Intestinal parasites, inflammation and nutritional status in Mexican children

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Artwork:

Cover: Fragment of “Civilización Huasteca” (1950) by Diego Rivera, mural of Palacio Nacional in México City

Chapter numbers: Fragments of “La Gran Tenochtitlan” (1945) by Diego Rivera, mural of Palacio Nacional in México City

Vrije Universiteit Amsterdam

Intestinal parasites, inflammation and nutritional status in Mexican children

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CHAPTER 

General Introduction

Intestinal parasitic infections and malnutrition are very common in children of low and middle income countries[1-3]. Intestinal parasitic infection, obesity and micronutrient deficiencies account for more than 10% of the global disability-adjusted-life-years (DALYs)[4-6]. Mexico is a case in point. At least half of the children are estimated to be infected with at least one species of intestinal parasites[7-9]. In addition, micronutrient deficiencies such as iron (34-39%) and zinc (19-24%) are highly prevalent while the combined prevalence of overweight and obesity in children is more than 30%[10, 11]. These co-existing conditions place a heavy burden on the health care system in Mexico[12, 13]. Thus, it is important to study their relationship for the planning of future public health programs in the country. In this thesis, we study the associations between intestinal parasitic infections and nutritional outcomes in a pediatric population with high rates of overweight and obesity.

Intestinal parasites

Intestinal parasites are a broad number of organisms that require a host for their own survival and that live in the gastro-intestinal tract. These include two main groups: Intestinal protozoa and soil transmitted helminths (STHs). School-aged children from rural communities are at highest risk of infection, due to the lack of health, sanitation and water supply services and due to behavioral risks, such as frequent outdoor exposure and poor personal hygiene[7, 14]. Several techniques are available to diagnose STHs and intestinal protozoa infections. In this thesis we used Kato-Katz for the detection of STHs and direct wet smear for the detection of protozoa[15, 16]. These two techniques have in common that fecal samples are studied by microscopy, and the number of eggs (in case of STH) and trophozoites/cyst (in case of intestinal protozoa) are recorded to determine the intensity of infection.

Intestinal Protozoa

Intestinal protozoa have a direct life cycle where transmission typically occurs via the fecal-oral route[17]. They can be classified according to their effect on human health as "pathogenic" or "non-pathogenic". Pathogenic protozoa such as *Giardia lamblia, Entamoeba histolytica and Cryptosporidium* can cause acute diarrhea and dysentery, malabsorption, blood loss and reduced growth[18, 19]. "Non-pathogenic" protozoa such as *Entamoeba coli* (*E.coli*) and *Endolimax nana*, are usually not associated with illness or clinical symptoms. According to epidemiological studies in different regions of Mexico, the most common intestinal protozoan is *E. coli* [7, 20]. Figure 1 shows the national combined incidence (2012) of *E. coli, Balantidium coli, Endolimax nana and Cryptosporidium*, which are referred to as "other intestinal protozoa"[21]. Using information from the Epidemiological Surveillance System of Mexico (SINAVE)[21], the only nationwide data available on intestinal protozoa.

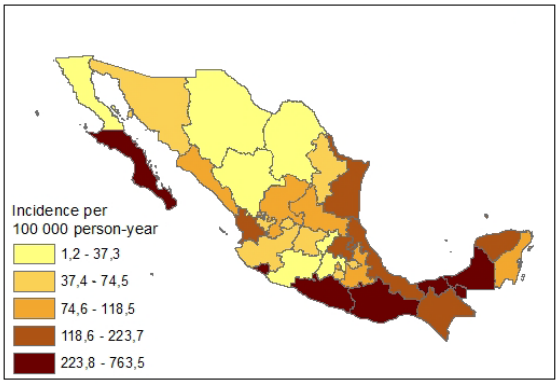

Figure 1. Combined incidence of "other intestinal protozoa" *(Entamoeba coli, Balantidium coli, Endolimax nana and Cryptosporidium)* in Mexico, according to SINAVE, 2012

Soil transmitted helminths

STHs refer to the intestinal worms that are transmitted through contaminated soil. Infection occurs by ingestion of eggs (e.g. *A. lumbricoides* and *T. trichiura*) or by penetration of the skin (e.g. by hookworm larvae)[18, 22]. The Global Burden of Disease Study 2010 estimated that STHs rank first among all neglected tropical diseases in DALYs, with 5.1 years lived with disability[23]. These DALYs were estimated using multiple studies associating STHs with moderate and severe anemia, micronutrient deficiencies and reduced growth[24, 25]. It was estimated that more than 1.8 billion people in the world were infected with one or more species of STHs in 2013[26]. The most common STHs in the world are roundworm (i.e. *Ascaris lumbricoides)*, whipworm (i.e. *Trichuris trichiura)* and hookworms (i.e. *Necator americanus* and *Ancylostoma duodenale)*. As *A. lumbricoides* is the most common STH in Mexico, SINAVE reports *A. lumbricoides* infections separately[27-30]; other helminth infections are referred to as "other helminth infections" which include the combined incidence of more than 15 different helminth infections. The distribution of *A. lumbricoides* in Mexico is presented in Figure 2[21].

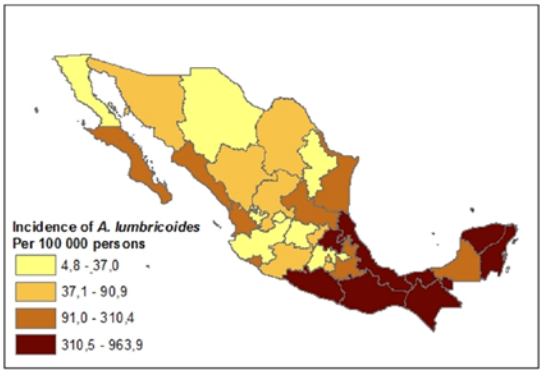

Figure 2. Incidence of *A. lumbricoides* in Mexico, according to SINAVE, 2012

Nutritional status

Nutritional status indicators aim to obtain an objective estimate to whether an individual or a population is well-nourished, under-nourished or over-nourished[31,32]. In chapters 2-6 we tested the associations between endemic intestinal parasites in Mexico and different outcomes related to nutritional status. In the following sections the details of these outcomes will be introduced and briefly discussed.

Body composition

In this thesis we used dual X-ray absorptiometry (DXA) to measure body fat content. This test is one of the reference methods to determine body fat percentage[33]. Anthropometric measurements and indices such as waist circumference, body mass index (BMI), body mass index for age z-score (BMIz) and height for age-z-score (HAZ) have proven to be effective as measurements of obesity, underweight or stunting (low height for age)[34]. Therefore these were also selected as part of the outcomes set.

Micronutrient status

Micronutrient deficiency (i.e. lack of essential vitamins or minerals) is a form of malnutrition, which in most cases is not visible and therefore overlooked[35]. Micronutrients are elements (i.e. minerals) or compounds (i.e. vitamins) that cannot be synthetized by the body and are essential for a wide range of body functions such as growth, metabolism, structure, immunity and cognition[35]. There are more than 17 essential minerals and more than 15 vitamins. The most prevalent deficiencies in Mexico are iron, folate, vitamin B12 and zinc[36].

Diet

Diet assessment is used to evaluate the intake of macro and micronutrients, both at the individual and population level[37]. For this purpose different methods have been implemented depending on the outcome of interest. For instance, the 24 hour recall (24hR) records an individual's intake from the previous day. The 24hR gives rich detail about the type and amounts of foods consumed[37]. However it fails to provide information on the variety of food around the year (particularly important in countries with a high intake of seasonal fruits and vegetables)[38]. On the other hand, food frequency questionnaires (FFQs) can provide information about food intake over a specific period of time (i.e. one year) and measure long-term behavior in a relatively inexpensive way. However, FFQs have a limited list of items (i.e. foods and beverages) and are hampered by the inability of individuals to accurately report their food intake retrospectively over a long period of time[37]. For these reasons in chapter 4 we used both methods capturing short and long term intake of different food individual items and food groups.

Inflammation

Even when inflammation is not *per se* a nutritional status outcome, it is one of the mechanisms through which nutritional status outcomes are associated with each other[39, 40]. For instance body fat is correlated with concentrations of leptin and tumor necrosis factor alpha, which are inflammatory molecules that affect food intake and micronutrient concentrations[41]. For this reason different research groups have measured inflammation as part of the nutritional status assessment[42, 43]. In this thesis we measured the most important inflammatory markers that have shown to play a role in these associations and will be discussed in depth in chapter 6.

Intestinal parasites and nutritional status

Intestinal parasites and body composition

STHs and pathogenic protozoa have previously been associated with a lower HAZ and BMIz[44-46]. The underlying mechanisms have been described as decreased appetite, lower food intake, malabsorption of macro and micronutrients, blood loss, competition for nutrients, impaired immune response and changes in the gut microbiome[2,47]. These factors result in a lower concentration of available nutrients for the children, which has been proposed as the main cause of stunting and underweight in infected children[48].

