RANDOMIZED CONTROLLED TRIAL OF MEBENDAZOLE PLUS IRON SUPPLEMENTATION VERSUS PLACEBO PLUS IRON SUPPLEMENTATION DURING PREGNANCY

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SHORT TITLE PROPOSED

A double-blind randomized controlled trial of antenatal mebendazole

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ABSTRACT

OBJECTIVE The aim of the study was to assess the effectiveness of antenatal mebendazole plus iron supplementation versus placebo plus iron supplementation on birthweight in a highly hookworm-endemic area.

METHODS This study was a double-blind randomized controlled trial set in rural and peri-urban communities in the Peruvian Amazon region. A total of 1042 second trimester pregnant women between the ages of 18 and 44 years were recruited from April to November 2003, and followed to July 2004. Women were randomly assigned to receive either mebendazole (500 mg single dose) plus iron supplements (60 mg elemental iron daily) or placebo plus iron supplements. The primary outcome was mean infant birthweight and secondary measures included proportion of low birthweight (<2500g) and maternal anemia. Adverse birth outcomes were also recorded.

RESULTS The prevalence of hookworm infection was 47.5%. There were no differences between intervention groups in mean birthweight (3104 g vs 3090 g, p=0.629), proportion of low birthweight (8.1% vs 8.7%, p=0.755) or maternal anemia in the 3rd trimester (33.0% (158/479) vs 32.3% (152/471), p=0.815). However, the proportion of very low birthweight (<1500g) was significantly lower in the mebendazole group (0% (0/479) vs 1.5% (7/471), p=0.007). No statistically significant difference (p=0.664) in adverse birth outcomes (miscarriages, malformations, stillbirths, early neonatal deaths and premature babies) was found between the mebendazole group and the placebo group (28 versus 31, respectively).

CONCLUSION This trial provides additional evidence for the use of anthelminthics, over and above iron supplementation, within antenatal care programs in hookworm-endemic areas.

RESUME

OBJECTIF: L'objectif de cette recherche était de mesurer l'efficacité d'un traitement prénatal de mebendazole plus suppléments de fer versus un placebo plus suppléments de fer sur le poids à la naissance dans une zone fortement endémique en ankylostomes.

METHODES: Cette étude était un essai clinique randomisé à double insu qui prenait place dans des communautés rurales et péri-urbaines de la région Amazonienne du Pérou. Un total de 1042 femmes enceintes dans leur deuxième trimestre, entre 18 et 44 ans, ont été recrutées d'avril à novembre 2003, et furent suivies jusqu'à juillet 2004. Les femmes ont été assignées aléatoirement à un traitement de mebendazole (500 mg dose unique) plus suppléments de fer (60 mg de fer élémentaire par jour) ou à un placebo plus suppléments de fer. Le résultat primaire était le poids moyen à la naissance et les résultats secondaires incluaient les proportions de faible poids à la naissance (<2500g) et anémie maternelle. Les effets adverses chez le bébé à la naissance ont aussi été notés.

RESULTATS : La prévalence d'infection d'ankylostome était de 47.5%. Il n'y avait aucune différence entre les groupes d'intervention sur le poids moyen à la naissance (3104 g vs 3090 g, p=0.629), sur la proportion de faible poids à la naissance (8.1% vs 8.7%, p=0.755) ou sur l'anémie maternelle dans le $3^{\text{ième}}$ trimestre (33.0% (158/479) vs 32.3% (152/471), p=0.815) entre le groupe mebendazole versus le groupe placebo, respectivement. Cependant, la proportion de très faible poids à la naissance (<1500g) était significativement plus basse dans le groupe mebendazole (0% (0/479) vs 1.5% (7/471), p=0.007). Aucune différence statistiquement significative (p=0.664) a été trouvée entre le groupe

mebendazole et placebo en ce qui concerne le nombre de fausse-couches, malformations, mort-nés, mortalité néonatale et prématurité (28 versus 31, respectivement).

CONCLUSION : Cet essai apporte des preuves additionnelles pour l'utilisation des anti-helminthiques, en plus des suppléments de fer, à l'intérieur des programmes prénataux dans les régions endémiques en ankylostomes.

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DEDICATION

To Renato and Ema

CONTRIBUTIONS OF THE CO-AUTHORS

Renee Larocque MSc (RL) wrote this thesis and was the principal author of five of the seven manuscripts (five manuscripts are in the thesis (Manuscripts A-E) and two are in the Appendices (Manuscripts F and G). For the five manuscripts included in the thesis, RL was directly involved in the study design, implementation and execution of the research project and participated in obtaining funding and approval from ethics review committees both in Canada and Peru. She performed all of the data analyses for all five manuscripts. On site (Iquitos, Peru) she acted as study director and was responsible for data collection, oversight of quality control and management of the research team. **Theresa W. Gyorkos PhD**, (co-author on all manuscripts) was the principal investigator of the research project and RL's thesis supervisor. She contributed greatly in every step of the implementation of this project: from initial study design and funding application to dissemination of the results. She visited the study site on many occasions in order to monitor the work done and was accessible daily via e-mail. **Dr. Eduardo Gotuzzo MD**, (Manuscripts B, C, D, F and G) director of the Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia (Lima) and **Dr. Martin Casapia MD**, (Manuscripts B, C, D and G) director of the "Asociacion Civil Selva Amazonica" (Iquitos), were the Peruvian-based co-investigators on the research project. They worked on the design, facilitated the implementation of the study and participated in the process of manuscript development and publication. Their help was essential in obtaining mebendazole tablets and for arranging ethical review and approval by Peruvian government health authorities. **Dr. J. Dick MacLean MD** (Manuscript C) and **Dr. Julio Soto MD, PhD** (Manuscript C), who were members of my thesis committee, participated in the clinical aspects of the development of the study design as well as with reviewing the manuscript. **Dr. Elham Rahme PhD,** (Manuscripts C and E) also a member of my thesis committee, guided me through the statistical analysis of the data and reviewed the manuscripts for publication.

Hannah Shenker BSc, (Manuscript F) a Canadian medical student, and **Rosario Ugaz MD**, (Manuscript F) a Peruvian medical resident, participated in the data collection for this manuscript and assisted in its preparation for publication.

ORIGINALITY STATEMENT

In 1994, the World Health Organization (WHO) formulated their recommendation on antenatal deworming based on limited empirical evidence from observational studies of non-pregnant populations and expert opinion. Expert opinion is primarily collected through informal consultations where prominent researchers from around the world are invited to discuss and reach consensus on a particular issue. Therefore, even though WHO already recommends the use of antenatal deworming, this randomized controlled trial will fill an important gap in knowledge.

In the published literature, only two observational studies have examined the effects on birthweight of the use of albendazole or mebendazole during pregnancy. Additionally, three studies (one randomized trial and two non-randomized trials) have examined the effects of deworming on maternal anemia. The only randomized controlled trial (RCT) of a benzimidazole conducted to date had a small sample size; infant outcomes were not measured; and sub-standard iron supplement concentrations were administered.

There has been no randomized controlled trial examining the effect of antenatal anthelminthic treatment added to iron supplements on infant birthweight. In addition, this trial is the largest trial to date of a benzimidazole administered to pregnant women examining both infant and maternal outcomes.

There are three observational studies that reported on adverse birth outcomes following the use of mebendazole. However, no RCT has yet reported details of adverse birth outcomes, especially on malformations, an additional novel contribution of our RCT.

The relationship between intensity of soil-transmitted helminth infections and anemia during pregnancy has previously been examined in two studies in highly malaria endemic areas (prevalence of 20-25%) of the world. Our RCT was different in the fact that the population was almost malaria free (< 2%). The relationship between intensity of soil-transmitted helminth infections and anemia described in this thesis is therefore not confounded by malaria and, as such, provides a unique contribution to existing scientific evidence.

Different summary measures (eg. arithmetic means and geometric means) have been reported in the literature to measure the intensity (eggs per gram) of intestinal helminth infection. However, these measures have limitations in their ability to estimate appropriately the true mean in the population. In this thesis, a simple alternative method is proposed: the weighted geometric mean. This method overcomes the limitations of other summary measures and could potentially change the way researchers summarize parasite intensities.

LIST OF ACRONYMS AND ABBREVIATIONS

95%CI	95% confidence interval
ACC/SCN	Sub-Committee on Nutrition of the Administrative Committee on Coordination
β	Beta
dL	decilitre
CIHR	Canadian Institutes of Health Research
CPHA	Canadian Public Health Association
CR	cure rate
CRH	corticotropin-releasing hormone
CVMP	Committee on Veterinary Medicinal Products
DALY	disability adjusted life years
DISA	Direccion de la salud (Peru)
ENDES	Encuesta Demographica y de Salud Familiar (Peru)
Epg	eggs per gram
ERR	eggs reduction rate
FRSQ	Fonds de la recherche en santé du Québec
g	gram
Hb	hemoglobin
HiCN	cyanmethaemoglobin
IDA	iron deficiency anemia
IFPRI	International Food Policy Research Institute
IGF	insulin growth factor

INACG	International Nutritional Anemia Consultative Group
IPPH	Institute of Population and Public Health (CIHR)
IUGR	intrauterine growth retardation
Kg	kilogram
L	litre
LBW	low birthweight
mg	milligram
MINSA	Ministerio de Salud (Peru)
OR	odds ratio
PAHO	Pan American Health Organization
post-D	post-delivery
®	registered
PRP	percentage reduction in prevalence
r	correlation coefficient
RCT	randomized controlled trial
r_s	Spearman correlation coefficient
SD	standard deviation
UN	United Nations
UNICEF	United Nations Children's Fund
USD	United States dollar
TM	trade mark
VLBW	very low birthweight
WHO	World Health Organization
X^2	chi square

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1 INTRODUCTION

Anthelminthics have been used widely for deworming in different populations but mostly in school-aged children. Until recently, pregnant women had been excluded from mass treatment programs for the prevention and control of helminth infections. It is known that hookworms cause blood loss and are stongly associated with lower hemoglobin levels. WHO, recognizing that pregnancy is a time of higher iron requirements, examined the benefits and risks of using anthelminthics in this population. WHO, following the 1994 WHO Informal Consultation on Hookworm Infection and Anemia in Girls and Women, recommended that a single oral dose of anthelminthic treatment (albendazole, levamisole, mebendazole or pyrantel), within a mass treatment strategy (and following a rapid assessment of prevalence (screening) in a small proportion of the population) be administered to pregnant women living in hookworm-endemic areas (> 20% prevalence), but, as a general rule, that these drugs be given after the first trimester (WHO, 2006; 1996a). This recommendation was formulated from expert opinion and evidence from published studies using anthelminthics in non-pregnant populations.

In human studies, to date, five have examined the benefits in pregnant women and infants following the use of albendazole or mebendazole during pregnancy (Christian *et al.*, 2004; Torlesse and Hodges, 2001; Abel *et al.*, 2000; de Silva *et al.*, 1999; Atukorala *et al.*, 1994); while three studies have examined the risks of adverse birth outcomes following the use of albendazole or mebendazole (Acs *et al.*, 2005; Diav-Citrin *et al.*, 2003; de Silva *et al.*, 1999).

Only two of these studies have used birthweight as an outcome (Christian *et al.*, 2004; de Silva *et al.*, 1999) and only one study was a randomized controlled trial (Torlesse and Hodges, 2001). However, this trial was of very small sample size (N=125 divided into 4 arms).

Despite WHO's recommendation, to our knowledge, only two countries currently include anthelminthics within government-sponsored antenatal care programs: Sri Lanka and Nepal. It is believed that the lack of scientific evidence on the benefits of deworming during pregnancy on birth outcomes, such as birthweight, and on the risk of adverse birth outcomes, such as malformations, from well-designed randomized controlled trials, may be one of the reasons for the still infrequent use of deworming during pregnancy in developing countries. WHO is often asked to make recommendations in the absence of a strong body of empirical evidence, which was the situation with anthelminthic use during pregnancy. Therefore, even though WHO already recommends the use of anthelminthics during pregnancy, there is still a gap in knowledge on the effects of hookworm infections in pregnant women and its treatment. A need for randomized trials of antenatal anthelminthics has been emphasized by the findings of observational studies and the scientific community.

We, therefore, designed a randomized controlled trial in a study population of pregnant women in Iquitos, Peru, a hookworm-endemic area. Women were randomly assigned to receive either mebendazole (500 mg single dose) plus iron supplements (60 mg elemental iron daily) or placebo plus iron supplements and outcomes such as birthweight and maternal anemia were measured and adverse birth outcomes recorded.

2 LITERATURE REVIEW

2.1 Hookworm infection

2.1.1 Life cycle and epidemiology

Hookworm infection is of public health importance in many developing countries. There are two species of human hookworm, *Necator americanus* and *Ancylostoma duodenale*. The distribution of these species overlap, therefore, many people suffer from mixed infections. Hookworm infections are frequently found concurrently with *Trichuris trichiura* and *Ascaris lumbricoides* infections. Because each of these parasites' life cycle involves a soil stage, they are commonly referred to as the soil-transmitted helminths.

Hookworm infection is acquired by penetration of the infective third-stage larvae through the skin (*A. duodenale* may also be acquired orally). After entering the skin, the larvae migrate via the lymphatic system and the blood stream to capillaries in the lungs from where they will then ascend the bronchioles, bronchi and trachea to finally be swallowed and reach the small intestine. Here the larvae will molt into adult worms. Six to eight weeks will pass from the time the infective larvae penetrate the skin until they reach sexual maturity and mate. Each adult female hookworm will produce thousands of eggs per day. Hookworm eggs exit the body in the feces and contaminate the external environment. Outside the human host, the eggs will hatch within 24-48 hours if in adequate conditions (warm, humid and shady); they will then develop into first-stage larvae and after two molts they will develop into infective third-stage larvae. Infection is diagnosed by detecting hookworm eggs in human stool samples by microscopic examination.

In the human host, adult hookworms attach to the intestinal wall and cause blood loss directly through ingestion of, and mechanical damage, to the mucosa, and indirectly, by affecting the supply of nutrients necessary for erythropoiesis (Crompton, 2000; Banwell and Schad, 1978). Therefore symptoms of this infection will vary in proportion to the degree of blood loss caused by the worms.

An estimated 1,298 million people harbor hookworm infections; 1,049 million are infected with *Trichuris trichiura* and approximately 1,472 million are infected with *Ascaris lumbricoides* (Crompton and Nesheim, 2002). Extending Murray and Lopez's approach to calculating DALYs (disability-adjusted life years lost) for specific parasite infections, Chan *et al.* (1997) have estimated that the impact of hookworm infection is 22.1 million DALYs, *Trichuris,* 6.4 million and *Ascaris,* 10.5 million. When taken together, the total number of DALYs for these three soil-transmitted helminth infections totals approximately 39 million, which exceeds that estimated for malaria (35.7 million) (Murray and Lopez, 1994).

The prevalence of hookworms has been reported to reach as high as 80% in rural environments in the tropics where soil contamination results from lack of adequate hygiene, sanitation and waste management (Stoltzfus *et al.*, 1997b). The prevalence of hookworm infection increases with age, typically reaching its peak in late adolescence/early adulthood and then maintaining this level throughout adulthood. The intensity of infection on average is found to be higher in adults than children (Bundy *et al.*, 1990). *Trichuris* and *Ascaris*, unlike hookworms, have their prevalence and intensity peak during childhood. Almost every aspect of the health impact of soil-transmitted helminths is related to the intensity of infection; the greater an individual's worm burden, the greater the associated morbidity. Especially in hookworm infection, the degree of severity of morbidity varies not only according to the number of worms present but also according to host age, hookworm species and nutritional intake of iron (Crompton and Nesheim, 2002).

Which hookworm species causes infection can be important since blood losses have been determined to be much higher in infections of *Ancylostoma duodenale* (up to 0.15 ml/worm/day) than in those of *Necator americanus* (up to 0.03 ml/worm/day) (Stephenson, 1987). In 1998, WHO proposed a classification for intensities for each soil-transmitted helminth infection (based on quantitative egg counts obtained using the Kato-Katz method) (WHO, 1998) (Table 1). The WHO-defined intensity classes for hookworm were based on data published by Stoltzfus *et al.* (1996) using fecal blood loss as measured in Zanzibari children infected mainly with *N. americanus*.

Table 1. Thresholds of intensity for soil-transmitted helminth infections as suggested by WHO (1998)

	Light intensity infection	Moderate intensity infection	Heavy intensity infection
A. lumbricoides	1-4,999 epg	5,000-49,999 epg	≥50,000 epg
T. trichiura	1- 999 epg	1,000- 9,999 epg	≥10,000 epg
Hookworms*	1-1,999 epg	2,000- 3,999 epg	≥ 4,000 epg

*The hookworm categories are based on the fecal loss of hemoglobin in African children infected mainly with *N. americanus* (Stoltzfus *et al.*, 1996).

2.1.2 Hookworm and anemia

In developing countries, both nutritional deficiencies and parasitic infection, specifically hookworm infection, contribute most to anemia. In fact, hookworm infections are recognized as the leading cause of pathological blood loss in tropical and sub-tropical countries (Pawlowski *et al.*, 1991). It has been estimated that, on average, blood losses into the feces (measured as fecal hemoglobin) increase by 0.825 mg per gram of feces for every increment of 1,000 hookworm eggs per gram (epg) in Zanzibari school-aged children (Stoltzfus *et al.*, 1996).

Numerous studies have found a significant correlation between lower hemoglobin (Hb) levels and higher fecal egg counts of hookworm (Brooker *et al.*, 1999; Olsen *et al.*, 1998; Stoltzfus *et al.*, 1997a; Lwambo *et al.*, 1992; Latham *et al.*, 1983; Shield *et al.*, 1981). Stoltzfus and colleagues (1997a) reported that the relationship between hookworm infection and Hb concentration may be apparent only above a threshold worm burden. However, studies in the past have demonstrated that it is impossible to determine exactly which hookworm intensity level universally causes anemia. For hookworm infection the degree of severity of morbidity varies with age, hookworm species and nutritional status, and therefore WHO has not defined fixed thresholds (WHO, 1998).

Foy and Nelson (1963) had reported that only high hookworm intensities (>100 *Necator* worms) had an impact on hemoglobin, while Pritchard (1991) (in Papua New Guinea) and Hercberg *et al.* (1986) (in Benin) found no significant relationship between egg count and Hb level. These results contrast with the findings of Brooker *et al.* (1999), Olsen *et al.* (1998) and Latham *et al.* (1983) (all three in Kenya) who showed that even light hookworm infections (as low as 200 epg) contributed significantly to low hemoglobin in Kenyan adults. Others have identified the hookworm intensities where anemia occurs to be between 2500 and 8000 epg (Lwambo *et al.*, 1992 (in Thailand, Kenya, Venezuela and Nigeria); Shield *et al.*, 1981 (in Papua New Guinea)). Stoltzfus *et al.* (1997a) found that the intensity of hookworm infection was the strongest explanatory variable for both Hb and serum ferritin in Zanzibari schoolchildren. Furthermore, they reported that 73% of severe anemia was attributable to hookworm infection.

2.1.3 Hookworm and anemia in pregnancy

Persons most at risk from hookworm morbidity are those with impaired iron status or iron-deficiency anemia. Pregnant women have a higher risk of developing iron deficiency because of the higher iron requirements during pregnancy (Stoltzfus *et al.*, 1997b).

Bundy and colleagues (1995) have estimated that approximately one-third of all pregnant women in developing countries are infected with hookworm. It has been suggested that the impact of hookworm infection on maternal morbidity 'may well be very considerable' (Partnership for Child Development, 1997). Hookworm-attributable anemia, induced by deficiencies of iron, total energy, protein and possibly folate and zinc, is a significant cause of intrauterine growth retardation and low birthweight (Stephenson *et al.*, 2000; Cline *et al.*, 1984). It has been suggested that the likely causative pathway involves appetite inhibition resulting in low pregnancy weight gain (Cline *et al.*, 1984), but a comprehensive understanding is still lacking.

In 1996, Shulman *et al.* (1996) reported that pregnant women with higher hookworm egg counts were significantly more anemic, with egg counts tending to be higher in multigravidae. Results from a case-control study (N = 479) undertaken in Nepal indicated that the prevalence of hookworm infection was associated with a significantly increased risk of severe anemia during pregnancy (OR = 5.43; 95%CI: 1.20, 24.61) (Bondevik *et al.*, 2000). Also in Nepal, a cross-sectional study of 336 pregnant women found that hookworm intensity was the strongest predictor of both low hemoglobin and serum ferritin (Dreyfuss *et al.*, 2000). These researchers suggested that hookworm infection (all intensities) was responsible for 54% of the cases of moderate to severe anemia.

More recently, a cross-sectional survey of Peruvian pregnant women living in the Amazon region found that, while prevalences of soil-transmitted helminth infections were not associated with anemia, higher intensity infections were (Larocque *et al.*, 2005). Pregnant women infected with moderate and heavy intensities of hookworm infection (OR = 1.84; 95%CI: 1.06, 3.17) and those with moderate and heavy intensities of both hookworm and *Trichuris* infections (OR = 2.13; 95%CI: 1.10, 4.13) were more likely to suffer from anemia than women having no or light intensity infections.

2.1.4 Drugs and pharmacokinetics

WHO (1996b) recommends four anthelminthic drugs in the treatment of soil-transmitted helminth infection: albendazole, levamisole, mebendazole and pyrantel. The benzimidazoles, mebendazole and albendazole, introduced in 1972 and 1983, respectively, are safe, effective and inexpensive broad-spectrum anthelminthics used in community-based helminth control programs. Of the WHO-recommended anthelminthics, only mebendazole and albendazole are offered in a single-dose format (mebendazole 500 mg and albendazole 400 mg). In its single-dose format, mebendazole and albendazole are similarily efficacious in the treatment of *A. lumbricoides* (cure rates (CR) 98-100%, egg reduction rates (ERR) 99-100%) and *T. trichuria* (CR 14-70%, ERR 80-89%) (WHO, 1996b). For the treatment of hookworms, albendazole (CR 57-100%, ERR 73-100%) is more efficacious than mebendazole (CR 22-30%, ERR 70-82%) (WHO, 1996b). It is known that mebendazole in its multiple-dose format is more efficacious than its single-dose format for all three soil-transmitted helminth infections (Bennett and Guyatt, 2000). Because of the ease of use of their single-dose format, the benzimidazoles are the drugs most widely used in mass treatment programs for helminth infections (Montresor *et al.*, 2002; Bradley and Horton, 2001; de Silva *et al.*, 1999).

A study in Mali reported failure of mebendazole in treatment of human hookworm (De Clercq *et al.*, 1997). It was suggested that one possibility for this failure was the development of resistance to the benzimidazoles. Albonico *et al.* (2002) also indicated that hookworms may be developing mebendazole resistance on Pemba Island as a result of intense exposure to the drug. Although reduced efficacy of the benzimidazoles has been reported, evidence of resistance has not been demonstrated by genomic analysis. Monitoring of anthelminthic drug efficacy to detect possible resistance is therefore a component of all deworming programs (WHO, 2002).

The detailed mechanisms of action of the benzimidazoles remain unclear; however experimental evidence shows that the parasites suffer from metabolic disruption at a number of different sites which are mostly implicated in the energy production of the parasite (Lacey, 1988). It is believed that the primary mode of action of these drugs is binding to beta-tubulin, thereby inhibiting the formation of microtubules and so stopping cell division (Lacey, 1990). One consequence is impairment of nutrient uptake by the parasite. These drugs are believed to have both larvicidal and ovicidal effects on the soil-transmitted helminths (Tracy and Webster, 1996).

Benzimidazoles have only limited solubility in water and are poorly absorbed from the gastrointestinal tract. The absorbed fraction is quickly metabolized by the liver and excreted in the bile. Plasma concentrations of mebendazole are detectable only at very high dosage (1.5 g) while the plasma concentration of albendazole is 15-49 times higher than that of mebendazole (WHO, 1996b).

While the initial animal-based studies testing these drugs in rats indicated some teratogenicity at high dosages, studies in rabbits, pigs, dogs, cats, sheep and horses did not show any teratogenetic effects (EMEA, 2001, 1999, 1997; van den Bossche *et al.*, 1982).

WHO (1996a), following the WHO Informal Consultation on Hookworm Infection and Anemia in Girls and Women, recommended that a single oral dose of anthelminthic treatment be given to pregnant women living in hookworm-endemic areas (> 20-30% prevalence), but, as a general rule, that these drugs be given after the first trimester.

Since the WHO recommendation on the use of anthelminthics in pregnancy, four studies in humans have examined adverse birth outcomes following the use of albendazole or mebendazole during pregnancy. (To avoid redundancy in the epidemiological details of these studies, a brief summary is presented here because further details are presented in Manuscript D.) A very large cross-sectional survey (N=7087) found that there was no statistically significant difference in proportions of major birth defects (1.8% vs 1.5%, p=0.39) after mebendazole intake compared to women who did not take any mebendazole (de Silva *et al.*, 1999). Moreover, proportions of combined stillbirths and perinatal deaths were lower in women who had taken mebendazole compared to women who had not taken any mebendazole (1.9% vs 3.3%, p=0.0004). Torlesse and Hodges (2001), who reported hemoglobin concentration during pregnancy after receiving albendazole therapy in a randomized controlled trial, did not examine adverse birth outcomes but do report one case of bilateral supernumerary fingers (a relatively common congenital abnormality in the study area). A prospective controlled cohort study in Israel found that there was no statistically significant increase in the rate of major birth malformations between women receiving mebendazole during their pregnancy compared to women who did not take any mebendazole (3.3% vs 1.7%, p= 0.478) (Diav-Citrin *et al.*, 2003). It is important to note that in the group of pregnant women who had taken mebendazole, most had taken it during the first trimester of pregnancy (71.5%), which is a contraindication of its use (WHO, 2006; 1996a). Finally, in a matched case-control study undertaken in Hungary, among 22 843 women who had newborns or fetuses with congenital anomalies, 14 (0.06%) mothers had been treated with mebendazole compared to 14 (0.04%) mothers among the 38 151 mothers who had newborns without any defects (controls) (OR = 1.8; 95%CI: 0.7, 4.2) (Acs *et al.*, 2005).

2.1.5 Anthelminthics in pregnancy

In Manuscript A, the evidence in the medical literature on the use of anthelminthics during pregnancy is reviewed. Published articles of original data were retrieved and the question "Should deworming be included in antenatal packages in hookworm-endemic areas of developing countries?" was answered and future research questions proposed.

The results of this manuscript were presented at the Canadian Public Health Association Conference (2005) and the abstract was selected for the CIHR-IPPH/CPHA Dr. John Hastings PhD Student Award.

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2.1.5.1 MANUSCRIPT A

Should deworming be included in antenatal packages in hookworm-endemic areas of developing countries?

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Abstract

Background: WHO recommends antenatal (after the first trimester) deworming for pregnant women who live in areas where the prevalence of hookworm infection exceeds 20-30%. However, deworming has not been included within antenatal care packages in most developing countries.

Methods: A review of articles publishing original data identified primarily through Medline using subject heading terms and textwords for "deworming", "pregnant women", "hookworm", "anthleminthic", "anthelmintic", "albendazole", "mebendazole", "pregnancy" and their combinations was conducted. Bibliographies of retrieved articles were scanned to identify any additional relevant documents.

Results: Five articles examined the benefits of antenatal deworming. All provided evidence favourable to deworming, in terms of both maternal and infant outcomes. Comparison of outcome measures could be improved with a more standardized approach to outcome ascertainment and reporting.

Conclusion: The evidence base for the inclusion of deworming in antenatal care packages (after the first trimester) in hookworm-endemic areas is mostly observational in nature. Future research should be directed towards 1) strengthening the evidence base with empirical data from randomized controlled trials and 2) furthering our understanding related to government uptake of the WHO policy on deworming.

Introduction

Nutritional iron deficiency is probably the main cause of anemia in pregnancy; however intestinal parasite (worm) infections, especially hookworm infections, contribute to anemia by causing blood loss and by affecting the supply of nutrients necessary for erythropoiesis. Hookworm infections are considered to be a leading cause of pathological blood loss in tropical and sub-tropical countries.[1] At any one time, one third of all pregnant women in developing countries are estimated to be infected with hookworms.[2] In 1996, the World Health Organization[3] recommended that deworming (using either albendazole, levamisole, mebendazole or pyrantel) be given to pregnant women after the first trimester in areas where the prevalence of hookworm infection exceeds 20-30%. This recommendation was based primarily on expert opinion and published accounts of research in non-pregnant populations. However, despite this recommendation, routine deworming within government-sponsored antenatal care programs remains infrequent in most developing countries. Our objective therefore was to review published evidence from research conducted in pregnant populations in order to document the specific benefits and/or risks of antenatal deworming.

Methods

A Medline search was carried out to identify reports of original data published between January 1966 to February 2006 using subject heading terms and textwords for "deworming", "pregnant women", "hookworm", "anthelminthic", "anthelmintic", "albendazole", "mebendazole", "pregnancy" and their combinations. The search made no restriction to language of publication, but only papers published in English, French or Spanish were read fully. No other restrictions were made. Further references not captured by this search were sought from the bibliographies of the retrieved articles.

Results

A total of five studies have examined the benefits of antenatal deworming in developing countries (Table 1). Three were observational studies (two investigating mebendazole (both in Sri Lanka)[4,5] and one, albendazole (in Nepal)[6]); one was a pre-post experimental study (mebendazole) (in India)[7]; and one was a randomized controlled trial of albendazole (in Sierra Leone)[8].

One of the first studies to investigate micronutrient supplementation and deworming in pregnant women was reported in 1994 in Sri Lanka among 115 pregnant women working on a tea plantation.[4] The government of Sri Lanka had included deworming in addition to iron-folate supplementation as a routine component of its antenatal care program. In the program, pregnant women received iron and folate in their first trimester and were offered a single dose of mebendazole in their second trimester. An evaluation of this program found that receiving the combination of mebendazole with iron-folate supplementation was more effective in improving women's iron status during pregnancy than receiving iron-folate supplementation alone. One limitation of this study was that its sample size was small.

In 1996, again in Sri Lanka, the effect of mebendazole treatment during pregnancy on birth outcome was investigated through a cross-sectional study.[5]

The reported combined proportions of stillbirths and perinatal deaths (1.9% vs 3.3%) were significantly lower in the mebendazole group, as was the proportion of very low birthweight babies (< 1500g) (1.1% vs 2.3%). The sample size was very large (N=7 087). The information about mebendazole intake had been assessed by a questionnaire administered to the mothers on the second (for vaginal deliveries) or third day (for cesarean sections) post-partum. Although several biases could have been operating (recall bias, report bias, selection bias), this study provided meaningful evidence of the potential benefits of deworming during pregnancy. An even greater effect might have been demonstrated if the analysis had included a comparison of all low birthweight infants (<2500g), but these data were not published.

