricoides* (i.e., roundworm) and *Trichuris trichiura* (i.e., whipworm) on mineral content in human breast milk; however, due to numerous limitations, including small sample size, high risk of selection bias, inconsistent procedures by study site, high rate of attrition (i.e., 36%), inappropriate comparisons between groups, and lack of adjustment for confounders, no meaningful conclusion can be drawn (33).

The median concentration of retinol in breast milk reported in the present study was similar to those described by de Pee *et al.* in Indonesia (34), Rice *et al.* in Bangladesh (35), and de Azeredo *et al.* in Brazil (36) over the first 6 mo of lactation, but less than half those reported in other studies of adult lactating women from low-and-middle-income countries (LMICs) (37-40). In the present study, 88% and 94% of mothers had low breast milk vitamin A (≤1.05 μmol/L) at 1 and 6 mo postpartum, respectively. These prevalences are comparable to those reported in Bangladesh (35), India (41), and Brazil (36). The high prevalence of low breast milk vitamin A is consistent with the WHO classification of a severe public health problem (defined as ≥ 25% of women with low breast milk vitamin A), and suggests that concentrations may be inadequate for meeting the metabolic needs of breastfeeding infants as well as building liver vitamin A stores (27). A recent cross-sectional study in Peru found that nearly half of infants under the age of 5 years had vitamin A deficiency (i.e., 44.6%), and that the risk of deficiency was highest in the

Amazon regions (42). The median concentration of β-carotene was significantly lower than those reported in a multinational study of adult lactating women (39), but similar to those reported by Azeredo *et al.* and Meneses and Trugo in Brazil (36, 37). The α-tocopherol and γ-tocopherol concentrations reported in the current study are comparable to those observed in adult lactating women in high-income countries (43-45), but higher than those reported in other Latin American countries (36, 40).

There is evidence that concentrations of B vitamins significantly differ across milk sampling collection methods, laboratory methods, stages of lactation, dietary intake, and geographic origins (21, 46), and large variability exists among studies reporting their concentrations in breast milk. In the present study, breast milk concentrations of total thiamin were comparable to those reported in Japan (47) and Cameroon (21), but lower than those reported among adult women in Cambodia (48), Malawi (21), and the United States (49-51). Total riboflavin concentrations were two to eight times lower than those reported in other LMICs and industrialized countries (21, 22, 47, 49-53). Milk concentrations of nicotinamide were in accordance with those reported by Sakuri *et al.* in Japan (47), and Hampel *et al.* in India, but higher than those of adult lactating women in China, Malawi, and Cameroon (21). Significantly lower concentrations of pyridoxal were found compared to other studies (21), but results were comparable to those reported in Japan (47). At 1 and 6 mo postpartum, the concentration of cobalamin was lower than what is considered the normal range of cobalamin in human milk (150–700 pmol/L) (54). These concentrations were significantly less than those reported in Guatemala, Mexico, and Brazil (55-57), but similar to cobalamin concentrations described in Bangladesh (23).

Reported concentrations of the minerals copper, iron, and zinc also show a wide variation in the literature. In the present study, mineral concentrations in breast milk were within the ranges commonly reported (58, 59). Two studies conducted in Lima, Peru in women with confirmed febrile illness found similar concentrations of copper, but higher concentrations of iron (60, 61) compared to those reported in the present study. Iron concentrations presented here were in accordance with those reported by Domellöf *et al*. in Honduras (61). Milk concentrations of zinc

were similar to those reported in Spain, but lower than those described in Honduras (61), Finland (62), and Poland (63).

Estimation of the proportion of median vitamin and mineral concentrations meeting AI values suggests that a large number of infants may not be receiving adequate nutritional content from breast milk for most of the micronutrients measured in the present study. These calculations estimate that less than 15% of median concentrations met AIs for riboflavin; between 20-30% met AIs for retinol; and that less than 50% met AIs for iron. However, due to inconsistencies in the breast milk collection procedures (i.e., time of day, stage of lactation, method of collection) in data used to set AI values, as well as small sample sizes of individual studies (64), it is unknown if current AIs are generalizable to the trial population of the present study. AIs are based on a mean assumed intake of 0.78 L/d for healthy, exclusively breastfed infants up to 6 mo of age. In the current study, about half of mothers practiced exclusive breastfeeding up to 1 mo postpartum, and nearly all mothers had introduced food-based liquids or complementary foods by 6 mo. Therefore, many infants in the present study had alternative sources for nutrient intakes, other than breast milk.

The study was limited by the lack of detailed dietary information in mothers, and the inability to determine the effect of diet on breast milk micronutrient concentrations. It is well established that diets high in animal products, fruits, and vegetables provide preformed vitamin A and carotenoids (65). Additionally, as biochemical indicators in serum were not assessed, the presence of micronutrient deficiencies could not be determined. Lastly, the baseline prevalence of STH infection (48.4%) (66) in the total trial population was lower than anticipated, and is likely to have led to effect dilution (i.e., both STH-infected and STH-uninfected mothers were present in the experimental group). This caveat may be responsible for the lack of treatment effect on the concentration of micronutrients in breast milk.

This is the first study to evaluate the effects of anthelminthic treatment on breast milk quality indicators. As the relationship between STH infection and breast milk composition has not been adequately studied, a broad panel of indicators was measured. Overall, this study offers important insights into the quality of breast milk in mothers living in Iquitos, Peru.

By comparing recommendations for AIs of micronutrients for infants developed by the Institute of Medicine with estimates of infant nutrient intake in our study population, it is clear that the quality of breast milk in mothers living in Iquitos may not be sufficient to meet the nutritional needs of their infants. Further research is needed to better understand the biological mechanisms responsible for malnutrition in the presence of STH infection. In particular, the effects of deworming should be investigated in populations of lactating women living in areas of higher STH endemicity and having different nutritional profiles.

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STATEMENT OF AUTHORS' CONTRIBUTIONS TO MANUSCRIPT

LSM, TWG, and BB were responsible for trial conception and design, with critical input from MC, AM, ER, GSM, and LHA. LSM and LP were responsible for on-site trial management, including logistics, supervision of data collection, and ensuring data quality. LHA, SSF, and DH were responsible for assisting with milk collection procedures and the analyses of breast milk samples. LSM, TWG, and ER were involved in data analysis. LSM and TWG were responsible for manuscript preparation. All authors contributed to the interpretation of study findings and revision of the manuscript.

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Table 1: Biochemical indicators of breast milk quality and laboratory analysis method.[1]

Biochemical indicator	Units	Method	Detectable range	Coefficient of variation
Fat-soluble vitamins and precursors				
Vitamin A (retinol)	μmol/L	HPLC-DAD	0.008-2	10.4%
Carotenoid (β-carotene)	μmol/L	HPLC-DAD	0.006-1.5	22.6%
Vitamin E (α-tocopherol)	mg/L	HPLC-DAD	0.12-30	10.5%
Vitamin E (γ-tocopherol)	mg/L	HPLC-DAD	0.08-20.0	12.7%
Water-soluble vitamins and vitamers				
Vitamin B_1 (total thiamin[2])	μg/L	Thiochrome HPLC-FL	1-250	Free thiamin (5.6%); TMP (4.4%); TPP (14.0%)
Vitamin B_2 (riboflavin)	μg/L	UPLC–MS/MS	1-500	11.0%
Vitamin B_2 (FAD)	μg/L	UPLC–MS/MS	1-500	19.1%
Vitamin B_2 (total riboflavin)	μg/L	Riboflavin + FAD[3]		
Vitamin B_3 (nicotinamide)	μg/L	UPLC–MS/MS	1-500	5.3%
Vitamin B_6 (pyridoxal)	μg/L	UPLC–MS/MS	1-500	2.7%
Vitamin B_{12} (cobalamin)	pmol/L	IMMULITE	111-885[4]	10.0

Minerals				
Copper	mg/L	ICP-AES	0.0125-1	1.3%
Iron	mg/L	ICP-AES	0.0125-1	3.9%
Zinc	mg/L	ICP-AES	0.0125-1	1.6%

[1]FAD= flavin adenine dinucleotide; HPLC-DAD= high performance liquid chromatography-diode array detector; HPLC-FL= high performance liquid chromatography-fluorescence detection; UPLC–MS/MS = ultra-performance liquid-chromatography tandem mass spectrometry; ICP-AES= inductively coupled plasma-atomic emission spectroscopy

[2]Total thiamin is calculated from free thiamin, thiamin monophosphate (TMP), and thiamin pyrophosphate (TPP).

[3]Total concentration of vitamin B_2 was calculated using the formula: Total riboflavin= riboflavin + (FAD × 0.479).

[4]Values lower than the detectable range are extrapolated using a standard curve established using diluted breast milk samples, as described previously (23).