While most studies have focused on undernutrition, recently it was found that viral and bacterial infection may also contribute to obesity[25, 49-52]. Additionally a study in a Venezuelan population reported that intestinal parasite infection was associated with living in a dual burden household (i.e. co-existing overweight/obesity and stunting in the same household)[53]. The hypothesis that intestinal parasitic infection is associated with obesity was tested and discussed in chapters 4 and 5.

Intestinal parasites and food intake

The relationship between intestinal parasites and nutritional status is often explained by a reduced appetite and food intake[47]. However most studies testing this hypothesis have been carried out in animal models and with species of intestinal parasites that only affect animals[54-56]. The few studies done in humans concentrated on anti-helminthic treatment and its effect on appetite, measured with an appetite questionnaire and a single meal (*at libitum)* intake in the morning[62, 63]. In this thesis (chapter 4) the association of each individual parasite with two validated measurements of food intake (i.e. three time-points 24hR and one FFQ) are reported for the first time.

Intestinal parasites and micronutrients

While most studies have found an association between intestinal parasitic infection and micronutrient deficiencies, some studies have found no association[44, 57, 58]. Some of the mechanisms presented may be specific to a particular micronutrient or intestinal parasite. For instance: iron deficiency has been associated with blood loss caused by hookworm[59] and *A. lumbricoides and Giardia lamblia* have been associated with a decreased vitamin A absorption[60]. Few studies have been performed in Mexico evaluating the relationship between STHs and micronutrients focusing on iron, zinc and vitamin A[61], while associations between STHs and micronutrients such as vitamin D, E and C remain under-studied. Chapter 5 explores the associations between zinc, iron an vitamins A, D, E, C and B12 with intestinal parasites in children from rural Mexico.

Intestinal parasites and inflammation

The relationship between STHs and inflammation markers has been studied in animal and human studies, with conflicting results[62, 63]. *In vitro* studies have shown that STHs regulate systemic inflammation through an anti-inflammatory response dominated by the cytokine IL-10. IL-10 regulates the concentration of the inflammatory cytokines secreted by the adipose tissue (e.g. TNF-α, IL-6 and leptin)[64-66]. Interestingly these molecules are responsible for insulin resistance and progression to chronic disease[62, 67]. In contrast to STHs, pathogenic intestinal protozoa such as *Blastocystis* and *Entamoeba histolytica* have been associated with higher concentrations of TNF-α promoting a Th1 systemic inflammatory response[68]. This response involves effector cells such as granulocytes and lymphocytes, and signaling molecules such as IL-6 and leptin[54, 69-71]. The relationship between intestinal parasites with inflammatory markers are explored and discussed in chapter 6.

Research setting and databases

We performed a cross-sectional and an ecological study to test the associations of intestinal parasitic infections with body composition, food intake, micronutrient concentrations and inflammatory markers.

Cross sectional data

A cross-section study was performed in 2 rural communities “Santa Cruz” and “Santa Maria Begoña” in the municipality of El Marques, State of Queretaro (Figure 3). A total of 301 school-aged children (6-10 years of age) participated in this study that was performed in spring 2013. Participants were randomly selected from the schools located in the two communities (less than 2 km apart). Caretakers received oral and written information about the study and were asked to sign an informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures involving human subjects were approved by the Bioethics Committee of the School of Natural Sciences at the Universidad Autónoma de Querétaro (UAQ). Children’s parents or legal guardians were asked to attend their local health clinic to answer a medical history and a socioeconomic status questionnaire validated in previous studies[72]. Anthropometry (weight, height, waist circumference) and body fat percentage were measured in all children. A fresh stool sample was collected from each child and analyzed for parasites. Dietary intake was determined using both a FFQ and three 24hR. Zinc, iron and vitamins A, C, D, E, C-reactive protein, interleukin-6, interleukin-10, tumor necrosis factor α, leptin were determined from a fasting blood sample. The data of this study was used in chapters 2,4,5 and 6.

Figure 3. Map of Mexico with the location of Queretaro (reproduced from travelbymexico)

Ecological data

To test the association between intestinal parasitic infection and BMIz in the Mexican population we constructed an ecological database. Individual level data on height, weight and age was obtained from the 2012 National Health and Nutrition Survey (ENSANUT 2012). This survey of over 50,000 households is representative for the Mexican population at national and state level[73]. Statewide data on STHs and intestinal protozoa in 2012, 2006 and 2000 was obtained from the SINAVE [21]. The statewide data on demographic and socioeconomic variables was obtained from the National Institute of Statistics and Geography (INEGI) 2012 report[74]. This database was used in chapter 3.

Thesis outline

This thesis investigates the associations between the most common intestinal parasitic infections and nutritional status in children of rural Mexico. The main research questions of this thesis are: Are intestinal parasitic infections associated with: 1) body composition; 2) food intake; 3) micronutrient status; and, 4) intestinal and systemic inflammation?

Chapter 2 explores the association between endemic intestinal parasitic infections with the percentage of body fat in school age children of a rural community in Mexico. In chapter 3 we used an ecological approach to analyze the current and long term (i.e. association between past infection and current BMIz) association between the most common intestinal parasites and BMIz in children and adolescents of Mexico. Chapter 4 studies the relationship between food intake and endemic intestinal parasites in children of rural Mexico. Chapter 5 analyses the association between specific intestinal parasites and micronutrient concentrations. In addition it evaluates how this association is affected by percentage of body fat. Chapter 6 studies the association between specific intestinal parasite infections and local and intestinal inflammation markers. Finally chapter 7 summarizes and discusses the results of the studies from chapters 2-6 according to the objectives. In addition, the implications of the study results for public health and the remaining research gaps will be addressed.

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CHAPTER 2

Children with moderate-high Infection with Entamoeba coli have higher percentage of body and abdominal fat than non-infected children

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Abstract

Background: Intestinal parasites, virus and bacterial infections are positively associated with obesity and adiposity *in vitro* and in animal models, but conclusive evidence of this relationship in humans is lacking. The aim of this cross-sectional study was to determine differences in adiposity between infected and non-infected children, with a high prevalence of intestinal parasitic infection and obesity. **Methods:** A total of 296 school-aged children (8.0 ± 1.5 years) from a rural area in Querétaro, Mexico, participated in this study. Anthropometry (weight, height, waist circumference) and body fat (DXA) were measured in all children. A fresh stool sample was collected from each child and analyzed for parasites. Questionnaires related to socioeconomic status and clinical history were completed by caretakers. **Results:** Approximately 11% of the children were obese and 19% were overweight. The overall prevalence of infection was 61%. *Ascaris lumbricoides* was the most prevalent soil transmitted helminth (16%) followed by hookworm. *Entamoeba coli* was the predominant protozoa (20%) followed by *Endolimax nana, Balantidium coli, Entamoeba histolytica/dispar, Iodamoeba Bütschlii* and *Giardia lamblia*. Children with moderate-heavy infection of *Entamoeba coli* had significantly higher waist circumference, waist to height ratio, body and abdominal fat than children not infected or with light intensity infection ($p<0.05$). **Conclusion:** These findings raise the possibility that a moderate or heavy infection with *E. coli* may contribute to fat deposition and thereby have long term consequences on human health. Further studies are needed to better understand if *E. coli* contributes directly to fat deposition and possible mechanisms.

Introduction

Intestinal parasitic infections are a global public health problem, with the heaviest burden occurring in developing countries, especially in tropical and subtropical climates[1]. It is estimated that more than 3.5 billion people are infected. School-aged children have the highest burden of intestinal parasitic infection across age groups, partly due to behavioral risk, such as frequent outdoor exposure and poor personal hygiene[2]. Soil Transmitted Helminths (STH) together with intestinal protozoa are the most common parasites dwelling the intestine of children and may lead to persistent colonization[2].

Infectobesity is a term used to define obesity caused by infectious disease. Different pathogens, such as virus and parasites, have shown to be associated to fat deposition in animal models[3-5]. For instance, intestinal protozoan induced markers related to metabolic syndrome on a dragonfly model and intestinal helminths were associated with anorexia in sheep[6, 7].

No previous studies have tested the association between parasite infection and body fat in humans. Most studies have focused instead on the relationship between intestinal parasites and markers of under-nutrition, such as stunting or underweight. A recent study in a Venezuelan population found an association between infection with intestinal parasites and living in a dual burden household (i.e. co-existing overweight/obesity and stunting in the same household)[8].

Intestinal parasitic infections have been associated with micronutrient deficiencies and changes in gut microbiota and mucosa. Both micronutrient deficiencies and gut microbiota have been related to higher risk of obesity[9]. In addition, helminths and protozoans may have a differential effect on leptin secretion[10], inflammation, food intake and nutrient absorption and metabolism[11]. Helminths cause abdominal pain, anorexia, trigger a (Type 2) anti-inflammatory response and compromise the intestinal epithelial cells[12]. In contrast, *Entamoeba coli (E. coli)* and other protozoan parasites are usually asymptomatic, but can lead to malabsorption and promote a (Type 1) pro-inflammatory response[13].