A community-based study was carried out in India.[7] The intervention group received an education program focusing on anemia, iron supplementation (60 mg elemental iron), and 100 mg mebendazole tablets taken twice daily for 3 days. It was reported that the control group received the usual government-sponsored prophylaxis program but this program was not described. (It is likely that it would not have included deworming.) Therefore, interpretation of these results is difficult. The authors reported a significant decrease in the prevalence of anemia and increased mean hemoglobin in both the second and third trimester in those receiving the education plus iron plus mebendazole intervention.

Subsequently, a randomized placebo-controlled trial was conducted in Sierra Leone to measure the impact of a single dose (400 mg) of albendazole and daily iron-folate supplements (36 mg iron and 5 mg folate) on hemoglobin concentration during pregnancy.[8] After controlling for baseline hemoglobin concentration (obtained during the first trimester), the mean benefit of deworming relative to the placebo group on the change between baseline and third trimester was 6.6 g/L hemoglobin. In addition, it was found that the combined effect of deworming and iron-folate supplements was additive. These findings suggested that deworming could be included in strategies to control anemia during pregnancy in Sierra Leone and in other developing countries. Unfortunately, this trial was limited by a small sample size (N=125; 4 arms; between 29 and 35 women per arm) and the lower than standard amount of elemental iron (ie. 36 mg instead of 60 mg).[9]

Finally, within a micronutrient randomized trial conducted in 30 villages in Nepal, a 400 mg single dose of albendazole was offered to pregnant women at mid-gestation and again at late gestation.[6] Women receiving albendazole in the second trimester were found to have increased hemoglobin levels and a lower proportion of severe anemia in the third trimester. The birthweights of infants born to women who had received two doses of albendazole were higher by 59 g (compared to women who had not taken the albendazole). It was also reported that infant mortality at 6 months fell by 41%, however, this number must be used carefully because of small numbers. It is also important to add that these results, while adjusted for micronutrient supplement intake, were not randomized to intake of albendazole.

Two additional studies in Israel and in Hungary have examined adverse birth outcomes following the use of mebendazole during pregnancy.[10,11] These studies, in addition to the five mentioned above, did not find significantly more adverse outcomes in the dewormed group compared to the comparison group.

It should be noted that these reports provide different outcome measures and so a rigorous and direct comparison of outcomes cannot be undertaken. Efforts to use standardized outcome measures would be helpful.

Discussion

The results of this review provide evidence of the benefits of antenatal deworming both for the mothers and for the infants. In addition, there were no reports of increased proportions in adverse birth outcomes in infants of mothers who had been dewormed compared to mothers who had not. Therefore, cumulative evidence to date, which also includes policy statements from WHO based on several Informal Consultations over a number of years, with concurring policy uptake by other agencies such as Unicef, would suggest that there is convincing evidence for the inclusion of deworming in antenatal care packages (after the first trimester) in any country where hookworm infection is of public health importance.

The deworming drugs, albendazole and mebendazole, have several features which make them attractive to their inclusion within government-sponsored antenatal programs. First and foremost, they are inexpensive, at less than .02 $ USD per tablet in most countries.[12] Iron supplementation, which has been estimated at $21.39 USD per pregnant woman per year in South America, has been estimated to be significantly more expensive than deworming.[13] Additionally, areas participating in the Global Programme for the Elimination of Lymphatic Filariasis receive albendazole free-of-charge.[14] Finally, its distribution and administration is simplified by the fact that they are available in single dose format, and do not require weights for dosing.

At the present time, only Sri Lanka and Nepal have included deworming in their routine antenatal care programs. The weakness of the evidence base may have been one reason why uptake of this policy by other governments has been hindered. Future research therefore should be directed towards 1) strengthening the evidence base with empirical data from randomized controlled trials and 2) furthering our understanding of barriers and of enabling factors related to government uptake of the WHO policy on deworming.

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Table 1: Studies that have examined the benefits of deworming in antenatal care in developing countries and their main results (based on Medline search 1966-2006).

Study, date, location	Study design	Deworming drug	Sample size	Deworming-attributable result
Atukorala *et al.* 1994 Sri Lanka	Non-randomized effectiveness trial	Anthelminthic (probably mebendazole)	115	Increase in women's iron status
de Silva *et al.* 1999 Sri Lanka	Cross-sectional survey	mebendazole	7087	Decrease in proportions of stillbirths, perinatal deaths and very low birthweight
Abel *et al.* 2000 India	Pre-post experimental community-based	mebendazole	828	Decrease in prevalence of anemia; increase in mean hemoglobin in second and third trimesters

	study			
Torlesse and Hodges 2001 Sierra Leone	Randomized placebo-controlled factorial trial	albendazole	125 (4 arms)	Decrease in hemoglobin lowest in albendazole group; effects of albendazole and iron-folate supplements additive
Christian *et al.* 2004 Nepal	Non-randomized community-based study	albendazole	3325	Decrease in severe anemia in third trimester; increase in birthweight and decrease in 6-month infant mortality after 2 doses

2.2 Anemia

2.2.1 Description of anemia and its measurements

Anemia is regarded worldwide as a matter deserving of sustainable public health intervention. Anemia is defined as low hemoglobin (Hb) concentration and in pregnant women Hb levels less than 110 g/L constitute anemia (WHO, 1996a). Anemia is usually caused by iron deficiency, which is the most common nutrient deficiency in the world (Hallberg, 2001); however, other nutrient deficiencies can also impair Hb synthesis, such as vitamin A and folate. Iron is essential for the production of Hb, which is used to deliver oxygen from the lungs to body tissues, transport electrons in cells and synthesize iron enzymes that are required to utilize oxygen for the production of cellular energy.

The reduction of iron in the body has three main stages: (1) iron depletion; the earliest evidence of iron deficiency is the decrease of iron stores without a decline in the level of functional iron compounds; (2) iron deficient erythropoeisis; iron storage is depleted and there is insufficient iron absorption to counteract normal body losses even though there is no detectable anemia yet, and (3) iron deficiency anemia; the third and final stage is characterized by a decrease in functional iron and the onset of clinical symptoms (Hoffman *et al.*, 2005). Iron deficiency anemia produces the signs and symptoms common to all anemias which are pallor, headache, weakness, dizziness, easy fatigability, breathlessness, heart palpitations, difficulty concentrating and difficulty sleeping.

Functional consequences of iron deficiency are the result of inadequate oxygen delivery. These consequences are impaired mental development and physical coordination in infants, poor school performance in school-aged children and a restricted activity to execute tasks requiring physical activity at all ages (Akman *et al.*, 2004; Kordas et al., 2004; Beard, 2003; Beard *et al.*, 1988).

Iron deficiency, anemia and iron deficiency anemia are sometimes mistakenly used interchangeably. Iron deficiency (without anemia) is defined as a reduction in total body iron (also referred to as reduction in iron stores) owing to a prolonged imbalance between iron supply and iron requirements; however the deficit in iron is not sufficiently large to decrease the hemoglobin below the normal level. Although several indicators (eg. plasma transferrin concentration, serum transferrin saturation, erythrocyte protoporphyrin and soluble transferrin receptor concentration) can be used to provide an accurate diagnosis of iron deficiency, it is common to use serum ferritin, a protein that stores iron in the body, to measure the depletion of iron storage. Serum ferritin gives a quantitative estimate of the size of the iron stores (Cook *et al.*, 2003). Serum ferritin levels may be affected by factors other than iron stores such as infections or inflammatory disorders. Therefore, the use of serum ferritin as an indicator of iron stores must be carefully considered in each study population.

Anemia is the most easily identifiable manifestation of functional iron deficiency. It appears when hemoglobin concentrations fall below age and lifestage thresholds. The only methods generally recommended for use in surveys to determine the population prevalence of anemia by hemoglobinometry are the cyanmethemoglobin method and the Hemo-Cue® system. The cyanmethemoglobin method is the best laboratory method for the quantitative determination of hemoglobin and serves as a reference for comparison and standardization of other methods (WHO, 2001). The Hemo-Cue system®, which consists of a portable device, is a reliable quantitative method for measuring hemoglobin concentrations in the field in an easy and simple way using only a fingerprick blood sample.

Iron deficiency anemia is diagnosed on the basis of a combined reduced hemoglobin concentration and abnormal values of the indicators for iron deficiency (low serum ferritin), indicating depleted iron stores (Weiss and Grodeuk, 2005).

2.2.2 Epidemiology of anemia

WHO estimates that about 2 billion individuals or about 40% of the world's population suffer from anemia (WHO, 2000). It has been estimated that, at any one time, in developing countries, half of the population (mainly children and women of reproductive age) are affected by anemia (Hercberg and Galan, 1992). The average prevalence of anemia in pregnant women in developing countries is estimated to be 56% compared to 18% in industrial countries (WHO, 2000). In developing countries, prevalences of anemia in pregnant women range from 35% to 75% (WHO, 1992). In Peru, 35% of women of childbearing age and 50% of pregnant women have been reported to have anemia while prevalences among preschoolers in Peru range from 27% to 53% (PAHO, 1994; Zavaleta *et al.*, 1993; WHO, 1992). A more recent cross-sectional study conducted in the Peruvian Amazon found a prevalence of anemia of 45% in the overall population (Roshanravan *et al.*, 2003). During pregnancy, approximately 75% of all anemias diagnosed are due to iron deficiency (Sifakis and Pharmakides, 2000). Lack of iron in the diet, especially in rural and poor communities is critical during pregnancy. In one rural community, Gyorkos *et al.* (2004) found that the available iron intake was only 17.8% of that required by a pregnant woman (in the 2nd trimester) in a rural area of Peru (see Appendix 1).

Inadequate absorption of dietary iron has been suggested to be the main explanation for the much higher prevalence of anemia in developing countries where diets are usually low in bioavailable iron (Zavaleta *et al.*, 2000). Also, in developing countries, the additional effects of parasite infection need to be considered (Crompton and Nesheim, 2002).

2.2.3 Anemia and pregnancy

Iron deficiency is most common in population sub-groups that are at peak rates of growth; namely, infants, young children and pregnant women (Stoltzfus *et al.*, 1997b). Pregnancy is a time during which the risk for developing iron deficiency anemia is greatest as iron requirements are substantially higher than average. Allen (1997) calculated that the demands for iron during pregnancy increase from 0.8 to 7.5 mg of absorbed iron per day while Stoltzfus *et al.* (1997b) estimated that the basal requirement of 0.7 mg of daily iron is augmented by an additional requirement of 2.14 mg of iron per day during pregnancy. It has been found that Hb concentration falls progressively during pregnancy until 35 to 36 weeks after which the Hb concentration remains fairly constant (Whittaker *et al.*, 1996).

WHO (1996a) estimates that women in developing countries may be pregnant for as much as one half of their reproductive lives, suggesting that women of reproductive age in these countries are at constant risk of anemia.

2.2.4 Iron supplementation in pregnancy

WHO (1998) recommends iron supplementation (60 mg elemental iron daily) for all pregnant women for six months in pregnancy and three months post-partum in areas where the prevalence of anemia is ≥ 40%. (Elemental iron is the amount of iron in a supplement that is available for absorption). In two meta-analyses of randomized trials of routine iron and folate supplementation versus routine iron supplementation only in pregnant women, it was found that Hb and iron status improved with iron doses around 100 mg/day (Mahomed, 2002; 2000). In a review on the effects of iron supplementation on maternal hematologic status in pregnancy, Sloan *et al.* (2002) reported that iron supplementation did raise hemoglobin levels and that its effects are dose-dependent. However, it is known that side effects are also dose-dependent (Solvell, 1970). A recent randomized study evaluated the effect of various dosages of iron (20, 40, 60 and 80 mg) and found that 40 mg was sufficient in preventing iron deficiency during pregnancy; however the population studied were healthy Danish pregnant women (Milman *et al.*, 2005). Results could be very different in developing countries where pregnant women are very often anemic before becoming pregnant. Daily supplementation has been found to be most effective for preventing anemia in India, especially severe anemia during pregnancy compared to weekly supplementation (Mukhopadhyay *et al.*, 2004).

2.2.5 Anemia and low birthweight

Anemia in pregnancy has been associated with low birthweight (<2500 g). Various researchers (Yazdani *et al.*, 2004; Malhotra *et al.*, 2002; Zhou *et al.*, 1998; Murphy *et al.*, 1986) showed that low birthweight (LBW) has a U-shaped distribution with higher proportions at both ends of the hemoglobin range. Zhou *et al.* (1998) in a cohort study (N=829) found that the risk of LBW in anemic women (<110 g/L) was tripled compared to the reference group (110-119 g/L). Malhotra *et al.* (2002) (N=447) observed a 10.5 fold (95%CI: 4.5, 24.5) higher risk of having LBW babies in women with severe anemia (defined in this study as <75 g/L). In a cohort study (N=629) in Pakistan, it was found that a LBW outcome in anemic pregnant women (<110 g/L) was 1.9 (95%CI: 1.0, 3.4) times higher than in non-anemic women (Lone *et al.*, 2004). Similarily, in a cohort study (N=405) in China, it was reported that the risk of LBW was 6.5 (95%CI: 1.6, 26.7) times greater among women with moderate anemia (<95 g/L) compared with those without anemia (≥120 g/L) (Ronnenberg *et al.*, 2004). It is important to note that the lack of standardization within the definitions of anemia and severe anemia make the interpretation of the cumulative evidence difficult.

Nonetheless, even though a positive association between anemia and low birthweight had been reported in the majority of studies, causality has been difficult to ascertain. Rasmussen (2001) undertook an extensive literature review in order to determine whether iron deficiency, iron-deficiency anemia or anemia was causally related to low birthweight or preterm birth. He highlighted the lack of appropriate experimental designs in the published studies (Rasmussen, 2001). Of the intervention studies examining birthweight as an outcome, most were conducted in populations with adequate hemoglobin and birthweight values, and, as such, provided little insight into anemia and LBW. Furthermore, no intervention studies taking gestational age into account had been conducted in developing countries or in populations of anemic women. Similarly, the Cochrane review of iron and folate supplementation in pregnancy reported that little information was available on the effect of iron supplementation on maternal or fetal outcomes, hence no conclusions could be drawn (Mahomed, 2002). Mahomed (2002) concluded that more trials were needed to establish the most appropriate strategies for reducing anemia in populations where it is a serious problem and to establish how anemia affects maternal and fetal outcomes.

Iron supplementation has consistently demonstrated positive effects on maternal iron status. However does iron supplementation have any effects on increasing birthweight? One of the first clinical trials was conducted in 1991 in Sri Lanka (Agarwal *et al.*, 1991). The researchers examined the effect on infant birthweight of two supplementation regimens given to pregnant women: those given daily iron (60 mg) and folic acid (500 μg) and those given no supplementation. Mean birthweight was found to be significantly higher in the treated group when compared to the control group (2.88 kg vs 2.59 kg, $p<0.01$). In addition, the proportion of low birthweight was significantly lower in the treated group compared to the control group (20.4% vs 37.9%, $p<0.05$). In the Gambia, another randomized, double-blind, placebo-controlled trial (N=550) of antenatal iron supplementation (60 mg elemental iron) or placebo was conducted (Menendez *et al.*, 1994). The birthweight of infants born to women who took iron supplementation was increased by an average of 56g (3103 g vs 3047 g, $p=0.14$) and the percentage of low birthweight babies was less in the iron supplementation group (3% vs 5%, $p=0.2$), however, neither of these differences reached statistical significance. Preziosi *et al.* (1997), in a randomized controlled trial of 100 mg elemental iron vs placebo in pregnant Nigerian women, also did not find a statistically significant difference in birthweight (3046 g ± 450 vs 3016 g ± 408, $p>0.05$). However, Cogswell *et al.* (2003), found a statistically significant lower incidence of low birthweight babies (4% vs 17%, $p=0.003$) in non-anemic pregnant women receiving a daily 30 mg iron supplement versus non-anemic women receiving a placebo. In a double-blinded randomized community trial in Nepal, Christian *et al.* (2003), determined that iron plus folic acid supplementation during pregnancy increased birthweight by 37 g (95%CI: 16, 90) compared to vitamin A supplementation (control group). A cross-sectional survey (N=3559) in Zimbabwe was recently undertaken and found that babies born to mothers who received iron supplementation (60 mg elemental iron) during pregnancy were 103 g (95%CI: 42, 64) heavier on average than babies born to mothers not receiving iron supplementation (Mishra *et al.*, 2005).

Anemia in pregnancy has also been associated with increased maternal morbidity and mortality (Guidotti 2000; McDermott *et al.*, 1996; WHO, 1996a). Viteri (1994) has estimated that anemia is the major contributory or sole cause of death in 20-40% of the 500 000 maternal deaths/year. WHO estimates that 16% of all maternal deaths are specifically associated with iron deficiency anemia (IDA) (WHO, 2000). Crompton (2000) believes that as much as 20% of all maternal deaths in the world are associated with iron deficiency anemia. Despite the variation in estimates, it is clear that anemia and in particular, IDA, is responsible for an important proportion of maternal deaths worldwide.

2.2.6 Framework for potential mechanisms between anemia and low birthweight

The two principal causes of LBW is prematurity (< 37 weeks of gestation) and intrauterine growth retardation (IUGR). It is believed that there is considerable overlap between the risk factors for preterm delivery and IUGR and that there are strong links in the underlying biological mechanisms associated with these two outcomes (Allen, 2001). In order to understand the biological mechanisms through which iron deficiency or anemia might cause poor fetal growth and preterm deliveries, it is necessary to understand that it is primarily the corticotropin-releasing hormone (CRH) (which is produced in the maternal and fetal compartment of the placenta) that regulates the process of delivery (Allen, 2001). In a longitudinal cohort of 485 pregnant women, abnormally high CRH concentrations early in pregnancy were highly associated with preterm deliveries (McLean *et al.*, 1995).

Moreover, among hormones that influence fetal growth, the insulin-like growth factor (IGF) system is probably the most important, and IGF-1 is the most important hormone (Gluckman and Harding, 1997). In animal models, maternal starvation lowered fetal IGF-1 concentrations which then also lowered fetal growth (Bassett *et al.*, 1990). Cortisol is also thought to have a role in fetal growth. In sheep, cortisol inhibits longitudinal growth of the fetus (Fowden *et al.*, 1996). Allen (1997) provides three possible explanations for the effects of iron deficiency or anemia on birthweight.

1) Iron deficiency or anemia may increase the stress hormone, norepinephrine, and cortisol:

Anemia, through hypoxia, and iron deficiency through increase of norepinephrine concentrations, can induce maternal and fetal stress, which stimulates the release of corticotropin-releasing hormone (CRH) (Calogero *et al.*, 1988). Chronic hypoxia can be the result of low hemoglobin concentrations. This state is presumably worsened during pregnancy when demands for oxygen are higher. It is thought that even though the changes in transplacental oxygen transfer are small when maternal hemoglobin concentrations fall, oxygen transfer to the fetus is probably reduced in anemic women (Viteri, 1994). One study found that arterial oxygen levels of women living at high altitude were directly related to infant birthweight, and more specifically to IUGR, but not to preterm deliveries (Moore *et al.*, 1982). Hypoxia is also thought to stimulate CRH production in placental cells (Smith, 1998). Both hypoxia and iron deficiency increase norepinephrine levels (Gulmezoglu *et al.*, 1996; Dallman, 1986), which is a strong stimulus for the secretion of CRH (Calegaro *et al.*, 1988). Moreover, it was found that norepinephrine infusion into pregnant sheep caused a reduction in fetal protein synthesis thus unfavourably affecting fetal growth (Milley, 1997). CRH also stimulates the production of fetal cortisol, which is thought to hinder longitudinal growth of the fetus (Allen, 2001).

2) Iron deficiency anemia may increase oxidative stress.

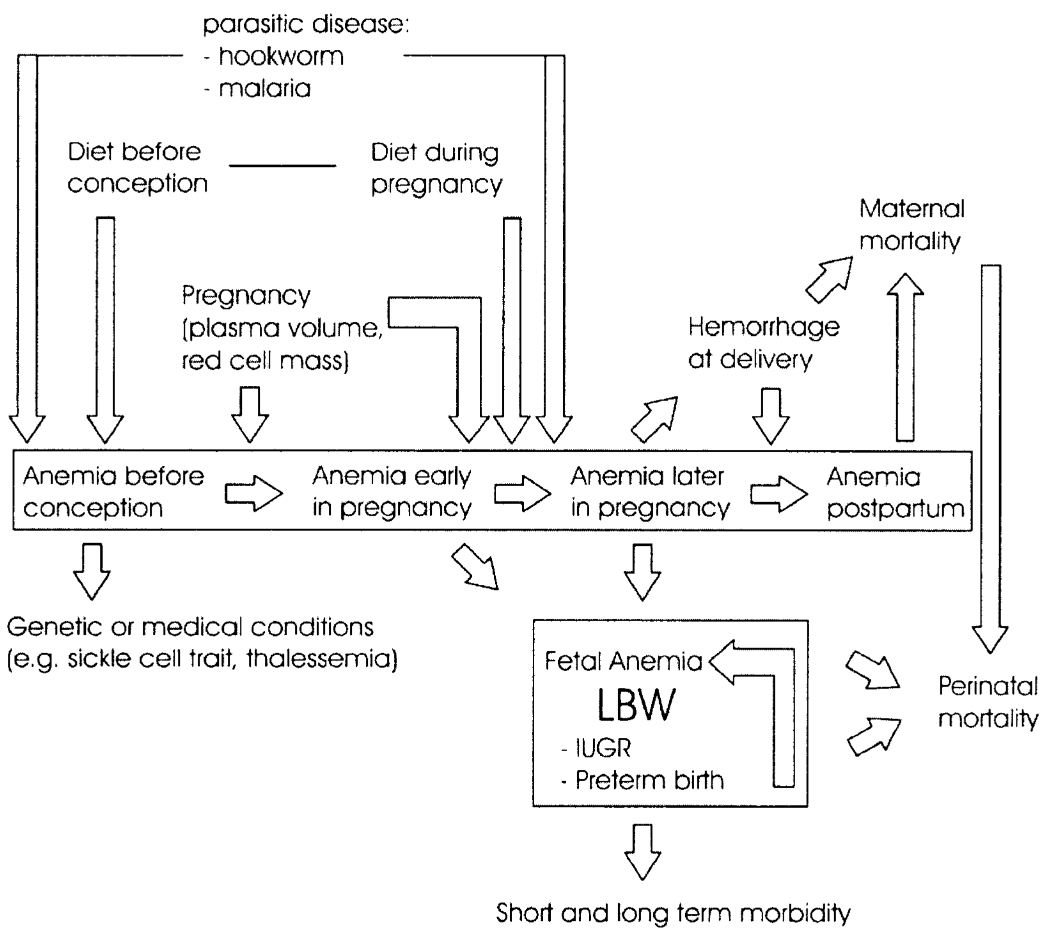
Iron deficiency may also intensify oxidative stress because it causes erythrocytes to be more susceptible to oxidative damage. It is believed that the fetoplacental unit is very susceptible to oxidative damage (Allen, 2001). Oxidative stress is one mechanism thought to cause preeclampsia and pregnancy-induced hypertension (Poranen *et al.*, 1996; Cester *et al.*, 1994). It is also believed that oxidative stress plays a role during pregnancy and in initiation of preterm labor (Pressman *et al.*, 2003). There exists a possibility an antioxidant nutrient might improve pregnancy outcome by reducing oxidative stress (Scholl and Stein, 2001).

3) Iron deficiency may increase the risk of maternal infections

Finally, there is some evidence that iron deficiency may depress the immune system through altering the proliferation of T and B cells, reducing the killing activity of phagocytes and neutrophils and lower bactericidal and natural killer cell activity (Allen, 2001). Iron deficiency may therefore increase the risk of maternal infections that are directly related to preterm deliveries (Allen, 2001).

Overall, the general theoretical framework linking maternal anemia to LBW is illustrated in Figure 1.

Figure 1. General theoretical framework linking maternal anemia to LBW. Diagram modified from Rasmussen, 2001. (Hb=hemoglobin, LBW=low birthweight, IUGR=intrauterine growth retardation).



2.3 Low birthweight

2.3.1 Description of the problem

Low birthweight (LBW) defined as birthweight <2500 g is a significant health problem in many parts of the world (Ramakrishnan, 2004). Prematurity (< 37 weeks of gestation) and intrauterine growth retardation (IUGR) are the two main causes of LBW. IUGR implies that fetal growth is inhibited and as such the fetus does not attain its full growth potential. There is no standard definition for IUGR but it may be defined as (1) a birthweight > 2SD below the median for gestational age; (2) birthweight less than 2500 g and a gestational age greater than or equal to 37 weeks; or (3) a birthweight less than the 10^{th} (or 5^{th}) percentile for gestational age (Kramer, 1987). In developing countries, the majority of LBW is due to IUGR, while in industrial countries the majority of LBW is due to preterm birth (Vilar and Belizan, 1982). Therefore, determinants of LBW are likely to be different in a developing country as compared to a developed country. LBW is often used as a proxy to quantify the magnitude of IUGR in developing countries because valid assessment of gestational age is generally not available.

At least 17 million LBW infants are born every year, representing about 16% of all newborns in developing countries (ACC/SCN, 2000). In Latin America LBW proportions are thought to be around 12% (UNICEF, 2003); UNICEF (2004) data for Peru reports 11%.

Birthweight is an extremely powerful predictor of infant survival and growth. Low birthweight is associated with a range of both short and long-term adverse consequences. LBW infants are more likely to be iron-deficient (Olivares *et al.*, 1992) and have low Apgar scores (McIntire *et al.*, 1999; Herceg *et al.*, 1994). Birthweight also affects postnatal growth and development (Steer, 2000). Infants born with LBW are more likely to die during the neonatal period (McCormick, 1985). Furthermore, countries with the highest infant mortality also tend to have the highest rates of LBW (WHO, 1995). Because immune function is severely compromised in low birthweight infants, low birthweight infants have more illnesses than infants of normal birthweight (Saha *et al.*, 1983). A Brazilian study compared morbidity, specifically intestinal and respiratory infections, and mortality rates of low birthweight and appropriate birthweight infants born at term (Lira *et al.*, 1996). The low birthweight infants experienced a sevenfold higher mortality rate and a fourfold higher rate of hospitalization than appropriate birthweight infants. The low birthweight infants also experienced a 33% increase in the number of days with diarrhea and a 32% increase in the number of days with vomiting. These results supported previous findings that reported rates of acute diarrhea and hospitalization almost two to four times greater in LBW infants as compared to normal birthweight babies (Bukenya *et al.*, 1991; Ittiravivongs *et al.*, 1991; Victora *et al.*, 1990; Victora *et al.*, 1989). A more recent study conducted in Brazil concluded that low birthweight infants were especially vulnerable to the effects of diarrhea, and suggested that the greater frequency and differential effects of diarrheal episodes, when compared to normal birthweight infants, explained the poorer development of low birthweight infants (Morris *et al.*, 1999). Low birthweight also appears to be an important risk factor for childhood pneumonia (Victora *et al.*, 1999; Fonseca *et al.*, 1996; Bartlett *et al.*, 1991). As acute respiratory infections are one of the major causes of infant mortality in developing countries (the second ranked cause in Peru) (PAHO, 2000), low birthweight is considered to be a major health problem. Most importantly, the risk of neonatal death (up to 28 days) for infants of birthweight between 2000-2499 g is four times higher compared to infants weighing between 2500-2999g, and ten times higher compared to infants weighing between 3000-3499 g (Ashworth *et al.*, 1998). Moreover, it has been found that adults who themselves had been born LBW are at increased risk of chronic diseases such as high blood pressure, non-insulin dependent diabetes mellitus, coronary heart disease and stroke (Hardy *et al.*, 2004; Hattersley and Tooke, 1999; Barker, 1998).

2.3.2 Determinants of low birthweight

In a review of the determinants of LBW, Kramer (1987) identified maternal undernutrition, which includes low gestational weight gain, low pre-pregnancy body mass index and short maternal stature as the major determinants of LBW in developing countries. Other determinants of LBW in developing countries include young maternal age, cigarette smoking during pregnancy, having malaria infection during pregnancy, socio-economic status, pre-pregnancy weight, and most frequently, anemia (with ORs of up to ≈ 5) (Deshmukh *et al.*, 1998; Lawoyin, 1997; Dinh *et al.*, 1996; Matteelli *et al.*, 1996; Hirve and Ganatra, 1994; Vega *et al.*, 1993; Mavalankar *et al.*, 1992; Kramer, 1987). Most of the existing research on the determinants of LBW focuses primarily on nutritional determinants in populations from industrialized countries. The role of maternal intestinal parasitic infections on birthweight is less well known.

2.3.3 Low birthweight and deworming

Only two studies have investigated the effect of anthelminthics on birth outcomes and none have studied the combined effect of anthelminthics and iron supplementation.

In 1996, in Sri Lanka, the effect of mebendazole treatment during pregnancy on birth outcome was investigated using a cross-sectional survey design (de Silva *et al.*, 1999). The information about mebendazole intake was assessed by a questionnaire administered to the 7087 mothers on the second (vaginal deliveries) or third day (cesarean sections) post-partum. The proportions of stillbirths and perinatal deaths were significantly lower in the mebendazole group (1.9% vs 3.3%; 95%CI: 0.40, 0.77), as was the proportion of very low birthweight babies (< 1500 g) (1.1% vs 2.3 %; 95%CI: 0.32, 0.71). Although several biases could have been operating (recall bias, selection bias) and despite having only a univariate analysis, this study provided impressive evidence of the potential benefits of anthelminthic treatment during pregnancy. An even greater effect could have been demonstrated if all low birthweight infants (< 2500 g) would have been analyzed, but these data were not published.

More recently, within a randomized trial of different micronutrient combinations conducted in 30 villages in Nepal, a 400 mg single dose of albendazole was offered to pregnant women at mid-gestation and again at late gestation (Christian *et al.*, 2004). This study showed the positive effects of two doses of anthelminthic (albendazole) given to pregnant women in increasing mean infant birthweight. The birthweights of infants born to women who had received two doses of albendazole were higher by 59 g (95%CI: 19, 98) (compared to women who had not taken any albendazole). However, mean birthweight did not significantly increase in women who had received only one dose of albendazole compared to women who had not received any albendazole. These results were obtained by multivariate modelling adjusting for nutrient supplement group, maternal parity, tobacco smoking, early pregnancy weight, height, ethnic group, literacy, gestational duration of pregnancy, and social status.

Surprisingly, although iron supplementation is considered standard antenatal care in most countries, there has been no randomized trial on the effect of anthelminthic treatment added to iron supplementation on infant birthweight.

2.3.4 Use of birthweight as an outcome measure

Two different expressions of birthweight are usually used in research: mean birthweight and expressing birthweight as a proportion (ie. proportion of LBW and/or proportion of VLBW). Using birthweight as a continuous outcome has the advantage of incorporating all of the information available and provides the opportunity to categorize or dichotomize the data later on should the need arise. The mean also provides more precise information with smaller standard errors.