Table 2: Baseline characteristics of mother-infant pairs enrolled in breast milk quality substudy, by intervention group, Iquitos, Peru for the recruitment period of February to August 2014.[1]

Characteristic	Albendazole n=116	Placebo n=99
Maternal characteristics		
Age (y) means ±SD	24.4 ±6.7	26.0 ±7.7
Married or cohabiting *n* (%)	103 (88.8)	88 (88.9)
Primigravida *n* (%)	32 (27.6)	24 (24.2)
Less than secondary school education *n* (%)	71 (61.2)	67 (67.7)
Vaginal delivery *n* (%)	98 (84.5)	77 (77.8)
Infant characteristics		
Male n (%)	59 (50.9)	51 (51.5)
Birthweight (kg) means ±SD	3.2 ±0.4	3.2 ±0.4
Birth length (cm) means ±SD	48.7 ±1.8	48.6 ±1.7
Birth head circumference (cm) means ±SD	33.6 ±1.3	33.7 ±1.1
Gestational age (wk) means ±SD	38.7 ±0.8	38.6 ±1.0
Apgar score at 5 minutes	9.9 ±0.4	9.8 ±0.6
Household characteristics		
Access to potable water in home *n* (%)	83 (71.6)	75 (75.8)
Home with dirt or wooden floor *n* (%)	71 (61.2)	70 (70.7)
Number of people residing in household means ±SD	6.2 ±2.7	6.2 ±2.6

[1]SD = standard deviation

Table 3: Distribution of breast milk quality indicators at 1 and 6 mo postpartum among those with complete data, Iquitos, Peru, starting from the first day of the 1-mo substudy visit in April 2014 to the last day of the 6-mo substudy visit in December 2014.[1]

Indicator	***1 mo postpartum***				***6 mo postpartum***			
	Albendazole n=109		**Placebo n=91**		**Albendazole n=108**		**Placebo n=92**	
Retinol µmol/L	0.5	(0.3 - 0.8)	0.5	(0.4 - 0.8)	0.4	(0.2 - 0.5)	0.4	(0.2 - 0.6)
β-carotene µmol/L	0.02	(0.01 - 0.02)	0.02	(0.01 - 0.02)	0.02	(0.01 - 0.02)	0.02	(0.01 - 0.02)
α-tocopherol mg/L	3.2	(2.1 - 4.5)	3.2	(2.3 - 4.3)	3.0	(1.9 - 4.1)	3.0	(2.0 - 4.6)
γ-tocopherol mg/L	1.7	(1.3 - 2.1)	1.6	(1.2 - 2.1)	1.5	(1.1 - 2.1)	1.5	(1.2 - 2.1)
Thiamin[2] µg/L	123.5	(110.2 - 139.5)	129.5	(107.7 - 143.1)	123.5	(110.5 - 139.5)	129.5	(107.7 - 143.1)
Riboflavin µg/L	27.1	(15.6 - 39.6)	28.0	(17.1 - 36.9)	35.0	(22.9 - 45.4)	30.0	(22.7 - 38.6)
FAD µg/L	25.0	(19.1 - 32.7)	24.9	(19.0 - 34.5)	37.8	(25.8 - 57.0)	42.2	(30.9 - 50.4)
Total riboflavin µg/L	42.1	(28.5 - 53.5)	42.7	(29.6 - 52.1)	54.9	(39.8 - 74.9)	51.1	(41.3 - 66.1)
Nicotinamide µg/L	366.7	(262.3 - 601.5)	386.9	(258.5 - 549.4)	238.1	(177.7 - 359.3)	245.1	(174.6 - 352.1)
Pyridoxal µg/L	46.9	(37.0 - 71.2)	52.9	(31.1 - 81.4)	68.5	(50.2 - 93.0)	74.5	(49.4 - 105.7)
Cobalamin[3] pmol/L	106.4	(64.1 - 203.9)	104.0	(59.1 - 186.1)	102.2	(58.8 - 187.4)	98.2	(57.8 - 176.1)
Copper mg/L	0.3	(0.3 - 0.4)	0.3	(0.2 - 0.3)	0.2	(0.1 - 0.2)	0.2	(0.1 - 0.2)
Iron[4] mg/L	0.2	(0.1 - 0.2)	0.2	(0.1 - 0.2)	0.1	(0.06 - 0.2)	0.1	(0.07 - 0.2)
Zinc mg/L	2.9	(2.3 - 3.4)	2.6	(2.1 - 3.2)	1.2	(0.9 - 1.8)	1.3	(0.9 - 1.7)

[1]Data expressed as median (interquartile range); FAD= flavin adenine dinucleotide

[2]At 1 mo, thiamin concentration was not detectable in 1 participant.

[3]At 1 mo, cobalamin concentrations were not detectable in 2 participants; at 6 mo, cobalamin concentrations were not detectable in 3 participants.

[4]At 1 mo, iron concentrations were not detectable in 2 participants; at 6 mo, iron concentrations were not detectable in 4 participants.

Table 4: Effect of maternal postpartum deworming on breast milk quality indicators at 1 and 6 mo postpartum (N=215[1]), Iquitos, Peru starting from the first day of the 1-mo substudy visit in April 2014 to the last day of the 6-mo substudy visit in December 2014.[2]

Indicator	***1 mo postpartum***				***6 mo postpartum***			
	Difference$_{unadjusted}$		**Difference$_{adjusted}$[3]**		**Difference$_{unadjusted}$**		**Difference$_{adjusted}$[3]**	
		(95% CI)		**(95% CI)**		**(95% CI)**		**(95% CI)**
Retinol µmol/L	-0.01	(-0.2, 0.2)	-0.02	(-0.2, 0.2)	-0.1	(-0.3, 0.09)	-0.1	(-0.3, 0.1)
β-carotene µmol/L	-0.007	(-0.1, 0.1)	-0.003	(-0.1, 0.1)	0.02	(-0.1, 0.2)	0.03	(-0.1, 0.2)
α-tocopherol mg/L	-0.02	(-0.2, 0.1)	-0.03	(-0.2, 0.1)	-0.08	(-0.3, 0.1)	-0.1	(-0.3, 0.1)
γ-tocopherol mg/L	-0.03	(-0.2, 0.2)	-0.04	(-0.2, 0.2)	0.07	(-0.2, 0.3)	0.04	(-0.2, 0.3)
Thiamin[4] µg/L	-1.7	(-9.6, 6.3)	-1.4	(-9.5, 6.7)	-0.7	(-8.3, 7.0)	-0.3	(-8.1, 7.4)
Riboflavin µg/L	-0.09	(-0.3, 0.2)	-0.1	(-0.4, 0.1)	0.06	(-0.1, 0.3)	0.07	(-0.1, 0.3)
FAD µg/L	-0.006	(-0.1, 0.1)	-0.01	(-0.2, 0.1)	-0.04	(-0.2, 0.1)	-0.03	(-0.2, 0.1)
Total riboflavin µg/L	-0.4	(-5.6, 4.9)	-1.4	(-6.6, 3.9)	3.3	(-3.3, 10.0)	3.9	(-2.8, 10.7)
Nicotinamide µg/L	-0.006	(-0.2, 0.2)	-0.02	(-0.2, 0.2)	-0.006	(-0.2, 0.2)	0.02	(-0.2, 0.2)
Pyridoxal µg/L	-0.04	(-0.2, 0.2)	-0.05	(-0.2, 0.1)	-0.03	(-0.2, 0.1)	-0.05	(-0.2, 0.09)
Cobalamin[5] pmol/L	0.09	(-0.3, 0.5)	0.05	(-0.3, 0.4)	-0.1	(-0.6, 0.3)	0.1	(-0.5, 0.3)
Copper mg/L	0.02	(-0.006, 0.04)	0.01	(-0.009, 0.03)	-0.008	(-0.03, 0.01)	-0.008	(-0.03, 0.01)
Iron[6] mg/L	0.2	(-0.04, 0.5)	0.2	(-0.05, 0.5)	-0.2	(-0.6, 0.1)	-0.2	(-0.6, 0.1)
Zinc mg/L	0.2	(-0.08, 0.4)	0.2	(-0.07, 0.4)	0.05	(-0.1, 0.2)	0.05	(-0.1, 0.2)

[1]Intention-to-treat analysis includes data from 185 participants who had breast milk composition outcome data at 1 and 6 mo postpartum, as well as 15 participants at 1 mo and 15 participants at 6 mo whose outcome data were imputed using multiple imputation.

[2]CI = confidence interval; FAD= flavin adenine dinucleotide; Retinol, β-carotene, α-tocopherol, riboflavin, FAD, nicotinamide, pyridoxal, cobalamin and iron, were log-transformed (natural log)

[3]Adjusted for maternal age, education, socioeconomic index, and infant sex

[4]At 1 mo, thiamin concentration was not detectable in 1 sample. Thiamin concentrations that were not detectable were set to a nominal value (0.001) for all analyses.