In Mexico, both parasitic infections and obesity are considered public health problems. More than 30% of the population is infected with intestinal parasites in urban areas and almost 60% in rural areas[14]. In children alone, the combined prevalence of overweight and obesity is over 30%[15].

Evidence from animal and human studies have led us to the hypothesis of an association between infection with intestinal parasites and obesity. Thus, the objective of this cross-sectional study was to evaluate differences in adiposity between infected and non-infected school-aged children from a rural area in Queretaro, Mexico.

Methods

Subjects

A total of 296 school-aged children (6 -10 years of age) participated in the study that was performed on spring 2013. Participants were randomly selected from two schools located in two rural communities less than 2 km apart, situated in the municipality of El Marques, in the State of Queretaro. This municipality has a semi-arid climate. The main ethnicity of the population is mestizo and the main economic activity is agriculture. Both communities had a population of 3,902 (in the last survey 2010) and are 35 km from a metropolitan center. In this region, the typical diet includes foods such as corn-tortilla and beans; however, corn based beverage “atole”, chicken, pork, eggs, dairy products, seasonal fruits and vegetables are commonly consumed. Locals usually get their food from family-owned convenience stores called “tienditas” where children have access to caloric dense foods such as soft drinks, sweets and salty deep fried “churros” at relatively low prices.

Study design

Children's caretakers received both oral and written information about the study and were asked to sign an informed consent. Children with any physical or mental disability and those that received any vitamin or mineral supplementation, deworming treatment, probiotics or drugs that could affect the intestinal microbiota in the last 4 months were excluded from the study. The study was approved by the Bioethics Committee of the Universidad Autónoma de Querétaro (UAQ). A total sample size of 284 was needed in order to identify statistical differences on body fat of at least 7% between infected and non-infected children. The expected prevalence of infected children was 25% and the estimated standard deviation of body fat was 6%, assuming a type I error of 5% and a statistical power of 80%.

Once the children were included in the study, parents or legal guardians were asked to answer a medical history and a socioeconomic status questionnaire. In that survey, additional questions were asked related to the participant's age, crowding (defined as the total number of co-residents per household) and years of education of the child's caretaker. The same day, a labeled, sterile polypropylene stool cup was provided by the staff with written instructions on how to collect the stool sample. Fecal samples were collected on a second visit.

Anthropometry and Body Fat Evaluations

Children were transported from their local schools to the Nutrition Clinic at UAQ where weight, height and waist circumference were measured in duplicate. By personnel trained following the World Health Organization (WHO) procedures[16]. Children were weighed barefoot and wearing light clothing using a calibrated digital scale (SECA, mod 813 Hamburg, Germany) with a precision of 0.1 g. Height was measured to the nearest 0.1 cm using a stadiometer (SECA, mod 206 Hamburg, Germany). Waist circumference was measured to the nearest 0.1 cm at the narrowest part of the torso using a flexible fiber glass anthropometric tape (SECA, mod 206 Hamburg, Germany). Waist to height ratio was calculated as waist circumference (cm) divided by the height (cm). Body Mass Index for Age z-score (BAZ) and Height for Age z-score (HAZ) were calculated using the AnthroPlus software (Geneva: WHO, 2009) based on the WHO criteria of BMI-for-age for children aged 5-19 years. Underweight was defined as two z-scores below the WHO reference median, overweight as one standard deviation above the WHO reference median and obese as two standard deviations above the reference median of the BMI-for-age z-score. Stunting was defined as two z-scores below the WHO reference median height-for-age[17].

On the same day as the anthropometric measures, whole body composition was also measured by a certified technician using Dual-energy X-ray absorptiometry (DXA) (Hologic Mod Explorer, 4500 C/W QDR, INC 35 Crosby Drive, Bedford, MA 01730, USA). Body fat percent and body fat content (Kg) were recorded from the values provided by the DXA. Abdominal fat percent and abdominal fat content (Kg) were estimated following procedures described by Hill et al[18].

Stool Analysis

The stool sample was analyzed the same day of the children's visit to the UAQ Nutrition Clinic. A direct coproparasitological test consisting of a wet mount with iodine staining of slides was performed to screen for the presence of protozoan intestinal parasites, as described by WHO[19]. Samples without protozoan trophozoites or cysts were classified as non-infected, samples with less than 90 protozoan trophozoites or cysts per observation field were considered as having a light intensity infection, samples with more than 90 protozoa trophozoites or cysts per observation field were considered as having a moderate-heavy intensity infection. Next, a Kato-Katz smear (2 x 25 mg = 50 mg) was performed according to standard procedures to determine the presence and quantify the number of eggs of STH. Children were characterized following the WHO recommendations[20]. Absence of protozoa or eggs were considered as non-infected. Samples with 1-4999 of *A. lumbricoides* eggs per gram of

feces (epg) were considered with light-intensity infection, and samples with 5000 or more epg were considered as moderate-heavy intensity infection. For hookworm, 1 – 1999 epg were considered with light intensity infection and with 2000 or more were considered with moderate-heavy intensity infection. Children with 1 – 999 epg of *T. trichiura* were categorized with light intensity infection and with 1000 or more as moderate-heavy intensity infection.

Data Analysis

A descriptive analysis of all the variables was performed. The distribution of dependent variables in each analysis was explored to confirm a normal distribution. A chi-squared test was used to determine differences in the prevalence of infection between girls and boys and between communities. An analysis of covariance (ANCOVA) adjusted by crowding, age, community and mother´s education level was performed to determine differences between the non-infected and infected children (overall, helminthes and protozoa) in the anthropometric and body fat variables. Parasites with a prevalence higher than 15% were stratified into three levels depending on severity of infection (non-infected, light intensity infection and moderate-heavy intensity infection) and analyzed separately to determine differences between the infection severity groups in the anthropometry and body fat variables. All statistical analyses were performed by SPSS 18.0 (SPSS, Chicago IL).

Results

General characteristics of the subjects are summarized in Table 1. The prevalence of underweight and stunting was 2% and 6%, respectively. In contrast, we found a high prevalence of overweight (19%) and obesity (10%). No statistical differences were found between communities in any of the anthropometric, socioeconomic or body fat variables (data not shown).

As seen in Table 1, 61% of the children in the study were positive for one or more parasites (overall parasitic infection). The prevalence of helminth infection (STH) was 19%, *A. lumbricoides* had the highest prevalence (16%), followed by hookworm (2%); *T. trichiura* infection was not found. Of the studied children, 47% were positive for protozoan infection. Six different types of protozoa were found: *E. coli* was the most common (20%), followed by *E. nana* (16%) and *B. coli* (13%). The prevalences of *Entamoeba histolytica, Iodamoeba Bütschlii* and *Giardia lamblia* were below 6% and only 4% of the children were infected with both helmiths and protozoa parasites.

Table 1. Main characteristics of the studied children according to gender

	boys (n=137)	girls (n=159)	Overall (n=296)	p
Age (years)[1]	8.0 ± 1.5	7.9 ± 1.6	8.0 ± 1.6	0.64
Weight (Kg)[1]	27.5 ± 7.9	27.5 ± 8.5	27.7 ± 8.3	0.96
Height (cm)[1]	126.2 ± 9.7	126.1 ± 10.1	126.2 ± 9.9	0.91
Waist Circumference (cm)[1]	59.1 ± 8.6	60.2 ± 9.6	60.0 ± 9.2	0.29
Height-for-age (Z-Score)[1]	-0.7 ± 1.0	-0.5 ± 0.9	-0.6 ± 0.9	0.18
BMI-for-Age (Z-Score)[1]	0.4 ± 1.4	0.3 ± 1.1	0.3 ± 1.2	0.49
Body Fat (%)[1]	26.3 ± 6.7	31.2 ± 6.0	29.0 ± 6.8	<0.01
Abdominal fat (%)[1]	24.7 ± 9.3	30.5 ± 9.1	28.0 ± 9.6	<0.01
Body Fat (Kg)[1]	7672 ± 4117	9022 ± 4185	8399 ± 4201	0.01
Abdominal fat (Kg)[1]	356 ± 247	445 ± 264	404 ± 259	<0.01
Stunting %[2]	3.0 %	2.7 %	5.7 %	0.37
Underweight %[2]	1.0 %	0.7 %	1.7 %	0.43
Overweight %[2]	11.5 %	7.1 %	18.4 %	0.10
Obesity %[2]	5.8 %	4.1 %	9.8 %	0.05
Parasitic Infection				
Overall Prevalence %[2]	30.1 %	31.4 %	61.0 %	0.13
Helminth (overall) %[2]	10.8 %	7.8 %	19.0 %	0.03
Hookworm %[2]	1.0 %	1.4 %	2.0 %	0.59
Ascaris lumbricoides %[2]	9.1 %	6.8 %	16.0 %	0.06
Protozoa (overall) %[2]	21.3 %	26.0 %	47.0 %	0.40
Entamoeba coli %[2]	9.1 %	10.5 %	20.0 %	0.53
Entamoeba histolytica %[2]	2.7 %	2.7 %	5.0 %	0.47
Endolimax nana %[2]	6.4 %	9.8 %	16.0 %	0.20
Iodamoeba Bütschlii %[2]	0.3 %	0.3 %	1.0 %	0.71
Balantidium coli %[2]	7.8 %	4.7 %	13.0 %	0.03
Giardia lamblia %[2]	1.0 %	2.0 %	3.0 %	0.33

[1] Mean ± S.D. t test for independent samples, [2] chi squared test

No differences were observed between infected and non-infected children in overall parasitic infection, protozoa infection and helminth infection in any of the body fat or anthropometric variables (Table 2).