It is known that low birthweight is highly associated with infant mortality as well as morbidity. However, in recent years many countries (Argentina, Brazil, Canada, and Uruguay) have reported declines in infant mortality despite increases or little change in the prevalence of LBW (Kramer *et al.*, 2005; Barros *et al.*, 2001; Silva *et al.*, 1998). Wilcox (2001) suggests that the proportion of LBW is not a good indicator of a population's infant risk of death because changes in the predominant distribution of birthweight, which do not accurately predict infants' risk of death, easily affect LBW. Herz-Piciotto (2001) mentions that even when birthweight is not an indicator of higher mortality risk, it might inform us about morbidity. Additionally, she mentions that Wilcox's hypothesis should be evaluated in studies examining birthweight or problems in childhood and beyond, such as infectious disease. Kabir (2002), reported that the approaches discussed by Wilcox may have limited application in a majority of developing countries and that the <2500 g cut-off for LBW might help identify high risk babies in an efficient and cost-effective way.

Mean birthweight as an overall comparison outcome measure between two groups is therefore the most informative measure. In order to provide comparisons with published accounts of past research, proportions of birthweight should also be analyzed and reported.

3 OBJECTIVES

3.1 General objective

The main objective of this study was to assess the effectiveness of antenatal mebendazole plus iron supplements versus placebo plus iron supplements on mean birthweight in a highly hookworm-endemic area.

3.2 Secondary objectives

Secondary objectives investigated the effectiveness of antenatal mebendazole plus iron supplements versus placebo plus iron supplements on different proportions of birthweight (low birthweight < 2500 g or very low birthweight < 1500 g) and on maternal anemia (mean hemoglobin and proportions of anemic women (< 110 g/L)). Frequencies of adverse birth outcomes between the two intervention groups were also compared.

4 METHODS

4.1 Study location and population

The present study was carried out in Iquitos, Peru, a tropical and humid jungle area. Iquitos is situated in the Department of Loreto, which comprises nearly one-fourth of the landmass of Peru and has the ecologic characteristics of the Amazon lowlands (see Figure 1 on page 108). Iquitos is the only large urban centre in Loreto and has a population of 345 000 inhabitants. Its rural population is clustered in towns and villages throughout the Amazon tributary system. In the Department of Loreto, 24% of the population is comprised of women of reproductive age (ENDES, 2000). In 2003, 14 874 deliveries took place in Loreto, of which 60% were reported to have taken place in a hospital, 37% in a health center and 3% at home (Unpublished data from "Ministerio de Salud del Peru" (MINSA)). Hospital deliveries were high in 2003 because the government had newly established a cost-free in-hospital delivery program. Only 63% of pregnant women in Loreto attended an antenatal care program from 1995 to 1999 (ENDES, 2000). The adjusted maternal mortality ratio in Peru is 410/100 000 live births (Unicef, 2000), while the infant mortality rate is 26/1000 live births (Unicef, 2003).

Soil-transmitted helminth infections, malaria and anemia are endemic in the area. Living conditions are characterized by poor sanitation and limited access to potable water. Maternal and Child Programs are run at the hospitals (Hospital Apoyo de Iquitos and Hospital Regional de Loreto) and at various "posta medica" (health centre) in the towns and villages.

4.2 Sample size estimation

Sample size calculations were based on the formula for the comparison of two means for the principal outcome of interest, infant birthweight. In addition to issues related to statistical power, the calculation of the number of subjects per group was based on the ability to investigate subgroup differences for four different levels of intensity of hookworm infection.

For the primary outcome of mean infant birthweight, a standard deviation of 500g was used. This figure was obtained from a study on mebendazole and birth outcome in Sri Lanka (de Silva *et al*, 1999). From available data, it was determined that Sri Lanka has a very similar percentage of low birthweight infants compared to Peru; as such, and in the absence of any other published data, the assumption that the distribution of birthweight would likely be similar was made. It was also determined that it would be important to be able to measure an effect difference of at least 200 g between the two groups -- a difference which is judged to be clinically significant. To detect this difference with a power of 80% and an alpha level of 0.05 for a two-sided test, a sample size of 100 subjects per subgroup would be required. The total sample size therefore was calculated to be 400 subjects per intervention group for a total of 800 women. Assuming a loss-to-follow-up of not more than 10%, based on results obtained during the pilot study (Larocque *et al.*, 2004, see Appendix 2), it was expected that approximately 900 women would need to be recruited to meet sample size requirements.

4.3 Design and randomization

A double-blind randomized controlled trial design was used. Computer-generated randomly-ordered blocks of four, six and eight were used to randomly allocate women to each intervention group. Two researchers not otherwise involved in the trial prepared sealed envelopes containing the intervention assignment. The research "obstetrices" (i.e. nurses specialized in obstetric care) were given a supply of envelopes each day for administration to participating pregnant women, until the sample size was achieved.

The local project director, the field workers, the obstetrices, the laboratory technologists, and the participating pregnant women were all blind to the intervention assignment. The intervention assignment list was kept in Canada while all the recruitment and data collection took place in Peru.

4.4 Recruitment techniques and inclusion and exclusion criteria

Pregnant women were recruited from 12 health centres in the Iquitos region from April 2003 to November 2003. Recruitment also included canvassing door-to-door in neighbourhoods in the more rural catchment areas of the health centres because it was known that many pregnant women did not seek antenatal care. The inclusion criteria were: second trimester ($\geq$ 18 weeks; < 26 weeks) pregnant women between 18 to 44 years of age (gestational age of the woman was determined using a combination of the fundal height and the first day of the last menstrual period); not having received anthelminthic treatment in the last six months prior to recruitment; residing in rural or peri-urban areas (defined as having no running water or flushing toilet facility in the home) and giving consent. Exclusion criteria were: having severe anemia (hemoglobin < 70 g/L) or having a medical condition for which follow-up was required.

4.5 Interventions

Once inclusion criteria were satisfied, each woman was given a plastic container and asked to return the next day with a single stool specimen. The container was then collected and fingerprick blood was obtained to measure hemoglobin (by Hemo-Cue®) and presence/absence of malaria (by thick and thin smear). Following blood drawing, women received either a single dose of mebendazole (500 mg) plus an iron supplement (60 mg elemental iron) or a placebo tablet (similar appearance, smell and taste to the mebendazole tablet) plus an iron supplement (60 mg elemental iron). Both mebendazole and placebo tablets were purchased from Janssen-Cilag (Brazil). Iron was 300 mg ferrous sulphate (PharmaScience, Canada). Women were given containers having a one-month supply of iron supplements and instructed to take one supplement per day. Women returned every month for their antenatal check-up and, at the same time, received another month's supply of iron. The standard-of-care for pregnant women in the study area included iron supplements.

A pink identification card was also attached to their antenatal carnet (a carnet that includes the pregnant women's maternal health information and that they bring with them to all their health clinic or hospital visits). The woman's name, probable date of delivery and next appointment date were written on the card. The cards were particularly helpful in the delivery division of the hospitals to identify the women participating in the project. The pink identification card also had the phone numbers of the research coordinator and research director in case of any problems or emergencies.

4.6 Measurements

Each woman was followed until delivery (approximately 5 months). Assessments were made at baseline (2^{nd} trimester), at 3^{rd} trimester and at delivery. They included: 1) anemia, 2) malaria, and 3) hookworm and other soil-transmitted helminth infections.

On the same day women were randomized to either intervention regimen, a questionnaire was administered by the study obstetriz to obtain socio-demographic information (e.g. age, address, schooling, marital status), reproductive health characteristics (e.g. parity (number of children), gravida (number of pregnancies), approximate gestational age and previous pre-term delivery, medication history (current medication intake), and environmental characteristics (e.g. type of housing, type of latrine and water availability) (see Appendix 3 for copy of the questionnaire).

The stool specimens and malaria smears were examined by two experienced laboratory technologists on the same day they were received.

The Kato-Katz technique for the quantification of intestinal helminth infection was used. Intensity of infection was expressed as eggs per gram (epg) of feces. The Kato-Katz method is the preferred technique for assessment of intestinal helminth infection in the field (WHO, 1998). Briefly, a small amount of fecal material was used to fill the hole of a plastic template. The template was then removed and the remaining amount of feces was covered with a malachite green pre-stained cellophane strip. The fecal sample was pressed firmly, by inverting the slide on the lab counter, against the cellophane strip to spread evenly. The slide was kept at room temperature for 30 minutes prior to microscopic examination in order to clear fecal material. Hookworm eggs become transparent quickly; therefore it was important to respect the time limits.

Examination of thick and thin blood smears of fingerprick blood is the preferred technique for field assessment of malaria infection (Kokoskin, 2001). Briefly, two small drops of fingerprick blood were placed on the slide; one on one side and the other in the middle. The one on the side of the slide is used for the thick smear. Using the corner of another slide, the drop is spread in a circular pattern until it is the size of a dime. The one on the centre of the slide is used for the thin smear. Another slide was angled up to the drop at a 30-45° angle, allowing the drop to spread along the contact line of the two slides. The second slide (spreader) was used to quickly push the blood toward the unfrosted end of the lower slide. The slides were allowed to dry and were then fixed in methanol. Malaria detection followed routine Giemsa staining the next morning.

Hemoglobin concentration was obtained using Hemo-Cue® assessment from blood fingerprick of pregnant women. The accuracy of the Hemo-Cue® assessment has been determined to be within +/- 1.5% compared to the Gold Standard Cyanmethaemologin (HiCN) method (Kwant *et al.,* 1987). The Hemo-Cue® devices were calibrated daily. Briefly, a microcuvette (specific for the Hemo-Cue®) was applied to a drop of fingerprick blood. The correct amount of blood is drawn into the cuvette by capillary action. The cuvettes were placed in the cuvette holder into the Hemo-Cue® analyzer and a reading would appear within a few seconds.

Birthweight was measured at birth in the delivery hospital by the attending nurse using a baby electronic scale (Seca model 334) (Seca corp, Baltimore, Maryland USA). For domiciliary births, study obstetrices used portable baby electronic scales (Seca model 345) to obtain birthweights as soon after birth as possible. The accuracy of the digital scale is better than ± 0.3% or ± 10g (insert from the manufacturer). Scales were calibrated on a weekly basis to ensure that they were functioning efficiently. The standard definitions of low birthweight (LBW) (< 2500 g) and very low birthweight (VLBW) (< 1500 g) were used. Recumbent crown-heel length of the baby was measured using a measuring mat (Seca model 210). Infants whose birthweights were obtained more than two days after birth were excluded from the analysis.

4.7 Training and quality control

Both technologists received a specialized two-week 'refresher' training in the parasitology laboratory of the Instituto de Medicina Tropical "Alexander von Humboldt" at the Universidad Peruana Cayetano Heredia in Lima, Peru. The first week of the training consisted of knowledge strengthening on general parasitology and parasitology techniques while the second week focused mostly on the familiarization with the Kato-Katz technique for quantification of intestinal helminth infection. Reading of slides and quantification of eggs were repeated various times in order to increase inter-observer reliability between both technologists.

Once in Iquitos, quality control consisted of: 1) revision of 10% of all stool specimens by a technical expert from the Instituto de Medicina Tropical "Alexander von Humboldt" every 3 months; 2) daily review of the lab log and immediate appropriate remedy of errors or abnormal observations; 3) daily supervision of slide preparation by the project director; 4) daily reading of all negative slides by the second technologist; and 5) storage of all fecal samples in 10% formalin for three months should remedial review be required.

The quality control for malaria detection consisted of: 1) revision of all positive slides by the second technologist; 2) daily review of the lab log and immediate daily remedy of errors or abnormal observations; 3) constant review of the quality of the smears; 4) daily supervision of smear preparation; and 5) storage of all stained smears should remedial review be required.

Data collection activities during fieldwork were regularly supervised to ensure that forms were being correctly completed. The local project director (RL) checked all forms immediately after data collection at the end of each day. If there were discrepancies or unclear answers, a discussion and explanation of the question and eligible answers took place to avoid making errors the next day. Corrections were made, if warranted.

Nurses working at the two government-run hospitals were trained to use the digital scale. An informative session was given by the project director at the neonatal division. The obstetrices working on the project were in contact with these nurses on a daily basis to answer any questions or to attend to any problems with the scales.

4.8 Data entry and management

Only one person was in charge of data entry into the Excel software (Microsoft Office XP) and it was the project director (RL). Data were entered on a daily basis in order to detect errors as quickly as possible and remedy the situation rapidly, if warranted. Newly entered data were sent weekly to the principal investigator (TWG) in Canada. No other person had access to the computer other than the project director and the principal investigator. A password was necessary to have access to the files containing the data. Once a woman had completed her participation in the project her whole questionnaire was reviewed completely in order to ensure that there were no data entry mistakes. Once the study was finalized, all the questionnaires, consent forms and informative material were brought to Canada. Any other material was destroyed to ensure maintenance of confidentiality of the women's medical and personal information.

4.9 Statistical analysis

Descriptive analyses were conducted using means (± SD) and proportions by treatment group. Data were analyzed on an intention-to-treat basis. Means were compared using the two-sample t test and proportions, the X^2 test or Fisher's exact test, when appropriate. When comparing before and after intervention measurements in the same group of women, the paired t-test was used when comparing means while McNemar's test was used when comparing proportions. The analysis for birthweight was restricted to singleton live births. Crude odds ratios (ORs) and 95% confidence intervals (95%CIs) were computed for each categorical variable. Although the pregnant women in this study were randomized to either intervention group, which should control for known and unknown confounding, there was some loss to follow-up and so multivariate analyses were undertaken to ensure control of all potential confounding. Therefore, multivariate linear models were developed to compare mean birthweight between intervention groups controlling for characteristics that differed between groups or that were known confounders. Exact logistic regression models were used to compare the proportions of LBW and VLBW between the groups. Exact logistic regression is adequate when data are skewed, which was the case with our data.

The cure rates (CR), percentage reductions in prevalence (PRP), geometric means and egg reduction rates (ERR) were calculated for all three soil-transmitted helminth infections (hookworm, *Trichuris* and *Ascaris*) at the third trimester assessment (approximately 76 days following treatment) (Albonico *et al.*, 2003). All analyses were done with SAS version 6.0 (SAS Institute, Cary, NC).

4.10 Ethical consideration

Informed written consent was obtained from each pregnant woman invited to participate in the study (see Appendix 4). The women signed the information sheet as a testimony that they had been informed of all the essential study information and wanted to participate. Confidentiality was assured at all times. Women having severe anemia (Hb levels < 70 g/L) were excluded from the study and referred to the local health authorities for immediate treatment. Women could withdraw from the study at any time for whatever reason.

Ethics approval was obtained from these ethics review committees: Research Institute of the McGill University Health Centre (Montreal, Canada); the "Comite Institucional de Etica de la Universidad Peruana Cayetano Heredia" (Lima, Peru); and the "Comite Etica de la Direccion General de Salud de las Personas del Ministerio de Salud de Peru" (Lima, Peru) (See certificates in Appendix 5).

WHO's recommendation states that single-dose anthelminthic treatment can be given to second trimester pregnant women in hookworm-endemic areas (prevalence >20-30%). This recommendation was based primarily on expert opinion and published accounts of research in non-pregnant populations. Even with these recommendations many doctors and health professionals are not convinced that anthleminthics should be given during pregnancy. Therefore a state of clinical equipoise was declared meaning that there was a failure of consensus within the clinical community (Freedman, 1987). Clinical equipoise describes a genuine uncertainty on the part of the expert medical community about the comparative merits of each arm of a clinical trial (Freedman, 1987).

PREFACE TO MANUSCRIPT B

In the randomized controlled trial that we conducted in Iquitos, Peru, baseline data on prevalence and intensity of soil-transmitted helminths and prevalence of anemia and hemoglobin levels were available for 1042 pregnant women. Numerous published studies have found a significant correlation between lower hemoglobin levels and higher fecal egg counts of hookworm; however most of these studies were conducted in populations of children or non-pregnant adult women. In this manuscript, we report on the relationship between the intensity of soil-transmitted helminth infection (hookworm, *Trichuris* and *Ascaris*) and anemia in a population of pregnant women.

These results have been presented at scientific conferences including: The American Society of Tropical Medicine and Hygiene Annual Meeting (2003, 2004) and The Canadian Conference on International Health (2004).

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5 MANUSCRIPT B

LRH: LAROCQUE AND OTHERS

RRH: HELMINTHS AND ANEMIA IN PREGNANCY

RELATIONSHIP BETWEEN INTENSITY OF SOIL-TRANSMITTED HELMINTH INFECTIONS AND ANEMIA DURING PREGNANCY

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Abstract

A direct relationship exists between the intensity of hookworm infection and blood loss. Other parasites may also contribute to blood loss. Our objective was to assess the relationship between the intensity of soil-transmitted helminth infections and anemia in pregnant women in a highly endemic area of Peru. Recruitment occurred between April and November 2003. Overall, 47.31% of 1042 women had anemia (hemoglobin < 11 g/dL), 47.22% were infected with hookworm and 82.25% with *Trichuris*. Prevalences of infections were not associated with anemia. However, those infected with moderate and heavy intensities of hookworm infection (OR = 1.84; 95%CI: 1.06, 3.17) and those with moderate and heavy intensities of both hookworm and *Trichuris* infections (OR = 2.13; 95%CI: 1.10, 4.13) were more likely to suffer from anemia than women having no or light intensities. These results support routine anthelminthic treatment within prenatal care programs in highly endemic areas.

INTRODUCTION

Anemia is regarded worldwide as a medical condition deserving of sustained public health intervention. Anemia in pregnant women is defined as hemoglobin levels less than 11 g/dL.[1] It is usually caused by iron deficiency, which is the most common nutrient deficiency in the world. It has been estimated that, at any one time in developing countries, half of the population (mainly children and women of reproductive age) is affected by anemia.[2] During pregnancy, approximately 75% of all anemias diagnosed are due to iron deficiency.[3] In Peru, 35% of women of reproductive age and 50% of pregnant women were found to be anemic.[4]

Iron deficiency is highest in population sub-groups that are at peak rates of growth; namely, infants, young children and pregnant women. Pregnancy is a time during which the risk for developing iron deficiency anemia is greatest as iron requirements are substantially higher than average.[5,6] Furthermore, WHO considers that women in developing countries may be pregnant for as much as one half of their reproductive lives, and therefore are at increased risk of anemia during this time.[1]

Anemia in pregnancy has been associated with poor birth outcome, such as low birthweight[7-10] and increased maternal morbidity and mortality.[1,11,12] It has been reported that close to 500,000 maternal deaths occur every year, the vast majority taking place in the developing world. Anemia is thought to be the major contributory cause of death in 20--40% of these maternal deaths.[13] Furthermore, it has been estimated that 16% -- 20% of all maternal deaths are associated with iron deficiency anemia.[14]

In developing countries, both nutritional deficiencies and parasitic infection, specifically hookworm and malaria infection, contribute most to anemia. In fact, hookworm infections are recognized as the leading cause of pathological blood loss in tropical and sub-tropical countries.[15] Hookworm infections contribute to anemia by causing blood loss directly through ingestion and mechanical damage of the mucosa, and indirectly, by affecting the supply of nutrients necessary for erythropoiesis.[14,16] It has been estimated that, on average, blood losses into the feces (measured as fecal hemoglobin) increase by 0.825 mg per gram of feces for every increment of 1,000 hookworm eggs per gram of feces (epg).[17] Lack of iron in the diet, especially in rural and poor communities, is critical during pregnancy.[18]

Bundy and colleagues[19] have estimated that approximately one-third of all pregnant women in developing countries are infected with hookworm infection (44 million out of 124 million pregnancies). It has been suggested that the impact of hookworm infection on maternal morbidity 'may well be very considerable'.[20] Hookworm-attributable anemia, induced by deficiencies of iron, total energy, protein and possibly folate and zinc, is a significant cause of intrauterine growth retardation and low birthweight.[21,22] Evidence also suggests that both iron deficiency anemia and separately, hookworm infection, will inhibit appetite resulting in low pregnancy weight gain and intrauterine growth retardation followed by low infant birthweight.[21] It has even been suggested that the hookworm-attributable burden of disease in girls and women, especially during pregnancy, may constitute the most important component of their global disease burden.[22] Added to this is the impact of concurrent parasitic co-infections and other co-morbid conditions.

In 1998, WHO proposed a classification for intensities for each soil-transmitted helminth infection (based on quantitative counts obtained using the Kato-Katz method)[23]. Especially in hookworm infection, the degree of severity of morbidity varies not only according to the number of worms present but also according to determinants of the host (eg. age), parasite (eg. species) and host-parasite interaction (eg. nutritional intake of iron). The intensity classes for hookworm set by WHO were based on data published by Stoltzfus *et al*[17] using fecal blood loss as measured in Zanzibari children. There exists the possibility that the epg limits of the different classes could change in different populations and with different proportions of hookworm species. Indeed, in a study population which included both children and adults in western Kenya, Olsen *et al*[24] found that a cut-off of 300 epg differentiated anemic from non-anemic individuals.

Numerous studies have found a significant correlation between lower hemoglobin levels and higher fecal egg counts of hookworm.[24-27] Stoltzfus and colleagues[28] reported that the relationship between hookworm infection and hemoglobin concentration may be apparent only above a threshold worm burden. Heavy intensity *Trichuris* infection, which is associated with decreased food intake and blood loss, has also been associated with anemia.[29,30]

In this study, we assess the relationship between the intensity of soil-transmitted helminths (hookworm, *Trichuris* and *Ascaris*) and anemia in a population of pregnant women in Iquitos, Peru, a highly hookworm-endemic area.

MATERIALS AND METHODS

Study area. The present study was carried out in Iquitos, Peru, a tropical and humid jungle area. Iquitos is situated in the Department of Loreto, which comprises nearly one-fourth of the landmass of Peru and has the ecologic characteristics of the Amazon lowlands (Figure 1).

Soil-transmitted helminth infections, malaria and anemia are endemic. Living conditions are characterized by poor sanitation and limited access to tap water. Maternal and Child Programs are run at the hospitals (Hospital Apoyo de Iquitos and Hospital Regional de Iquitos) and at various "posta medica" (health centers) in the towns and villages. In 2000, approximately 40% of pregnant women in Loreto failed to make at least one visit to a health center before delivery.[31]

Study design and population. This baseline survey took place between April 2003 and November 2003. The inclusion criteria were: second trimester pregnant women aged between 18 to 44 years; not having received anthelminthic treatment for six months prior to recruitment into the study; residence in rural or semi-urban areas (defined as having no running water or toilet facilities in their house) and consent to participate. Exclusion criteria were: having severe anemia (< 7 mg/dL)[1] or having a medical condition for which follow-up was required. All pregnant women attending health centers for their periodic pre-natal care visits who met the inclusion criteria were invited to participate in the study. Recruitment also included canvassing (door-to-door) in neighborhoods in the more rural catchment areas because it was known that many pregnant women did not seek pre-natal care.

Questionnaire. An interviewer-administered questionnaire was used to obtain socio-demographic information, reproductive health characteristics, medication history) and environmental characteristics. It was adapted from two which had previously been used in recent studies on pregnant women in Peru.[18,32] It was first written in English and then translated and pre-tested in Spanish. Interviewers were qualified midwives (ie. "obstetrices") and were trained in questionnaire administration in order to attain standardization and maximize inter-interviewer reliability. Quality control was performed by daily review of each questionnaire by the project director (RL) and immediate remedy of any error or problem.

Measurement of anemia. Hemoglobin concentration was obtained from fingerprick blood using Hemo-Cue® assessment. The accuracy of the battery-operated Hemo-Cue® photometer has been determined to be within +/- 1.5%.[33] Each Hemo-Cue® photometer was checked for calibration every morning before their use.

Measurement of parasite infections. Containers for stool specimens were given to each woman at the time of questionnaire administration. Women were asked to return the next day with the fecal sample. The fecal samples were examined on the same day they were returned by two trained biologists using two study-dedicated Nikon microscopes. The biologists had a total of over 25 years of laboratory and microscopy experience and, in addition, they received a specialized two-week training in the parasitology laboratory of the "Instituto de Medicina Tropical Alexander von Humboldt" at the Universidad Peruana Cayetano Heredia in Lima, Peru. The Kato-Katz technique for quantification of helminth infection was used.[34] Intensity of infection was expressed as eggs per gram of feces. The

WHO[23] categories for light, moderate and heavy levels of intensities of soil-transmitted helminth infections were used. Because of the extremely hot and humid weather in Iquitos which accelerated the clearing process for hookworm eggs, the slides were read within 25 minutes. Quality control measures (eg. daily supervision, re-reading of all negative slides, external evaluator, storage of slides) were strictly enforced.

Presence of malaria infection was determined from fingerprick blood. Examination of Giemsa-stained thick and thin smears was used to determine the presence/absence of the malaria parasite. Interviewers were trained by the biologists to make the smears. Again, strict quality control measures were put in place.

Statistical methods. Descriptive statistics included frequencies and means (and standard deviations). Frequencies of socio-demographic variables were also presented in sub-groups defined by the presence or absence of anemia and presence or absence of hookworm infection. P values based on chi-square test (for proportions) and t-test (for means) were calculated. For univariate analysis, odds ratios (ORs) and 95% confidence intervals (95%CI) were computed for each categorical variable; Pearson's correlation coefficients were examined for continuous variables; and Spearman's correlation coefficients (r_s) were calculated for correlations between continuous and categorical variables. The correlation between parasite egg counts and hemoglobin was also examined at various egg count thresholds (> 1000 epg, > 2000 epg, > 3000 epg and > 5000 epg). A chi-square test for trend was used to compare proportions of anemic women with proportions of non-anemic women in terms of categories of intensity (none and light versus moderate versus heavy).

The association between anemia (Hb < 11 g/dL or Hb $\geq$ 11 g/dL) and its determinants was examined by univariate and multivariate logistic regression analysis. A multiple linear regression analysis was also performed to identify predictors of hemoglobin concentration. A stepwise approach was used with variables significant at the $P < 0.05$ being kept in the final model. Variables were included in the multivariate model if, in the univariate analysis, they had a *P* value of ≤ 0.25 or if they had been previously identified as potential confounders or effect modifiers in the published literature. When variables were found to be highly correlated, the most informative variable was kept for further modeling. Age and marital status were correlated ($r_s = 0.20$, $P < 0.0001$) so age was selected for model-building. Additionally, type of housing and presence of toilet facilities in the home were highly correlated with the environment variable (rural or peri-urban area) ($P = 0.0016$ and $P = 0.020$, respectively); therefore only environment was used for further modeling. Wearing sandals inside the house was correlated with wearing sandals outside the house ($P < .001$); therefore wearing sandals outside the house was retained. Parasite egg intensities were summarized as geometric means and log transformed (base 10) for comparison and analyses. All statistical analyses were carried out using the SAS software package (SAS Institute, Cary, NC, USA).

Ethics approval. Ethics approval was obtained from these ethics review committees: Research Institute of the McGill University Health Centre (Canada); the "Comite Institucional de Etica de la Universidad Peruana Cayetano Heredia";

and the “Comite Etica de la Direccion General de Salud de las Personas del Ministerio de Salud de Peru”.

RESULTS

Population characteristics. A total of 1042 second trimester pregnant women were recruited. The socio-demographic characteristics of this study population are summarized in Table 1. The women were on average 25.2 years old (range: 18--42), had less than a secondary school education, were married or ‘conviviente’ (i.e. living with partner but not married) were in their fourth month of pregnancy and lived in a peri-urban type of environment. Most lived in homes with roofs made out of tree leaves and floors of earth. Access to drinking water was primarily from community installations, such as pipes of water functioning only at certain times of the day.

Anemia. The prevalence of anemia was 47.31%; the mean hemoglobin level was 11.04 ± 1.10 g/dL (range: 7.0--14.4 g/dL). More single women were anemic than married (or ‘conviviente’) women (Table 1). Increasingly higher proportions of anemia were found with longer gestational age. Women who used latrines had a higher level of anemia than women who had more modern types of toilet facilities in their house. Women living in concrete houses were found to be more anemic than women living in either brick or wooden houses. Lastly, women who always wore sandals either inside or outside their houses had a higher prevalence of anemia than women who reported not wearing sandals either inside or outside of their houses.

Prevalence and intensity of parasites. The overall prevalences were 47.22% for hookworm, 82.25% for *Trichuris* and 63.92% for *Ascaris* (Table 2). Only 9.31% of the pregnant women were free of any parasite infection; 20.25% of the women had a single infection, 38.96% had two infections and 31.48% were infected with all three worm infections. The prevalence of *Trichuris* and hookworm co-infections was 44.05%.

Several determinants influenced the prevalence of hookworm infection. Women living in rural areas had a significantly higher prevalence of hookworm infection compared to women living in peri-urban areas (Table 1). Hookworm-infected women were generally married or 'conviviente' and multiparous, with less than a secondary school education. Women with animals in their house were found to have a significantly lower prevalence than women not having animals in their house. Women not wearing sandals either inside or outside of their houses had significantly higher hookworm prevalence than women wearing sandals. Hookworm was also more prevalent in women living in houses constructed of palm leaves, where the floor was of wood or earth, and where there was no toilet facility or nearby latrine.

Only 1.63% of women had heavy intensity hookworm infection, while 3.84% had a moderate intensity infection, and 41.75% had a light infection (52.78% having no infection) (Table 2). Geometric mean egg counts among infected women were 347 for hookworm (range: 24--13 848 epg), 571 for *Trichuris* (range: 24--25 200 epg), and 3490 for *Ascaris* (range: 24--166 800 epg). When intensity categories were combined into none and light versus moderate and heavy, 5.47% of women had moderate to heavy hookworm infection. As for

Trichuris infection, only 2.21% had a heavy infection, 26.78% had a moderate infection and 53.26% had a light infection (17.75% having no infection). A total of 28.98% of women had a moderate to heavy *Trichuris* infection. Only 3.84% (n = 40), however, had both moderate and heavy hookworm and moderate and heavy *Trichuris* infections. In *Ascaris* infection, only 2.11% of women had a heavy infection, while 30.61% had a moderate infection and 31.19 had a light infection (36.08% having no infection). Approximately one third (32.73%) of women had a moderate to heavy *Ascaris* infection.

Even though this region of Peru is known as a malaria-endemic zone, only 2 women were found to be smear-positive, while 13 were taking chloroquine treatment. Malaria therefore was not included as a variable in any analysis.

Relationship between soil-transmitted helminths and anemia. No single prevalence of any parasite infection was associated with anemia. However, several intensity measurements were found to be associated with anemia. There was a significant correlation between increasing hookworm egg counts and decreasing hemoglobin levels, however, this correlation was weak ($r = -0.12$, $P < 0.001$). No correlation was found between either *Trichuris* or *Ascaris* egg counts and hemoglobin. Examining egg count thresholds, the strongest statistically significant association with hemoglobin was found at the > 2000 epg threshold for hookworm ($r = -0.30$, $P = 0.020$). No significant association was found at any threshold for *Trichuris* and *Ascaris*. When using the log-transformed eggs per gram count data, no significant association with hemoglobin was found for any of the three parasite species.