[5]At 1 mo, cobalamin concentrations were not detectable in 2 samples; at 6 mo, cobalamin concentrations were not detectable in 3 samples. Cobalamin concentrations that were not detectable were set to a nominal value (0.001) for all analyses.

[6]At 1 mo, iron concentrations were not detectable in 2 samples; at 6 mo, iron concentrations were not detectable in 4 samples. Iron concentrations that were not detectable were set to a nominal value (0.000125) for all analyses.

Table 5: Effect of deworming on the prevalence of low breast milk vitamin A at 1 and 6 mo postpartum (N=215[1]), Iquitos, Peru starting from the first day of the 1-mo substudy visit in April 2014 to the last day of the 6-mo substudy visit in December 2014.[2]

Outcome	**Albendazole n=116**	**Placebo n=99**
1 mo postpartum		
Prevalence breast milk retinol ≤ 1.05 µmol/L (95% CI)	87.5 (81.2, 93.8)	87.8 (81.0, 94.5)
Unadjusted RR (95% CI)	1.0 (0.9, 1.1)	*reference*
p value	0.953	
Adjusted[3] RR (95 % CI)	1.0 (0.9, 1.1)	*reference*
p value	0.919	
6 mo postpartum[4]		
Prevalence breast milk retinol ≤ 1.05 µmol/L (95% CI)	94.3 (89.8, 98.7)	92.9 (87.3, 98.4)
Unadjusted RR (95% CI)	1.0 (0.9, 1.1)	*reference*
p value	0.698	
Adjusted[3] RR (95 % CI)	1.0 (0.9, 1.1)	*reference*
p value	0.822	

[1]Intention-to-treat analysis includes data from 185 participants who had breast milk composition outcome data at 1 and 6 mo postpartum, as well as 15 participants at 1 mo and 15 participants at 6 mo whose outcome data were imputed using multiple imputation.

[2]RR = risk ratio; CI = confidence interval

[3]Adjusted for maternal age, education, socioeconomic index, and infant sex

[4]At 6 mo, fat concentration could not be analyzed from 1 sample. Fat concentrations that could not be analyzed were imputed using multiple imputation methods described above.

Table 6: Median micronutrient concentrations and adequate intakes of micronutrients at 1 and 6 mo postpartum by intervention group among participants with complete data, Iquitos, Peru starting from the first day of the 1-mo substudy visit in April 2014 to the last day of the 6-mo substudy visit in December 2014.

Indicator	Adequate intakes[1]	Albendazole		Placebo	
		Median concentration[2]	% AI	Median concentration[2]	% AI
Retinol µg/d[3]					
1 mo	400	122.6	30.7	113.7	28.4
6 mo	400	80.0	20.0	93.6	23.4
α-tocopherol mg/d					
1 mo	4	2.5	62.9	2.5	61.5
6 mo	4	2.3	57.7	2.4	58.9
Thiamin mg/d					
1 mo	0.2	0.1	48.1	0.1	50.5
6 mo	0.2	0.1	48.2	0.1	50.5
Total riboflavin mg/d					
1 mo	0.3	0.03	10.9	0.03	11.1
6 mo	0.3	0.04	14.3	0.04	13.3
Pyridoxal mg/d					
1 mo	0.1	0.04	36.6	0.04	41.2
6 mo	0.1	0.05	53.4	0.06	58.1
Cobalamin µg/d[4]					
1 mo	0.4	0.1	28.1	0.1	27.5
6 mo	0.4	0.1	27.0	0.1	26.0
Copper µg/d					
1 mo	200	240.7	120.4	224.4	112.2
6 mo	200	141.7	70.8	137.6	68.8
Iron mg/d					
1 mo	0.27	0.1	47.4	0.1	44.4
6 mo	0.27	0.08	30.3	0.1	36.4

Zinc mg/d					
1 mo	2	2.3	112.9	2.0	100.2
6 mo	2	0.9	47.2	1.0	50.4

[1]Source: Otten et al., 2006 (31)

[2]Values assume a healthy breast milk intake of 0.78 L/d (32)

[3]Vitamin A conversion factor 1 μg/dL retinol = 0.0349 μmol/L (67)

[4]Vitamin B_{12} conversion factor 1 pg/mL cobalamin = 0.738 pmol/L (67)

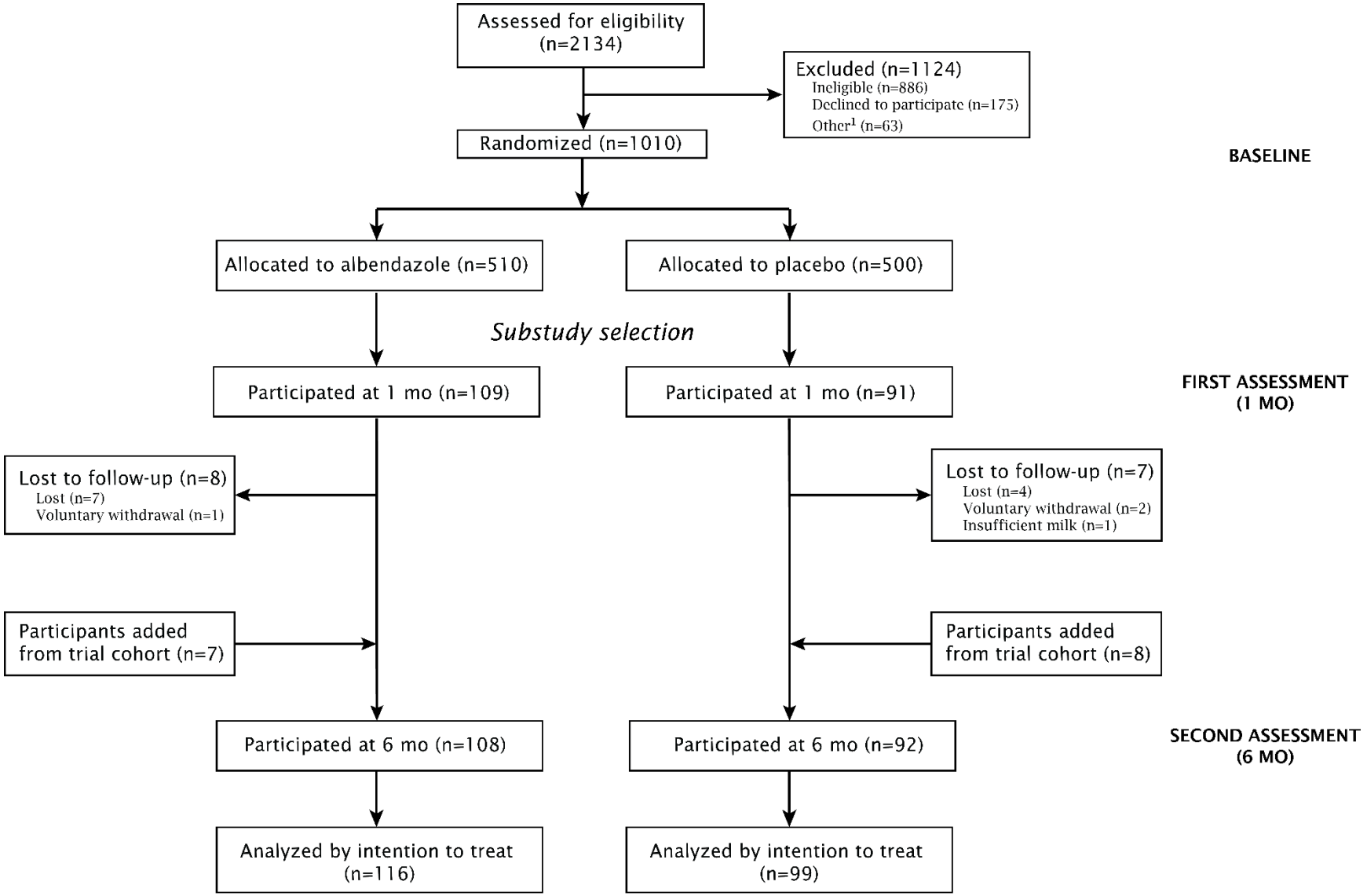

Figure 1: Substudy flow diagram showing recruitment, enrolment (randomization), and follow-up visits

[1]63 women were screened but were not enrolled because the sample size of the trial was already met.

CHAPTER 8: GENERAL DISCUSSION

8.1 Summary of findings

This body of work presents findings of the first RCT on deworming during lactation. In 1994, WHO held an informal consultation, bringing together world experts for discussions about the burden of hookworm infection and anemia in WRA (WHO 1996b). Following this meeting, recommendations were established for including adolescent girls, pregnant women, and lactating women in deworming programs where STHs endemicity levels exceed 20% and where anemia is also prevalent (WHO 1996b; 2002; 2006a). The group also called for research on the impact of STH infections and deworming in populations of pregnant and lactating women. While there have been a few studies in pregnant women, evidence in lactating women has been non-existent. As the goal to eliminate STH infections as a public health problem by 2020 is currently receiving global attention (WHO 2012), findings from the current study can contribute timely empirical evidence to inform strategic prevention and control plans.