Table 2. Anthropometric and body fat variables according to helminth, protozoan and overall infection[1]

	Overall			Helminth			Protozoa		
	Non-Infected (n=114)	Infected (n=182)	p	Non-Infected (n=241)	Infected (n=55)	p	Non-Infected (n=156)	Infected (n=140)	p
Waist Circumference (cm)	59.2 ± 9.4	59.6 ± 8.9	0.80	59.6 ± 9.0	60.3 ± 10	0.17	60.4 ± 9.7	59.1 ± 8.5	0.15
Waist-Height Ratio	0.5 ± 0.1	0.5 ± 0.1	0.95	0.5 ± 0.1	0.5 ± 0	0.21	0.5 ± 0.1	0.5 ± 0.1	0.12
Height-for-Age (z-score)	-0.7 ± 1.0	-0.6 ± 1.0	0.76	-0.7 ± 1.0	-0.4 ± 1	0.32	-0.6 ± 1.0	-0.7 ± 1.0	0.59
BMI-for-Age (z-score)	0.2 ± 1.3	0.4 ± 1.2	0.28	0.3 ± 1.3	0.4 ± 1	0.35	0.3 ± 1.3	0.3 ± 1.2	0.96
Body Fat (%)	29.1 ± 7.2	28.8 ± 6.5	0.97	29.0 ± 6.6	28.5 ± 8	0.73	29.1 ± 7.3	28.7 ± 6.1	0.58
Abdominal Fat (%)	28.0 ± 10	27.8 ± 9.1	0.92	28.0 ± 9.4	26.9 ± 11	0.67	28.3 ± 10.5	27.4 ± 8.5	0.40
Body Fat (Kg)	8.3 ± 4.5	8.5 ± 4.0	0.87	8.4 ± 4.1	8.4 ± 5	0.32	8.7 ± 4.7	8.1 ± 3.6	0.19
Abdominal Fat (Kg)	0.4 ± 0.3	0.4 ± 0.2	0.64	0.4 ± 0.3	0.4 ± 0	0.24	0.4 ± 0.3	0.4 ± 0.2	0.12

[1]Mean ± S.D. ANCOVA adjusted by age, sex and mother´s educational level.

Prevalence of infection with *A. lumbricoides, E. coli* and *E. nana* was above 15% and were further analyzed, stratifying by the severity of infection. There were no children with moderate-heavy infection of *A. Lumbricoides* and no differences between the non-infected and light intensity infection groups were found in any of the anthropometry and body fat variables (Table 3). Children with no infection, light intensity infection and moderate-heavy intensity infection with *E. nana* had similar anthropometry and body fat results (Table 3). However, the moderate-heavy intensity infection group with *E. coli* had significantly higher waist circumference, waist-to-height ratio, body fat and abdominal fat compared with children that were not infected or with a light intensity infection (P <0.05). No differences between the non-infection, light-infection and moderate-heavy infection groups were found in the height-for-age z-score or BMI-for-age z-score variables (Table 3).

Table 3. Anthropometric and body fat variables according to the severity of parasitic infection[1]

	No Infection	Light Infection	Moderate-Heavy Infection	P
Entamoeba Coli	(n=238)	(n=37)	(n=21)	
Waist Circumference (cm)	60.16 ± 9.12	59.61 ± 6.99	64.65* ± 11.05	0.03
Waist-Height Ratio (Units)	0.47 ± 0.05	0.47 ± 0.04	0.50 * ± 0.05	0.04
Height for Age Z-Score	-0.05 ± 1.02	-0.37 ± 0.88	-0.26 ± 0.91	0.44
BMI for Age (Units)	0.26 ± 1.30	0.24 ± 1.11	0.70 ± 1.07	0.33
Body Fat (%)	28.84 ± 6.90	27.86 ± 5.04	32.66* ± 7.14	0.03
Abdominal Fat (%)	27.44 ± 9.79	26.27 ± 6.98	32.58* ± 10.23	0.04
Body Fat (K)	8.33 ± 4.28	7.90 ± 2.74	10.05* ± 4.84	0.04
Abdominal fat (K)	0.40 ± 0.27	0.37 ± 0.17	0.51* ± 0.29	0.04
Endolimax nana	(n=247)	(n=34)	(n=15)	
Waist Circumference (cm)	59.98 ± 9.44	58.71 ± 7.61	61.31 ± 7.22	0.50
Waist-Height Ratio (Units)	0.48 ± 0.05	0.46 ± 0.04	0.47 ± 0.03	0.35
Height for Age Z-Score	-0.61 ± 0.94	-0.68 ± 1.39	-0.75 ± 0.78	0.82
BMI for Age (Units)	0.32 ± 1.22	0.35 ± 1.72	0.19 ± 0.88	0.93
Body Fat (%)	29.01 ± 6.86	28.27 ± 6.48	28.90 ± 6.41	0.81
Abdominal Fat (%)	27.95 ± 9.88	27.05 ± 8.48	27.78 ± 7.83	0.86
Body Fat (K)	8.50 ± 4.32	7.96 ± 3.77	7.74 ± 3.24	0.58
Abdominal fat (K)	0.41 ± 0.27	0.37 ± 0.22	0.36 ± 0.18	0.53
Ascaris lumbricoides	(n=250)	(n=46)	(n=0)	
Waist Circumference (cm)	59.43 ± 9.14	61.46 ± 9.15	-	0.10
Waist-Height Ratio (Units)	0.47 ± 0.05	0.48 ± 0.05	-	0.18
Height for Age Z-Score	-0.67 ± 1.01	-0.37 ± 0.91	-	0.05
BMI for Age (Units)	0.29 ± 1.28	0.43 ± 1.25	-	0.49
Body Fat (%)	28.87 ± 6.71	29.20 ± 7.16	-	0.74
Abdominal Fat (%)	27.78 ± 9.50	28.15 ± 10.25	-	0.47
Body Fat (K)	8.33 ± 4.21	8.75 ± 4.20	-	0.79
Abdominal fat (K)	0.40 ± 0.26	0.42 ± 0.27	-	0.54

[1]Mean ± S.D. *are statistically different in ANCOVA adjusted by age, sex and Mother´s educational level.

Discussion

In the present study, the overall prevalence of parasitic infection (61%) and the combined prevalence of overweight and obesity (29%) were high. These results are similar to other Hispanic populations[21]. The overall prevalence of infection, helminth infection and protozoan infection as well as the prevalence of *E. coli, Entamoeba histolytica*, *Iodamoeba bütschlii* and *Giardia lamblia* found in this study was similar with those found in other rural areas of Mexico. For instance in a study in a Mexican rural population Quihui-Cota et al. found 68% of the population had a helminth or protozoan infection, (16% helminth, 63% protozoan, and 26% with *E. Coli*[22].

In the population studied, children with a moderate-heavy intensity infection with *E. coli* had significantly higher waist circumference, waist to height ratio, percentage of body and abdominal fat than non-infected children. The association of *E.coli* with anthropometric and body composition variables may be explained through different pathways. *E. coli* may be promoting fat deposition and a higher waist circumference through inflammatory response mechanisms[23]. However the metabolic and immune consequences of being infected with *E.coli* still needs to be investigated. In addition, intestinal protozoan parasites and microbiota are both present in the gut, and may alter the microbiota composition[24]. Changes in gut microbiota have shown to have an effect on food intake, appetite, and metabolic functions[25]. For instance, modified gut microbiota can increase caloric uptake from the diet and can modulate host genes that affect energy deposition in adipocytes and thereby increase the risk of diet-induced obesity[26].

Furthermore, a heavy infection with *E. coli* may lead to micronutrient deficiencies in the host as *E.coli* might deplete the available micronutrients in the gut. Micronutrient deficiencies, in turn, have been described as a risk factor for obesity[27]. Additionally, it is also possible that waist circumference and waist to height ratio of moderate-heavy infected children with *E. coli*, may have been affected by the abdominal distention that intestinal infection promotes. However results on percentage of abdominal and body fat are not influenced by abdominal distention and those results showed the same pattern.

It is not possible to compare our findings related to *E. coli* with other studies because to our knowledge, this is the first study to investigate the association of *E. coli* on obesity and adiposity markers. However, *E. coli* may be understudied because it has previously been described as a non-pathogenic parasite that presents no symptoms in infected individuals[28].