The mean hemoglobin of pregnant women having moderate and heavy intensity hookworm infection was significantly lower than women with no or light hookworm infection (10.75 g/dL vs. 11.05 g/dL; $P = 0.042$). Similarly, a higher proportion of women having moderate and heavy infections of hookworm were anemic than women having no or light infection (OR = 1.84; 95%CI: 1.06, 3.18) (Table 3). Additionally, we found increasing proportions of anemic women with increasing intensity categories of hookworm infection ($X^2_{(trend)} = 7.08$; $P = 0.027$). Women having moderate and heavy *Trichuris* infection were just as likely to have anemia as women having no infection or light *Trichuris* infection (OR = 1.26; 95%CI: 0.96, 1.65). Women having moderate and heavy *Ascaris* infection were less likely to have anemia than women having no or light *Ascaris* infection (OR = 0.75; 95%CI: 0.58, 0.98).

Women with moderate and heavy intensities of both hookworm and *Trichuris* infections were more than twice as likely to have anemia (OR = 2.14; 95%CI: 1.10, 4.14). No other combination of co-infections or combinations of intensities of co-infections had a strong association with anemia.

Two parasite variables were found to be highly statistically significantly associated with anemia (moderate and heavy hookworm infection and co-infection of moderate and heavy hookworm and *Trichuris* infections) (Table 3). As these two variables were also highly correlated ($P < 0.001$), they were used separately in two models to examine the determinants of anemia.

On multivariate analysis, and considering moderate and heavy hookworm infection, after adjusting for age, education, environment, gestational age, wearing sandals outside the house and taking iron supplements, the sole determinant of anemia was found to be moderate and heavy hookworm infection (OR = 1.84; 95%CI: 1.06, 3.17). Similarly, after adjustment, co-infection of moderate and to heavy hookworm and *Trichuris* infections was found to be statistically significantly associated with anemia (OR = 2.13; 95%CI: 1.10, 4.13).

In the multivariate linear regression analysis, where hemoglobin was the dependent variable, hookworm intensity in addition to age and gestational age was retained in the final model (Table 4). The regression coefficient of hookworm epg corresponded to a 0.107 g/dL decline in hemoglobin per 1000 epg increase. The estimated effect of gestational age (weeks) corresponded to a 0.027 g/dL decrease in hemoglobin per additional week.

DISCUSSION

To our knowledge this is one of the largest studies of the occurrence of helminth infection in pregnant women in South America. It confirms the high prevalence of soil-transmitted helminth infections, particularly that of hookworm infection, in this vulnerable population. It also confirms the critical importance of ascertaining the intensity of infection. The higher the intensity of hookworm infection, the higher the proportion of anemic women. No association was found with prevalence. Although over 90% of our population had no or light infections, we found a statistically significant association between moderate and heavy hookworm infection and anemia. This type of association has previously been demonstrated in Nepal[35] and in Kenya[36]. In both these studies, it appeared that intensity was associated with anemia after a threshold of infection had been reached (2000 epg and 1000 epg, respectively). We also observed a threshold at 2000 epg in our study. The 2000 epg threshold is consistent with the lower bound of the WHO category of moderate hookworm infection. Therefore, although the WHO categories of hookworm intensity are based on data obtained in child populations, our data suggest that the same categories may be applicable in pregnant populations.

The association between moderate and heavy hookworm infection and anemia was strengthened when there was co-infection with moderate and heavy *Trichuris* infection. It is of interest to note that, after adjusting for known covariates, these 'infection' determinants were the only statistically significant determinants of anemia. This result also highlights the potential role of *Trichuris* infection in anemia. Being able to rule out malaria as a determinant of anemia in our study population likely contributed to these observations.

Further understanding of parasite determinants of anemia would be beneficial. In particular, it would be important to corroborate our findings in other pregnant populations in other geographical areas. This would produce evidence from populations having different hookworm species (and in different ratios), different intensities of hookworm (and other soil-transmitted helminths) and different anemia profiles (eg. proportion iron-deficiency) among other concurrent host, parasite and host-parasite interaction determinants.

Our results provide additional evidence in support of the recommendation of WHO, UNICEF and INACG to include anthelminthic treatment in prenatal programs, in areas where the prevalence of hookworm infection exceeds 20-30%.[37] Anthelminthic therapy is inexpensive, and in areas participating in the

Global Programme for the Elimination of Lymphatic Filariasis, albendazole is free of charge. An evaluation of the cost-effectiveness of including anthelminthics within prenatal care programs is urgently required. Benefits would also follow from an increased understanding of the benefits of prenatal anthelminthic treatment on infant outcomes such as birthweight, growth and development.

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Table 1. Prevalence of anemia and hookworm infection in 1042 second-trimester pregnant women living in Iquitos, Peru, 2003, by sociodemographic characteristics.

Variable	N	Frequency (%)	Prevalence anemia (%)	*P* value*	Prevalence hookworm (%)	*P* value*
Total	1042	----	47.31	----	47.22	----
Age						
< 25 years	556	53.36	48.92		46.04	
≥ 25 years	486	46.64	46.64	0.462	48.56	0.417
Environment						
Live in peri-urban area	968	92.90	47.73		46.07	
Live in rural area	74	7.10	40.54	0.229	62.16	0.007
Marital status						
Married or 'conviviente'	970	93.09	46.39		47.84	
Single or other	72	6.91	58.33	0.051	38.89	0.138
Gestational age						
Fourth month	532	51.06	44.55		46.43	

Fifth month	337	32.34	48.66	0.237	47.77	0.700
Sixth month	173	16.60	52.60	0.066	48.55	0.628
Iron supplementation						
Yes	154	14.78	53.25		43.51	
No	888	85.22	46.17	0.105	47.86	0.317
1st pregnancy						
Yes	195	18.71	47.18		37.44	
No	847	81.29	47.23	0.990	49.47	0.002
Have animals						
No	514	49.33	47.76		51.07	
Yes	528	50.67	46.78	0.752	43.56	0.015
Wear sandals outside the house						
Yes	950	91.17	48.11		46.53	
No	92	8.80	37.90	0.058	54.95	0.123
Wear sandals inside the house						
Yes	724	69.55	48.62		44.34	

No	318	30.45	44.34	0.202	54.09	0.004
House						
Concrete	97	9.31	49.48		25.77	
Brick	65	6.24	38.46	0.164	41.54	0.039
Wood	347	33.30	33.30	0.005	42.07	0.002
Palm tree material	533	51.15	48.22	0.819	55.16	< 0.001
Floor						
Concrete	172	16.51	44.19		35.47	
Wood	345	33.11	46.38	0.637	56.52	< 0.001
Dirt	525	50.38	48.76	0.296	44.95	0.026
Water						
Bottled water	60	5.76	46.67		33.33	
River	78	7.50	52.56	0.494	57.69	0.004
Well	360	34.55	49.17	0.720	42.78	0.158
Drinkable water from truck	123	11.80	54.47	0.322	42.28	0.238
Drinkable community water	421	40.40	42.52	0.868	52.49	0.005
Type of toilet facilities						

Modern facilities	23	2.21	34.78		26.09	
Latrines	945	90.69	47.62	0.217	47.09	0.031
Nothing	74	7.10	45.95	0.339	55.41	0.01
Schooling						
Secondary completed	263	25.19	48.09		24.81	
< secondary completed	779	74.81	46.92	0.742	54.76	< 0.001

* *P* values were based on chi-square test

Table 2. Prevalence and intensity of hookworm infection, and prevalence of anemia, in 1042 second trimester pregnant women living in Iquitos, Peru, 2003.

Intensity categories	N	Prevalence of hookworm (%)	Prevalence of anemia (%)	*P* value
Presence of infection	492	47.22	48.78	0.340
Absence of infection	550	52.78	45.82	
None	550	52.78	45.82	
Light	435	41.75	47.13	0.683
Moderate	40	3.84	55.00	0.265
Heavy	17	1.63	76.47	0.008
None and light	985	94.53	46.40	
Moderate and heavy	57	5.47	61.40	0.027

Table 3. Relationship between intensity of soil-transmitted helminth infections and anemia(< 11g/dL) during pregnancy, based on data from 1042 second trimester pregnant women living in Iquitos, Peru, 2003.

Variable	Reference group	Crude		Adjusted	
		OR	95% CI	OR	95% CI
Any hookworm	No hookworm	1.13	0.88, 1.44		
Any *Trichuris trichuria*	No *Trichuris*	1.01	0.73, 1.39		
Any *Ascaris lumbricoides*	No *Ascaris*	0.83	0.64, 1.06		
Moderate and heavy hookworm	None and light	1.84	1.06, 3.18	1.84	1.06, 3.17
Moderate and heavy *Trichuris*	None and light	1.26	0.96, 1.65		
Moderate and heavy hookworm and *Trichuris*	Not moderate and heavy hookworm and *Trichuris*	2.14	1.10, 4.14	2.13	1.10, 4.13

Table 4. Regression coefficients (β) and corresponding P values of variables found to be determinants of hemoglobin (g/dL) in 1042 pregnant women living in Iquitos, Peru (2003).

Variable	β	95% CI	*P* value
Hookworm egg count (per 1000 epg)	-0.107	-0.162 , -0.052	0.0002
Age (per year)	0.012	-0.0001 , 0.023	0.0527
Gestational age (per week)	-0.027	-0.053 , -0.001	0.0405

PREFACE TO MANUSCRIPT C

Manuscript A summarized the published evidence on the benefits and risks of antenatal anthelminthics during pregnancy. Thereafter, Manuscript B described in detail the population of pregnant women included in the randomized controlled trial. Using socio-demographic and epidemiologic information obtained through questionnaires and biological measurements, the association between the intensity of helminth infection and hemoglobin levels was examined.

Manuscript C presents the main results of this thesis research. It focuses on the effectiveness of antenatal mebendazole plus iron supplements versus placebo plus iron supplements on birthweight in a population of pregnant women living in a highly hookworm-endemic area.

This trial is the largest conducted to date of a benzimidazole administered to pregnant women. The results fill an important gap of knowledge and will be used by WHO and national governments as they review deworming programs targetted to pregnant women.

These results have been presented at: The American Society of Tropical Medicine and Hygiene Annual Meeting (2004, 2005), The Global Forum for Health Research (2004) and the International Congress on Tropical Diseases and Malaria (2005).

This manuscript has been accepted for publication in the *Journal of Tropical Medicine and International Health.*

6 MANUSCRIPT C

A double-blind randomized controlled trial of antenatal mebendazole to reduce low birthweight in a hookworm-endemic area of Peru

Abbreviated running title: RCT of antenatal mebendazole

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Abstract

OBJECTIVE The aim of the study was to assess the effectiveness of antenatal mebendazole plus iron versus placebo plus iron on birthweight in a highly hookworm-endemic area.

METHODS This trial was a double-blind randomized controlled trial set in rural and peri-urban communities in the Peruvian Amazon region. A total of 1042 second trimester pregnant women between the ages of 18 and 44 years were recruited from April to November 2003, and followed to July 2004. Women were randomly assigned to receive either mebendazole (500 mg single dose) plus iron supplements (60 mg elemental iron daily) or placebo plus iron supplements. The primary outcome was mean infant birthweight and secondary measures included proportion of low birthweitht babies and maternal anaemia.

RESULTS The prevalence of hookworm infection was 47.5%. There were no differences between intervention groups in mean birthweight (3104 g vs 3090 g, P=0.629), proportion of low birthweight (<2500g) (8.1% vs 8.7%, P=0.755) or maternal anaemia in the 3rd trimester (33.0% (158/479) vs 32.3% (152/471), P=0.815). However, the proportion of very low birthweight (<1500g) was significantly lower in the mebendazole group (0% (0/479) vs 1.5% (7/471), P=0.007).

CONCLUSION This trial provides additional evidence for the use of anthelmintics, over and above iron supplementation, within antenatal care programs in hookworm-endemic areas. Benefits of de-worming may be higher in

countries not having an antenatal iron supplementation program or where intensity of hookworm infections are higher.

Trial Registration www.controlled-trials.com Identifier: ISRCTN08446014

Keywords mebendazole, pregnant women, hookworm, birthweight, anaemia, randomized-controlled trial

Introduction

Birthweight is an extremely powerful predictor of infant growth and survival. Low birthweight (LBW) (< 2500 g) infants are more likely to be iron-deficient (Olivares *et al.* 1992) and have low Apgar scores (McIntire *et al.* 1999). Birthweight also affects postnatal growth and development (Steer 2000). LBW infants have higher rates of intestinal and respiratory infections, higher rates of hospitalization, and higher mortality rates than appropriate weight infants (Ashworth 1998, Lira *et al.* 1996, Bartlett *et al.* 1991, Bukenya *et al.* 1991, Victora *et al.* 1990). Moreover, adults who were born LBW are thought to be at increased risk of chronic diseases such as hypertension, non-insulin dependent diabetes mellitus, coronary heart disease and stroke (Barker 1997).

A major determinant of LBW in developing countries is maternal undernutrition which is associated with low gestational weight gain, low pre-pregnancy body mass index and short maternal stature (Kramer 1987). Young age at first pregnancy, cigarette smoking during pregnancy, and having malaria during pregnancy are also associated with LBW (Kramer 1987).

Significant correlations between maternal anaemia and LBW and between severe iron deficiency anaemia and LBW have been reported (Yazdani *et al.* 2004, Singla *et al.* 1997, Scholl & Hediger 1994; Viteri 1994).

One possible approach to reducing LBW is to reduce maternal anaemia during pregnancy. In developing countries both nutritional iron deficiency and parasitic infections (mostly hookworm infections, but also to a lesser extent, *Trichuris* infections), are two primary causes of anaemia (Dreyfuss *et al.* 2000;

Larocque *et al.* 2005) . One study of second trimester pregnant women in Peru found that iron intake was only 17.8% of the recommended requirements (Gyorkos *et al.* 2004). Although nutritional iron deficiency is strongly associated with anaemia in pregnancy, intestinal helminth infections, especially hookworm infections, contribute to anaemia by causing blood loss and by affecting the supply of nutrients necessary for erythropoiesis. In fact, hookworm infections have been identified as the leading cause of pathological blood loss in tropical and sub-tropical countries (Pawlowski *et al.* 1991). Bundy and colleagues (1995) have estimated that approximately one-third of all pregnant women in developing countries are infected with hookworms. The World Health Organization (1996) recommends that anthelmintic treatment be given to pregnant women after the first trimester in areas where the prevalence of hookworm infection exceeds 20-30%. However, use of antenatal anthelmintic treatment remains uncommon in most developing countries, and research on its effects has been limited.

In a large retrospective cross-sectional survey in Sri Lanka (N=7087), the proportion of very low birthweight (VLBW) was found to be lower in women who reported taking mebendazole during their pregnancy (de Silva *et al.* 1999). In a randomized factorial trial of 125 pregnant women in Sierra Leone, maternal anaemia was most reduced by the combination of albendazole plus iron-folate supplements (Torlesse & Hodges 2001). More recently, observations on antenatal anthelmintic use within a micronutrient trial in Nepal (N=3327) indicated positive effects of two doses of albendazole in reducing both LBW and 6-month infant mortality (Christian *et al.* 2004).

To date, there has been no randomized controlled trial on the effect of antenatal anthelmintic treatment added to iron supplements on infant birthweight. We therefore designed a double-blind, randomized controlled trial to compare the effectiveness of antenatal mebendazole plus iron supplements versus placebo plus iron supplements on birthweight and maternal anaemia in a hookworm-endemic area.

Methods

Study location and population

The trial was conducted in Iquitos, Peru, a tropical jungle area. Iquitos is situated in the Department of Loreto, which comprises nearly one-fourth of the landmass of Peru and has the ecologic characteristics of the Amazon lowlands (see Figure 1).

Soil-transmitted helminth infections, malaria and anaemia are endemic in the area. Living conditions are characterized by poor sanitation and limited access to potable water. Maternal and Child Programs are active at the two main hospitals (Hospital Apoyo de Iquitos and Hospital Regional de Iquitos) and at various health centers ("posta medica") in the towns and villages. Only 63% of pregnant women in Loreto attended an antenatal care program in 2000 (Endes 2000). In Peru, it is estimated that 35% of women of childbearing age and 50% of pregnant women have anaemia (Zavaleta et al. 1993). The adjusted maternal mortality ratio in 2000 in Peru was 410/100 000 live births and the infant mortality rate was 26/1000 live births (Unicef 2003).

Pregnant women were recruited from 12 health centres in the Iquitos region from April 2003 to November 2003. Recruitment also included canvassing door-to-door in neighbourhoods in the more rural catchment areas of the health centres because it was known that many pregnant women did not seek antenatal care. The inclusion criteria were: second trimester (≥ 18 weeks; < 26 weeks) pregnant women between 18 to 44 years of age (gestational age of the woman was determined using a combination of the fundal height and the first day of the last menstruation period); not having received anthelmintic treatment for six months prior to recruitment; residing in rural or peri-urban areas (defined as having no running water or flushing toilet facility in the home) and giving consent. Exclusion criteria were: having severe anaemia (< 7 g/dL) (20) or having a medical condition for which follow-up was required.

Randomization and study interventions

A double-blind randomized controlled trial design was used. Computer-generated randomly-ordered blocks of four, six and eight were used to randomly allocate women to each intervention group. Two researchers not otherwise involved in the trial prepared sealed envelopes containing the intervention assignment. The designated "obstetriz" (i.e. health professional specialized in obstetric care) in each participating health centre was given a supply of envelopes. Envelopes were distributed among the recruitment sites until the sample size was achieved.

Once inclusion criteria were satisfied, each woman was given a plastic container and asked to return the next day with a single stool specimen. The container was then collected and fingerprick blood was obtained to measure haemoglobin (by Hemo-Cue®) and presence/absence of malaria by thick and thin smear. The accuracy of the battery-operated Hemo-Cue® photometer has been determined to be within +/- 1.5% of the HiCN method (the gold standard cyanmethaemoglobin method) (Kwant *et al.* 1987). Each Hemo-Cue® photometer was checked for calibration every morning before use.

Following blood drawing, women received either a single dose of mebendazole (500 mg) plus an iron supplement (60 mg elemental iron) or a single dose placebo (similar appearance, smell and taste to the mebendazole tablet) plus an iron supplement (60 mg elemental iron). Both mebendazole and placebo tablets were purchased from Janssen-Cilag (Brazil). Iron was ferrous sulphate (PharmaScience, Canada). Women were given containers having a one-month supply of iron supplements and instructed to take one supplement per day. Women returned every month for their antenatal check-up and, at the same time, received another month's supply of iron. The standard-of-care for pregnant women in the study area was iron supplements.

The local project director (RL), field workers, obstetrices, laboratory technologists, and pregnant women were all blind to the group assignment. The group assignment list was kept in Canada while all the recruitment and data collection took place in Peru.

On the same day women were randomized, a questionnaire was administered by the study obstetriz to obtain pertinent socio-demographic and epidemiologic information. The stool specimens and malaria smears were examined by two trained laboratory technologists on the same day they were received. Both technologists had previously received a specialized two-week training in the parasitology laboratory of the Instituto de Medicina Tropical "Alexander von Humboldt" at the Universidad Peruana Cayetano Heredia in Lima, Peru. The Kato-Katz technique for quantification of intestinal helminth infection was used. Intensity of infection was expressed as eggs per gram (epg) of faeces. Quality control consisted of: 1) revision of 10% of all stool specimens by a technical expert from the Instituto de Medicina Tropical "Alexander von Humboldt"; 2) daily review of the lab log and immediate appropriate remedy of errors or abnormal observations; 3) daily supervision of slide preparation; 4) re-reading of all negative slides by the second technologist; and 5) storage of all faecal samples in 10% formalin for three months should remedial review be required. Malaria detection followed Giemsa staining. The quality control for malaria detection consisted of: 1) re-reading of all positive slides by the second technologist; 2) daily review of the lab log and immediate appropriate remedy of errors or abnormal observations; 3) daily check of the quality of the smears with remedial training of obstetrics when required; and 4) storage of all smears should remedial review be required.

Each woman was followed until delivery (approximately 5 months). Assessments were made at baseline (2nd trimester), at 3rd trimester and at delivery and included: 1) anaemia, 2) malaria, and 3) hookworm and other soil-transmitted helminth infections.

Data collection activities during fieldwork were regularly supervised to ensure that forms were being correctly completed. The local project director checked all forms at the end of each day. If there were discrepancies or unclear answers, a discussion and explanation of the question and eligible answers took place to avoid making errors the next day. Corrections were made, if warranted.

Birthweight was ascertained at birth in the delivery hospital by the attending nurse using a baby electronic scale (Seca model 334) (Seca corp, Baltimore, Maryland USA) provided by our study. Nurses were trained to use the scale. For domiciliary births, study obstetrices used portable baby electronic scales (Seca model 345) (Seca corp, Baltimore, Maryland USA) to obtain birthweights as soon after birth as possible. The accuracy of the digital scale is better than ± 0.3% or ± 10g (insert from the manufacturer). Scales were calibrated on a weekly basis. Recumbent crown-heel length of the baby was measured using a measuring mat (Seca model 210) (Seca corp, Baltimore, Maryland USA). Infants whose birthweight was obtained more than two days after delivery were excluded from the analyses.

Outcome measures

The primary outcome measure was mean infant birthweight. Additionally, LBW (< 2500 g vs ≥ 2500 g) and VLBW (< 1500 g vs ≥ 1500 g) comparisons were also examined. The secondary outcome was maternal anaemia in the 3^{rd} trimester measured by 1) mean haemoglobin and 2) haemoglobin < 11 g/dL vs ≥ 11 g/dL.

Sample size calculation

We estimated that 100 pregnant women per group would allow us to detect a difference in mean birthweight of 200g with 80 % power at a 5% significance level. However, in order to determine the effect difference in sub-groups according to hookworm intensity using the four levels of WHO (1996), 100 subjects per sub-group would be needed (ie. 400 pregnant women per intervention group, for a total of 800 women). The final number of pregnant women was determined to be 960, assuming a loss-to-follow-up of approximately 20%.

Statistical analyses

Descriptive analyses were conducted using means and proportions. Data were analyzed on an intention-to-treat basis. Means were compared using the two-sample t test and the paired t-test; proportions, X^2 tests, Fisher's exact test, and McNemar's test, when appropriate. The analysis for birthweight was restricted to singleton live births. Crude odds ratios (ORs) and 95% confidence intervals (95% CI) were computed for each categorical variable. A general linear model was used to compare mean birthweight in the two intervention groups by intensity level of hookworm infection. Multivariate linear regression was performed to compare mean birthweight between intervention groups controlling for factors that differed between groups or that were known effect modifiers. Similarly, logistic regression models were used to compare proportions of LBW and exact logistic regression models to compare proportions of VLBW between the groups.

Geometric means were calculated from epg count data. Cure rates (CR) were calculated for all three soil-transmitted helminths (hookworm, *Trichuris* and *Ascaris*) at the 3rd trimester assessment. Percentage reductions in prevalence (PRP) and egg reduction rates (ERR) were obtained by comparing values between the third and second trimesters.

All analyses were carried out using SAS version 6.0 (SAS Institute, Cary, NC).

Ethical consideration

Ethics approval was obtained from these ethics review committees: Research Institute of the McGill University Health Centre (Canada); the "Comite Institucional de Etica de la Universidad Peruana Cayetano Heredia" (Peru); and the "Comite Etica de la Direccion General de Salud de las Personas del Ministerio de Salud de Peru" (Peru). Written informed consent was obtained from all women.

Results

Baseline Characteristics

Between April 2003 and November 2003, 1042 women were enrolled and randomized (figure 2). Of 522 women allocated to the mebendazole group, 17 (3.3%) were lost to follow-up; in the placebo group, 19 (3.6%) women were lost to follow-up. There was a total of 492 singleton livebirths in both groups. The numbers of twin pregnancies were comparable in the two groups (3 vs 2). There was a total of 17 miscarriages and stillbirths (1.7%) (10 in the mebendazole group and 7 in the placebo group). Of the 984 liveborn singletons, 34 (3.5%) either did not have a birthweight recorded or had their birthweight measured after 48 hours and were excluded from further analysis.

Table 1 shows that women in the two groups were similar in terms of socio-demographic characteristics and pregnancy-related variables. The proportions of home deliveries were similar in both groups (8.4% vs 8.1%). The groups were similar in terms of the prevalence of anaemia (47.4% (222/479) vs 48.0% (226/471)) and all three soil-transmitted helminth infections (Table 2). In addition, similar proportions of women in both groups had moderate and heavy intensities of helminth infections. Most women harboured light hookworm infections, with only 5.2 % (n = 49) having moderate and heavy intensity hookworm infection (>2000 epg); 29.1 % (n = 276) had moderate and heavy intensity *Trichuris* infection (>1000 epg) and 33.0 % (n = 313) had moderate and heavy intensity *Ascaris* infection (>5000 epg). Culture analysis confirmed that both hookworm species, *N. americanus* and *A. duodenale,* were present in our study population.

Response to treatment

The CRs, PRPs and ERRs for each parasite species are shown in Table 3. Mebendazole had the greatest effect on *Ascaris* infections and the lowest on hookworm infections. The prevalences and mean intensities of soil-transmitted helminth infections at each assessment are shown in Figures 3 and 4, respectively. Post-intervention, both prevalences and mean intensities were significantly lower in the mebendazole group compared to the placebo group. In addition, the proportion of moderate and heavy intensity hookworm infection decreased significantly throughout the study period in the mebendazole group (from 5.9% (28/479) at baseline to 2.1% (10/479) post-delivery).

Even though this region of Peru is known as a malaria-endemic region, only two women were found to be smear-positive at baseline (1 case of *Plasmodium vivax* and 1case of mixed *P. vivax* and *P. falciparum* infection, both in the mebendazole group). Two more cases were detected during the measurements in the third trimester: one case of *P. falciparum* in the mebendazole group and one case of *P. vivax* in the placebo group. And finally, two additional cases were detected during measurements at delivery: one case of *P. vivax* in the mebendazole group and one case of *P. falciparum* in the placebo group.

There was a statistically significant reduction in anaemia between the second and third trimesters within each group; however, there was no between-group difference in the proportions of anaemia (33.0% (158/479) vs 32.3% (152/471), *P*=0.815).

We observed no statistically significant difference between the mebendazole and placebo groups in terms of mean birthweight (3104 g ± 441.54 vs 3090 g ± 481.62, *P*=0.629) (Table 4). Similarly, the proportion of LBW was not statistically significantly different between the two groups (8.1% (39/479) vs 8.7% (41/471), *P*=0.755) (Table 4) with an odds ratio of 0.93 (95% CI: 0.59, 1.47). There was also no between-group difference in mean birthweight or proportion of LBW by intensity strata of hookworm infection (F = 0.82; *P*=0.511). After adjusting for maternal age, the mean difference in birthweight between the two groups was 16.83 g (95% CI: -41.49, 75.15). The proportion of VLBW was, however, statistically significantly lower in the mebendazole group (0% (n = 0) vs 1.5% (n = 7), *P*=0.007) (Table 4) with an odds ratio of 0.10 (95% CI: 0.0, 0.68). When adjusted for maternal age, marital status, previous intake of iron, environment and schooling, the odds ratio for VLBW remained the same (OR=0.10; 95% CI: 0.0, 0.69). In an extensive review of the medical charts of the seven VLBW babies, no unusual particular characteristic was noted.

The frequency of adverse birth outcomes was similar in the two groups (P=0.664). Of a total of 28 (5.6%) adverse events in the mebendazole group, there were 2 miscarriages, 8 stillbirths, 4 malformations, 3 early neonatal deaths and 11 premature deliveries. Of a total of 31 (6.2%) adverse events in the placebo group, there were 3 miscarriages, 4 stillbirths, 5 malformations, 6 early neonatal deaths and 13 premature deliveries.

Discussion

This trial is the largest trial to date of a benzimidazole administered to pregnant women. WHO (1996) guidelines recommend that anthelmintics be part of routine antenatal care in areas where hookworm prevalence exceeds 20-30%. Mebendazole was chosen as the anthelmintic for this trial because it is more commonly used in Peru than the other WHO-recommended anthelmintics (ie. albendazole, levamisole and pyrantel). It should be noted that the benzimidazoles, albendazole and mebendazole, are available in single dose format and consequently are preferred in large scale de-worming programs. Standard-of-care necessitated the concurrent administration of iron supplements, and so, in our trial, the benefits of mebendazole, over and above those of iron supplementation, were assessed.

We have shown that antenatal mebendazole (500 mg) plus iron supplements (60 mg elemental iron) significantly reduces the proportion of very low birthweight babies (< 1500 g) when compared to placebo plus iron supplements. Our results confirm previous observations from a large retrospective cohort study conducted in Sri Lanka that also found a beneficial effect of antenatal mebendazole on very low birthweight (de Silva *et al.* 1999). When considering the millions of women who give birth every year in hookworm-endemic areas, it is clear that the magnitude of the benefit of de-worming in terms of reducing numbers of very low birthweight babies, might well be considerable.

Other birthweight measures were not found to be different between our two groups (ie. mean birthweight; proportion low birthweight (< 2500 g)). Although one would expect that the effects of mebendazole would be most clearly observed in women with moderate and heavy intensity hookworm infection, we were unable to demonstrate an important differential benefit. Furthermore, while maternal anaemia decreased significantly in each group between the second and third trimesters, we found no difference between the two groups. This result shows the possible powerful effect of iron supplementation. Indeed, iron supplementation has been estimated to far exceed benefits derived from iron fortification, vitamin A supplementation and de-worming, with the possible exception of de-worming of high intensity levels of hookworm infection, particularly those caused by *Ancylostoma duodenale* (WHO 1996). Our proportions of low birthweight are lower in both groups (8.1 % and 8.7 %, respectively), than the proportion reported by Unicef for Peru (11 %) (Unicef 2003). This is probably due to the effects of the iron supplementation.

In the mebendazole plus iron group, hookworm egg reduction rates were found to be acceptable (slightly over 60%); however, cure rates were found to be lower than expected (approximately 30%)(WHO 2002). Because of the timing of our second assessment (ie. approximately 76 days after mebendazole administration), it is likely that some re-infection had already occurred, and therefore any efficacy measurement is likely to be underestimated. The use of albendazole, which is known to have higher cure rates than mebendazole, might overcome this limitation.

WHO recommends that, if hookworm prevalence is over 50%, anthelmintic administration should be repeated 12 weeks after the first dose (Stoltzfus & Dreyfuss 1998). Our study population had a baseline prevalence of 46.4%, and may have benefited from a two-dose regimen. Benefits, in terms of 6-month mortality rates and mean birthweight, have recently been reported in Nepal following receipt of two antenatal doses of albendazole (Christian *et al.* 2004). Although the Nepal study was large (N=3327), it was designed as a trial of different micronutrients, not anthelmintics, and the results must be further corroborated.