This study's results demonstrate a high prevalence of maternal undernutrition, especially with respect to iron and vitamin A status. Blood Hb concentrations and vitamin A levels in breast milk are considered to be valid indicators for determining nutritional status at a population-level (de Benoist et al. 2008; WHO 1996a). At both the 1 and 6 month study visits, the prevalences of maternal anemia (i.e., > 45%) and low breast milk retinol (i.e., > 85%) exceeded those indicative of a severe public health problem as defined by WHO (de Benoist et al. 2008; WHO 1996a). The prevalence of postpartum anemia in the present study was similar to reported prevalences of anemia during lactation in other LMICs (Milman 2011), although mild-to-moderate anemia was the most common in our study population. The prevalence of low breast milk vitamin A was comparable to prevalences reported in Bangladesh (Rice et al. 1999), India (Vinutha et al. 2000), and Brazil (de Azeredo and Trugo 2008) but higher than prevalences found in other studies conducted in LMICs (Bahl et al. 2002; Basu et al. 2003; Engle-Stone et al. 2014; Klevor et al. 2016). Explanations for this variability likely reflect local differences in diet and supplementation use, which would have provided additional context to our results but their

adequate study was outside the scope of the current trial. It would be beneficial to include this aspect in future research.

The prevalence of STH infection at 6 months postpartum among mothers randomized to the control group (i.e., 50.2%), can be considered representative of the overall STH infection status in our population, and is substantially lower than STH prevalences reported among pregnant women in other studies (Larocque et al. 2006; Navitsky et al. 1998; Ndibazza et al. 2010; Ndyomugyenyi et al. 2008; Torlesse and Hodges 2001). Most surprising is the dramatic fall in STH prevalence compared to the 91% reported in Larocque *et al.* (2006) from the same study (in Peru) area ten years earlier. However, since the trial conducted by Larocque *et al.*, a school-based deworming program has been implemented in the region of Loreto (as of 2012). The sharp decline in prevalence among WRA may be the result of both improvements in sanitation over the ten year interim period and a reduction in environmental contamination from treating school-aged children, leading to positive spill-over effects in WRA.

In our study population of infants, the prevalence of stunting more than doubled between birth and 6 months of age. Over the first 6 months of life, z-scores for WAZ, LAZ, and HCAZ showed a negative deviation compared to the WHO growth standard population. Among South American countries, Peru has been described as having one of the highest prevalences of stunting in children under 5 years of age (UNICEF 2009). The prevalences of stunting experienced by infants in our study population are within the range reported in other LMICs (Lundeen et al. 2014). These findings are consistent with the critical period of development, between conception and the first two years of life, at which time growth faltering can have long-standing consequences on future health trajectories (Victora et al. 2010). The practice of exclusive breastfeeding in the trial population was suboptimal, with the prevalence decreasing from 49.0% at 1 month postpartum to 1.0% by the 6-month study visit. In 2014, national statistics from Peru reported that 70.6% of infants under 6 months of age were exclusively breastfed, and that the median duration of exclusive breastfeeding in the region of Loreto was 5.4 months (ENDES 2014). Study findings also suggest that breastfed infants may not be receiving adequate nutritional content from breast milk, especially in terms of vitamin A, B vitamins, and iron.

Together, these results demonstrate a high risk of malnutrition in infants living in Iquitos, and a need to implement early nutritional interventions.

The causal relationship between maternal STH infection and infant malnutrition was explored by evaluating the impact of providing deworming to women during early lactation on infant health outcomes over the first 6 months of life. Overall, we found no statistically significant difference in growth outcomes between intervention groups. While one-time, single-dose deworming did have a positive impact on reducing STH infections in mothers, no benefits were found, 6 months postpartum, in terms of mean maternal Hb concentration or the prevalence of anemia in our study population. One reason for this finding could be the low baseline prevalences of hookworm and *T. trichiura* – which are known to cause blood loss and iron deficiency anemia (Brooker et al. 2008). Similarly, postpartum deworming had no effect on maternal fatigue. This may be due to a lack of significant effect of the deworming intervention compared to the control group in terms of anemia (which may be a mediating factor between STH infection and fatigue) (Sobrero et al. 2001). The trial was not able to detect a statistically significant benefit of maternal postpartum deworming on breast milk composition.

8.2 Strengths and limitations

The main strength of the study is its RCT design, where the effects of deworming could be ascertained while, at the same time, the influence of measured and unmeasured confounding by external factors could be minimized. Overall, baseline characteristics of the trial population were similar between intervention groups, demonstrating that the randomization procedure was successful. Enrollment occurred within a short time window following delivery (i.e., within 3 days), and thus the study population comprised a homogenous group of women in terms of stage of lactation. As the intervention was single-dose, and ingestion was directly observed by research assistants at bedside, a high rate of compliance was achieved. Although anthelmintics are readily available in Iquitos without a prescription, they are not frequently used among adults. In this trial, use of deworming outside of the trial protocol was low and therefore the risk of contamination through external treatment was minimal.

The large sample size of more than 1000 mother-infant pairs was well-powered to detect treatment effects not only in the primary outcome of infant weight gain between birth and 6 months of age but also in secondary infant and maternal health outcomes. Minimization of selection bias through differential loss-to-follow up was achieved through a high follow-up rate (> 95%), which was similar in the two intervention groups. Allocation concealment and blinding were maintained throughout the trial to minimize the chance of selection bias and ascertainment bias. The presence of random error in outcome ascertainment was minimized through intensive training of research personnel, standardization of operating procedures, calibration of equipment, and ensuring that participants were visited as close as possible to their target follow-up date. High internal validity was maintained throughout the trial by ensuring data collected were of high quality. The completeness and coherency of data were well-monitored through the use of a real-time electronic data collection tool, on-site monitoring of study visits, and daily review of data with research assistants. The integrity of biological specimens was ensured through proper handling, labelling, storage, and shipment. Laboratory analyses were performed according to established preparation, examination, and quality assurance techniques. In the case of breast milk quality assessment, samples were run together with internal and external standards.

According to CONSORT guidelines (Moher et al. 2001; Schulz et al. 2010), all analyses were first performed according to the ITT principle, in addition to complete-case and per-protocol analyses. Missing outcome values were imputed using multiple imputation, so that participants could be analyzed by the groups to which they were randomized, irrespective of losses, noncompliance, and protocol deviations. Consistency of results between different analytic approaches demonstrates the robustness of the findings, and suggests that participants who were followed-up at 6 months were likely representative of the trial population at enrollment.

The present study was limited by the lower than expected prevalence of STH infection which may have contributed to the null findings, due to the enrolment of a large proportion of uninfected women who would not have benefitted from deworming. A recent Cochrane review (Taylor-Robinson et al. 2015) found, that among trials of children known to be infected with STH, deworming had benefits on growth outcomes, but among trials of whole communities endemic for STHs (which would have included both infected and uninfected persons),

deworming did not have an effect. To reflect operational constraints of real-world deworming programs and due to ethical constraints, eligibility in the present trial was not restricted to those infected with STHs, only that individuals reside in STH-endemic areas. To estimate baseline STH infection, only stool specimens from those randomized to the experimental group were analyzed. It was believed that this method would allow for accurate estimation of STH infection status prior to enrolment (because, by design, women randomized to the experimental group should have been representative of the total study population), and would satisfy ethical guidelines (because, as per the trial protocol, these women would be treated). Research assistants actively tried to collect stool specimens from all participants in the hospital. However, the proportion of women in the experimental group who were able to provide a stool specimen in the hospital (64/510) was suboptimal because: a) many women were concurrently taking iron supplementation, which has side effects of constipation; b) women were advised during antenatal care visits to take laxatives prior to arriving at the hospital for delivery; c) following birth, consumption of solid foods by mothers is uncommon; and d) women were not comfortable providing a stool specimen during labour or shortly after birth. Though the women who provided baseline stool specimens seemed to be similar to the other women participating in the trial, it is unknown whether the prevalence and intensity of STH infection, and, in particular, the prevalence and intensity of the different STH species, was truly representative of the entire trial population at baseline. Additionally, without accurate diagnosis of STH infection in the control group, subgroup analysis in those infected with STHs at baseline (Montresor et al. 2015b) was not possible.