We found that overall parasitic infection was not associated with body fat in rural children of the state of Queretaro. Similarly, Orden et al. found no difference in BMI for age z-score between infected and non-infected children in Buenos Aires, Argentina[29]. The lack of association between overall, helminth and protozoan infection in our study may be due to the differential effect that each parasite may have in the regulation of inflammation pathways, gut microbiota composition and energy intake[30]. For instance, *E. coli* and *A. lumbricoides* may change the composition of gut microbiota by different mechanisms. The overall, helminth and protozoan infection variables are each composed of multiple parasites. In this study each specific parasite has a different association with body composition variables and in some cases these associations are in opposite directions. Separate analysis of each parasite is the best approach to test the associations between parasites and body fat variables.

Some limitations of the study need to be addressed. First, due to the cross-sectional design of the study we cannot determine if *E. coli* infection is triggering fat deposition, or if a high fat percentage is making children more susceptible to *E. coli* infection. A controlled intervention trial could help determine, more accurately, the relationship between of *E. coli* infection and obesity. Additionally, stool samples were only collected from one day, thus the number of infected children is likely to be underestimated. However, the prevalence of infection found in this study was high and similar to that reported previously in rural Mexico.

These results show evidence of an association between a moderate-heavy infection with *E. coli* and a higher body and abdominal fat. These results were specific to *E. coli* infection. Further studies are needed to better understand whether *E. coli* contributes directly to fat deposition or if the associations found here are true elsewhere, and whether these driven by unmeasured confounders. However, these findings raise the possibility that a moderate or heavy *E. coli* may contribute to changes in body composition and thereby have long term consequences on human health.

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CHAPTER 3

Are intestinal parasitic infections associations with obesity in Mexican children and adolescents ?

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Abstract

Background: Obesity is a worldwide healthcare challenge. Recent studies have shown an association of viral and bacterial infection with obesity. However, studies on the association between parasitic infections and obesity are scarce. In this ecological study, we examined the relationship between infection with intestinal helminths and protozoa with obesity in Mexico. **Methods:**We used publicly available individual-level data for BMI for age z-score (BMIz) in 2012 as a measure of obesity and state-level data on incidence of infection with helminths (*Ascaris lumbricoides*) and protozoa (*Entamoeba coli, Balantidium coli, Endolimax nana, Cryptosporidium*) in 2000, 2006 and 2012 as a proxy for the probability of intestinal parasitic infection. **Results:** A higher probability of infection with *Ascaris lumbricoides* and protozoan infections in 2000 and 2006 were associated with an higher BMIz in 2012. Furthermore, a higher probability of protozoan infection in 2012 was associated with a higher BMIz in 2012, while a higher probability of *Ascaris lumbricoides* infection in 2012 was associated with a lower BMIz in 2012. **Conclusion:** Our results suggest that a higher probability of infection with *Ascaris lumbricoides* or protozoa was associated with a higher BMIz later in life. The association between current intestinal parasite infection and BMIz is opposite for *Ascaris lumbricoides* and protozoa. These findings may have important implications for Mexico, given the context of a high prevalence of parasitic infection and an emerging obesity epidemic.

Introduction

Obesity and associated morbidities are public health challenges that many low and middle income countries are facing[1, 2]. Often in these countries, infections are common as well, especially in children[3, 4]. Recent studies have shown that certain viral and bacterial infections are associated with obesity (described as "infectobesity")[5-8]. However, studies on the association between parasite infections and obesity are still scarce[9-11].

We hypothesize that intestinal parasites may be associated with obesity by two plausible mechanisms. The first possibility is that both obesity and parasitic infections are positively associated with poverty, as reported previously in different low and middle income countries[12, 13]. However, a second alternative may be that intestinal parasites have an effect on the metabolism by the alteration of the gut microbiome composition.

It is assumed that "stress factors" or "insults" such as infectious diseases and under-nutrition presented during a "critical window" of development (i.e. childhood or puberty) can lead to changes in the gut microbiome composition[14-16]. These changes may be reflected as alterations in metabolism and may lead to fat deposition over time[17-20]. For instance, modified gut microbiota can increase caloric uptake from the diet and can modulate host genes that affect energy deposition in adipocytes and thereby increase the risk of diet-induced obesity[19].

According to the last health and nutrition survey of Mexico (ENSANUT 2012) Mexico has a combined prevalence of overweight and obesity of approximately 70% in adults and 30% in children[21, 22]. Approximately 50% of the total population is infected with one or more species of intestinal parasites[23, 24] with *A. lumbricoides* being the most common intestinal helminth infection, and *E. coli* the most prevalent intestinal protozoan infection[11, 25]. In a previous study, we found that *Entamoeba coli* (*E. coli*) infection was associated with a higher percentage of body fat and food intake, while *Ascaris lumbricoides* (*A. lumbricoides*) infection was associated with a lower food intake in children[11]. The objective of this ecological study is to evaluate the association between the probability of infection with intestinal helminths (*A. lumbricoides*) and intestinal protozoa *(Entamoeba coli, Balantidium coli, Endolimax nana, Cryptosporidium*) with BMI for age z-score in the Mexican population.

Methods

Datasets:

We used individual and state-wide data (32 states) collected by three different federal organizations of Mexico. Individual level data on height, weight and age was obtained from the 2012 National Health and Nutrition Survey (ENSANUT 2012). This survey of over 50,000 households is representative for the Mexican population at national and state level. The data is available at http://ensanut.insp.mx and the methodology is described elsewhere[26]. Statewide data on intestinal helminths and protozoa in 2012, 2006 and 2000 was obtained from the National System for Epidemiological Surveillance (SINAVE)[27]. The data is available at: http://www.epidemiologia.salud.gob.mx. Finally we used state-wide data on demographic and socioeconomic variables from the National Institute of Statistics and Geography (INEGI) 2012 report, available at http://www.inegi.org.mx/[28].

Dependent Variable: BMI for age z-score

Using data from ENSANUT 2012, we calculated the BMI for age z-score (BMIz) for all individuals. BMIz for individuals above 19 years was calculated using the last available point of the WHO grow charts for children (228 months). The calculations were made using the World Health Organization (WHO) SPSS anthroplus macro for children 5-19 years (WHO, Geneva, Switzerland). The macro is publicly available at http://www.who.int/childgrowth/software/en/.

Independent variables: infection with intestinal parasites

Information on intestinal helminths and protozoa was extracted from SINAVE, which is the only publicly available data source on intestinal parasite infection across states. In this system, data is reported annually as the incidence per 100 000 person-years in different age groups (i.e. less than 1y, 1-5y, 6-10y, 11-19y) following the same procedures in each of the 32 states of Mexico[27]. *A. lumbricoides* infection, the most common helminth infection in Mexico, is reported as the incidence of *A. lumbricoides* infection, and *E. coli*, the most common protozoa is reported in the group of "non-pathogenic protozoa" as the grouped incidence of *Entamoeba coli, Balantidium coli, Endolimax nana and Cryptosporidium*.

We used the SINAVE incidence data as a proxy for the probability of infection, in function of the state and the age of the subject at a particular time point. For instance, the probability of infection in 2012 of an individual of 15 years old was approximated by the incidence of the intestinal parasite infection in his/her state of residence in 2012 for his/her corresponding age group. In addition, the same individual's probability of infection in 2006 and in 2000, was determined by the incidence of each intestinal parasitic infection in his/her state and for his/her age group at that given year.

Additional covariates

In order to control for confounding we included both individual-level covariates from the ENSANUT 2012 survey and state-level covariates from the INEGI survey in 2012. The individual-level covariates used in the analysis were: sex (male/female), age (years), place of residence (rural/urban) and "marginality" (marginalized/not-marginalized). Marginality indicates whether a child is from a marginalized socioeconomic status or not based on indicators such as parents education level, access to sanitation facilities, access to drinking water, income and population size of the community, as described in detail elsewhere[26]. State-level covariates were: population with health coverage (%), education level of adults (mean number of years in school), households without sanitation facilities (%) and the poverty rate (population living in poverty). The poverty rate was determined by the "poverty index", which indicates whether a household is poor or not. In addition to income, the poverty index also takes into consideration access to healthcare, social security, material of the roof, floor and walls of the household, access to basic services and food, according to standard procedures which are described elsewhere[29].

Statistical analysis

As shown in figure 1 the population was stratified into three age groups depending on the age of individuals in 2000, 2006 and 2012. For the cross sectional analysis on the association between infection and obesity in 2012 we extracted data on individuals aged 1-5y (n=8,927), 6-10y (n=16,347) and 11-19y (n=13,992) in 2012. For the analysis on the association between infection in 2006 and obesity in 2012 we selected those individuals from the ENSANUT survey of 2012 who were aged 1-5y (n=9,523), 6-10y (n=13,025), and 11-19y (n=7,845) in 2006. Likewise for the analysis concerning infection in 2000 we selected those individuals from the ENSANUT survey of 2012 who were aged 1-5y (n=6,625), 6-10y (n=7,580), and 11-19y (n=5,623) in 2000.