The only other trial of a benzimidazole was that of albendazole and iron in pregnant women in Sierre Leone (Torlesse & Hodges 2001). However, this study was small in size (N $\approx$ 30 in each of four intervention groups) and the iron supplements contained only 36 mg of elemental iron (compared to the recommended 60 mg). The combined albendazole and iron group had the greatest effect on reducing maternal anaemia. Future research examining the benefits of deworming alone (for example, when iron supplementation is not available or is not accessible) would make an important contribution.

The benzimidazoles, albendazole and mebendazole, have several features which make them attractive to local governments. First, they are inexpensive, at less than .02 $ USD per tablet (WHO 2002). They are considerably less expensive than iron supplementation which has been estimated at $21.39 USD per pregnant woman per year in South America (Baltussen *et al.* 2004). Additionally, albendazole is free-of-charge in areas participating in the Global Program for the Elimination of Lymphatic Filariasis (Molyneux *et al.* 2003). Second, they are easy to administer, being available in single dose format, and do not need weights for dosing. They also have an effect on concurrent soil-transmitted helminth infections such as *Ascaris* and *Trichuris* infections, thereby reducing the risk of complications associated with ectopic migration of larvae or worms (MacLeod 1988).

Strengths and limitations of the study

The main strength of this study is that it is the largest RCT to date examining the effect of antenatal mebendazole on infant birthweight and maternal anaemia. Additionally, loss to follow-up was minimal and similar in the two groups. Therefore, if any bias was present, it would be very small and non-differential. Our population had mostly light intensity hookworm infections, therefore our results are generalizable to other pregnant populations with similar intensity levels. It may well be that this represents the intensity profile of most hookworm-endemic areas, although this would have to be confirmed.

One limitation of our study is that recruitment was limited to pregnant women between 18 and 44 years of age. Results are therefore generalizable only to this age range. However, it is expected that similar results would be obtained in pregnant women of other ages.

Implications

Our results emphasize the need for further research into anthelmintic effects in pregnant women and their offspring. It would be important to investigate these effects in different geographic regions having different co-morbidities of anaemia, hookworm infections and malaria; in regions having different ratios of *Necator* and *Ancylostoma* infections; and in regions having different intensities of hookworm infection (especially where this would result in two doses being administered). Cost-effectiveness analyses and modeling approaches should also be explored to ensure the transfer of as timely and comprehensive information as possible to local decision makers.

At the present time, only Sri Lanka and Nepal have included anthelmintics in their routine antenatal care programs. Our results provide additional evidence for the continuing implementation of these programs and contribute to the growing body of evidence documenting the benefits of deworming.

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Zavaleta N, Berlanga R, Lonnerdal B, *et al.* (1993) Prevalence and determinants of iron deficiency anemia in a representative sample of pregnant women in Lima, Peru. Final report presented to the Pan American Health Organization. Washington DC: Pan American Health Organization.

Figure 1. Iquitos, Department of Loreto, Peru.

Figure 2. Trial profile.

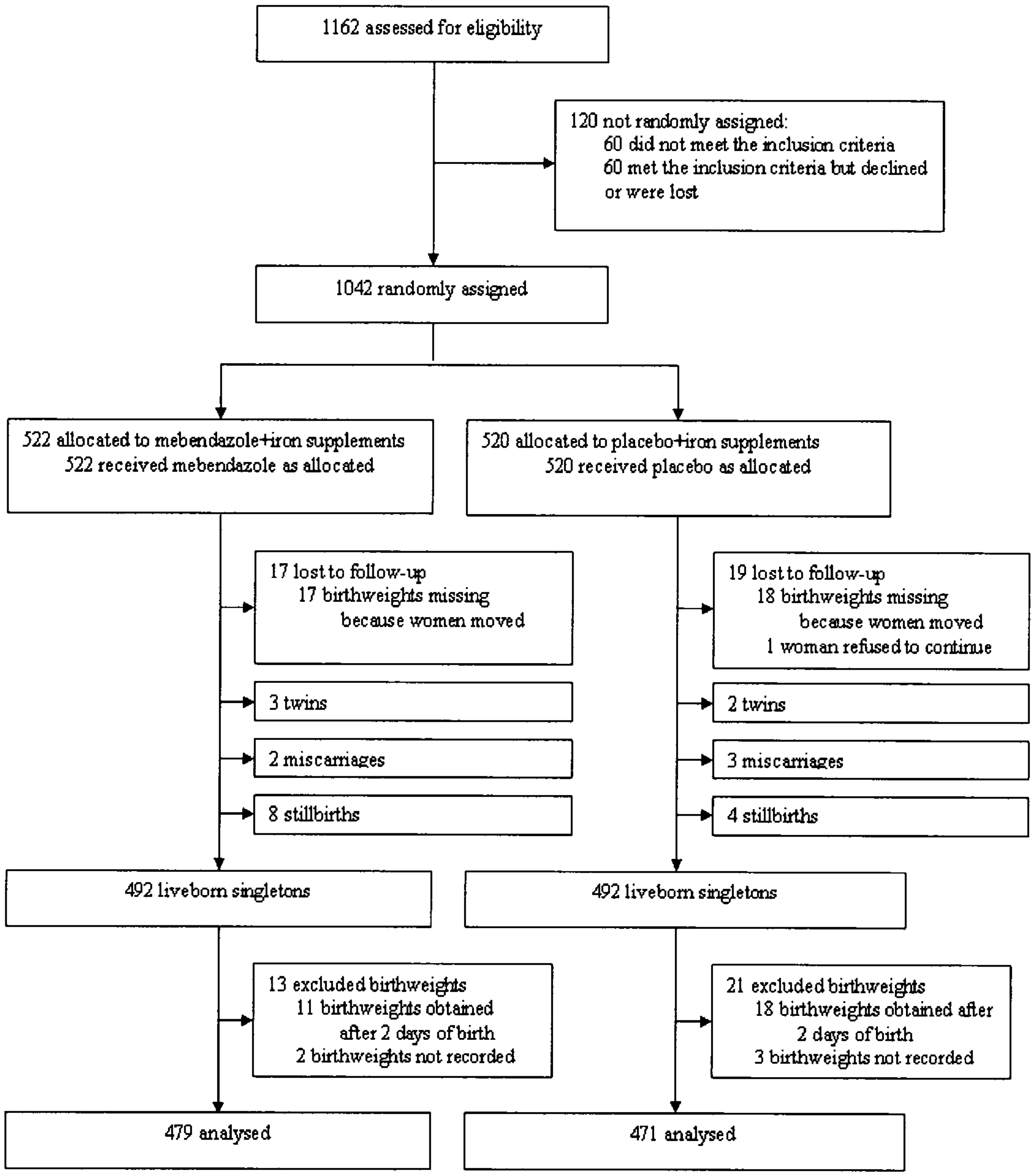
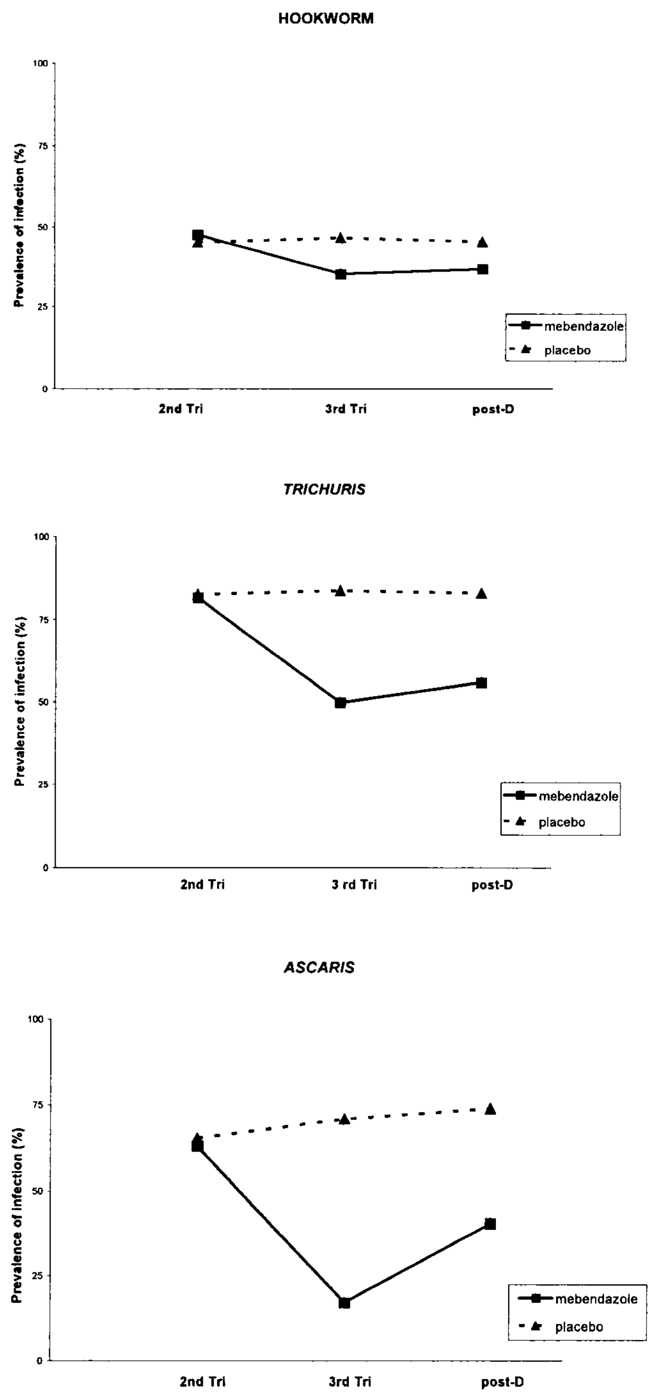
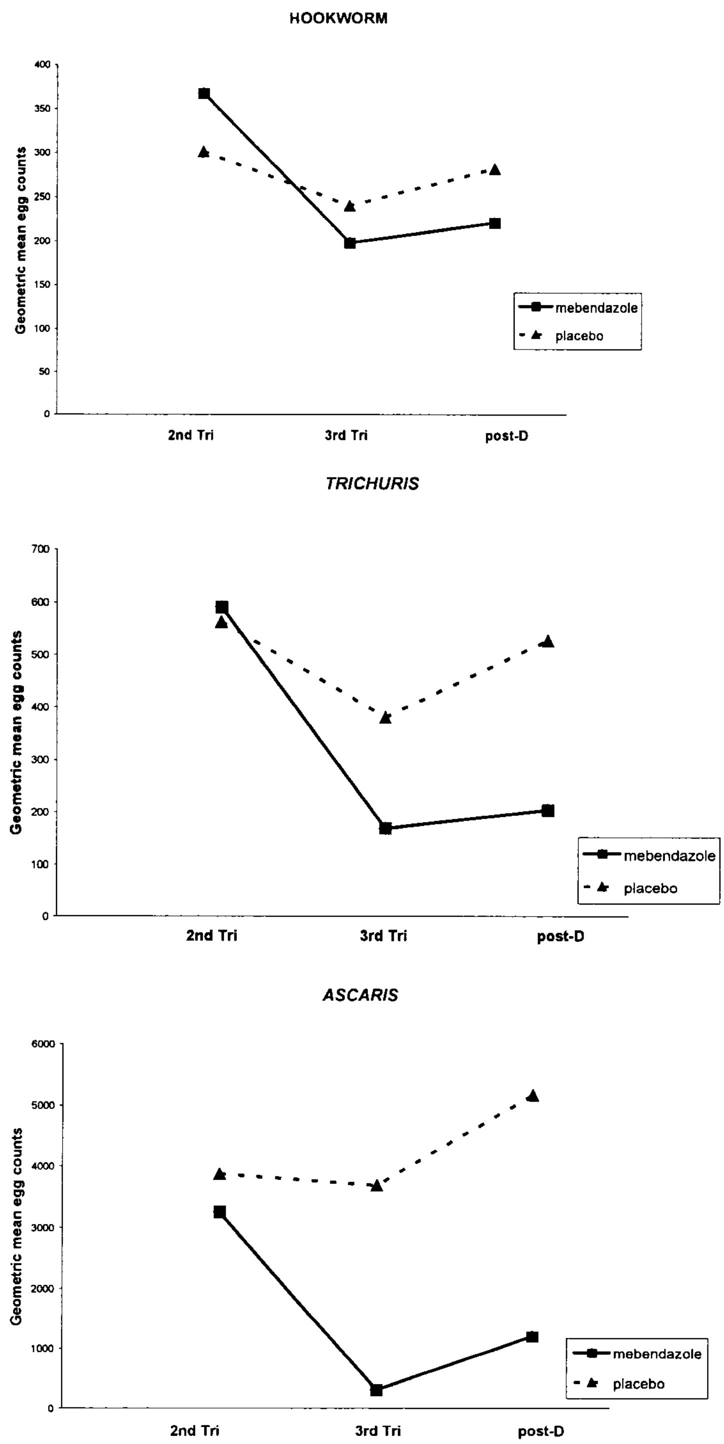


Figure 3. Prevalence of hookworm, *Trichuris* and *Ascaris* infection during pregnancy.



2nd Tri=second trimester (baseline); 3rd Tri=third trimester; post-D=post-delivery.

Figure 4. Geometric mean egg counts of hookworm, *Trichuris* and *Ascaris* infection during pregnancy.



2nd Tri=second trimester (baseline); 3rd Tri=third trimester; post-D=post-delivery.

Table 1. Baseline characteristics of 950 second trimester women randomized to either mebendazole plus iron or placebo plus iron.

	Mebendazole + iron (n=479)	**Placebo + iron (n=471)**
Age (years)	25.2 (5.8)	25.4 (5.5)
Gestational age (weeks)	20.4 (2.6)	20.4 (2.6)
Living with partner	441 (92.7)	441 (93.6)
Environment		
Residence in urban areas	433 (90.4)	440 (93.4)
Residence in rural areas	46 (9.6)	31 (6.6)
Schooling completed		
Less than primary	79 (16.5)	62 (13.2)
Primary completed	281 (58.7)	285 (60.5)
Secondary completed	119 (24.8)	124 (26.3)
Primigravidae	87 (18.2)	88 (18.7)
Women already taking iron supplementation	74 (15.5)	73 (15.5)
Housing		
Concrete	50 (10.4)	42 (8.9)
Bricks	31 (6.5)	24 (5.1)
Wood	151 (31.5)	165 (35.0)
Rustic material	247 (51.6)	240 (51.0)
Floor		
Concrete	89 (18.6)	59 (12.5)
Wood	157 (32.8)	157 (33.3)
Dirt	233 (48.6)	255 (54.14)
Toilet facility		
Modern non-flushing toilet	13 (2.7)	6 (1.3)

Latrines	430 (89.8)	437 (92.8)
Nothing	36 (7.5)	28 (5.9)
Water		
Treated	31 (6.5)	32 (6.8)
Cistern trucks	60 (12.5)	51 (10.8)
Community pipe	178 (37.2)	198 (42.0)
Wells	167 (34.9)	166 (35.2)
Other	43 (9.0)	24 (5.1)

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

Table 2. Baseline prevalences and intensities of soil-transmitted helminth infections and baseline prevalence of anaemia and mean hemoglobin levels.

	Mebendazole + iron (n = 479)	**Placebo + iron (n = 471)**
Soil-transmitted helminth prevalences		
Hookworm	228 (47.6)	213 (45.2)
Trichuris	391 (81.6)	389 (82.6)
Ascaris	302 (63.1)	308 (65.4)
Co-infection hookworm/*Trichuris*	213 (44.5)	200 (42.5)
Soil-transmitted helminth intensities*		
Moderate/heavy hookworm	28 (5.9)	21 (4.5)
Moderate/heavy *Trichuris*	145 (30.3)	131 (27.8)
Moderate/heavy *Ascaris*	144 (30.1)	169 (35.9)
Moderate/heavy co-infection hookworm/*Trichuris*	19 (4.0)	14 (3.0)
Anaemia (< 11 g/L)	222 (47.4)	226 (48.0)
Haemoglobin level (g/L)	11.1 (1.1)	11.1 (1.1)

Results are expressed as means (SD) or numbers (%). *intensity classification based on WHO [20].

Table 3. Cure rates, percentage reductions in prevalence and egg reduction rates at approximately 76 days following mebendazole administration (N=479).

	Cure rate (CR)	**Percentage reduction in prevalence (PRP)**	**Egg reduction rate (ERR)**
Hookworm	30.7	25.8	60.8
Trichuris	39.1	38.9	92.9
Ascaris	72.5	72.8	98.3

Results are expressed as percentages

PREFACE TO MANUSCRIPT D

Manuscript D completes the description of adverse birth outcomes observed in the RCT. While Manuscript C documented the benefits following the use of mebendazole in pregnancy, Mansucript D focuses on details relating to risks associated with the use of mebendazole during pregnancy. In this manuscript, numbers and types of adverse birth outcomes are compared between the two intervention groups (mebendazole plus iron supplements versus placebo plus iron supplements). More specifically, the occurrence of neonatal mortality, miscarriages, malformations, stillbirths, early neonatal deaths and premature babies are compared between the groups.

The results of this manuscript were presented at The American Society of Tropical Medicine and Hygiene Annual Meeting (2005).

This manuscript has been accepted for publication in the *Pediatric Infectious Disease Journal.*

7 MANUSCRIPT D

Lack of risk of adverse birth outcomes following deworming in pregnant women

Abbreviated title: Fewer adverse birth events after deworming

Running head: Birth risks of deworming

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Keywords: benzimidazoles, mebendazole, adverse birth outcomes, pregnancy, hookworm

ABSTRACT

BACKGROUND: Pregnant women who live in hookworm-endemic areas may benefit from deworming during their pregnancy. The benefit derives from reducing anemia, primarily iron-deficiency anemia caused by hookworm infection-attributable blood loss. Where the prevalence of hookworm is more than 20-30%, the WHO recommends that pregnant women receive anthelminthic treatment (either mebendazole, albendazole, levamisole or pyrantel), after their first trimester. The objective of this study is to report, describe and compare the occurrence of adverse birth outcomes in a large randomized controlled trial of antenatal mebendazole (500 mg single dose) plus iron supplements versus placebo plus iron supplements conducted between April 2003 and June 2004 in the Amazon region of Peru.

METHODS: Physician-recorded data on adverse birth outcomes occurring during the trial (N=1042) were obtained. Proportions were compared using chi-square analysis.

RESULTS: No statistically significant difference ($p = 0.664$) was found between the mebendazole group and the placebo group in terms of numbers of miscarriages, malformations, stillbirths, early neonatal deaths and premature babies (28 versus 31, respectively).

CONCLUSIONS: The evidence provided by this large randomized controlled trial of mebendazole administered during pregnancy indicates that deworming can be safely included in antenatal care programs in hookworm-endemic areas.

INTRODUCTION

In developing countries, it is estimated that 56% of pregnant women have anemia (compared with 18% in developed countries) and that approximately 20% of maternal deaths are either directly or indirectly related to anemia.[1, 2] The causes of anemia during pregnancy are multifactorial but one of the primary causes in developing countries is infection with hookworms. The age-prevalence and age-intensity curves of hookworm infection reach their peak in young adulthood and remain at this level throughout life.[3] Pregnant women therefore constitute a high risk group for anemia and hookworm infection. It has been estimated that one-third of all pregnant women in developing countries are infected with hookworms.[4] Anemia has important adverse consequences on the health of both the pregnant women and the fetus.[5] Deworming during pregnancy is therefore considered as an effective tool in reducing anemia in areas where hookworm prevalence exceeds 20-30%.[6, 7]

WHO recommends any of the following four drugs for the treatment of hookworm infection in pregnancy: albendazole, levamisole, mebendazole and pyrantel.[7] These drugs, administered after the first trimester, have been found to be safe and effective, having few and minor, if any, side effects.[2,8,9] Because of the ease of use of their single dose format, the benzimidazoles (albendazole and mebendazole) are the drugs most widely used in helminth control programs targeted to school-aged children and pregnant women.[10-12] Despite the recognized benefits of deworming, it is possible that fear of adverse birth outcomes has limited its inclusion in routine antenatal care.

To date, four studies have examined adverse birth outcomes following use of albendazole or mebendazole during pregnancy (Table 1). Three were observational studies examining mebendazole[10, 13, 14] and one was a randomized controlled trial of albendazole[15]. None of these studies found significantly more adverse outcomes in the benzimidazole group compared with the comparison group. However, because of limitations in study design and methodology (eg. small sample sizes, unknown or non-standard dosages, possibility of information and selection biases), and a lack of detail in the ascertainment or reporting of the adverse outcomes, a new and rigorous evaluation was warranted.

We therefore report here on the adverse birth outcomes from a large double-blinded, placebo-controlled randomized trial we conducted in Iquitos, Peru, a highly hookworm-endemic area. Baseline data confirmed a high prevalence of hookworm infection (47.2%) in the pregnant women participating in the trial[16] thereby meeting the WHO threshold of over 20-30% for routine de-worming in antenatal care.

METHODS

Details of the double-blind randomized controlled trial comparing iron plus mebendazole versus iron plus placebo appear in the international trial registry #ISRCTN08446014. This trial received ethics approval from the McGill University Health Centre in Montreal, Canada, the Ministerio de Salud in Iquitos, Peru and the Universidad Cayetano Peruana Heredia in Lima, Peru; and satisfied all ethics questions of the funding agency (The Canadian Institutes of Health Research). All participating women signed an informed consent form. Briefly, between April and November 2003, a total of 1042 pregnant women were recruited in their second trimester and randomized to receive either a single 500 mg dose of mebendazole (Nemasole^{TM}) or placebo (Jansen-Cilag Pharmaceutical (Brazil)), together with a 30-day supply of iron supplements (ferrous sulphate; 60 mg elemental iron; PharmaScience, Canada). Following randomization, women in both groups received 30-day supplies of iron supplements throughout their pregnancy. The mebendazole and placebo were single dose tablets of 500 mg and were administered by the interviewer to the women at the time of the initial interview. Interviewers were blind to treatment assignment. Interviewers were qualified nurse midwives or 'obstetrices' with membership in the 'Colegio de Obstetrices de Loreto' whose university-based training included five years of nursing, specializing in obstetrics. Women were eligible to participate in the trial if they were older than 18 years of age, were in their second trimester of pregnancy, lived in a peri-urban or rural area (did not have water or sanitation facilities in the home), had not taken any anthelminthic treatment in the previous six months and gave consent.

Gestational age was estimated from information provided by the woman on date of last menstrual period and on measurement of fundal height as ascertained by the obstetriz. Birth outcome and birthweight were recorded at the hospital or the woman's home (in the case of a domiciliary birth). Birthweight was measured using digital stationary or portable scales (Secacorp, USA) that were supplied by the project. In the case of a domiciliary birth, the baby was observed by the research obstetriz within 48 hours of birth for assessment of clinical outcome. Adverse birth outcomes were recorded as miscarriage, malformation, stillbirth, early neonatal death (death at < 7 days) and premature birth (birth < 37 weeks gestation). The attending physician and nurses at delivery were blind to the treatment assignment of the woman. Details on the type of malformation and cause of early neonatal death were obtained from medical charts in the study hospitals. In all cases of adverse birth outcomes the diagnosis was confirmed by the hospital neonatologist.

Twins were excluded from all analyses. A comparison of the proportions of adverse outcomes in the two groups was tested using the chi-square test. Perinatal mortality was calculated as follows: (number of stillbirths + number of early neonatal deaths)/1 000 births.

RESULTS

Mebendazole and placebo were administered at an average gestational age of 20.44 ± 2.61 and 20.35 ± 2.56 weeks, respectively. There was no reported use of anthelminthic medication other than that provided by the trial. There were no differences between the two groups in terms of age (25.2 yr ± 5.8 vs 25.4yr ± 5.5), gestational age (20.4 wk ±. 6 vs 20.4 wk ± 2.6), parity (primigravidae: 18.2% vs 18.7%), marital status (living with partner: 92.7% vs 93.6%) or schooling (< secondary schooling: 75.2% vs 73.7%), in addition to other sociodemographic variables. Moreover, hemoglobin levels at baseline were similar in both groups (11.05 g/dL ± 1.10 vs 11.01 g/dL ± 1.06) (data not shown). There were 22 outcomes excluded in the mebendazole group (n=522): 3 were twins, 17 were lost to follow-up and 2 had missing birthweight and outcome information. There were 24 outcomes excluded in the placebo group (n=520): 2 were twins, 19 were lost to follow-up and 3 had missing birthweight and outcome information. Overall, loss to follow-up and incomplete information accounted for 3.95% (41/1037) of the total population, excluding twins (ie. 3.7 % and 4.2%, in the mebendazole and placebo groups, respectively).

Birthweight outcomes (mean birthweight, low birthweight and very low birthweight) are detailed in Larocque et al (unpublished data). There was a total of 28 (5.60%) adverse birth outcomes in the mebendazole group compared with 31 (6.25%) in the placebo group (Table 2). This difference was not statistically significant ($p = 0.664$). Although there was a greater number of stillbirths in the mebendazole group (8 vs 4), there were less early neonatal deaths (3 vs 6). The number of malformations in the mebendazole group was lower than in the placebo group (7 vs 8). The respective proportions of malformations were 1.40% (7/500) and 1.61% (8/496), a difference that was not statistically significant ($p = 0.783$). No clinically significant difference was found between the two groups in terms of miscarriages (2 versus 3) ($p = 0.647$). None of these differences were considered to be clinically significant. Details of the type of malformations are given in Table 3.

The perinatal mortality rate was 22/1000 births in the mebendazole group compared with 20.2/1 000 births in the placebo group. These rates were not found to be either clinically or statistically significantly different ($p = 0.840$).

Issues of compliance are not of concern here because both the mebendazole and the placebo were administered as single 500 mg tablets and consumption was directly observed by the research obstetrices. Daily compliance with the iron supplements was difficult to ascertain but it was considered to be similar in the two groups, based on attendance at follow-up visits and self-reporting of consumption at the time of provision of monthly refills.

DISCUSSION

These data on adverse birth outcomes were obtained from the largest placebo-controlled randomized trial of a benzimidazole administered during pregnancy. The perinatal mortality rates in this study were similar to those reported for the Americas in 1999 (ie. 22/1 000 livebirths)[17] whereas our malformation rates were lower (ie. 1.5% versus 2-3%[18]). Loss to follow-up was minimal and equivalent in the two groups, further substantiating the internal validity of the results and the use of univariate statistical testing.

Our results provide rigorous evidence and additional support to those of previous studies which have reported no additional important adverse effects of either mebendazole or albendazole on birth outcomes following use during pregnancy, after the first trimester [11,13-15]. National governments, health care practitioners, and helminth control program managers should be confident in the routine inclusion of these anthelminthics in antenatal care programs in areas where hookworm infection meets the threshold prevalence set by WHO (i.e. > 20-30%).

Future research from randomized controlled trials on this issue would further strengthen the evidence. In particular, it would be helpful to understand if a similar finding holds for each of the four WHO-recommended deworming drugs in areas where the intensity of hookworm infection varies and in areas where the species (or ratios between the species) of hookworm differs (ie. *Necator* *americanus* versus *Ancylostoma duodenale*). Lastly, promotion of deworming in pregnant women is warranted, especially when hookworm prevalence or the proportion of heavy intensity hookworm infection greatly exceeds the threshold. To date, only Sri Lanka and Nepal include deworming within routine antenatal care. Active dissemination and translation of peer-reviewed published research is urgently needed to spur national governments to include deworming in antenatal programs.

ACKNOWLEGEMENTS

We are pleased to acknowledge the cooperation of the maternity and neonatal departments of the Hospital Regional de Iquitos, the Hospital de Apoyo de Iquitos, and the Centro de Salud San Juan, all in Iquitos, Peru. The dedication of Obstetriz Carmen Nunez de Rojas, coordinator of the project, is also gratefully recognized. This project was funded by the Canadian Institutes of Health Research (Grant # MCT-53575).

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Table 1. Previous studies documenting adverse birth outcomes following deworming during pregnancy, after the first trimester.

First author, year, place	**Study design**	**Dosage and drug**	**No. pregnant women**	**Adverse birth outcome**
Acs 2005 [14] Hungary	Matched case-control	-2x100 mg for 3 consecutive days, mebendazole	Cases of congenital anomalies[1] = 22843	0.06% used mebendazole
			Controls = 38151	0.04% used mebendazole
Diav-Citrin 2002[13] Israel	Matched Cohort	-No mebendazole	55	0 major birth defects
		-Mebendazole (single 100 mg dose - 29%, repeated single 100 mg dose – 36%, 100 mg dose for 3 consecutive days – 35%)[2]	55	5 (only 1of the 5 occurred following consumption of mebendazole in the second trimester; the other 4 were in the first trimester)
Torlesse 2001[15]	RCT	-400 mg albendazole + 36 mg iron	32	1[3] malformation
		-400 mg albendazole + 36 mg placebo	29	0

Sierra Leone		-36 mg iron + 400 mg placebo	35	0
		-400 mg placebo + 36 mg placebo	29	0
de Silva 1999[11] Sri Lanka	Survey	-No anthelminthics	1737	1.5% Major birth defects – 3.3% Stillbirths and perinatal deaths – 2.3% very low birthweight (<1500g)
		-Unknown dose mebendazole	5275	1.8% Major birth defects – 1.9% Stillbirths and perinatal deaths – 1.1% very low birthweight

1- Congenital anomalies excluding Down syndrome (no further details provided)

2- Only 28.5% of the 192 women in the mebendazole group had taken mebendazole after the first trimester; in all other cases, mebendazole had been taken in the first trimester.

3- One case of bilateral supernumerary fingers was found in a woman who had taken albendazole. It was not reported if this case was in the albendazole plus iron group or the albendazole plus placebo group.

Table 2. Occurrence of adverse birth outcomes during a randomized double-blinded placebo-controlled trial of iron plus mebendazole versus iron plus placebo in 1042 pregnant women in Iquitos, Peru, 2003-2004.

Adverse Birth Outcomes	**Mebendazole plus iron group (n = 522)**	**Placebo plus iron group (n = 520)**
Excluded: Twins	3	2
Excluded: Lost-to-follow-up	17	19
Excluded: Missing birthweight and outcome information	2	3
Miscarriages	2	3
Stillbirths	8	4
premature	4 (2)*	1
term	4	3
Early neonatal deaths	3	6
premature	0	4 (1)
term	3 (1)	2 (2)
Malformations		
term	4	5
Premature births	11	13
TOTAL	28	31

* ( ) indicates number of malformations

Table 3. Types of malformations occurring during a randomized double-blinded placebo-controlled trial of iron plus mebendazole versus iron plus placebo in 1042 pregnant women in Iquitos, Peru, 2003-2004.