Another potential weakness of the trial was that infant morbidity (i.e., episodes of diarrhea, respiratory illness, fever, ear infection) relied on report by the mother and was not clinically confirmed by a health care professional. This may have resulted in misclassification of infant morbidity; however, there is no reason to believe that self-reporting differed by intervention group, and thus misclassification was likely non-differential. Neither Hb concentration nor fatigue were assessed at baseline, because these measures are not thought to be reliable during the peripartum period. For this reason, it was not possible to assess the impact of deworming on the change in Hb or fatigue score over the first 6 months postpartum. Lastly, as data on maternal

dietary intake were not collected, the influence of diet on breast milk composition could not be assessed.

8.3 Generalizability

The results of this trial are generalizable to other populations of lactating women who reside in STH-endemic areas where antenatal and postnatal health services are available. Findings are applicable to other areas with similar STH prevalence and intensity profiles, where micronutrient deficiencies are also common (i.e., poor iron status and vitamin A deficiency). However, study findings may not be generalizable to areas with higher burdens of STH infection, or areas where other tropical diseases are of public health importance (e.g., malaria, schistosomiasis).

8.4 Results dissemination

The three manuscripts containing trial results have been prepared for submission to international scientific peer-reviewed journals. Presentations on trial findings are planned in 2016 at the American Society of Tropical Medicine and Hygiene, and in 2017 at the European Congress of Tropical Medicine and International Health. Findings will also be sent to newsletters such as Action against Worms and to global initiatives such as Deworm the World, Save the Children, Partners for Parasite Control, and Partnership for Child Development, among others. LSM received an award from the Regroupement Stratégique Santé Mondiale du RRSPQ to support results dissemination activities to various governmental, academic, and civil society fora within Peru. Results will also be communicated to international agencies like WHO and the Pan American Health Organization who have taken a leadership role in developing global health policies pertaining to deworming in high risk population groups.

8.5 Implications and impact of research

Over the past two decades, deworming has been at the centre of public health interventions aimed at improving growth and development trajectories of children living in LMICs. Since the

2020 goal was set for elimination of STHs, considerable effort has been made to increase coverage of preschool and school-aged children in endemic countries. Cumulative evidence suggests that infected members of communities act as hidden reservoirs of infection, and that deworming, while being a cost-effective strategy to avert morbidity in those treated, can also indirectly affect the population as a whole by reducing overall transmission (Lo et al. 2015; Lo et al. 2016). Thus, apart from benefits to the women themselves, targeting WRA in deworming programs may also help to reduce STH prevalence and intensity in children, and contribute to meeting the 2020 targets for STH control (Coffeng et al. 2015).

This thesis provides novel information about the benefits of integrating deworming into maternal postpartum care on infant and maternal health outcomes, and contributes to the growing knowledge base on the effectiveness of deworming in endemic areas. Taken together, these results can be used by international organizations, such as WHO, and ministries of health in endemic regions to help guide priorities for resource allocation and decision-making. As many countries are actively promoting delivery in health care facilities, integration of deworming into routine postpartum care may be seen as a feasible strategy for targeting WRA periodically throughout their most reproductive years, with little to no additional cost. Overall, the findings of this study provide empirical evidence to governments in the 100 worm-endemic countries globally.

8.6 Conclusion

Overall, this trial provides operational insights into the inclusion of an important subgroup of WRA in deworming activities. In populations suffering from underlying poor nutritional status, single-dose deworming may not be sufficient to combat high prevalences of anemia and micronutrient deficiencies, with the hopes of improving overall maternal and infant health, and so other strategies should be considered. Future research would benefit from studying the effectiveness of maternal postpartum deworming in populations with higher prevalences and intensities of STH infections, over longer periods of time, and with repeated administration intervals. It would be especially important to study benefits in areas where hookworm and *Trichuris* infections predominate, because of their effects on blood loss and anemia. Future

evaluations of deworming may also benefit from considering how deworming can best be incorporated into other nutritional interventions, (e.g., high-dose micronutrient supplementation). In order to maximize the benefits of deworming in STH-endemic areas, single-dose deworming should be offered periodically at scheduled intervals. Due to ethical constraints limiting accurate detection of baseline STH prevalence, the standard RCT design may not be appropriate to evaluate the effectiveness of deworming programs. Moving forward, more novel study designs, such as the stepped-wedge RCT design, should be considered when evaluating deworming programs.

CHAPTER 9: REFERENCES

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APPENDIX A

Published trial protocol:

Maternal deworming research study (MADRES) protocol:
a double-blind, placebo-controlled randomised trial to determine the effectiveness of deworming in the immediate postpartum period

BMJ Open

Maternal Deworming Research Study (MADRES) protocol: a double-blind, placebo-controlled randomised trial to determine the effectiveness of deworming in the immediate postpartum period

Layla S Mofid,[1] Martín Casapía,[3] Antonio Montresor,[4] Elham Rahme,[2] William D Fraser,[5] Grace S Marquis,[6] Jozef Vercruysse,[7] Lindsay H Allen,[8] Theresa W Gyorkos[1,2]

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For numbered affiliations see end of article.

Correspondence to
Dr Theresa W Gyorkos;
theresa.gyorkos@mcgill.ca

ABSTRACT

Introduction: Soil-transmitted helminth infections are endemic in 114 countries worldwide, and cause the highest burden of disease among all neglected tropical diseases. The WHO includes women of reproductive age as a high-risk group for infection. The primary consequence of infection in this population is anaemia. During lactation, anaemia may contribute to reduced quality and quantity of milk, decreasing the duration of exclusive breastfeeding and lowering the age at weaning. To date, no study has investigated the effects of maternal postpartum deworming on infant or maternal health outcomes.

Methods and analysis: A single-centre, parallel, double-blind, randomised, placebo-controlled trial will be carried out in Iquitos, Peru, to assess the effectiveness of integrating single-dose 400 mg albendazole into routine maternal postpartum care. A total of 1010 mother-infant pairs will be randomised to either the intervention or control arm, following inhospital delivery and prior to discharge. Participants will be visited in their homes at 1, 6, 12 and 24 months following delivery for outcome ascertainment. The primary outcome is infant mean weight gain between birth and 6 months of age. Secondary outcomes include other infant growth indicators and morbidity, maternal soil-transmitted helminth infection and intensity, anaemia, fatigue, and breastfeeding practices. All statistical analyses will be performed on an intention-to-treat basis.

Ethics and dissemination: Research ethics board approval has been obtained from the McGill University Health Centre (Canada), the Asociación Civil Impacta Salud y Educación (Peru) and the Instituto Nacional de Salud (Peru). A data safety and monitoring committee is in place to oversee study progression and evaluate adverse events. The results of the analyses will be published in peer-reviewed journals, and presented at national and international conferences.

Trial registration number: Clinicaltrials.gov: NCT01748929.

Strengths and limitations of this study

- This is the first study to evaluate the effectiveness of postpartum deworming on maternal and infant health outcomes.
- Results of this trial will provide empirical evidence to inform the WHO recommendation for deworming in women of reproductive age.
- A large sample size (n=1010) allows for high-powered primary and secondary analyses.
- This is a single-centre trial which can affect generalisability of the results to other areas with different prevalence profiles and transmission patterns of soil-transmitted helminth infections.
- The study is limited to soil-transmitted helminth infections and does not include the other parasitic infections.

INTRODUCTION

Background

Worldwide, over two billion people are infected with intestinal worms (hookworm, *Ascaris* and *Trichuris*), collectively referred to as the soil-transmitted helminths (STHs).[1] STH infections discriminate across socioeconomic strata, such that the most vulnerable individuals, who reside in areas where adverse health, social, and economic outcomes predominate, carry the greatest disease burden. The WHO and other organisations consider women of reproductive age to be a high-risk group for STH infection primarily because of the anaemia that is caused by hookworm[2] and *Trichuris*[3] (whipworm) infections. This exacerbates the already increasing iron requirements of pregnancy, with iron deficiencies extending into the early lactation period.[4] More than 50% of

pregnant women, especially in developing countries, are estimated to be anaemic.[5] During lactation, anaemia is thought to adversely affect milk production, which can decrease the duration of exclusive breastfeeding and lower the age at weaning,[6] and ultimately impact negatively on infant growth.

Lactating women in developing countries are at risk of suffering from a shortage of dietary fat and micronutrient deficiencies due to a suboptimal diet and parasitic infections, including STHs, which compete for micronutrients, like iron.[7] The consequences of co-occurring malnutrition and infection in a new mother may include an impact on breast milk composition[8]and subsequently, negatively affect the nutritional status of infants.[9] STH infections can cause reduced absorption of dietary fat leading to lower energy intake and inadequate absorption of vitamin A. Reduced transfer of vitamin A into breast milk may cause insufficient vitamin A acquisition in infant liver stores, leading to vitamin A deficiency over the first 6 months of life and beyond.[10] Although maternal iron stores do not directly affect breast milk concentrations of iron,[11] maternal anaemia may play a role in the frequency and duration of breastfeeding due to reduced energy levels.