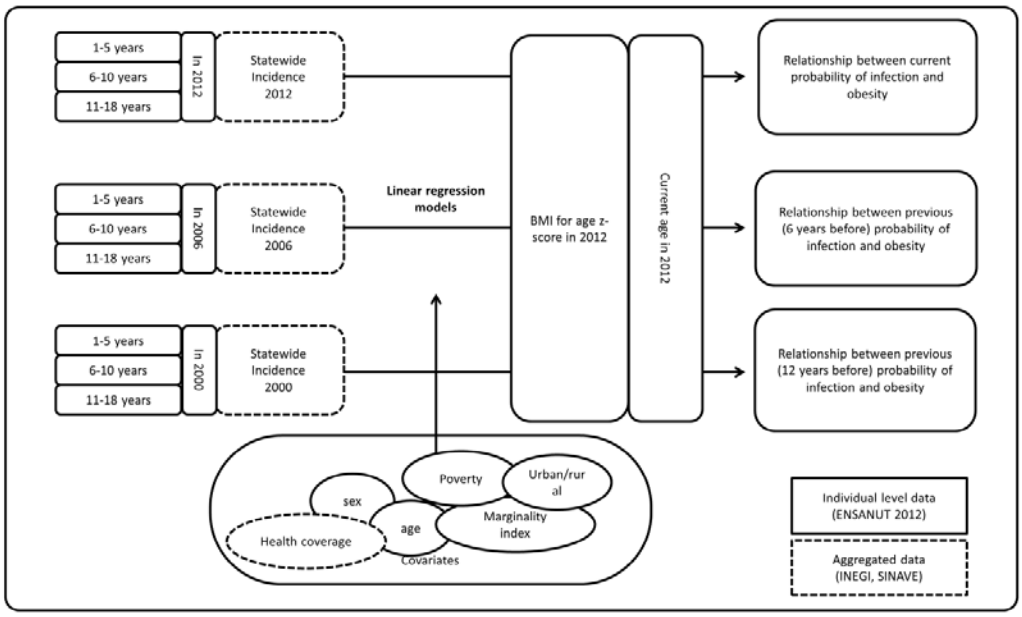

Figure1. Diagram of the study design

Linear regression models were used to determine the association between the probability of infection (2000, 2006 and 2012) with BMIz or in 2012. Associations were estimated for *A. lumbricoides* and protozoa for each of the three age groups separately. In order to facilitate interpretation of the results, the incidence of infection (proximate probability of infection) for the regression analysis was transformed from new cases in 100 000 person-years to new cases per 100 person-years. Findings bellow p value of 0.05 were considered significant.

Poverty rate and sex were explored as a possible effect modifiers [30]. For this purpose we performed the same analyses as described above, including an interaction term between sex or poverty rate and the probability of infection in each model. Models with statistically significant interaction terms ($p < 0.05$) were stratified in two groups, one above and one below the median of poverty rate.

In order to have a visual overview of the combined prevalence of overweight and obesity and the incidence of each parasitic infection in 2012, we mapped each variable stratified in quintiles. The unit of mapping was "the state" the largest administrative unit of Mexico, and the maps were generated using Arc GIS V10.1 (Redlands, CA).

Results

In 2012, the combined prevalence of overweight and obesity in Mexico was 10% for children aged 1-5y, 35% for children aged 6-10y, 36% for the age group of 11-19y (Figure 2). In total, 47% percent of the population lived in poverty. The health coverage was 62% and the rate of households without sanitation facilities was 12% (Table 1). The incidence of *A. lumbricoides* and protozoan infection decreased from 2000 to 2006 and from 2006 to 2012 for all age groups (Table 1).

Table 1. General Characteristics of the population

	Mean	S.D.	Minmu	Maximum	Source
A. lumbricoides 1 to 5 years					
Incidence in 2012	94 ±	129.3	3	625	SINAVE 2012
Incidence in 2006	404 ±	461.1	5	1925	SINAVE 2006
Incidence in 2000	1177 ±	1151.2	99	4559	SINAVE 2000
A. lumbricoides 6 to 10 years					
Incidence in 2012	67 ±	108.2	0	526	SINAVE 2012
Incidence in 2006	212 ±	301.8	3	1822	SINAVE 2006
Incidence in 2000	907 ±	999.8	45	3990	SINAVE 2000
A. lumbricoides 11 to 18 years					
Incidence in 2012	27 ±	42.7	0	219	SINAVE 2012
Incidence in 2006	90 ±	140.0	1	757	SINAVE 2006
Incidence in 2000	367 ±	421.5	18	1711	SINAVE 2000
Protozoa 1 to 5 years					
Incidence in 2012	93 ±	104.0	2	545	SINAVE 2012
Incidence in 2006	239 ±	275.2	9	1498	SINAVE 2006
Incidence in 2000	351 ±	215.5	73	905	SINAVE 2000
Protozoa 6 to 10 years					
Incidence in 2012	78 ±	93.7	1	568	SINAVE 2012
Incidence in 2006	150 ±	200.7	4	1590	SINAVE 2006
Incidence in 2000	212 ±	154.8	43	731	SINAVE 2000
Protozoa 11 to 18 years					
Incidence in 2012	48 ±	65.4	0	354	SINAVE 2012
Incidence in 2006	78 ±	97.5	2	902	SINAVE 2006
Incidence in 2000	113 ±	88.0	23	420	SINAVE 2000
Prevalence of ow/ob (1-5 y) 2012	10.1 ±	2.6	4.9	14.8	ENSANUT 2012
Prevalence of ow/ob (6-10 y) 2012	34.5 ±	6.3	22.5	51.5	ENSANUT 2012
Prevalence of ow/obs (11-18 y) 2012	35.9 ±	5.0	27.8	47.4	ENSANUT 2012
Females (%)	53.4	2.2	50.6	56.0	ENSANUT 2012
High marginality (%)	42.5	5.3	36.2	43.5	ENSANUT 2012
Poverty (%)	46.6 ±	13.3	21.0	78.5	INEGI 2012
Extreme Poverty (%)	11.2 ±	8.7	1.8	38.3	INEGI 2012
Years in school	8.6 ±	0.9	6.3	10.6	INEGI 2012
Health coverage (%)	62.4 ±	10.9	39.9	81.0	INEGI 2012

ow/ob: overweight/obesity

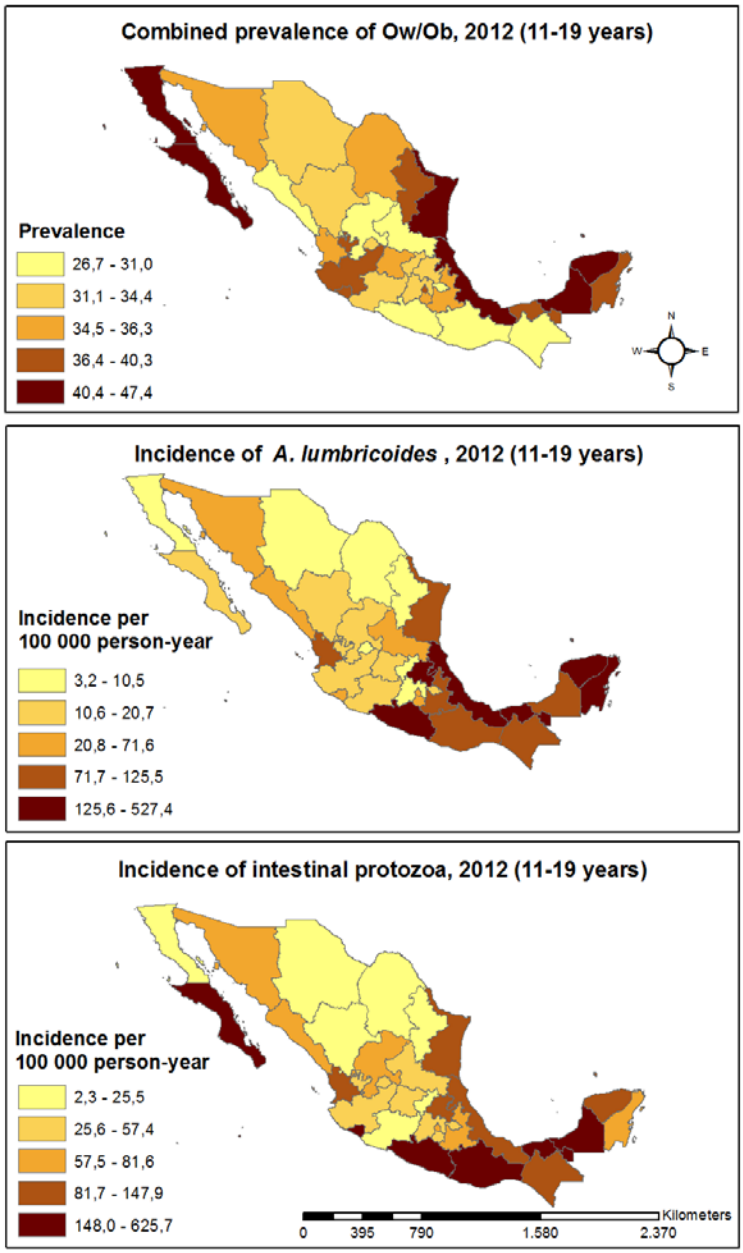

Figure 2. Map of combined prevalence of overweight and obesity and the incidence of Ascaris lumbricoides and protozoa in Mexico, 2012