Birth Outcomes	**Malformations in Mebendazole plus iron group**	**Malformations in Placebo plus iron group**
Stillbirths	Multiple, unspecified (premature – week 33)	
	Cleft palate (premature – week 26)	
Early neonatal death	Day 1:Down syndrome	Day 1: of the diaphragm
		Day 1: joined vagina and anus (premature – week 29)
		Day 2: Unspecified
Term births	Down syndrome	Hydrocephaly
	One additional finger	Club foot
	Facial deformation	Club foot
	Closed vagina	Cleft palate
		Missing left hand
Total	7	8

PREFACE TO MANUSCRIPT E

In Manuscript B and C, challenges appeared when we wanted to summarize the intensity (eggs per gram) of parasite infection. Different measures (eg. arithmetic means and geometric means) have been used in the published literature. Geometric means can be problematic, especially in statistical testing and when considering how to interpret the results. In Manuscript E, different methods that have been used to summarize the intensity of helminth infections are compared to a new proposed method "The weighted geometric mean method".

Preliminary results were presented at The American Society of Tropical Medicine and Hygiene Annual Meeting (2004).

A preliminary draft of a manuscript addressing these methods is being prepared for submission to the *International Journal of Parasitology.*

8 MANUSCRIPT E

SUMMARIZING EPG COUNT DATA USING A WEIGHTED GEOMETRIC MEAN

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INTRODUCTION

Eggs per gram (epg) are used as an indicator of the intensity of parasite infection. When summarizing the intensity of parasite infection within a population, both arithmetic and geometric means (GM) of epg have been used. GMs are frequently used because the distribution of epg data in most populations is highly skewed (usually characterized by a negative binomial distribution) (Anderson and May, 1991). The simple arithmetic mean calculation of such data would not be suitable for determining "average" conditions since the data distribution is not symmetrical. The relatively rare occurrences of very high intensity infections will result in an arithmetic mean considerably higher than a mean that would be reflective of the real population intensity profile. In such cases, logarithmic transformation of the data may be helpful for reducing the variability and producing a normal or near-normal shape. An arithmetic mean of the log-transformed data can then be calculated. Transforming this mean back to the original scale provides the GM. GMs are therefore appropriated measurements for skewed data. However, since the logarithm of 0 does not exitst, GMs can only be calculated for non-infected individuals. Clearly, in any population, many individuals will not be infected and will have zero epg values. Most parasitologists have dealt with this problem by adding a 1 to each epg count to permit calculation of the logarithm as shown below:

Geometric mean (0s replaced by 1s) = $\exp[\sum (\log_e(epg + 1))/n] - 1$

The calculation of the GM in parasitology research and in parasite prevention and control programs has been proposed by several researchers (Traub *et al.*, 2004; Albonico *et al.*, 2003) and WHO (1998). The GM requires log-transforming the data to produce data with a less skewed distribution and therefore less dominated by a small proportion of high epg values (Fulford, 1994). The distribution of the transformed data more closely approaches that of the normal bell-shaped curve than does the non-transformed data. This is a desirable property since the application of many statistical testing techniques requires the data to be normally distributed. Approximately normal distributions are therefore obtained from skewed data by converting numbers to their logarithms.

Through the use of bootstrap simulations, Fulford (1994) calculated the frequency with which the confidence intervals around the geometric mean and those around the arithmetic mean enclose the true mean (of the original dataset). He demonstrated that the geometric mean always outperforms the arithmetic mean by having its confidence intervals enclose the true mean with a higher frequency. However, the GM is not always preferable to the arithmetic mean. For example, Fulford (1994) found that in the case of infection with *Schistosoma mansoni*, the GMs were biased. This bias was attributed to the age-intensity relationship where it was proposed that log-transforming the data would artificially reduce the difference in intensity between two ag-groups. However, Fulford (1994) explains that if the researcher was interested in testing the hypothesis of the equality of two means, then using the GM would not bias the result.

Researchers examining synergistic associations between hookworm and other helminth infections in communities in rural Brazil used arithmetic means to express the intensity of infections (Fleming *et al.*, 2006). These researchers justified this calculation with the fact that the occurrence of clinical outcomes increases with the intensity of infections and that no saturation occurs at high intensities. In such cases, log-transforming the data would artificially reduce the difference in intensity between groups and would hide the true relationship between intensity of the infection and occurrence of outcomes. GMs would therefore be misleading in such situations. Some researchers do not express a preference for GM or arithmetic mean but choose to present both. For example, Tchuem Tchuente *et al.* (2003) have reported both arithmetic and GMs to give estimates of the parasite intensity in school children from Cameroon.

Other researchers have calculated the geometric mean using data only from the infected proportion of the study population (Larocque *et al.*, 2006; 2005; Ziem *et al.*, 2006; Tchuem Tchuente *et al.*, 2001).

We propose the use of a simple and alternative measurement for epg count data that overcomes the statistical limitations of both the arithmetic mean and the two previously constructed ways to calculate the GM. We call this alternative measurement a weighted geometric mean (wGM). The calculation of wGM takes into account the combination of a summary measure of epg count data in the infected proportion of a population with that of the magnitude of the infected proportion. As populations in different parts of the world have different hookworm prevalences and intensity distributions, the weighted geometric mean is an approach that can capture these differences.

Weighted geometric mean =

[% non-infecteds X $\exp\{\sum (\log_e epg)/n)/\text{non-infected}\}$] + [% infecteds X $\exp\{\sum (\log_e epg)/n\ /\text{infecteds}\}$]

The wGM sets the GM of the uninfected proportion of the population at 0 (since it is impossible to calculate the GM of a 0 value). The formula then reduces to wGM = % infecteds X exp [$\sum$ ($\log_e$epg)/number of infecteds]

The objective of this paper is to examine the suitability of this simple technique, the wGM, in summarizing epg count data and to compare this technique with those previously used (arithmetic mean; geometric mean replacing 0s with 1s; and geometric mean of infecteds only). In addition, these different means will be used in the context of statistical hypothesis-testing comparing the intensity of hookworm infection in relation to anemia in two groups using a recently obtained field data set.

METHODS

Data Source. Data on anemia and parasite infection from a baseline survey which took place in Iquitos, Peru, between April 2003 and November 2003 will be used. The study population included 1042 pregnant women.

Measurement of anemia. Hemoglobin concentration (g/dL) was obtained from blood fingerprick using Hemo-Cue® assessment. The cut-off of 11g/dL was used, as recommended by WHO (1996) to distinguish anemic from non-anemic women.

Measurement of hookworm infections. The Kato-Katz technique for quantification of intestinal helminth infection was used (Martin and Beaver, 1968). Intensity of infection was expressed as eggs per gram of feces. The WHO (1998) categories for light, moderate and heavy levels of intensities of hookworm infections were used: light infection = 1 - 1,999 epg, moderate infection = 2,000 - 3,999 epg and heavy infection $\geq$ 4,000 epg.

Statistical methods. The summary measures compared for the overall estimation of epg counts were: 1) the arithmetic mean ($\sum$epg/n); 2) the geometric mean (zeros replaced by 1) ($\exp[\sum (\log_e(epg + 1))/n] - 1$); 3) the geometric means in the infecteds only ($\exp[\sum (\log_e(epg_{infecteds}))/n]$; and 4) the proposed method: the weighted geometric mean (% infecteds x ($\exp[\sum (\log_e(epg_{infecteds}))/n]$).

In order to calculate standard deviations of the estimate of the weighted geometric mean, a moderate correlation (eg. 0.4) was assumed between the proportion of infecteds and the epg count in the infecteds.

RESULTS

The study population had a prevalence of hookworm infection of 47.2 %. Only 1.63% (N=17) of women had heavy intensity hookworm infection, while 3.84% (N=40) had a moderate intensity infection, and 41.75% (N=435) had a light infection, 52.78% (N=550), having no infection. Summary measures of epg counts from this population are reported in Figure 1. The weighted geometric mean (164 epg), fell between the arithmetic mean (427 epg) and the geometric mean (0s replaced by 1s) (16 epg). This indicates that in the weighted GM, the high values of epg have less of an influence than when measuring the arithmetic mean but that the weight of the zero values will depend on the proportion of uninfecteds in the population. If the prevalence of hookworm had been higher in the population the weighted geometric mean would have also been higher. For example, in a population having an 80% prevalence of hookworm infection, with the same arithmetic mean as our population (ie 427 epg), the weighted GM would be higher.

Is anemia related to hookworm intensity? Measures of mean epg counts are presented for both anemic pregnant women and non-anemic pregnant women in Table 1. When using the method geometric mean replacing 0s with 1s, the confidence intervals between anemic and non-anemic pregnant women overlap which would indicate that there is no difference between the intensity of hookworm infection between these two groups (in a univariate analysis). However, when using the weighted geometric mean, there is no overlap in the confidence interval between anemic and non-anemic pregnant women which would indicate that these two groups differ in their hookworm intensity and that anemia is related to hookworm intensity (again, in a univariate analysis).

DISCUSSION

Different measures (eg. arithmetic means and geometric means) have been used to summarize the intensity (eggs per gram) of parasite infection in the published literature. There are benefits and limitations for each measure. Even though the distribution of epg counts does not follow a normal distribution, researchers might still be interested in the value of the arithmetic mean because of its simplicity of interpretation and use in statistical testing. Log-transforming the epg counts when calculating the geometric mean adjusts for the skewness of epg parasite count distributions.

The proposed method, the weighted geometric mean, resolves some of the limitations of both the arithmetic and geometric means. It is a simple method which can be easily computed. This method attributes different weights to the epg counts depending on the prevalence of infection in each population. The weighted geometric mean can also be weighted by the proportions of different intensity categories of parasitic infection (ie. none, light, moderate and heavy). It can also be used in multivariate analyses. It is important to note that the answer to the question "is anemia related to hookworm intensity?" is different when using the geometric means replacing 0s with 1s and the weighted geometric mean. This should be further examined with other research questions and data from different populations.

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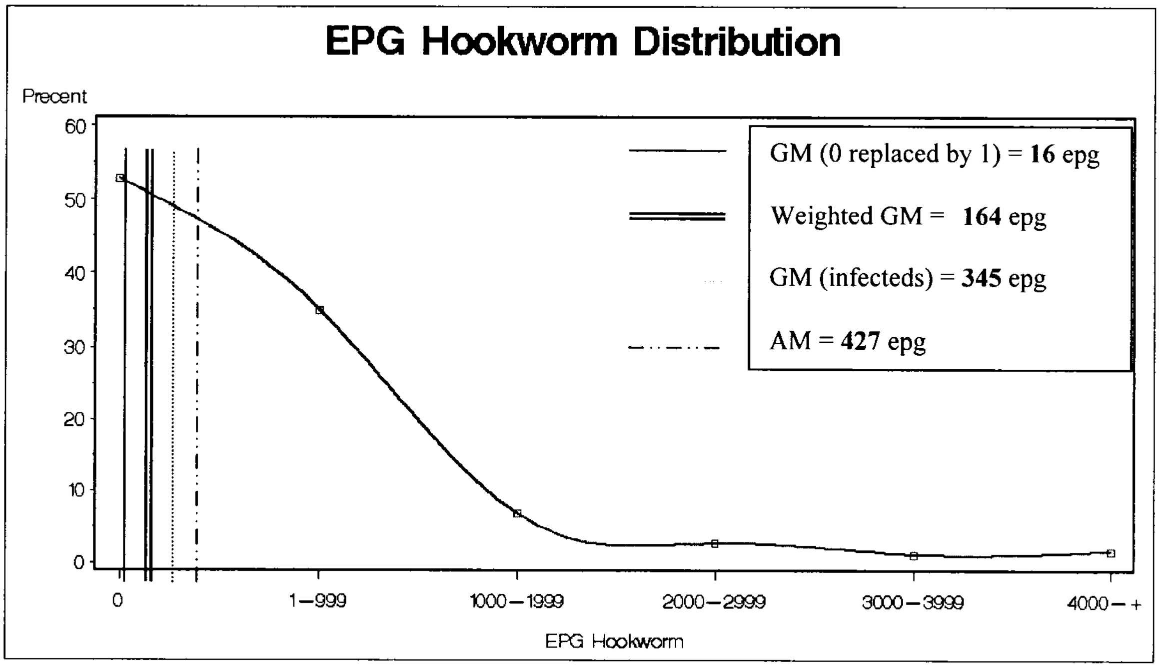
Table 1 – Summary measures of hookworm intensity in anemic and non-anemic pregnant women (N=1042) from Iquitos, Peru (2003).

	Anemic group N=550	**Non-anemic group** N=492
Method		
Arithmetic mean (AM)	569.85 (95%CI: 436, 703)	299.48 (95%CI: 232, 367)
Geometric mean (GM) (infecteds only)	432.87 (95%CI: 356,521)	280.69 (95%CI: 237, 328)
Geometric mean (0s replaced by 1s)	18.34 (95%CI: 15, 26)	12.26 (95%CI: 10, 17)
Weighted GM	211.15 (95%CI: 165, 268)	128.61 (95%CI: 103, 159)

Figure 1 – Results of different summary measures for hookworm intensity in a population of pregnant women (N=1042).



GM= Geometric mean

AM= Arithmetic mean

9 GENERAL DISCUSSION AND CONCLUSION

9.1 Benefits and risks associated with antenatal anthelminthics

This RCT is the largest trial to date examining the benefits and risks associated with the use of a benzimidazole during pregnancy in a hookworm-endemic area. WHO recommends the use of anthelminthics in areas where the prevalence of hookworm infection is over 20% (WHO, 2006; 1996a). In this trial, mebendazole was used because it was the anthelminthic most commonly used in Peru. Iron supplementation was the standard of care in the government-sponsored antenatal care package. Therefore our trial aimed to examine the effects of mebendazole use during pregnancy over and above the effects of iron supplementation.

The proportion of very low birthweight (VLBW) was statistically significantly lower in the women who had received mebendazole and iron compared to the women who received placebo and iron. Comparable results were observed in a large cross-sectional study in Sri Lanka (de Silva *et al.*, 1999). They also found that mebendazole use during pregnancy significantly decreased the proportion of VLBW. Considering the millions of women giving birth every year, the impact in terms of reducing VLBW that deworming could have is not to be overlooked.

The proportion of LBW was also lower in the mebendazole plus iron group but this difference was not statistically significant. Similarly, mean birthweight was higher in the mebendazole plus iron group compared to the placebo plus iron group but the difference was not statistically significant. A large non-randomized trial in Nepal also found that one dose of a benzimidazole (in this trial, albendazole) did not increase mean birthweight but that two antenatal doses of albendazole showed statistically significant increases in mean birthweight (Christian *et al.*, 2004). Although the Nepal study was large (N=3327), it was designed as a trial of different micronutrients, not anthelminthics, therefore the results must be further substantiated. WHO recommends two antenatal doses of anthelminthics in areas where hookworm prevalence is over 50% (Stoltzfus and Dreyfuss, 1998). Our study population had a baseline prevalence of 46.4%, and may have benefited from a two-dose regimen.

Anemia in our study population significantly decreased between the second and third trimester; however the decrease was similar between the two intervention groups. Considering that we found a statistically significant relationship between the intensity of hookworm infection and hemoglobin levels in our population at baseline (Larocque *et al.*, 2005), it was expected that the effects of mebendazole would be most clearly observed in women with moderate and heavy intensity hookworm infections; however, no differential benefits were observed. This relationship therefore should be further examined in pregnant populations where the proportion having moderate and heavy intensity hookworm infections is higher than in our study population.

The occurrence of adverse birth outcomes (malformations, stillbirth, early neonatal mortality, perinatal mortality, prematurity) was similar in both intervention groups. Therefore, our results demonstrate that there is no additional risk associated with the use of mebendazole during pregnancy. These findings support and corroborate the findings of previous studies which have reported no additional important adverse effects of either mebendazole or albendazole on birth outcomes following use during pregnancy, after the first trimester (Acs *et al.*, 2005; Diav-Citrin *et al.*, 2003; Torlesse and Hodges, 2001; de Silva *et al.*, 1999).

In the mebendazole plus iron group, hookworm egg reduction rates were found to be acceptable (slightly over 60%); however, cure rates were found to be lower than expected (approximately 30%) (WHO, 2002). Because of the timing of our second assessment (ie. approximately 76 days after mebendazole administration), it is likely that some re-infection had already occurred, and therefore any efficacy measurement is likely to be underestimated. The use of albendazole, which is known to have higher hookworm cure rates than mebendazole, might overcome this limitation. Only limited information on the use of levamisole and pyrantel in pregnancy is available; results from studies using these drugs would demonstrate if they could potentially be an effective alternative in pregnant women.

An additional benefit of using the benzimidazoles, is that they are relatively inexpensive (less than .02$ USD per tablet) (WHO, 2002). They are considerably less expensive than iron supplementation which has been estimated at 21.39 $ USD per pregnant woman per year in South America (Baltussen *et al.*, 2004). In areas participating in the Global Programme for the Elimination of Lymphatic Filariasis, albendazole is given free of charge (Molyneux *et al.*, 2003); this represents a great number of pregnant women who could receive anthelminthics at no additional cost to the local government.

9.2 Strengths and limitations of the study

The main strength of the study is that the results reported are from an RCT of antenatal anthelminthics with the largest sample size to date. Only one other study of a benzimidazole in pregnant women was a randomized controlled trial but it had a small sample size (N=125 divided within 4 arms) and sub-standard values of iron supplementation were used (36 mg instead of 60 mg of elemental iron) (Torlesse and Hodges, 2001). Additionally, our RCT is the only RCT examining infant outcomes (eg. birthweight, malformations and prematurity). Previous studies reporting infant outcomes were observational studies (Acs *et al.*, 2005; Christian *et al.*, 2004; Diav-Citrin *et al.*, 2003; de Silva *et al.*, 1999). Even though some of these studies had large sample sizes, several biases (eg. selection bias, recall bias, report bias) could have been operating.

In our trial, loss to follow-up was kept to a minimum and was similar in both arms of the trial. This ensured that, if any imbalance in terms of population characteristics occurred over the follow-up period, it was minimal and non-differential.

Furthermore, extensive measures of quality control were followed throughout this trial guaranteeing high internal validity. Results of this trial are generalizable to pregnant women living in areas of high prevalence of hookworm infections but of mostly low intensity. This scenario of prevalence and intensity of infection might represent most hookworm-endemic areas; however this would need further confirmation.

One limitation of our study is that women included were aged between 18 and 44 years of age. Therefore while the results are generalizable to these age groups, they may also be applicable to pregnant women aged less than 18 or older than 44 years. Another limitation of our study is the fact that a single stool sample was requested and analyzed to assess prevalence and intensity of helminth infections. Using a single stool sample may possibly underestimate prevalence and intensity, therefore the study population might be more infected and might have higher intensities of infection.

9.3 Implications

Our results demonstrate that the use of antenatal mebendazole significantly decreases the proportion of VLBW and that there is a lack of risk of additional adverse birth outcomes following deworming in second trimester pregnant women. These findings support the WHO recommendation of using antenatal anthelminthics in hookworm-endemic areas (> 20% prevalence), after the first trimester (WHO, 2006; 1996a). It would however be important to further investigate the use of different integrated intervention packages, which could include other vitamin and mineral supplements (vitamin A, folate, zinc, etc.). Additional information on the use of antenatal anthelminthics in different regions (different prevalence, intensity and parasite species) would be very informative to fill these knowledge gaps and to further develop antenatal packages better customized to each population. More empirical scientific evidence is also needed on the benefits and risks of albendazole during pregnancy, since this drug has been shown to have better cure rates in hookworm infection.

At the present time, only Sri Lanka and Nepal have included deworming in their routine antenatal care programs. The weakness of the evidence base may have been one reason why uptake of this policy by other governments has been hindered. Future research therefore should be directed towards 1) strengthening the evidence base with empirical data from randomized controlled trials of both albendazole and mebendazole (eg. one or two doses; both mother and infant outcomes, etc.) and 2) furthering our understanding of barriers and of enabling factors related to government uptake of the WHO policy on deworming.

One important step to take in order to break the barriers related to the uptake of such policies by government and health professionals is knowledge dissemination of the results of trials like this one. In addition to presenting our results to the scientific community, we also presented our results to health professionals and government authorities in Iquitos, Peru (in March 2006). A copy of the letter of invitation to that presentation can be found in Appendix 6.

The Millennium Development Goals aim at eradicating extreme poverty, reducing child mortality, improving maternal health, and combating HIV/AIDS, malaria and other diseases, by the year 2015 (UN Millennium Project, 2005). Deworming, especially in pregnant women, can help meet several Millennium Development Goals by improving birth outcomes and maternal health. The full advantage of antenatal deworming has yet to be measured but evidence to date suggests that benefits outweigh the risks.

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Appendix 1

Manuscript F

Gyorkos TW, Shenker H, Larocque R, Ugaz MR, Gotuzzo E. Sociodemographic and dietary correlates of anemia in pregnant women in Peru. *Ecology of Food and Nutrition* 2004; **43**:497-516.

SOCIODEMOGRAPHIC AND DIETARY CORRELATES OF ANEMIA IN PREGNANT WOMEN IN PERU

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The objective of this article is to identify sociodemographic and dietary correlates of iron-deficiency anemia among pregnant women in Chanchamayo, Peru. A dietary and socio-demographic survey was conducted among 206 pregnant women in July 2002. Blood analyses were performed to determine the presence of anemia (Hb < 110 g/L) among study participants. A higher prevalence of anemia was found in women from rural (51.2%), compared to semi-urban (32.8%), and urban (31.6%) areas. No significant difference in the prevalence of anemia was found between women with different intakes of various nutrients including total iron and heme iron. However, environmental risk factors were found to be statistically associated with anemia (OR = 2.21; 95%CI: 1.07, 4.53). Factors other than total caloric intake and daily iron intake must be further investigated to explain the significantly higher proportion of anemia in pregnant women living in rural areas. Our evidence suggests that environmental factors including parasitic infection could play an important etiological role.

KEYWORDS: Anemia, Pregnancy, Peru, Diet, Diet Surveys, Iron, Hookworm Infections, Developing Countries, Rural Population, Women

INTRODUCTION

Iron deficiency anemia is well recognized as one of the major nutritional disorders of the world and is especially prevalent among pregnant women (World Health Organization, 2002). An estimated 56% of pregnant women in developing countries suffer from anemia, whereas in developed countries it is estimated that 18% of pregnant women are anemic (WHO, 1996). In the latest Demographic and Family Health (ENDES) survey in Peru conducted in 2000, 32% of women of reproductive age (15–49 years) were found to have a hemoglobin measure of less than 11 g/dL (ENDES, 2000). Anemia in pregnancy has been associated with poor birth outcome (Allen, 2000; Rasmussen 2001; Scholl and Reilly, 2000) and increased maternal morbidity and mortality (Guidotti, 2000; WHO, 1996).

The etiology of anemia in pregnancy is multifactorial, although nutritional iron deficiency is often cited as a major contributing factor (van den Broek, 1998). The physiological iron requirement of premenopausal women is approximately 1.5 mg/day but can increase to 6 mg/day in the second and third trimesters of pregnancy (Food and Agriculture Organization, FAO/WHO, 1988). The ability of diet to meet these nutritional iron requirements depends on the iron content of the diet as well as the bioavailability of that iron (Stoltzfus and Dreyfuss,

1998). In developing countries where dietary iron sources are mainly low bioavailability, iron absorption can be as low as 5–10% (FAC WHO, 1988). It has been estimated that the physiologic demand for iri during pregnancy increases from 0.8 to 7.5 mg of absorbed iron per d (Allen, 1997). It has been argued that this increased iron requirement higher than can be met by any diet, regardless of the bioavailability the iron consumed (Bothwell, 2000). In normal pregnancy this discre ancy is compensated for partly by an increased absorption of dieta iron (Barrett et al., 1994), but mainly by a reliance on the woman's iri stores (Bothwell, 2000). However, it has been suggested that iron stoi are low or absent in the great majority of women in developing cou tries due to a diet low in iron, blood loss from parasitic infections, ai frequent and closely spaced pregnancies, thereby putting these wom at increased risk for anemia and its consequences (WHO, 2002).

The objective of this study was to explore the socio-demograpl and dietary correlates of anemia in a population of pregnant wom in the central jungle region of Chanchamayo, Peru.

METHODS

Design

A cross-sectional survey of pregnant women was undertaken at tl antenatal clinic of the *Hospital de Apoyo La Merced* in the province Chanchamayo, Peru. Chanchamayo is located approximately 3(kilometers east of Lima, in the high jungle (Figure 1). Informatic regarding diet and socio-demographic factors was collected during face-to-face interview. Serum hemoglobin was used to assess anemi which was defined as a hemoglobin level below 110 g/L (Stoltzf and Dreyfuss, 1998).

Subjects

The study protocol was approved by the research ethics committ of the Montreal General Hospital and by the Instituto de Medicii Tropical "Alexander von Humboldt"-Universidad Peruana Cayetai Heredia (Lima, Peru).

Peru is divided geographically into 24 "departments," each which is made up of provinces. Chanchamayo is one of nine provinc

FIGURE I
La Merced, Chanchamayo province, Peru.

in the Department of Junin. It is a jungle region at an altitude of 700 m and covers 4700 km^2. In Chanchamayo province, there are three hospitals and several nursing stations serving a total population of 22,000 (ENDES, 2000). At the time the survey was carried out, the antenatal clinic at the *Hospital de Apoyo La Merced* was following approximately 500 pregnant women from the urban center of La Merced and surrounding rural areas. All pregnant women who attended the hospitals antenatal clinic during the month of July 2002 were asked to participate in the survey. Of the 207 patients visiting the clinic in July, 206 (99.5%) consented to participate and were interviewed. The number of antenatal visits made prior to being surveyed ranged from one to four.

Data Collection

The questionnaire was developed in English, translated into Span and pretested on nonstudy subjects in La Merced before applicati The same interviewer conducted all interviews in morning clinics

The following socio-demographic information was obtai during the interview: age, birthdate, gestational age, marital sta previous pregnancies and outcome, level of education, employm status during and prior to pregnancy, income and whether i income was stable or inconsistent. Gestational age was self-repor and estimated in weeks.

The following additional information was obtained regard the woman's household: the level of education and employment s tus of her partner (if applicable), the number of people living in l household, their ages, and the employment status and incomes of household members, as well as whether this income was stable inconsistent. Finally, the following information was obtained regai ing environmental services and practices: type of environment (urb semi-urban, rural), human waste management (type of latrine a distance from house), source and storage of drinking water, garba disposal, presence of soap in the house, presence of animals in around the house, construction material of the house and its flo number of rooms in the family dwelling, presence of electricity in t house, exposure to agriculture or to a garden, and if so, whether fe tilizer is used, if footwear is always worn inside and outside of tl house or if the woman is at times barefoot.

Dietary information was obtained using a multiple pass 2 hour recall method (Johnson et al., 1996; Gibson and Ferguso 1999). Women were first asked to recall all foods eaten the day pri to the interview including all meals, snacks, and beverages. The li of foods was then reviewed and the women were probed for an additional food items consumed with those mentioned previously. A this time, women were also asked to clarify all methods of prepara tion of the foods consumed including all ingredients and cookin methods. The list of foods was then reviewed a third time and wome were asked to clarify food portion sizes by use of three-dimensiona common household measures such as cups, spoons, and dishes, a well as geometric shapes meant to represent different potential foo volumes. The use of local household utensils as measures was mean to maximize accuracy in portion size reporting. Finally, women were asked about any medications, vitamins, or dietary supplements taken, with special attention to the possibility of iron supplementation.

The multiple pass 24-hour recall method was chosen as the most appropriate method for dietary survey of the study population. It has been successful in estimating the overall energy and micronutrient intakes of populations around the world, and is an especially valid method for use in developing countries when time is limited and the diet of a population (not individuals, *per se*) is being studied (Johnson et al., 1996; Gibson and Ferguson, 1999). A study in Malawi found this method to be especially accurate in estimating the intakes of minerals such as iron, and accuracy was found to be independent of the level of education of the subjects interviewed (Ferguson et al., 1995). In Chanchamayo province, cultural factors and issues of food availability limit variety in food intake among individuals, regardless of pregnancy status and gestational age. In addition, poor compliance limited the possibility of more comprehensive individual dietary analyses. One multiple pass 24-hour recall of dietary intake was not taken to be representative of an individual study subject's overall diet, but rather was used to compile an average dietary intake for the study population as a whole. Finally, given low seasonal variation in diet in Chanchamayo province, the survey was undertaken in the month of July based on logistical considerations.

At the *Hospital de Apoyo La Merced*, blood analyses are performed as part of the standard antenatal exam and the costs are covered by the governmental health insurance that is available to all pregnant women in Peru. Hemoglobin values (from venous blood samples obtained at the woman's first visit to the clinic for the current pregnancy) were recorded from the medical chart. All blood analyses were performed in the hospital laboratory by hospital staff. Serum hemoglobin levels were available for 165 of the 206 women interviewed.

Dietary Data Analysis

The results of the multiple pass 24-hour recall interview were used to calculate the nutrient intakes of each woman for one 24-hour period. All foods involved were weighed in order to create volume-weight

equivalents for each item. These values were then used to convert the volumes of each food item consumed into weights consumed. Finally, nutrient composition tables for Peruvian foods (Ministerio de Salud, 1996; FAO/LATINFOODS, 2000) were used to calculate the nutritional contents of all food items.

Statistical Data Analysis

In order to identify important dietary and socio-demographic determinants of anemia among pregnant women, the statistical analysis was carried out using the SAS software package (SAS Institute, Cary, NC, US). For univariate analyses, a chi-square test was used to assess any potential association between anemia and any dichotomous or categorical variables and a Student's t-test was used for continuous variables. For bivariate analysis, odds ratio and confidential intervals were computed for each categorical variable. For assessment of predictors of anemia, multivariate analysis using logistic regression was performed. The main outcome, anemia, was used as a dichotomous variable (Hb < 110 g/L or Hb > = 110g/L). Spearman's correlation coefficients were calculated and, when variables were found to be statistically significantly correlated ($p < 0.05$), the most informative variable was kept for further modeling. In order to identify potential confounders, Mantel Haenszel's odds ratios obtained from a stratified analysis were compared to the crude odds ratios computed in the bivariate analysis. Variables were analyzed using a stepwise approach and were kept in the final model if the p value was less than 0.05. The following variables were evaluated for inclusion in the final model: environment (rural vs. semi-urban/urban), education (< completed secondary vs. > = completed secondary), age (< 25 years vs. > = 25), weight (< 58.9 kg vs. > = 58.9), number of persons per room (continuous), intake of total iron (mg/day), intake of total calories (mg/day), intake of heme iron (mg/day), number of living children (0–2 vs. 3–5, 6–7), number of dead children (0 vs. 1, 2), number of miscarriages (0 vs. 1, 2, 3), number of previous pregnancies (0–2 vs. 3–5, 6–8), gestational age (weeks), woman's employment status prior to pregnancy (employed vs. nonemployed), marital status (married/living with partner vs. other), electricity in the house (yes/no), presence of animals in the house (yes/no), wearing of shoes in the house (yes/no), wearing of shoes outside the house (yes/no), agricultural activities (yes/no), type of housing (cement vs. brick, wood, (mud, bamboo, aluminum)), type of floor (cement vs. wood, dirt), type of latrine (indoor vs. pipe to river, river, woods, ditch), type of water source (indoor tap vs. well, outdoor tap, river), and type of garbage disposal (picked up vs. burnt, woods, river).