To date, no study has investigated the effect of providing deworming treatment to women during the early postpartum period on infant or maternal health outcomes. In effect, the WHO recommendation to specifically include lactating women within the high-risk group of women of reproductive age in deworming campaigns[12] is based on expert opinion and not on empirical evidence. Systematic reviews on deworming have, however, been conducted on other subgroups of women in the reproductive age (eg, non-pregnant, non-lactating women; pregnant women). A 2010 systematic review on deworming in non-pregnant populations[13] included two studies of women in the reproductive age: one trial[14] that specifically excluded lactating women, and one observational study[15] that included lactating women, but in which lactation status was not ascertained. Four trials to date[16–19] have been conducted in pregnant populations, and are summarised in three systematic reviews.[20–22] None of these trials used the same intervention (ie, the same anthelminthic and micronutrient combination) or had the same follow-up time frame. While data from pregnant populations are of value, given the different nature of the interface between mother and child at the time of deworming, it is unclear whether the results are generalisable to lactating women.

Rationale

Malnutrition is the leading cause of mortality in children under 5 years of age in developing countries, with over 150 million children classified as underweight, stunted and/or wasted.[23] Poor nutrition predisposes children to infection, leading to increased risk of mortality,[24] higher risk of cognitive deficits, lower educational achievement and lower productivity as adults,[25] thus perpetuating the poverty cycle into future generations. The health of mothers and young children are intimately intertwined, and the 1000-days period from conception to the age of 2 years is a crucial time in shaping the health and development of children.[25 26] It is within this critical window when interventions can have the greatest impact on future health throughout the entire lifespan.

Deworming has been shown to be one of the safest and most cost-effective interventions for reducing disease burden in endemic countries, and is the cornerstone of prevention and control measures against STH infections.[27] There is evidence from veterinary research in ruminants that has shown that worm infections can negatively affect the quality of milk and impair production.[28] In addition to modifying the nutritional and immunological composition of milk,[29] worm infections also suppress energy and protein availability prior to parturition,[30 31] which can influence feeding behaviours. Administration of deworming treatment has been shown to improve milk production in cattle.[32 33]

The proposed deworming intervention is expected to improve capacity for breastfeeding, and quality and quantity of milk transfer to infants by reducing maternal anaemia, improving appetite and increasing energy levels. Mothers with improved nutritional status and enhanced energy levels may be more likely to initiate and continue exclusive breastfeeding to the 6-month recommended time point, and beyond. Additionally, improved maternal nutritional status may influence the passage of certain nutrients (eg, thiamin, riboflavin, vitamin B-6 and vitamin B-12, among others[34]) to breast milk during breastfeeding. This may, in turn, improve nutritional status of infants, and enhance their growth and development (figure 1). Integration of deworming into early postpartum care, therefore, has the potential to improve health outcomes for two vulnerable populations simultaneously.

The aim of the current study is to provide rigorous empirical evidence on the benefits and underlying biological mechanisms of maternal postpartum deworming in STH-endemic areas in order to inform global public health policy.

Research objectives

Primary research objective

To determine the effectiveness of maternal postpartum deworming on mean weight gain in infants between birth and six months of age.

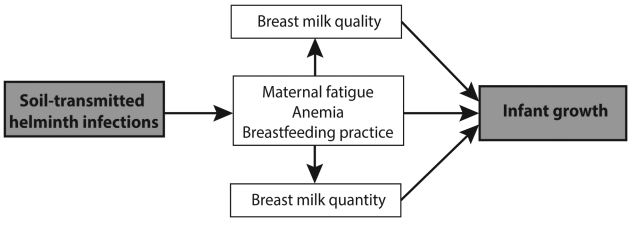

Figure 1 Proposed mechanism for the effect of soil-transmitted helminth infections on maternal and infant health.

Secondary research objectives

1. To determine the effectiveness of maternal postpartum deworming on the following infant outcomes: (A) weight and height indices (ie, z-scores for weight-for-age, weight-for-height and height-for-age); and (B) infant morbidity (ie, occurrence of diarrhoea, respiratory illness, fever, and ear infection in the previous 2 weeks).
2. To determine the effectiveness of maternal postpartum deworming on the following maternal outcomes: (A) STH infection and intensity; (B) anaemia; (C) self-reported fatigue; and (D) self-reported breastfeeding practice.
3. To determine the effectiveness of maternal postpartum deworming on indicators of breast milk quality, and quantity.

METHODS AND ANALYSIS

Study design and setting

A parallel, double-blind, randomised, placebo-controlled trial (RCT) will be used to examine the effectiveness of integrating single-dose albendazole administration into routine in-hospital maternal postpartum care on infant and maternal health outcomes, in Iquitos, Peru. Iquitos is the capital of the Department of Loreto in the Amazon Basin of northeastern Peru. Treatment allocation will take place within one centre, Hospital Iquitos 'Cesar Garayar Garcia'. This hospital has a catchment area that includes individuals who reside in the poor and highly STH-endemic district of Belén. In 2004, prevalences of 45% for hookworm, 60% for *Ascaris* and 80% for *Trichuris* were reported in over 1000 pregnant women enrolled in a previous RCT.[17]

Interventions

All women and children included in this trial will receive usual postpartum care (eg, examinations following delivery, nutritional counselling, infant vaccinations, etc), according to hospital standard of care and Ministry of Health guidelines. In addition, the following interventions with be compared.

Experimental group

A single-dose 400 mg albendazole tablet (oral deworming drug) will be given to women following delivery and prior to hospital discharge.

Control group

A single-dose 400 mg placebo tablet (identical to the experimental drug with respect to size, shape, colour, taste and smell) will be given to women following delivery and prior to hospital discharge.

Eligibility criteria

Inclusion criteria

Women are eligible to participate in the trial if they meet the following criteria: (1) they deliver at Hospital Iquitos 'Cesar Garayar Garcia'; and (2) they are likely to reside in Iquitos or a neighbouring area for the next 24 months.

Exclusion criteria

Women are ineligible to participate in the trial if: (1) they deliver twins or multiples; (2) they deliver a stillborn infant or an infant with a serious congenital medical condition; (3) they are transferred to another hospital prior to discharge; or if (4) they are unable to communicate in Spanish.

Randomisation

Prior to onset of the recruitment period, a statistician not otherwise involved in the trial will produce the randomisation sequence using a computer-generated permuted block design with randomly varying block sizes of 6 and 8 with a 1:1 allocation ratio. Albendazole and matching placebo will be packaged according to the randomisation sequence into opaque, sequentially-numbered envelopes by a pharmacist and a clinician not otherwise involved in the trial, and stored in a secure temperature-controlled pharmacy.

Outcomes

Primary outcome

The primary outcome is infant mean weight gain between birth and 6 months of age. Infant weight gain is thought to be the most accurate measure of breastfeeding adequacy and sufficient milk transfer from mother to breastfeeding infant.[6] It is also an important indicator of infant growth in the first year of life.[35 36]

Secondary outcomes

Secondary infant outcomes include weight and height indices (ie, z-scores for weight-for-age, weight-for-height and height-for-age) and WHO/UNICEF Integrated Management of Childhood Illness (IMCI) indicators of infant morbidity (ie, occurrence of diarrhoea, respiratory illness, fever, and ear infection) in the previous 2 weeks.[37] Maternal outcomes will include the prevalence and intensity of STH infection (combined and by species), anaemia, self-reported fatigue and breastfeeding practice, and breast milk quality and quantity. All secondary outcomes will be ascertained at 1, 6, 12 and 24 months following delivery.

Sample size and power calculations

The sample size is calculated based on the primary outcome, infant mean weight gain between birth and 6 months of age. An estimate of mean weight gain was obtained from data on children aged between 5 and 7 months residing in Belén in 2010. The estimate of 4.24 kg with a SD of 1.01 kg is the weight gain expected in the placebo group. Previous authors have claimed that an infant mean weight gain difference of approximately 500 g is clinically meaningful from trials on enriched formula feeding in infants.[36 38 39] However,

since the intervention for the proposed trial is given to women rather than infants, and since there is the possibility for effect dilution (ie, treating both STH infected and non-infected mothers), a mean weight gain difference of at least 200 g is expected between the two intervention groups. This difference in infant mean weight gain is also considered to be clinically significant.[40]

The sample size calculation is, therefore, based on an expected effect size of 0.2 kg and a SD of 1.01 kg, a significance level (α) of 0.05 and a power (1-β) of 0.80. As informed by a previous study in the same hospital population,[41] the final sample size takes into account a 20% loss to follow-up. Based on the above specifications and a two-sided independent t test, we estimate that 1010 participants is the total sample size needed to declare that infant weight gain is different between intervention groups.