Ascaris lumbricoides

Table 2 shows a positive association between the probability of *A. lumbricoides* infection in 2000 and 2006 with BMIz in 2012. In the adjusted model, an increase of 1% in the probability of infection in 2006 was associated with an increase of 0.13 in the BMIz in 2012 for age group 1-5y, 0.27 for age group 6-10y and 0.50 in BMIz for age group 11-19y. Furthermore, an increase of 1% in the probability of infection with *A. lumbricoides* in 2000 was associated with an increase of 0.10 in the BMIz in 2012 for age group 1-5y, 0.11 for age group 6-10y, and 0.25 for the 11-19y age group. In contrast table 3 shows that a higher probability of being infected with *A. lumbricoides* in 2012 was associated with a decrease of 0.32 in the BMIz for age group 1-5y and a decrease of 0.21 for age group 6-10y.

Table 2. Linear regression model between the proximate probability of infection in 2000 and 2006 with BMI for age z-score in 2012

	Ascaris lumbricoides						protozoa					
	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p
Incidence in 2006	Crude model			Adjusted model			Crude model			Adjusted model		
1 to 5 years (n=9523)	-0.03	(-0.04 - -0.03)	0.16	0.13	(0.12 - 0.13)	<0.01	0.00	(0.00 - 0.01)	0.79	0.00	(0.00 - 0.01)	0.93
6 to 10 years (n=13025)	0.04	(0.04 - 0.05)	0.14	0.27	(0.26 - 0.28)	<0.01	0.07	(0.06 - 0.08)	0.14	0.13	(0.12 - 0.13)	0.01
11 to 18 years (n=7845)	0.33	(0.32 - 0.34)	<0.01	0.50	(0.49 - 0.52)	<0.01	0.24	(0.23 - 0.26)	0.00	0.16	(0.15 - 0.18)	0.03
Incidence in 2000												
1 to 5 years (n=6625)	0.04	(0.04 - 0.05)	<0.01	0.10	(0.09 - 0.10)	<0.01	0.29	(0.28 - 0.30)	0.00	0.47	(0.46 - 0.48)	<0.01
6 to 10 years (n=7580)	0.08	(0.08 - 0.09)	<0.01	0.11	(0.11 - 0.11)	<0.01	0.58	(0.56 - 0.60)	0.00	0.61	(0.59 - 0.63)	<0.01
11 to 18 years (n=5623)	0.19	(0.18 - 0.19)	<0.01	0.25	(0.24 - 0.26)	<0.01	0.88	(0.85 - 0.90)	0.00	0.99	(0.96 - 1.02)	<0.01

Adjusted by: urban/rural strata, age, sex, marginality, poverty, health-coverage. Incidence per 100 person-year.

Table 3. Linear regression model between the proximate probability of infection in 2012 with BMI for age z-score in 2012

	Ascaris lumbricoides						Protozoa					
	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p
Incidence in 2012	Crude model			Adjusted model			Crude model			Adjusted model		
1 to 5 years (n=8927)	-0.17	(-0.18 - -0.16)	<0,01	-0.32	(-0.33 - -0.31)	<0,01	0.02	(0.01 - 0.03)	0.74	0.08	(0.06 - 0.10)	0.34
6 to 10 years (n=16347)	-0.15	(-0.16 - -0.14)	<0,01	-0.21	(-0.22 - -0.19)	0.01	0.19	(0.18 - 0.21)	0.02	0.61	(0.59 - 0.63)	<0,01
11 to 18 years (n=13992)	0.17	(0.16 - 0.19)	0.05	0.16	(0.13 - 0.18)	0.23	0.43	(0.42 - 0.45)	0.00	0.85	(0.83 - 0.88)	<0,01

Adjusted by: urban/rural strata, age, sex, marginality, poverty, health-coverage. Incidence per 100 person-year.

Table 4 shows the results stratified by poverty rate. In the states with low poverty rates in 2012, *A. lumbricoides* infection was associated with an increased BMIz in 2012 in all age groups. In states with high poverty rates *A. lumbricoides* infection differed between age strata; the probability of infection with *A. lumbricoides* was associated with a lower BMIz for age group 1-5y, no association in BMIz for age group 6-10y and a higher BMIz for age group 11-19y. Neither sex nor poverty rate were modifiers for the associations between the probability of *A. lumbricoides* infection in 2006 or 2000 with BMIz in 2012 for any of the age groups. Therefore no stratified analysis were performed for these years.

Table 4. Linear regression model between the proximate probability of infection with *A. lumbricoides* in 2012 with BMI for age z-score in 2012 acording to statewide poverty rates

	Low Poverty				High Poverty			
	β	95% C.I.		p	β	95% C.I.		p
***A. lumbricoides* Incidence in 2012**								
1 to 5 years	0.33	(0.31 -	0.35)	<0.01	-0.28	(-0.29 -	-0.27)	<0.01
6 to 10 years	0.51	(0.48 -	0.53)	<0.01	-0.05	(-0.07 -	-0.04)	0.42
11 to 18 years	1.14	(1.11 -	1.17)	<0.01	0.34	(0.31 -	0.36)	0.01

Adjusted by: urban/rural strata. age. sex. marginality. poverty and health-coverage. Incidence per 100 person-year.

Protozoa

Table 2 shows the associations between the probability of infection with protozoa in 2000 and 2006 and BMIz in 2012 across the three studied age groups. The probability of infection with protozoa in 2006 was associated with a higher BMIz in 2012 in the 6-10y and 11-19y age groups. An increase of 1% in the probability of infection in 2000 was associated with an increase in the BMIz in 2012 of 0.47 for age group 1-5y, 0.61 for age group 6-10y and 0.99 for age group 11-19y.

Table 3 shows the associations between the proximate probability of protozoan infection in 2012 and BMIz in 2012 for every age group. In the adjusted model an increase of 1% in the probability of protozoan infection was associated with an increase of 0.6 in the BMIz for age group 6-10y and an increase of 0.9 for age group 11-19y

Neither sex nor poverty rate were effect modifiers for the associations between the probability of protozoan infection in 2012, 2006 or 2000 with BMIz in 2012 for any of the age groups. Therefore no stratified analysis were performed.

Discussion

Our results indicate that children with a higher probability of *A. lumbricoides* or protozoan infection (*Entamoeba coli, Balantidium coli, Endolimax nana, Cryptosporidium*) are more likely to have a higher BMIz in the same year, 6, and 12 years later in life. This finding is consistent with other studies showing early child "insults" including infections to be associated to later overweight and obesity[31-33]. This finding could be related to changes in the gut microbiota and inflammatory reactions due to parasitic infection that may lead to changes in appetite, food intake and thereby BMIz[34-36]. In line with this hypothesis, we recently found that *E. coli* infection was associated with a higher percentage of body fat and food intake in children[11] (Zavala et al., *submitted)*. Similarly Schilder et al., in a firefly model, observed that an intestinal protozoa common in insects caused fat deposition in the thorax which is comparable to obesity in mammals[9, 10]. Longitudinal studies are needed to assess the temporal association of intestinal parasites on obesity over time.

While a higher probability of protozoan infection in 2012 was associated with a higher BMIz in the same year, we found the opposite for *A. lumbricoides* infection. If the associations would have been explained purely by poverty, the same trend and direction on the associations would have been observed for both parasitic infection incidence, which was not the case either in the crude or adjusted model. We also found an association between BMIz and *A. lumbricoides* for the age groups 1-5y and 6-10y, and not for the oldest age group (11-19y). The difference between age groups might be explained by the fact that *A. lumbricoides* infection-related symptoms are more common in younger children[37]. Children infected with *A. lumbricoides* may experience abdominal pain, nausea and discomfort, which may lead to a lower food intake and therefore lower BMIz [38]. The results of our previous study in Mexican schoolchildren supports this hypothesis, as we found a negative association between *A. lumbricoides* infection and food intake (Zavala et al., submitted).

We found opposite associations between BMIz and *A. lumbricoides* incidence in states with high and low poverty rates. These differences may be explained with previous studies, as shown in a review by Guerrant et. al[33] in which children living in poverty were more likely to be malnourished, but also more likely to have stronger symptoms when infected.