RESULTS

Population Characteristics

The mean age of the women interviewed was 25.2 years with ages ranging from 12 to 41 years. The mean gestational age of the women was 25 weeks, ranging from 3 to 36 weeks. The mean number of previous pregnancies was 1.6, ranging from 0 to 8.

Almost 81% of the women were married or living with their partner, while the remainder were single (17%) or separated (2.5%). Nineteen percent had some form of post-secondary education, 19.4% had completed secondary school only, 44.2% had completed primary school only, and 15% had an incomplete primary level education. Five had no formal education whatsoever.

Many women were unable to estimate the income of their husbands or that of other household members. It was therefore difficult to estimate household incomes. Most women had not been employed before their pregnancy (56%), while 28% reported some type of employment, mainly as agricultural workers. Only 8% of the women were able to say that they had steady employment at the time of the interview. Twenty-six percent of the study participants reported that their spouse was employed regularly and 48% reported that their spouse worked once in a while.

The mean household size was five people (1.6±1.3 children under 15 years and 3.5±1.8 adults aged 15 years and older), with a range of 1–17 people. Almost one-third (30%) of the families lived together in one room.

Home Environment

Based on the address provided, 70 women were classified as living in the urban centers of La Merced or neighboring San Ramon, 82 women were classified as living in the densely populated "semi-urban"

settlements surrounding these urban centres, and 54 women w classified as living in the rural areas of the province of Ch chamayo.

Most of the women interviewed lived in homes constructed cement (48.5%) or wood (33%). Seventy-eight percent of the wom from rural environments lived in homes with dirt floors, wher 33% of the women from semi-urban or urban communities had c floors. When asked about their source of drinking water, 54% rural women reported that their drinking water came directly fror river or stream without filtering or treatment, whereas only 2% the women from semi-urban or urban communities cited suc water source. The remainder of the women reported getting th drinking water from a ground well or from a tap. Approximat 45% of the women from semi-urban or urban communities repor indoor plumbing as their source of drinking water, whereas only 4 of the women from a rural environment claimed to have suc water source. Five women reported the use of an outdoor water st age tank, four of which reported that the tank was covered.

Prevalence of Anemia

At 700 meters above sea level, the effect of altitude on hemoglobi insignificant (WHO, 2001). Anemia was therefore defined as a m sured hemoglobin below 110 g/L (WHO, 1992). By this definiti 37% of study participants for whom hemoglobin levels were av able were found to be anemic. Table I shows the prevalence of a mia among study participants by levels of independent soc demographic correlates. The prevalence of anemia among stu participants living in a rural environment was found to be sign cantly higher than those living in semi-urban ($p = 0.022$) and urb environments ($p = 0.025$). Furthermore, the prevalence of anem was found to be significantly higher among those women who h primary level education or less as compared to those who had co pleted secondary school ($p = 0.011$). The mean weight of wom interviewed was 58.9 kg and there was a significantly higher prev lence of anemia among women whose weight was below the mean compared to those who weighed more than the mean ($p = 0.04$ The prevalence of anemia was not significantly different amo women of different marital status, gestational age, or employme

TABLE I
Prevalence of Anemia and Socio-Demographic Characteristics of 165 Women Attending the Antenatal Clinic at *Hospital de Apoyo La Merced* in Chanchamayo region, Peru, July 2002

	Number of Women	Mean Hemoglobin	Prevalence of Anemia	P Value
Total	165	11.4 ± 1.29	0.370	
Age (mean age 25.2 ± 6.8)				
<25 years	94	11.5 ± 1.29	0.351	0.568
≥25 years	71	11.4 ± 1.54	0.394	
Weight (mean weight 58.9 ± 9.7 kg)				
<58.9 kg	85	11.2 ± 1.44	0.447	0.045
>58.9 kg	78	11.6 ± 1.34	0.295	
Gestational age				
First trimester	25	11.9 ± 1.50	0.320	0.480
Second trimester	70	11.5 ± 1.49	0.357	0.601
Third trimester	70	11.2 ± 1.24	0.400	
Education				
Less than secondary completed	101	11.2 ± 1.27	0.446	0.011
Secondary completed	64	11.8 ± 1.53	0.250	
Marital status				
Single	33	11.3 ± 1.59	0.394	0.747
Living with partner/married	132	11.4 ± 1.35	0.364	
Woman employed prior to pregnancy				
No	139	11.4 ± 1.43	0.353	0.291
Yes	26	11.5 ± 1.54	0.462	
Electricity				
No	41	11.4 ± 1.49	0.439	0.289
Yes	124	11.5 ± 1.37	0.347	
Construction material of house				
Cement	85	11.6 ± 1.39	0.306	0.271
Other (wood, brick, mud, bamboo, plastic or aluminum sheet, canvas)	80	11.3 ± 1.40	0.438	
Construction material of floor				
Dirt	73	11.5 ± 1.38	0.384	0.742
Wood/cement	92	11.4 ± 1.42	0.359	

TABLE I
Continued

	Number of Women	Mean Hemoglobin	Prevalence of Anemia	P Valu
Total	165	11.4 ± 1.29	0.370	
Latrine				
No	58	11.2 ± 1.29	0.431	0.229
Yes	107	11.5 ± 1.45	0.336	
Water source				
River	22	11.3 ± 1.07	0.455	
Well (open or covered)	29	11.7 ± 1.51	0.310	0.291
Plumbing	114	11.4 ± 1.43	0.368	0.447
Ever barefoot outdoors				
Yes	37	11.3 ± 1.36	0.405	0.609
No	128	11.5 ± 1.41	0.359	
Garbage disposal				
To district dump (picked up)	97	11.5 ± 1.40	0.340	0.213
River/woods/burnt	64	11.3 ± 1.40	0.438	
Environment				
Urban	57	11.6 ± 1.35	0.316	0.025
Semi-urban	67	11.5 ± 1.45	0.328	0.022
Rural	41	11.1 ± 1.37	0.512	

status. There was no significant difference in the prevalence of ane mia found among women with exposure to different environment variables such as construction material of the house and floor, pre ence of electricity, water source, method of human waste manage ment, method of garbage disposal, and whether or not the woma walked barefoot outside.

Dietary Analysis

The mean caloric intake of the total population was calculated to b 1961 kcal/day. Based on a mean weight of 58.9 kg and assumin low-to-moderate activity, the required caloric intake of the popula tion was estimated to be 2240 kcal/day (Ministerio de Salud, 1996 The reported caloric intake therefore represents 88% of the est mated average required caloric intake of the population. Compar son of the mean intakes of carbohydrate, protein, fat, and variou vitamins and minerals with the daily intakes recommended by WHO are shown in Table II (Ministerio de Salud, 1996; WHO, 1985).

The mean dietary iron intake of the study population was 10.7 (+/– 5.4) mg/day. Of this iron, the mean intake of animal derived dietary iron was 1.6 (+/– 2.6) mg/day, while the remainder was plant-derived. Iron-rich foods commonly consumed by study participants included animal sources such as beef, poultry, and eggs as well as plant sources such as green peas, lentils, and other types of local beans. The iron content of these foods, their bioavailability, as well as the proportion of study participants who consumed them in the 24 hours prior to the interview are shown in Table III.

Table IV shows the prevalence of anemia by tertiles of the dietary variables presented in Table II. When nutrient intakes were divided into tertiles, there was no significant difference in the prevalence

TABLE II

Mean Nutrient Intakes of 206 Women Attending the Antenatal Clinic at *Hospital de Apoyo La Merced* in Chanchamayo Region, Peru, with RDI's* for Pregnant Women, July 2002

Nutrient	Minimum and Maximum Intakes	Mean Daily Intake	SD	WHO RDI[a,b]	% of WHO RDI
Energy (kcal)	504.6–4417.4	1961.0	720.3	2,240	88%
Protein (g)	10.5–182.2	52.8	24.2	47.25	111
Fat (g)	0.7–119.0	41.4	21.0		
Carbohydrate (g)	108.6–857.5	344.7	127.0		
Calcium (mg)	79.1–1820.6	563.3	379.8	1100	56
Total iron (mg)	1.9–28.7	10.7	5.4	15–60**	18–71**
Heme iron (mg)	0–18.1	1.6	2.6		
Thiamine (mg)	0.1–3.1	0.69	0.47	1	69%
Riboflavin (mg)	0.2–14.3	1.6	1.8	1.5	107
Niacin (mg)	1.6–61.6	11.3	6.5	16.8	67
Ascorbic acid (mg)	1.0–1239.3	149.0	175.7	30	497
Vitamin A (μg)	0–1247.4	373.6	347.4	750	50%

*RDI = recommended daily intake by WHO.
**RDI values for iron depend on gestational age and bioavailability of iron.
[a]Ferguson et al., 1995.
[b]WHO, 1985.

TABLE III

Main Dietary Sources of Iron Among Foods Consumed by 206 Women Attending the Antenatal Clinic at *Hospital de Apoyo La Merced* in Chanchamayo region, Peru, July 2002

Food Item	Iron Content (mg/g)	Bioavailability[b,c](%)	Study Participan Who Ate Food it in 24 Hs Prior t Interview (%)
Heme iron			
Beef (lungs)	6.5	15–35	1.0
Beef (liver)	5.4	15–35	0.5
Beef (heart)	3.6	15–35	0.5
Beef (muscle/fat)	2.1	15–35	27.2
Poultry	1.5	15–35	45.1
Fish	1.5	7–17	11.7
Eggs	1.1	1.5–4	31.1
Non-heme iron			
"*huacatay*"[a]	8.7	2–20	0.5
Castilla beans	7.5	1.5–4	1.9
Panamito beans	6.3	1.5–4	3.9
Spinach	4.6	1.5–4	5.3
Wheat	4.6	6–8	3.4
Quinua	3.7	2–20	5.3
Green peas	1.7	2–20	21.8
Lentils	1.7	2–20	21.4

[a]Locally produced tuber.
[b]Gibson and Ferguson, 1999.
[c]Layrisse and Garcia-Casal, 1997.

of anemia among women in the different tertiles for any of t dietary variables examined including total iron and heme iron.

The diets of rural, semi-urban, and urban populations women in the study are described in Table V. No significant diff ences in total caloric intake were found between these three pc ulations. However, the proportion of calories coming frc carbohydrate was found to be significantly higher among rural pc ulations than among both semi-urban ($p = 0.029$) and urban pc ulations ($p = 0.013$), while the proportion of calories coming frc fat was found to be significantly lower among rural populatic than among both semi-urban ($p < 0.001$) and urban populatic

TABLE IV
Prevalence of Anemia by Tertiles of Nutrient intake Among 165 Women Attending the Antenatal Clinic at *Hospital de Apoyo La Merced* in Chanchamayo Region, Peru, July 2002

Nutrient	Range of Intake	Number of Women	Number Anemic	Prevalence of Anemia	P Value[b]
Energy (kcal)	504.6–1808.9	71	30	0.423	0.643
	1809.0–3113.1	84	26	0.310	0.226
	3113.2–4417.4	10	5	0.500	
Protein (g)	10.5–67.7	134	50	0.373	0.948
	67.8–124.9	30	11	0.367	
	125.0–182.2	1[a]	0	N/A	
Fat (g)	0.7–40.1	81	33	0.407	0.399
	40.2–79.6	77	24	0.312	0.163
	79.7–119.0	7	4	0.571	
Carbohydrate (g)	108.6–358.2	97	38	0.392	0.599
	358.3–607.9	62	20	0.323	0.380
	608.0–857.5	6	3	0.500	
Calcium (mg)	79.1–659.6	110	40	0.364	0.254
	659.7–1240.1	46	16	0.348	0.241
	1240.2–1820.6	9	5	0.556	
Total iron (mg)	1.9–10.8	98	39	0.398	0.346
	10.9–19.8	56	16	0.286	0.094
	19.9–28.7	11	6	0.545	
Heme iron (mg)	0.0–6.0	156	57	0.365	0.503
	6.1–12.1	6	3	0.500	
	12.2–18.1	3[a]	1	0.333	
Thiamine (mg)	0.1–1.1	139	51	0.367	0.094
	1.2–2.1	24	9	0.375	
	2.2–3.1	2[a]	1	N/A	
Riboflavin (mg)	0.2–4.9	159	58	0.365	
	5.0–9.6	3[a]	2	N/A	
	9.7–14.3	3[a]	1	N/A	
Niacin (mg)	1.6–21.6	158	57	0.361	0.128
	21.7–41.6	6	4	0.667	
	41.7–61.6	1[a]	0	N/A	
Ascorbic acid (mg)	1.0–413.8	155	56	0.361	0.428
	413.9–826.6	8	4	0.500	
	826.7–1239.3	2[a]	1	N/A	
Vitamin A (μg)	0–415.8	100	40	0.400	0.540
	415.9–831.6	55	16	0.291	0.193
	831.7–1247.4	10	5	0.500	

[a]tertiles in which the number of women is less than 5.
[b]student t test.

TABLE V
Dietary Comparison of Rural, Uban, and Semi-Urban Populations Among 206 Women Attending the Antenatal Clinic at *Hospital de Apoyo La Merced* in Chanchamayo Region, Peru, July 2002

	Rural	Semi-Urban	Urban	Total
Total calories (kcal)	1902±677	2004±777	1955±689	1961±72[illegible]
% calories from carbohydrate	73.5±9.0	70.5±8.6	70.0±7.7	71.1±8.6
% calories from protein	10.6±4.6	10.8±2.4	10.6±2.4	10.7±3.2
% calories from fat	15.9±6.1	19.3±7.0	20.6±7.8	18.8±7.3
Iron intake (mg)	10.2±4.8	11.2±5.8	10.6±5.4	10.7±5.4

($p < 0.001$). No differences were found between these populations ir the proportion of calories coming from protein. Similarly, no signifi- cant differences in total dietary iron intake were found between the three groups.

Multivariate Analysis

In the univariate analysis, the variables: type of floor, type of latrine, type of water source, type of garbage disposal, type of housing, electricity in house, agricultural activities were all found to be highly significantly correlated to the environment variable (rural or urban/ semi-urban). Therefore, the single variable environment was used in place of the other variables in the modeling procedure. Because women from semi-urban and urban areas had very similar levels of anemia, these groups were combined for analytical purposes. In the univariate analysis, only environment (odds ratio (OR) = 2.21; 95% CI: 1.07, 4.53) and education (OR = 2.41; 95% CI: 1.21, 4.80) were predictors of anemia. Because these two variables were found to be highly correlated (Spearman's coefficient = 0.343; $p < 0.0001$), either one could be used as a predictor variable for anemia.

DISCUSSION

The study population consisted of women living in rural, semi-urban, and urban communities in and around La Merced, in the Chanchamayo region of Peru. Despite a significantly higher prevalence of anemia found among women from rural communities, no significant difference in total caloric intake or total iron intake was found between these women and those from semi-urban and urban communities. In addition, when total and heme iron intake were divided into tertiles, no significant difference in the prevalence of anemia was found between groups. Despite small sample sizes, this suggests the presence of etiological factors other than iron intake and diet underlying the high prevalence of anemia in the study population. In fact, after adjustment for important covariates, environment was the sole variable that distinguished anemic from nonanemic women. Because of multi-colinearity, the environment variable used here incorporates not only rural and nonrural environments, but also related aspects such as type of floor, type of latrine, type of water system, type of garbage disposal, type of housing, presence of electricity, and agricultural exposure. All of these variables are known risk factors for parasitic infection.

Dietary Considerations

The calculated average daily caloric intake of the study population represents 88% of the estimated required caloric intake of the population. However, the estimated 2,240 kcal/day required caloric intake is based on the weights of the women on the day that they were interviewed, whereas in pregnancy, required caloric intake should be calculated based on prepregnancy weight (Roger et al., 1988). With a mean gestational age of 6.0 +/− 2.2 months, many of the women exceeded their prepregnancy weights at the time they were interviewed. Therefore, the estimated required caloric intake used for the purpose of this study is in fact an overestimate and the average reported caloric intake can therefore be assumed to be at the very least 88%, if not higher.

FAO and WHO have developed a model to classify the bioavailability of dietary iron. The model defines a meal as being of low-, intermediate-, or high-iron bioavailability depending on the source of dietary iron and the presence of iron absorption enhancers and inhibitors (Gibson, 1999). The diet described by the study population of pregnant women in Chanchamayo, Peru is one of intermediate bioavailability. By definition, this is a diet consisting mainly of cereals, roots, tubers, and minimal quantities of food of animal origin (Gibson, 1999). According to FAO/WHO, such a diet has an

average iron absorption of 10%. With a mean dietary iron intake 10.7 mg/day, the study population therefore has an average ava able iron intake of 1.07 mg/day. This represents 71.3% of the re ommended daily iron intake for premenopausal women, and or 17.8% of the iron required by a pregnant woman in her second t mester when iron requirements are at their peak.

In light of the increased iron requirements in pregnancy, ir supplements are prescribed daily for pregnant women in ma countries. However, in many developing countries iron suppleme tation programs fail due to low patient compliance, poor access health services, and inadequate supplies of supplement tablets (Yi 1996). In the present study, 26.2% of study participants report having taken iron supplements (600 mg/day) during their pre nancy, though it was not possible to estimate how many of the women were taking the supplements consistently.

In order to improve the iron intakes of pregnant women in ju gle regions of Peru, one must consider the causes of their dieta insufficiency. The diet is predominantly rice and root crop or tube based. The quantities of meat and poultry eaten by the women inte viewed were generally small (often less than 20g/serving), and oft consisted of parts of the animal sparse in micronutrients. For exar ple, it was common for a woman to have prepared poultry for h family but for her to have eaten the wings or feet of the chicke leaving the more nutritional parts for her husband and children.

In addition to animal products, ascorbic acid is a maj enhancer of iron absorption and was found in more than adequa amounts (497%) in the diets of study participants. Limes, orange mandarines, and papaya are all inexpensive and readily availab and are consumed daily in large quantities. However, there was high prevalence of iron absorption inhibitors such as phytates an inositol phosphates found in many bran products, rice, cereals, an soy products, as well as phenolic compounds found in tea, coffe cocoa, and certain spices such as oregano (FAO/WHO, 1988). I addition, the mean intake of vitamin A was found to be only 50% (that recommended by WHO, a deficiency which has been linked t anemia by some authors (Oski, 1983). Calcium was also found to b low (56% Recommended Daily Intake RDI of WHO), although th effects of calcium on iron absorption remain controversial (Redd and Cook, 1997). All of these factors are taken into account in th model for iron absorption mentioned above. Of note is that the folic acid and vitamin B12 content of the foods consumed by the women interviewed were not available in the nutrient composition tables for Peruvian foods used in the data analysis. It would be potentially useful to look for dietary folic acid and vitamin B12 deficiencies as contributing to nutritional anemia among pregnant women in Peru.

The causes of dietary iron insufficiency mentioned above represent not only cultural, but also socio-economic barriers. In an effort to combat poverty-induced dietary iron deficiency among pregnant women, a Peruvian nongovernmental organization has instituted a program to improve maternal nutrition through education and by donating various food items, including iron-rich lentils, to women in their third trimester of pregnancy. Forty of the 206 participants in the current study were benefiting from this program, as evidenced by the high proportion of study participants whose major source of iron was coming from lentils. As confirmed in our study, education was strongly associated with anemia so that it is likely that efforts to improve educational outcomes (e.g., improvement in retention; reduction in absenteeism) would produce health benefits. The upgrading of support programs, like the lentil supplementation program, with special emphasis on education, and critical evaluation of current prenatal iron supplementation programs, will help to reduce dietary iron deficiency in this population.

Environmental Risk Factors

A major cause of iron deficiency anemia in developing countries is blood loss due to parasitic infection, often by hookworms (Stoltzfus et al., 1997). Hookworm infections are prevalent throughout the tropics and subtropics, wherever there is fecal contamination of the environment, and are acquired mainly by skin contact with contaminated soil or vegetation. World Health Organization estimates that one-fourth of the world's population is infected with hookworm (WHO, 2002). In certain lowland regions of Peru, the incidence of hookworm infection has been cited as high as 69% (Diaz, 2000). In the present study, risk factors for infection with hookworms, consolidated into the "environment" variable, were prevalent among the women living in rural settings. This suggests that hookworm infection could be a significant etiological factor explaining the significant

association between environment and anemia in our study pop lation.

Future research should explore the environment-educatic anemia interaction as well as dietary insufficiencies in general order to better understand the underlying mechanisms and to mc appropriately apply cost-effective interventions to reduce anen during pregnancy.

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A PRELIMINARY ASSESSMENT OF THI NUTRITION AND FOOD-SYSTEM ENVIRONMENT OF ADULTS WITH INTELLECTUAL DISABILITIES LIVING IN SUPPORTED ARRANGEMENTS IN THE COMMUNITY

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This preliminary study characterized the adequacy of planned diets in two commu based, supported-living arrangements for adults with intellectual/developme disabilities in Montana. The goals were to clarify the residents' nutritional risks to identify appropriate areas and means for intervention. Observations, intervi and analyses of menus, shopping lists, and grocery receipts led to conclusions ab the residents' dietary adequacy. We conclude that the food systems, with t

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Appendix 2

Manuscript G

Larocque R, Gyorkos TW, Gotuzzo E. Conocimientos, actitudes y praticas de las mujeres embarazadas en Iquitos, Peru, acerca de la anemia, la uncinariasis y el uso de antiheminticos. *Revista Peruana de Enfermedades Infecciosas y Tropicales* 2004; **3**:3-8.

CONOCIMIENTOS, ACTITUDES Y PRÁCTICAS DE LAS MUJERES EMBARAZADAS EN IQUITOS, PERÚ, ACERCA DE LA ANEMIA, LA UNCINARIASIS Y EL USO DE ANTIHELMÍNTICOS

Título abreviado: CAP de anemia e infecciones de uncinariasis en gestantes
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RESUMEN

Un estudio sobre conocimientos, actitudes y prácticas (CAP) fue desarrollado en julio de 2001, acerca de 99 gestantes atendidas por la clínica prenatal del Hospital Apoyo de Iquitos, Perú, la anemia, la uncinariasis y el uso de antihelmínticos. 55 % sabían lo que la anemia era. Aunque el 53% sabían lo que eran parásitos, solo el 21% podían identificar un parásito por algún nombre. El 60% de las mujeres creían que tomar medicamentos durante el embarazo podría causar daños al bebe; sin embargo, de estas mujeres el 70% reportaron que quisieran ser tratadas contra parásitos mientras que están embarazadas si estuvieran infectadas. Está claro que el conocimiento de parásitos y la anemia en la población estudiada estaba limitada. Los resultados de este estudio necesitan ser combinados con evidencia en la prevalencia y la severidad de la anemia e infecciones parasitarias junto con la información sobre la dieta y los factores de riesgos con el objetivo de desarrollar un efectivo programa prenatal de salud y nutrición.
Palabras clave: Conocimiento, actitudes y prácticas, uncinariasis, anemia, embarazo, antihelmínticos.

SUMMARY

A KAP study was undertaken in July 2001 to assess the knowledge, attitudes and practices of 99 pregnant women attending the prenatal clinic of the Hospital de Apoyo Iquitos, Peru, with respect to anemia, hookworm infeciton and anthelminthic use. A total of 55% knew what anemia was. Although 53% knew what parasites were, only 21% could name a parasite. Sixty percent of the women believed that taking medication during pregnancy could harm the baby; however, of these, 70% reported that they would like to be treated for parasites while they were pregnant, if they were infected. It is clear that knowledge about both parasites and anemia in the study population was limited. Results of this study need to be combined with evidence on the prevalence and severity of anemia and parasitic infections in addition to dietary and risk factor information in order to develop an effective health and nutrition prenatal program.
Keywords: KAP study, hookworm infection, anemia, pregnancy, anthelminthics.

INTRODUCCIÓN

La anemia durante el embarazo continúa siendo el principal problema de salud pública en el mundo. Aproximadamente, un tercio de las gestantes de los países en vías de desarrollo sufren de anemia (hemoglobina < 110 g/L) y algunas veces de anemia severa (hemoglobina < 80 g/l). La Organización Mundial de la Salud (OMS) estimó que cerca del 56% de todas las mujeres de paises en vías de desarrollo estaban anémicas, comparadas con el 18% de paises industrializados[1]. en el Perú, el 35 % de las mujeres en edad reproductiva y el

50% de las gestantes, se ha estimado que están anémicas. En países en vías de desarrollo, la deficiencia nutricional de hierro e infecciones parasitarias, particularmente la malaria y la uncinariasis, son las causas más comunes de anemia[3].

Aunque la deficiencia nutricional de hierro es probablemente la principal causa de anemia en gestantes, las infecciones intestinales por helmintos, especialmente, la uncinariasis, puede contribuir con la anemia causando pérdidas de sangre y afectando el suministro de nutrientes necesarios para la eritropoyesis. La uncinariasis es la que encabeza las causas de la pérdida patológica de sangre en países tropicales y subtropicales[4]. Los valores de la prevalencia pueden alcanzar hasta 80% en condiciones rurales e insanitarias en los trópicos húmedos[4]. Un estudio epidemiológico realizado en la región amazónica del Perú encontró que 72% de los exámenes de heces eran positivos a uncinariasis, que representa tasas entre los más altos registrados en América del Sur[5]. En un estudio más reciente realizado en la región norte tropical de Perú, en poblaciones rurales, 70% de los análisis de heces fueron positivos a uncinariasis tanto en mujeres y en hombres[6]. En regiones donde la uncinariasis es endémica, es muy común en gestantes. Algunas estimaciones sugieren que cada año 44 millones de gestantes son infectadas con uncinariasis[7]. Las infecciones durante el embarazo afectan enormemente el nivel de hierro porque la demanda de hierro ya de por si es muy alta[4].

La correlación estadísticamente significativa entre la anemia materna y el peso al nacer del bebe ha sido reportada[8]. El estatus socioeconómico, el peso de la pre-gestación, y más frecuentemente, la anemia, han sido identificados como determinantes del bajo peso al nacer de bebes[9]. La anemia severa por deficiencia de hierro ha sido asociada con el incremento de la incidencia del bajo peso al nacer[10,13]. Estos descubrimientos sugieren que intervenciones selectivas en la etapa prenatal para reducir la anemia durante el embarazo podría ayudar a prevenir el bajo peso al nacer de los niños.

En áreas endémicas de uncinariasis, la deficiencia de hierro debido a infecciones de uncinariasis es considerada como crítica. Como uno de sus prioridades de investigación, la OMS ha resaltado la necesidad de estudiar las percepciones de las poblaciones en riesgo de los efectos de la uncinariasis y las consecuencias de su tratamiento durante el embarazo[14]. Uno de los estudios reportó la percepción de la anemia durante el embarazo en mujeres que viven en Dar-es-Salaam, Tanzania, pero no se miró en el conocimiento, actitudes o percepciones de la uncinariasis o su tratamiento[15]. Otro estudio evaluó la percepción de madres sobre los tratamientos contra gusanos intestinales en zonas rurales de Bangladesh pero los datos no fueron obtenidos de mujeres gestantes[16]. Similarmente, percepciones acerca del riesgo durante el embarazo fueron evaluadas entre mujeres de ciudades en el noreste de Brazil, pero no se evaluaron sobre la percepción de los tratamientos de antihelmínticos durante el embarazo[17]. Esta revisión de literatura publicada indica una falta de evidencia relacionada al conocimiento, actitudes y prácticas de las gestantes relacionada a sus causas y efectos.

Nosotros buscamos investigar los conocimientos, las actitudes y las prácticas de las gestantes con respecto a la uncinariasis, la medicación anti-uncinariasis usada y el suministro de hierro durante el embarazo en Iquitos, un área endémica de anemia y uncinariasis en el Perú. Esta área es típica de muchas áreas rurales en países en vías de desarrollo, donde la anemia y la uncinariasis conviven y son problemas conocidos de salud pública.

Materiales y métodos

Diseño de estudio

El diseño de estudio fue un estudio sobre conocimientos, actitudes y prácticas, que comprendió entrevistas personales con gestantes.

Población de estudio

Mujeres que asisten a la clínica de cuidado prenatal del Hospital de Apoyo en Iquitos fueron invitadas a participar en el estudio. Los criterios de inclusión fueron: edad entre 18 y 44 años, residencia en la región en los últimos 6 meses y consentimiento informado firmado.

Área de estudio

El estudio fue llevado a cabo durante el mes de julio del 2001, por un período de aproximadamente 3 semanas en Iquitos, Perú. Iquitos está situada en el departamento de Loreto, comprende cerca de un cuarto del territorio peruano y tiene las características ecológicas de las tierras bajas del Amazonas. Iquitos es el único gran centro urbano y la capital de Loreto, tiene aproximadamente 345 000 habitantes. La población rural está agrupada en pueblos y aldeas a lo largo del sistema de afluentes del Río Amazonas. La uncinariasis es endémica en esta región del Perú con una prevalencia del 70% tanto para mujeres y hombres[5,6]. La anemia y la malaria son también endémicas, los indicadores de salud son extremadamente pobres y el bajo peso al nacer representa un serio problema. Programas de Maternidad y la Niñez están presentes en los hospitales (2 hospitales-Hospital Apoyo de Iquitos y Hospital Regional de Iquitos) y en varias postas medicas de los pueblos y aldeas alrededor de Iquitos. El Hospital Apoyo de Iquitos fue seleccionado como el lugar del estudio porque tenía la más alta proporción de gestantes entre todos los centros de salud del lugar.

TABLA 1. CARACTERÍSTICAS SOCIO-DEMOGRÁFICAS DE 99 GESTANTES ATENDIDOS EN EL HOSPITAL APOYO DE IQUITOS, PERÚ, JULIO 2001.

Características socio-demográficas	Numero de mujeres (n=99)
Edad promedio (SD)*	25 (+/- 4.9)
Casada o conviviente	87
Escuela secundaria completa	60
Primera visita a la clínica prenatal	63
Servicio higiénico en la casa	83
Presencia de agua potable en la casa	71
Embarazo de primer hijo	41
En el 2do ó 3er trimestre	84

* SD = Desviación estándar

CUESTIONARIO

El cuestionario del estudio fue diseñado para obtener información sobre los conocimientos, actitudes y prácticas de las gestantes relacionadas a la anemia, la uncinariasis y el uso de antihelmínticos durante el embarazo. Esta incluye preguntas acerca de características socio-demográficas, como edad, educación y estatus marital; características de salud reproductiva, como gravidez, paridad y número de nacimientos pretérmino; y características ambientales, como la presencia de baños y agua potable en casa y manejo de los desperdicios. Información adicional fue buscada para establecer las actitudes y prácticas de las mujeres en salud y medicación en general. Información acerca del conocimiento de las gestantes sobre la anemia y la uncinariasis, sus causas y síntomas asociados fueron también buscados. Finalmente, también se les preguntó a las gestantes acerca su percepción sobre el suplemento de hierro y el uso de antihelmínticos durante el embarazo.