This trial will have sufficient power to detect a difference in proportions as low as 4% for moderate/heavy STH intensity (control group: 6% vs experimental group: 2%),[17] as well as differences in species-specific STH infection (ie, hookworm, *Ascaris*, *Trichuris*) and infant morbidity indicators (ie, recent occurrence of diarrhoea, respiratory illness, fever, and ear infection), since the differences in proportions between intervention groups for these outcomes are expected to be even larger.[17 42–44] The study will also have sufficient power to detect a clinically meaningful mean difference of 0.5 in z-scores for weight-for-age, weight-for-height and height-for-age,[45] and a difference in maternal haemoglobin levels similar to those observed in other studies on anthelmintic administration.[13 17 46] Sample size and power calculations were conducted using PS Power and Sample Size Calculations V.3.0 (Copyright 1997 by Dupont and Plummet).

Recruitment

Enumeration of pregnant women in their second and third trimester of pregnancy living in the study area was carried out in December 2013 and January of 2014 using rosters of pregnant women obtained from surrounding health centres and through door-to-door canvassing, in conjunction with the Regional Ministry of Health activities. A two-stage approach will be used for recruiting women into the study prior to delivery.

In stage one, research assistants will visit the homes of women in their third trimester of pregnancy to explain the research study, obtain informed consent from both women and their partners, and administer the baseline questionnaire. Women who are interested in participating in the study will be assessed for eligibility. Informed consent will be sought at this time (figure 2).

In stage two, women presenting for delivery in the labour room of Hospital Iquitos 'Cesar Garayar Garcia' will be approached by a research assistant, reminded of the study, and asked whether they are still interested in taking part. Research assistants will work rotating shifts, such that at least one research assistant will be present in the labour room at all times to recruit vaginal and caesarean-section deliveries (ie, in-hospital data collection will be ongoing 24 h/day).

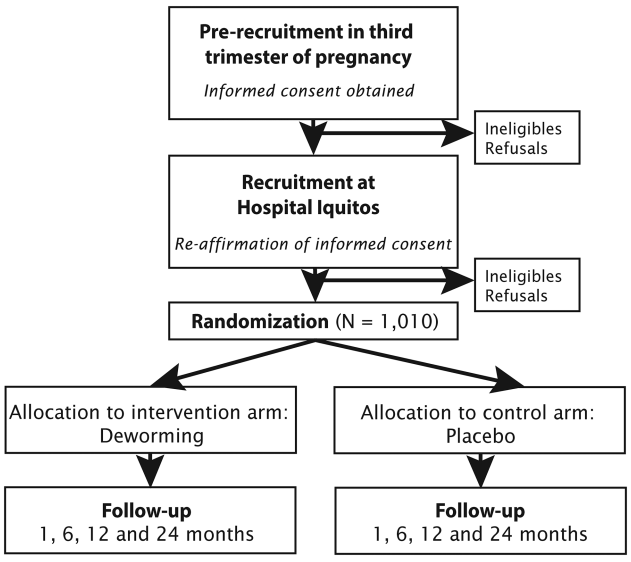

Figure 2 Flow diagram of the proposed randomised-controlled trial, including recruitment and follow-up. Duration of follow-up is 24 months.

Baseline assessments

Following informed consent procedures, research assistants will administer a standardised questionnaire to participants to obtain baseline information, including socio-demographics (eg, age, residence), obstetric and medical history (eg, parity, pregnancy complications), intended breastfeeding practices (eg, expected duration, exclusivity) and environmental exposures (eg, water source).

Following delivery, research assistants will extract birth date from hospital registries, including APGAR scores, gestational age, delivery type and presence of complications.

Treatment allocation

Mother-infant pairs are normally discharged from the hospital within 24 h of a vaginal delivery and within 72 h of a caesarean-section. Research assistants on duty will bring the sequentially numbered treatment envelopes, containing the single tablet of the randomly allocated intervention, to the maternity ward. Once women have their final medical examination and receive their hospital discharge papers, they will be visited by a research assistant at their bedside and receive the next numbered treatment envelope. The research assistant will directly observe ingestion of the tablet. Participants, research assistants, outcome assessors, data analysts, and coinvestigators will be blinded to the treatment allocation.

Outcome ascertainment

All mother-infant pairs will be visited in their home at the specified follow-up time points by a research assistant to ascertain primary and secondary outcomes

(figure 2). Data will be recorded on an application using mobile tablets, where collection and entry occurs in real time with automated question jump patterns, validation and prompts to research assistants.

Anthropometric measurements

Weight will be measured in duplicate at birth, 1, 6, 12 and 24 months using a portable electronic scale, accurate to the nearest 0.01 kg, calibrated daily using standard weights (Seca 354, Seca Corp., Baltimore, USA). Length will be measured in duplicate at birth, 1, 6, 12 and 24 months as recumbent crown-heel length on a flat surface using a measuring mat (Seca 417, Seca Corp., Baltimore, USA), accurate to the nearest millimetre. Both weight and length are measured in the unclothed infant. Head circumference (HC) will be measured in duplicate at birth, 1, 6, 12 and 24 months using a non-stretch Teflon measuring tape (Seca 212, Seca Corp., Baltimore, USA), accurate to the nearest millimetre. Mid-upper arm circumference (MUAC) will be measured in duplicate at 6, 12 and 24 months using a non-stretch measuring tape (UNICEF S0145620), accurate to the nearest millimetre. The mean of the first and second anthropometric measurements will be used in the analyses.

Infant morbidity

Research assistants will administer a questionnaire to the mothers at the 1, 6, 12 and 24 month study visits. The first component of the questionnaire is comprised of morbidity indicators modified from the IMCI Chart.[37] Mothers will be asked about episodes of diarrhoea, fever, cough and ear infection experienced by their infant in the previous 2 weeks. These indicators have been previously used for research purposes in developing countries.[17 43 47]

Breastfeeding practice and maternal energy levels

The second component of the questionnaire is designed to assess current breastfeeding practices (eg, duration of exclusive breastfeeding, timing of first introduction of complementary foods) and maternal fatigue. Questions on breastfeeding practice have been adapted from the WHO indicators for assessing infant and young child feeding practices.[48] Maternal fatigue is measured using the Multidimensional Assessment of Fatigue (MAF Basia Belza 1993, All rights reserved)[49] and the Fatigue Assessment Scale (FAS).[50]

Maternal haemoglobin

Maternal haemoglobin levels will be measured by research assistants at the 1, 6, 12 and 24-month home visits using a HemoCue machine, accurate to within 1.5% of the gold standard reference.[51] Blood for this test will be drawn from women by finger-prick using disposable lancets.

Maternal STH infection and intensity

Stool specimens will be obtained from women at baseline and at 6 months of follow-up in order to assess the prevalence and intensity of STH infections. In the case that a woman is unable to provide a stool specimen at the time of the visit, a small plastic container labelled with her unique study identification code will be left with her and a research assistant will return the following day for collection. Stool specimens will be transferred to the laboratory where these will be analysed using the Kato-Katz method by a trained microscopist. This technique is recommended for the assessment of STH prevalence and also to quantify the intensity of infection (ie, eggs per gram of stool).[52]

Substudy

During initial informed consent procedures, women will be asked if they would like to take part in a substudy to assess breast milk quality and quantity. Of those who agree to participate, a random sample of 200 mother-infant pairs will be selected. Breast milk quality and quantity will be assessed in the subsample at 1 and 6 months following delivery. Each time point will consist of six home visits over a 2-week period, on days 1, 2, 4, 5, 14 and 15.

Breast milk quality assessments

Quality of breast milk will be assessed by collecting a 50 mL milk sample from mothers. To standardise breast milk collection methods, every effort will be made to collect samples between 8:00 and noon to avoid extremes in diurnal variations.[34] Women will be assisted in providing a breast milk sample with a hospital-grade electronic breast pump. Collection will occur by pumping milk into a presterilised tube with a leak-proof seal from the breast from which the infant has not fed for 2 h. All milk samples will be transferred to the local laboratory on ice. Macronutrient assessment will be completed on the same day of collection using the MIRIS Human Milk Analyser (HMA, Miris, Uppsala, Sweden). This machine analyzes the macronutrient composition of breast milk, including fat, protein, lactose, energy and dry matter from a 1–3 mL sample in 60 s. The remainder of each sample will be divided into two presterilised tubes and stored at −80°C; these will then be transported on dry ice to Ghent University (Belgium) and to the Western Human Nutrition Research Center (WHNRC) (California) for micronutrient and immunological assessment. Breast milk quality indicators include: IgA, IgG, lactoferrin, lysozymes, vitamins A, B1, B2, B3, B6, B12, C and D, calcium, copper, iron, zinc, free fatty acids, triglycerides and phospholipids. At 1 month following delivery, the breast milk sample will be obtained on day 1 of the 2-week assessment. At 6 months following delivery, the breast milk sample will be obtained on day 15 of the 2-week assessment (figure 3).