El cuestionario fue preparado en español, e incluye 75 preguntas: 36 cerradas y 41 abiertas. Esta demora aproximadamente 15-20 minutos en ser tomada. En el cuestionamiento a gestantes, se usaron expresiones locales para gusanos (bichos) y anemia (pocheca) debido a que estas palabras eran más comúnmente entendidas que los términos específicos o científicos para parásitos intestinales y anemia. Un solo investigador de campo fue entrenado para administrar el cuestionario y entrevistar a todas las mujeres.

ANÁLISIS

Un análisis descriptivo fue hecho para obtener una visión general de la población del estudio. Entonces, los porcentajes de las respuestas de las mujeres para cada pregunta fueron calculadas. Se realizaron análisis bivariados para determinar si había alguna correlación entre las siguientes variables: edad, educación, conocimiento de parásitos, conocimiento de la transmisión parasitaria, conocimiento de la anemia, conocimiento de las causas de la anemia, presencia de servicios higiénicos en la casa y presencia de agua potable en la casa. Los coeficientes de correlación de Spearman y los valores p fueron obtenidos usando SAS[18].

RESULTADOS

Características socio-demográficas

De las 109 gestantes contactadas, 99 (91%) aceptaron ser entrevistadas. La mayoría de las entrevistadas tenían edades entre 20 y 27 años (rango: 18-38 años; media: 25.2 +/- 4.9 años) (Tabla 1). Casi todas las mujeres estaban comenzando su tercer trimestre de gestación (rango: 1-9 meses; 6 +/- 2.1 meses). La mayoría de las mujeres (64%) reportaban haber estado en el centro de salud prenatal para su primera visita. Tenían en promedio 1 hijo (rango: 0-9), con 59% siendo multíparas. Un gran número (67%) eran convivientes, una expresión local que significa vivir con su pareja sin estar casada; 21% estaban casadas; mientras el 12% estaba soltera. El nivel de educación fue relativamente alto con 17% habiendo terminado algún tipo de estudio post-secundario (Ej. diploma en computación, grado de bachiller), 44% habiendo terminado la escuela secundaria, 33% habiendo completado la escuela primaria, y solo el 6% no había completado la primaria. La mayoría de las mujeres (84%) reportaron tener un servicio higiénico en su casa (en contraposición a tener instalaciones externas) y el 72% reportó tener agua disponible en su casa.

Actitudes sobre la toma de drogas durante el embarazo

60 mujeres creyeron que tomar medicamentos durante el embarazo podría dañar al bebe, 11 no creyeron que sucediera esto, nueve no supieron si podría dañar al bebe, mientras que 19 reportaron que esto dependería principalmente sobre que tipo de medicación era prescrito por el doctor, en que etapa del embarazo los medicamentos eran tomados y de que medicamentos se trataba. Cuando se les pregunto si habían tomado medicamentos durante su embarazo, 39 respondieron que sí. La medicación mas frecuentemente tomada fue para trata las infecciones urinarias, las cuales son infecciones muy comunes entre las mujeres de esta región del Perú. La mayoría de mujeres (n=75) reportaron completar siempre el régimen prescrito del tratamiento. Aquellas que mencionaron no cumplir completamente con el tratamiento dieron las siguientes razones: insuficiente dinero, mal olor de la droga y mejoría en los síntomas.

ANEMIA

Casi todas de las mujeres entrevistadas (85%) reportaron saber lo que era la anemia pero solo 46 sabían el correcto significado (Tabla 2). Estas 46 mujeres dieron las siguientes respuestas cuando se les preguntó: ¿Qué es la anemia? 25 reportaron que era escasez de sangre, 19 que era escasez de hemoglobina y dos escasez de vitaminas. Treinta y cinco de 46 mujeres estaban en su primera visita, entre ellas 15 era primíparas y 20 multíparas. De las 11 que ya habían asistido al centro de salud al menos una vez, 8 fueron multíparas y 3 fueron primíparas. De las 53 mujeres que no sabían el significado de la anemia, 25 (incluida 15 multíparas) ya habían visitado el centro de salud al menos una vez antes y, de las que visitaban por primera vez por este embarazo, 15 eran multíparas.

Las causas de la anemia entendidas por la mujeres incluían la mala nutrición (n=62), no tomar vitaminas (n=36), y parásitos (n=12). Un total de 11 mujeres no tenían idea que causaba la anemia. Nueves mujeres mencionaron la sobre ingesta de alimentos ácidos como una causa de anemia. Debido a que 63 de las mujeres estaban en su primera visita al hospital, era imposible para ellas saber los resultados de su evaluación de hemoglobina para su actual embarazo, a pesar de eso 35(36%) reportaron haber sido diagnosticadas con anemia. De las 36 mujeres que no estaban en su primera visita, 9(25%) reportaron haber sido diagnosticadas con anemia.

Cuando fueron preguntadas acerca del beneficio del suplemento con hierro durante el embarazo, 59% de las mujeres respondieron saber cuales eran los beneficios. De esas mujeres, 31 (53%) reportaron que este podría curar la anemia, 16 (28%) reportaron que las harían más fuertes, 13 (22%) reportaron que les aumentaría el apetito, 6 (10%) reportaron que sería bueno para la salud del bebe, 4 (7%) reportaron que les proveería de vitaminas y 3 (5%) no sabían cuales eran los beneficios. Un total de 89 gestantes dijeron que les gustaría tomar hierro durante el embarazo, 5 mencionaron que esto dependería si el doctor los prescribiría, 4 no sabían y 1 dijo que no le gustaría tomar hierro. Un total de 47 entrevistadas reportaron tomar suplementos de hierro durante su actual embarazo. Entre ellas, 42 estaban dentro de las 63 que estaban en su primera visita al hospital (entre ellas 22 eran primíparas), mientras que 5 estaban entre las mujeres que ya habían visitado el centro de salud al menos una vez (3 siendo primíparas).

PARÁSITOS

Aunque 52 mujeres reportaron conocer que eran los parásitos, solo 12 (21%) de estas mujeres podían nombrar diferentes tipos de parásitos. Ninguna de las mujeres entrevistadas sabía que eran la uncinariasis. Cuando fueron preguntadas acerca de su conocimiento sobre la transmisión de parásitos, un total de 73 mencionó que sabían como se transmitían los parásitos. De estas mujeres, 30 (41%) tenían un conocimiento general de cómo los parásitos eran transmitidos, 31 (42%) tenían poco conocimiento, mientras 12 (16%) no sabían como los parásitos eran transmitidos. Algunas mujeres creían que la humedad influenciaba en como los parásitos eran transmitidos y algunas mujeres pensaban que los parásitos eran transmitidos a través de contactos sexuales entre hombres y mujeres.

Aproximadamente, la mitad (n=44) de las mujeres mencionó saber que hacían los parásitos en el cuerpo. Estas mujeres tuvieron las siguientes respuestas cuando fueron preguntadas sobre lo que los parásitos producían en el cuerpo: 26 (59%) dijeron que los parásitos chupaban sangre, 7 (16%) que los parásitos absorbían los alimentos o las vitaminas y 11 (25%) no sabían que hacían los parásitos en el cuerpo.

La mayoría de las mujeres (n=73) reportaron usar siempre zapatos en o alrededor de su casa. Un total de 24 mujeres reportaron usar zapatos solamente algunas veces mientras que 2 reportaron nunca usar zapatos.

Cuando fueron preguntadas si les gustaría ser tratadas contra parásitos mientras que estaban embarazadas,

TABLA 2. CONOCIMIENTOS, ACTITUDES Y PRÁCTICAS DE 99 GESTANTES EN IQUITOS, PERÚ, SOBRE LA ANEMIA, LOS PARÁSITOS Y EL USO DE ANTIHELMÍNTICOS DURANTE EL EMBARAZO, JULIO 2001

Item de información	Número de mujeres (n=99)
Conocían el correcto significado de la anemia	46
Reportaron haber sido diagnosticadas con anemia antes de su actual embarazo (n=63)*	35
Reportaron haber sido diagnosticadas con anemia en una visita previa por su actual embarazo (n=36)	9
Esta tomando actualmente suplementos de hierro	47
Le gustaría tomar suplementos de hierro	89
Sabe acerca de los beneficios del suplemento de hierro	58
Sabes lo que los parásitos son	52
Tiene un conocimiento general de como se transmiten los parásitos	30
Piensa que los parásitos chupan sangre	26
Siempre usa zapatos en la casa o alrededores	73
Le gustaría ser tratada contra parásitos durante el embarazo si es que estuviera infectada	69

* el número de mujeres que estaban en su primera visita en la clínica prenatal por su actual embarazo (y aún no han sido evaluadas para la anemia)

asumiendo que estaban infectadas, 70% dijeron que sí, 24% mencionaron que dependería si el doctor lo prescribía, 3% no sabían y otro 3% dijeron que no. Ninguna de las gestantes había sido previamente diagnosticada con parásitos intestinales. (Es importante hacer notar que este examen no es parte del programa prenatal).

CORRELACIONES

Los coeficientes de correlación de Spearman variaron de $r_s=0.002$ a $r_s=0.39$. Entre estas correlaciones algunas fueron estadísticamente significativas: la edad estaba correlacionada tanto con el conocimiento de la anemia ($r_s=0.21$, $p=0.042$) así como con el conocimiento de las causas de la anemia ($r_s=0.27$, $p=0.007$); la educación estaba correlacionada con el conocimiento de los parásitos ($r_s=0.37$, $p=<0.001$), el conocimiento de la transmisión parasitaria ($r_s=0.30$, $p=0.002$), la presencia de servicios higiénicos en la casa ($r_s=0.35$, $p=0.005$) y la presencia de agua potable en la casa ($r_s=0.23$, $p=0.021$); el conocimiento de los parásitos estaba correlacionado con el conocimiento de la transmisión parasitaria ($r_s=0.33$, $p=0.009$); y finalmente, la presencia de servicios higiénicos en la casa estaba correlacionada con la presencia de agua potable en el baño ($r_s=0.39$, $p<0.001$).

DISCUSIÓN

Debido a que la mayoría de las mujeres estaban en su tercer trimestre y porque el 64% estaban en su primera visita al hospital, la práctica de las mujeres en esta área del Perú es de buscar tardíamente cuidado prenatal en su embarazo. La clínica de cuidado prenatal recomienda que las mujeres realicen su visita lo más pronto posible, en su primer trimestre. No está claro porque esta práctica es tan diferente a las recomendaciones. Esta situación amerita mayor investigación.

Aunque la clínica del Hospital de Iquitos es conocida por recibir personas de bajos ingresos tanto de zonas urbanas y rurales, 84% de la población reportó tener un baño en casa y 71% tener agua disponible en su casa. Esto indica que nuestra población fue probablemente más urbana que rural. Sin embargo, esto también pudo haberse producido por alguna confusión en el cuestionario por las palabras baño, inodoro y letrina.

La mayoría de las mujeres de nuestro estudio (60%) creían que tomar medicamentos durante el embarazo puede dañar al bebé. Sin embargo, un total de 39 mujeres del estudio dijeron sí a tomar medicamentos durante su embarazo. También, cuando fueron preguntadas si querían ser tratadas contra parásitos mientras estaban embarazadas, asumiendo que estaban infectadas, 69 mujeres dijeron que sí. Esto indica que los parásitos pueden ser considerados como uno de las molestias importantes para las mujeres en nuestro estudio. Era también importante para las mujeres en nuestro estudio que sus hijos no estuvieran infectados con parásitos.

De las mujeres que se presentaron a la clínica para su primera visita, 56% reportaron haber estado diagnosticada con anemia, que corresponde con el reporte previo de 50% de gestantes sufriendo anemia en el Perú[2]. Sin embargo, solo el 25% de las mujeres que sabían definitivamente sabían acerca de su estatus de anemia (basado en los resultados de las muestras de sangre obtenidas en una visita previa), reportaron estar anémicas. Esta discrepancia también necesita mayor investigación.

Mientras que el 47% de las mujeres de nuestro estudio estaban tomando suplementos de hierro, la mayoría parece tener poco entendimiento sobre lo que la anemia es. La causa de la anemia mas frecuentemente mencionada estaba relacionada a la nutrición, mientras que los parásitos intestinales fueron mencionados por pocas mujeres. Un estudio realizado en Dar-es-Salaam, Tanzania, sobre las percepciones de gestantes frente a la anemia reportó similares porcentajes para las respuestas acerca de las causas de la anemia[15]. La mayoría de mujeres definió la anemia como escasez de sangre, un término que fue encontrado también usado entre mujeres del noreste de Brasil para definir anemia[17]. Aunque información sobre anemia es dado verbalmente al comienzo de cada sesión prenatal parece ser que hay que hacer mas para informar a las mujeres sobre que lo que la anemia es, cuales son sus causas y como puede ser tratada o prevenida. En nuestro estudio encontramos que esta faltando conocimiento sobre la anemia en mujeres que ya han sido previamente atendidas en la clínica, y sorprendentemente, también en mujeres multíparas.

Aunque 90% de las gestantes mencionaron que les gustaría tomar suplementos de hierro durante el embarazo, sólo el 47% lo estaban haciendo al momento del embarazo. Insuficiente dinero parece la razón más probable para esto. Un programa de suplemento gratuito de hierro implementado por el gobierno peruano fue iniciado en julio del 2001 pero sólo es ofrecido en determinados clínicas médicas, y no incluye el Hospital Apoyo de Iquitos que cuida por el mayor numero de gestantes entre todas las clínicas prenatales de Iquitos. Se habría mencionado que este programa es un programa piloto y su duración no estaba asegurada. Según conversaciones informales con funcionarios del Ministerio de Salud, fue mencionado que uno de los actuales retos es tener suficiente stock a la mano en cada clínica al mismo tiempo.

Un total de 53% de las mujeres reportaron conocer lo que los parásitos eran; sin embargo, de estas mujeres solo el 21% podían nombrar algún tipo de parásito. Las mujeres se referían a cualquier especie de parásito como «bichos», un término común que significa parásitos intestinales, colocando por lo tanto a todos los parásitos en la misma categoría. Nadie de las mujeres entrevistadas sabía lo que la uncinariasis era pero, interesantemente, 12% pensaba que los parásitos podrían ser una causa de anemia.

La mayoría de las mujeres reportaron siempre usar zapatos en la casa o alrededores. De observación directa, estaba claro que este número estaba inflado. Las mujeres saben que deben usar zapatos y si ellas no lo hacen, se sienten incómodas de mencionar esto.

Está claro que el conocimiento sobre los parásitos y la anemia están muy limitados en la población del estudio. Por lo tanto, existe una necesidad por mejorar los programas de educación y salud. En la clínica prenatal del Hospital Apoyo de Iquitos, a través del programa prenatal, las mujeres reciben alguna información sobre anemia pero no información acerca de uncinariasis u otros parásitos. Aunque existe educación sobre la anemia, la mayoría de mujeres reportaron no tener suficiente dinero para comprar suplementos de hierro. Educación en uncinariasis podría fácilmente ser incluida en el programa prenatal, con simples mensajes sobre como prevenir la transmisión de estos parásitos.

Ninguna de las variables estaba fuertemente correlacionada. Sin embargo, la educación fue significativa en conocimientos sobre parásitos y en reportar tener servicio higiénico en la casa. La educación parece ser bastante alta en esta población con 17% teniendo completa alguna forma de estudio post-secundario. Estas correlaciones eran esperadas debido a que existe alguna educación en parásitos e higiene en los colegios. Un alto nivel de educación puede también significar una mejor situación de trabajo acompañado con mejores condiciones de vida.

Todas las mujeres de nuestro estudio parecieron estar dispuestas a recibir tratamiento contra parásitos durante el embarazo. El tratamiento durante el embarazo es recomendado por la OMS para mujeres que viven en áreas donde los niveles endémicos de uncinariasis son más altos que 20-30%[1,19]. La UNICEF y la entidad: Internacional Nutricional Anemia Consultative Group (siglas en inglés: INACG) también hacen esta recomendación[19]. Con la excepción de Sri Lanka[14], no está claro el porque los tratamientos con antihelmínticos no han sido incluidos en los programas prenatales en áreas altamente endémicas de uncinariasis. Podría ser de interés documentar los beneficios del tratamiento con antihelmínticos tanto para gestantes así como para sus hijos en un intento por defender y apoyar las recomendaciones de WHO, UNICEF y INACG.

RECONOCIMIENTOS

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Appendix 3

Questionnaire

Nombre ______________________ Código de tratamiento: ________
Centro de salud: ______________ p.1

Inclusión o exclusión	
Inclusión o exclusión Fecha:___/___/________	
1. Edad:__________años Entre 18 y 44 años? Si ☐ No ☐	• < 18 años = **excluida** • > 44 años = **excluida**
2. Fecha de nacimiento:____/____/__________	
3. Dirección:______________________________	•Rural = **aceptada** •Peri-urbano = **aceptada con condición** •Urbano = **excluida**
4. Rural ☐ Peri-urbano ☐ Urbano ☐	Condición •Agua en la casa y desagüé = **excluida** •Agua en la casa pero no desagüé = **excluida** • Desagüé en la casa pero no agua = **excluida**
Tiene usted: 5. Agua potable en la casa? Si ☐ No ☐ 6. Desagüe a la red? Si ☐ No ☐	
7. Tiene usted: Diabetes ☐ Tuberculosis ☐ Hipertensión ☐ Cáncer ☐ Insuficiencia renal ☐ SIDA ☐	•Enfermedad seria = **excluida**
8. Ha tomado purgante en los últimos 6 meses? Si ☐ No ☐	• Purgante en los últimos 6 meses = **excluida**
9. Cuantos meses de embarazo tiene usted?____________	
10. Cual es la fecha de su ultima regla?____/____/________	
11. Semanas de embarazo:__________________FPP:______________	
12. Altura uterina:__________cm	
Trimestre 1 ☐ **regresa en el trimestre 2** →siguiente visita :____/____/________ **Trimestre 2 (18 a 26 semanas)** ☐ **aceptada** → realizar consentimiento y encuesta **Trimestre 3** ☐ **excluida del estudio**	

→ Ahora explicación del proyecto y CONSENTIMIENTO INFORMADO
→ Aplicar la encuesta
→ Dar un envase de plástico por la muestra de heces
→ Citar la señora por la entrega de la muestra el día siguiente
→ Esperar la muestra de heces de la gestante ANTES de realizar las medidas de salud

Codigo de tratamiento:__________________p

Encuesta

Fecha:___/___/_________

13. Estado civil: Casada o conviviente, viviendo con su pareja ☐ Soltera ☐
Casada o conviviente, no viviendo con su pareja ☐ Viuda ☐
Divorciada ☐ Separada ☐

14. Que grado de instrucción tiene usted: Nada ☐
Primaria incompleta ☐ Primaria completa ☐
Secundaria incompleta ☐ Secundaria completa ☐
Superior incompleto ☐ Superior completo ☐

15. Es su primer embarazo? Si ☐ No ☐

16. Cuantos hijos tiene usted excluyendo este embarazo:_____________

17. Cuantos embarazos ha tenido excluyendo este embarazo:________________

18. Ha tenido abortos? Si ☐ No ☐ Insegura ☐

19. Ha tenido hijos que han nacido antes de los 9 meses? Si ☐ No ☐ Insegura ☐

20. Esta tomando medicamentos ahora? Hierro ☐ Otros ☐_____________________

21. Que tipo de casa tiene usted? Material noble ☐ Madera ☐ Ladrillo ☐ Rustico ☐ Insegura ☐

22. Que tipo de piso tiene usted en su casa? Cemento ☐ Tablado ☐ Tierra ☐ Insegura ☐

23. Que tipo de baño tiene usted? Baño de material noble ☐ Letrina en la casa ☐ Otro ☐__________
Letrina a fuera de la casa ☐ Letrina con tasa ☐
Letrina comunitaria ☐ Balsas ☐ Monte ☐ Insegura ☐

24. Que tipo de desagüe tiene usted? Caño (libre) ☐ Sin desagüe ☐
Desagüé a la red (cerrado) ☐ Insegura ☐

25. Donde votan su basura? Casa ☐ Huerta ☐ Rió ☐ Otro ☐__________________
Patio ☐ Basurero ☐ Monte ☐ Insegura ☐

26. De donde toma su agua? Rió ☐ Pozo de la comunidad ☐ Pozo artesano ☐
Agua potable ☐ Agua de lluvia ☐ Agua de camión cisterna ☐
Agua tratada ☐ Insegura ☐ Otro ☐________________

27. Desinfecta el agua de su casa? Si ☐ No ☐ A veces ☐ Insegura ☐

28. Hace hervir el agua? Si ☐ No ☐ A veces ☐ Insegura ☐

29. Tiene usted animales: No ☐ Si ☐ Cual?______________________________________

30. Usa sandalias: Fuera de su casa: a veces ☐ todo el tiempo ☐ nunca ☐
Dentro de su casa: a veces ☐ todo el tiempo ☐ nunca ☐

A continuación...

31. Come usted ladrillo o tierra? Si ☐ No ☐ A veces ☐ Insegura ☐

32. Que cantidad?________________ 33. Cada cuanto tiempo?______________________________

34. Cuando fue la ultima vez que tuvo malaria?______/______/______-______

35. El examen de malaria fue confirmado por examen de laboratorio? Si ☐ No ☐ Insegura ☐

36. Que tipo de malaria tuvo usted? vivax ☐ falciparum ☐ mixto (falciparum y vivax) ☐

37. Cuantos veces tuvo malaria durante los 2 últimos años (que fueron confirmados por examen de laboratorio)? ____________________

Medidas de salud – Primera visita (Trimestre 2) **Fecha:**____/___/__________

38. Muestra de HECES Si ☐ No ☐ → NO TRATAMIENTO

39. Muestra de sangre- HEMOCUE Si ☐→ niveles de Hb:__________mg/dl
No ☐

40. Gota gruesa MALARIA Si ☐, No ☐ 41. Temperatura axial: _________ ⁰C

→ ***IMPORTANTE No tratamiento antes de recibir muestras de heces, sangre y temperatura***

42. **Código de tratamiento:**______________________________

43. Observaciones:____

Siguiente visita:____/____/__________

1-Control de sulfato ferroso: Cuantas pastillas ha tomado?________**Siguiente visita**_____/______/____
2-Control de sulfato ferroso: Cuantas pastillas ha tomado?________**Siguiente visita**_____/______/____

Medidas de salud - Segunda visita (Trimestre 3) **Fecha:**____/___/________

44. Muestra de heces Si ☐ No ☐

45. Muestra de sangre- HEMOCUE Si ☐→ niveles de Hb:__________mg/dl
No ☐

46. Gota gruesa MALARIA Si ☐ No ☐ 47. Temperatura axial: _________ ⁰C

48. Ha tomado sus pastillas de hierro? Si ☐ No ☐ Algunas ☐ cuantas?________________

49. Esta tomando algunos medicamentos? No ☐ Si ☐ cual? ______________________________

50. En que hospital o centro de salud, usted piensa dar luz?______________________________

51. Observaciones:____

Siguiente visita:____/____/__________

1-Control de sulfato ferroso: Cuantas pastillas ha tomado?________**Siguiente visita**_____/______/____
2-Control de sulfato ferroso: Cuantas pastillas ha tomado?________**Siguiente visita**_____/______/____

Medidas de salud - Tercera visita (parto) Fecha:___/___/_________

52. Muestra de heces Si ☐ No ☐

53. Muestra de sangre- HEMOCUE Si ☐→ niveles de Hb:__________ mg/dl
No ☐

54. Gota gruesa MALARIA Si ☐ No ☐ 55. Temperatura axial: __________ °C

56. Ha tomado sus pastillas de hierro? Si ☐ No ☐ Algunas ☐ cuantas?________________

57. Esta tomando algunos medicamento? No ☐ Si ☐ Cual? ____________________________

58. Peso del recién nacido__________________g 59. Talla del recién nacido_________________cm

60. Observaciones:____

____

Siguiente visita:___/___/_________

Medidas de salud – Cuarta visita (1 mes post-parto) fecha:___/___/_________

61. Peso del niño:_______________g 62. Talla del niño:___________cm

63. Salud general del niño: Sano ☐ Enfermo ☐ Insegura ☐

En el ultimo mes, ha tenido su hijo algunas de las siguientes problemas?

64. Convulsiones No ☐ Si ☐
65. Problema de alimentación No ☐ Si ☐ Cual?_____________________________________
66. Hoy su hijo ha tenido diarrea? No ☐ Si ☐ Por cuanto tiempo?________________________
67. Había sangre en sus heces? No ☐ Si ☐
68. En el ultimo mes, su hijo ha tenido diarrea? No ☐ Si ☐ Por cuanto tiempo?______________
69. Habia sangre en sus heces? No ☐ Si ☐
70. Vacunas apropiadas No ☐ Si ☐ Cual?_________________________(verificar tarjeta)
71. Patología post-natal No ☐ Si ☐ Cual?____
72. Episodios de fiebre No ☐ Si ☐ Cuantas veces tuvo fiebre?_______________________
73. Hospitalizaciones No ☐ Si ☐ Porque?_______________________________________

74. Entre el parto y ahora su niño esta tomando:
Solamente leche maternal ☐ Solamente leche artificial ☐ Leche mixto ☐ Otro ☐

75. Observaciones:____

____

Appendix 4

Informed Consent Forms

Appendix 5

Ethics approvals

5.1 McGill University Health Centre

5.2 Peru (Ministerio de Salud)

5.3 Universidad Peruana Cayetano Heredia

Appendix 6

Letter of invitation for the presentation of the final RCT results (Iquitos, March 27, 2006)

Appendix 7

Signed waivers from co-authors and publishers

PROJECTO FIERRO

of study: Reducing low infant birthweight in hookworm-endemic areas: an RCT of prenatal iron s iron plus mebendazole

Informed Consent Form

tigators and institutions: ***Canada:*** Dr. Theresa W. Gyorkos (McGill University Health Centre Research ıte and McGill University), and Renée Larocque (McGill University), Montreal, Quebec ***Peru:*** Dr. 'do Gotuzzo (Instituto de Medicina Tropical "Alexander von Humboldt"-Universidad Peruana Cayetano lia), Lima and Dr. Martin Casapia (Hospital de Apoyo de Iquitos and Instituto de Medicina Tropical :ander von Humboldt"-Universidad Peruana Cayetano Heredia), Iquitos.

ground Information:
ıia or having too low amounts of iron in your blood during pregnancy can have serious adverse effects. ıf them is low infant birthweight. There are several ways to reduce anemia during pregnancy and one of ost common is iron supplementation. However, in most developing countries, hookworm infections are rtant in causing anemia. We are therefore planning to conduct a study to understand more about the its of hookworm treatment (mebendazole) in addition to iron supplementation (tablets containing iron) in ant women.

arch Objectives:
ıurpose of this study is to determine whether or not there are added benefits of including hookworm nent, in addition to iron supplementation in prenatal care programs, on reducing low infant birthweight.

ecide to participate in the study, what will I have to do?
and questionnaire
will be asked for a fingerprick of blood and a stool container will be given to you for stool collection. The l sample is so that we can see if you are anemic or not and if you have malaria or not, while the stool le will indicate if you are infected with hookworms. Information on socio-demographic characteristics ılso be collected at this time such as age, address, schooling, number of children, number of pregnancies, cation intake, health of previous children, gestational age, etc.
tment
:her you receive iron plus placebo (inactive pill) or iron plus mebendazole, will be determined by chance. treatments and laboratory tests will be given to you free of charge for the whole duration of the study. ıer you nor the clinic staff will know which treatment you have been given.
ıw-up
all, you will be seeing us 4 times: once during your second trimester (between moth 4 and 6 of your ıancy) and once during your third trimester (between month 6 and 9 of your pregnancy), then at delivery ıt 1 month after the delivery. When you will be followed-up, the same tests (blood and stool, for anemia ıarasites) will be done in the third trimester and at delivery (anemia and parasites). After delivery, the weight of your baby will be measured and his/her health will be evaluated one month after birth.

ıt if I decline to participate?
cipation in this study is voluntary. We hope that you and your infant will participate in our study but you ot obliged to do so. Whether you decide to participate or not, this will not affect any health care you need ourself, your infant or your family. Whether you decide to participate or not, this will not affect the usual that you would otherwise receive.

are the benefits and risks involved?

g the study, information collected through questionnaires and results obtained from blood or stool samples e put in a special research file separate from your clinic file. The clinic will not have access to the ch file. The principal investigators of PROJECTO FIERRO will keep your research file. No person other he principal researchers will have access to your questionnaire and test results. Therefore, all information rovide to the researchers will be kept confidential. A study code will be used to identify you so that the atory work and analyses will be performed anonymously.

will receive iron supplementation free of charge and you will have the opportunity to receive hookworm nent, also free of charge. Iron supplementation will reduce your anemia during pregnancy and hookworm nent will free you of hookworm infection. In case of severe anemia, you will be excluded from the study with your permission, you would be referred to your treating physician. We believe that the risks iated with the treatment and examination procedures are minimal. There may be a potential risk if the iron nent is taken above the recommended dose. However, provided you adhere to the schedule as prescribed, isk is negligible. Furthermore, some mild gastrointestinal irritations may be experienced with the ingestion on, however, taking the iron with meals will alleviate this. Mebendazole may be given safely to pregnant en as of their 2nd trimester. There has been some concern related to adverse effects following mebendazole nistration found in rats but these have not been shown to occur in humans. Mebendazole is a well-known and effective drug that has been used widely throughout the world for the treatment of intestinal parasites.

there be any compensation for my participation in the study?

e will be a monetary compensation to cover transportation costs for all your extra visits to the clinic related is research project.

stions:

will be happy to respond to any questions you might have. Please note that you can contact the clinic nurse btain further information about this study. She will be able to answer any questions that you may have at time or at any time throughout the study period.

laration of Consent

ve read and understood the contents of the preceding from. I have the opportunity to ask questions, and all e been answered to my satisfaction. My participation in this study is free and voluntary. I also understand I can withdraw from this study at any time, without penalty in terms of my medical care. I have been given gned copy of this consent form.

____________________	____________________	__________
nature of participant	Printed name	Date (d/m/y)
____________________	____________________	__________
nature of mother or guardian (only in the e of a particpant under 18 years old)	Printed name	Date (d/m/y)

ttest that I have fully explained the study to the participant and s/he has understood this explanation.

____________________	____________________	__________
nature of interviewer (investigator)	Printed name	Date (d/m/y)