Breast milk quantity assessments

Quantity of breast milk transferred from the mother to the infant will be measured using the 'dose-to-the-mother' deuterium-oxide turnover technique.[53 54] In addition to

optimising feasibility, efficiency and accuracy, this method is the most appropriate because it provides an estimation of water intake from both breast milk and non-breast milk sources.

On day 1, a baseline 3 mL urine sample will be collected from mothers and their infants. Subsequent to urine collection, mothers will receive a preweighed 10 g oral dose of deuterium oxide (99.8% purity), accurate to the nearest 0.001 g. Research assistants will return to participants' homes to collect four urine samples from mothers on days 2, 5, 14 and 15, and five urine samples from infants on days 2, 4, 5, 14 and 15 (figure 3). Mothers will be asked to provide a urine sample in a small plastic container during the home visit. Infant urine samples will be obtained using paediatric urine bags. Samples will be transferred into 3 mL cryogenic tubes and stored at −20°C. Frozen urine samples will be transported to the Instituto de Nutrición y Tecnología de los Alimentos (INTA) at the University of Chile for laboratory analysis using isotope ratio mass spectrometry.

Infant water intake from breast milk and non-breast milk will be calculated by fitting isotope data to a model for water turnover in mothers and infants, and transfer of milk from mother to infant using assumptions and equations described in previous studies.[53 55 56] Breast milk intake and total water intake will be expressed as g/day. This measure will represent the mean grams of breast milk transferred from mother to infant per day during each of the two 14-day assessment periods.

Statistical analyses

Descriptive analyses

Study flow, including participant consent and confirmation, recruitment, eligibility, and losses to follow-up will be depicted in a Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Continuous and categorical variables at baseline will be expressed as means (with SDs) and proportions, as appropriate, for description of the study population and to allow for comparisons in prognostic variables between intervention groups. Primary and secondary analyses will be performed according to the intention-to-treat principle.

Primary analysis

The primary outcome, infant weight gain (in grams), will be compared between the two intervention groups using a Student's t test. In addition to estimation of crude intervention effects, multivariable linear regression analysis will be used to compare infant mean weight gain between intervention groups, while adjusting for baseline covariates that were a priori determined to be important confounders in the published literature.[57] These include maternal age, parity, deworming in the past 6 months, intention to breastfeed, and infant gestational age.[58] Adjusted analyses will be reported as mean differences and 95% CIs.

Secondary analyses

Continuous outcomes will be modelled using multivariable linear regression and dichotomous outcomes will be modelled using multivariable logistic and log-linear regression methods. Log-linear regression will be used instead of logistic regression when the prevalence of the outcome is high (ie, >20%), as it is more appropriate for this situation. Secondary analyses will be adjusted for the baseline covariates mentioned above.

Sex-specific weight-for-age, weight-for-height, height-for-age, MUAC-for-age and HC-for-age z-scores will be calculated using WHO Anthro software (V.3.2.2, 2011) and macros.[59] Underweight, wasting and stunting will be defined as z-scores for weight-for-age, weight-for-height, height-for-age, respectively of <2 SDs from the median of the WHO reference population, according to the WHO Child Growth Standards.[60]

Subgroup analyses

To evaluate the presence of potential effect measure modification due to infant sex, birthweight, and birth length on deworming, interaction terms will be included in separate multivariable linear regression models with the primary outcome, infant mean weight gain. These analyses will examine if there appears to be effect measure modification by sex, birthweight and birth length, and if so, they will estimate the added value of the intervention in potentially vulnerable subgroups. They will also provide insight into whether deworming can improve growth trajectories separately for boys and

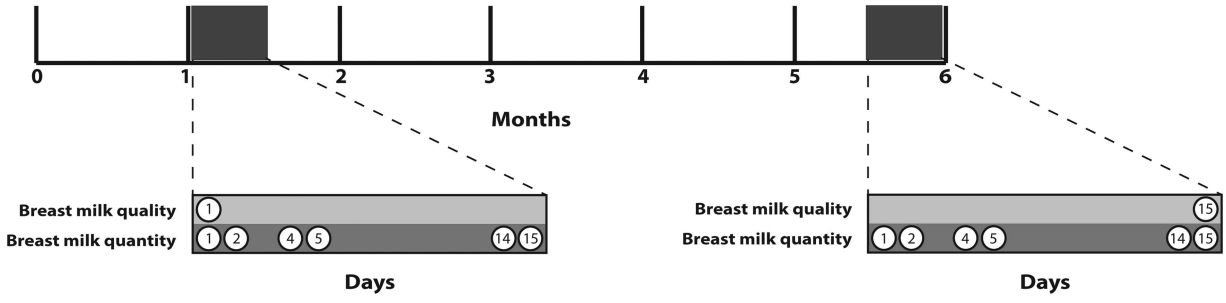

Figure 3 Timeline of data collection for milk quality and quantity in random sub-sample of participants. Each milk assessment is conducted over a 2-week period.

girls, as well as for infants born with low birthweight and birth length. If effect measure modification is found, results will be reported separately by subgroup.

DISSEMINATION

Trial registration

The trial is registered with clinicaltrials.gov (NCT01748929).

Information and informed consent

Prior to the onset of the study, meetings at health centres and the Hospital were held to inform the staff of the purpose of the trial, anticipated benefits and risks of participation, and study activities.

Written informed consent documents for participation in the trial were reviewed by the Research Ethics Boards of the McGill University Health Centre in Canada, and the Asociación Civil Impacta Salud y Educación and the Instituto Nacional de Salud in Peru. This document is written in Spanish at a basic literary level and describes the study with respect to its aims and expected contributions, the extent of participant involvement, potential benefits and inconveniences of participation, confidentiality, and the voluntary nature of initial and continued participation. During the informed consent procedure, research assistants will read the consent document to eligible women and their partners. At this time, women and their partners will have the opportunity to ask questions about the trial. Those who wish to participate in the study will be asked to provide consent by signature or fingerprint. For mothers/fathers under the age of 18 years, assent will be obtained and informed consent will be requested from their parent, guardian or spouse/partner over the age of 18 years. The Instituto Nacional de Salud in Peru requires consent from both mothers and fathers for infant participation in RCTs. Women who do not have a partner or whose partner is absent for an indefinite period of time (eg, works outside of Iquitos) will be asked to sign another document to declare the father's absence, in accordance with Peruvian ethics guidelines. Following informed consent, research assistants will administer a short 10-question evaluation to the mothers and their partners to confirm their comprehension of the document. If the mother/father responds incorrectly to a statement, the research assistant will reinforce the contents of the informed consent document.

Trial oversight

The Trial Steering Committee consists of the principal investigator and all coinvestigators. This committee will review the study protocol, ensure the trial is being conducted in accordance with the principles of good clinical practice, and appoint three international experts with expertise in deworming, biostatistics, or clinical trial methodology to comprise the Data Safety and Monitoring Committee (DSMC). The DSMC will act in accordance with internationally recognised guidelines, review the protocol prior to study initiation, and evaluate study conduct and the occurrence of adverse events at specific time points throughout the trial (ie, after 50% and 100% of recruitment, and after the completion of each follow-up visit).

Dissemination of results and data access

The results of this trial will be published in peer-reviewed journals, presented at various national and international fora, and communicated to international agencies, such as the WHO, who have taken a leadership role in developing global health policies pertaining to deworming in high-risk population groups. Following completion of the study, project data will initially be used by the research team to prepare the manuscripts and other standard scientific dissemination products. The final trial data set will be available for consultation and available via direct requests to the principal investigator (TWG).

Author affiliations
[1]Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montréal, Québec, Canada
[2]Research Institute of the McGill University Health Centre, Division of Clinical Epidemiology, Montréal, Québec, Canada
[3]Asociación Civil Selva Amazónica, Iquitos, Peru
[4]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland
[5]Département d'obstétrique et de gynécologie, Université de Sherbrooke, Sherbrooke, Québec, Canada
[6]School of Dietetics and Human Nutrition, McGill University, Ste. Anne-de-Bellevue, Québec, Canada
[7]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[8]USDA, ARS Western Human Nutrition Research Center, University of California, Davis, California, USA

Contributors All authors made a significant contribution to the formation and design of the study protocol. The protocol was written by LSM and TWG and was critically reviewed by MC, AM, ER, WDF, GSM, JV, and LHA. All authors gave approval for publication.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics approvals for protocol V.2.0 (26 August 2013) have been obtained from the Research Ethics Boards of the McGill University Health Centre in Canada; and the Asociación Civil Impacta and the Instituto Nacional de Salud (Peruvian Institute of Health) in Peru.

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

Data sharing statement Following completion of the study, project data will initially be used by the research team to prepare the manuscripts and other standard scientific dissemination products. The final trial data set will be available for consultation and publically available via direct requests to the principal investigator.

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