Our findings should be interpreted in the context of this being an ecological analysis and not an estimate on any causal effect of parasitic infection on obesity at individual level (ecological fallacy). However, we intended to minimize this issue using individual level data on BMIz and covariates. Although we adjusted for potential confounders, we cannot control for unknown or unmeasured factors such as food availability, diet, and physical activity, therefore the outcomes of this study should be interpreted with caution. In addition, it was not possible to take changes in the state of residence over the years in consideration, but according to INEGI, migration between states was relatively low. In the year 2000: 3,584 957 persons migrated between states, representing 3.6% of the population and in 2006: 2,406 454 persons migrated, corresponding to 2.3% of the population at that time[39]. We used incidence data of the studied parasites as a measure for the probability of infection[40], and the true prevalence of parasitic infection is most likely underestimated. A major strength of our study is that ENSANUT and INEGI surveys are representative of the Mexican population at national and state level. In addition the parasite

infection data of the SINAVE is collected following the same procedures nationwide and is therefore a good measuring tool for comparison purposes.

Conclusions

Our results suggest that children with a higher probability of infection with intestinal parasites have a higher BMIz later in life. The association between current intestinal parasite infection and BMIz is less straightforward, and seems to be opposite for *A. lumbricoides* and protozoa. Further research is needed to confirm these ecological associations and study possible mechanisms underlying the short-term and long-term consequences of intestinal parasite infections on health. These findings may have important implications for Mexico, given the context of a high prevalence of parasitic infection and an emerging obesity epidemic.

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CHAPTER 4

Energy and food intake are associated with specific intestinal parasitic infections in children of rural Mexico

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Abstract

Background: Intestinal parasites have been associated with nutritional status, however the underlying mechanisms are still unclear, the current study aims to investigate associations between *Entamoeba coli* and other intestinal parasitic infections with macronutrients and food group intake in school age children. **Methods**: 284 children (8.1 ± 1.6 y) from Queretaro, Mexico participated in this study. Anthropometric measurements were taken. Dietary intake was determined using both a food frequency questionnaire and three 24-h recalls. Intestinal parasitic infection was determined by microscopic examination of stool samples. MANCOVA adjusted by age, sex, and caretaker's educational level was performed to determine differences between infected and non-infected children in food intake. **Results:** Children infected with *Entamoeba coli* had a higher intake of energy and fat, and higher consumption of dairy, legumes, meat and cereals than non-infected children ($p<0.05$). In contrast, children infected with *Ascaris lumbricoides* had significantly lower intake of energy, carbohydrates and fiber, and lower consumption of dairy and meat compared with non-infected children ($p<0.05$). For the other intestinal parasite infections, no significant differences in energy or food intake were found. **Conclusions**: Our results shows that *A. lumbricoides* infection is associated with a lower reported food intake, while *E. coli* was associated with a higher reported food intake This finding suggests that alterations in food intake may represent an important link in the chain connecting intestinal parasite infection and nutritional status outcomes such as low height for age and micronutrient deficiencies.

Introduction

Intestinal parasitic infections are a major public health concern in many tropical and sub-tropical regions of the world, especially in rural communities with poor sanitation and hygiene[1-4]. While infection can occur at any age, school age children are most at risk for intestinal parasitic infection, due to behavioral and environmental factors[4]. Intestinal helminth infection is a well-established cause of child stunting[5, 6]. In contrast intestinal protozoa parasites have been positively associated with obesity and adiposity in vitro and in animal models, but conclusive evidence of this relationship in humans is lacking[7-9].

A decreased food intake is a pathway which is often mentioned to explain the relationship between intestinal parasites and nutritional status throughout the literature. However there are few references addressing this relationship, most studies have been carried out in animal models with animal species of intestinal parasites[10-12]. The few studies done in humans found that anti-helminthic treatment was associated with increased appetite. However in these studies appetite was also correlated to increased growth, and therefore is not possible to determine the independent effect of the intestinal parasites on appetite[13, 14]. Other limitation is that these studies did not addressed the effect of intestinal protozoa-treatment on appetite.

Intestinal parasites can modify circulating levels of certain hormones to generate specific changes that may be reflected in the host eating behavior[15]. For instance, in mouse models *Taenia taeniaformis* is associated with a lower concentration of leptin, a hormone that has a critical role in regulation of appetite, and food intake[10]. Also, sheep infected with *Teladorsagia circumcincta* had a lower consumption of food *ad libitum*[11], and a significant reduction in appetite resulting in weight loss[12]. Furthermore, intestinal parasite infections have been shown to modify gut microbiota composition in humans[16], and may thus modulate/affect appetite, energy absorption, food intake, and other metabolic processes[17-19].

The aim of this study was to evaluate the potential association between intestinal parasitic infections (helminths and protozoa) with energy, carbohydrate, protein, fat, fiber and food group intake in school age children living in rural communities of the state of Queretaro in Mexico.

Methods

Subjects

A total of 284 school age children between 6 and 10 years of age of two rural communities in the municipality of El Marques in Queretaro, Mexico, participated in the study. Clinical history was recorded via a standardized questionnaire applied to each participant's caretaker. The presence and history of any relevant co-morbidities and parasitic-medication intake was recorded. Children with any physical or mental disability, with any illness that related to food intake, that had been given any deworming treatment in the last 4 months before inclusion, or that were under a prescribed physical activity or diet regimen at the time of inclusion were excluded from the study. Demographic information (child's age, sex) and caretaker's educational level (as a measure of socioeconomic status) were collected by questionnaire[20, 21].

Anthropometry

Children were transported from their local communities to the Nutrition Clinic at the UAQ. Weight, height and waist circumference were measured twice in all children by trained personnel following the World Health Organization (WHO) procedures[22]. Children were weighed barefoot, wearing light clothing, using a calibrated digital scale (SECA, mod 813 Hamburg, Germany) with a precision of 0.1 g. Height was measured with +/- 0.1 cm precision using a stadiometer (SECA, mod 206 Hamburg, Germany). Nutritional status was calculated based on the WHO criteria of BMI-for-age for children aged 5-19 years using the Anthroplus software (Geneva: WHO, 2009).

Children were considered to be underweight if they had 2 z-scores below, overweight if they had 1 z-score above and obese if they had 2 z-scores above the reference median of the BMI-for-age z-score and were considered to be stunted if they had 2 z-scores below the WHO reference median of height for age z-score[23]. Body fat content (Kg) and body fat percentage was measured in all children by a certified technician using Dual-energy X-ray absorptiometry (DEXA) (Hologic Mod Explorer, 4500 C/W QDR, INC 35 Crosby Drive, Bedford, MA 01730, USA).

Parasitological Examination

A direct wet mount with iodine staining of slides was performed to determine the presence of protozoa as described by WHO[24]. Samples without protozoan trophozoites or cysts were classified as non-infected, samples with less than 90 protozoan trophozoites or cysts per observation field were considered as having a light intensity infection, samples with more than 90 protozoa trophozoites or cysts per observation field were considered as having a moderate-heavy intensity infection. Kato-Katz examination was performed to determine the presence of helminth eggs and the intensity of infection[25]. The intensity of infection was determined using the WHO guidelines[26]: Samples with 1-4999 of *A. lumbricoides* eggs per gram of feces (epg) were considered with light-intensity infection, and samples with 5000 or more epg were considered as moderate-heavy intensity infection. Children positive for any parasitic infection were sent to their local clinic for treatment.

Diet Evaluation

Food intake was assessed using both a qualitative food frequency questionnaire (FFQ) and three 24 hour recalls (24hR). The FFQ was designed and validated for the Mexican population[27]. Children's caretakers in the presence of the children were asked to report the frequency of consumption of 134 food and beverage items during the past year. Caretakers were also asked to indicate whether the children had changed their dietary habits during the 12 last months.

Three 24hR were applied by trained interviewers. Each caretaker was asked on three separate occasions to recall and describe in detail all types, amounts and preparation of foods and beverages consumed by the children in the previous 24 hours. The 24hRs were taken on two week days and one weekend day. In addition to the foods consumed, intake was also evaluated in terms of total energy and specifically for macronutrient composition and fiber. Therefore, in addition to daily caloric intake (kcal), carbohydrate (g), protein (g), fat (g) and fiber(g) intake were calculated using food composition tables from the United States Department of Agriculture (USDA) and from the National Institute of Medical Science and Nutrition "Salvador Zubiran"[28, 29]. Foods of the 24hR and FFQ were classified into food groups as defined in Table 1. Foods of the 24hR were reported as servings per day (spd).

Table 1. Definition of food groups in the study for FFQ and 24hR

Food Group	Foods per group
Fruits	All fresh and dried fruits
Vegetables	All vegetables and mushrooms
Dairy	Milk, milk products (including milk products with fat and sugar), cheese, yogurt
Legumes	Beans, white beans, chickpea, lentil, soy
Meat	Beef, lamb, chicken, fish, liver, brain, all sausages
Cereal	Ready-to-eat cereals, white bread, all grain bread, corn tortilla, tamale, corn
Fat	Avocado, olive oil, oil, lard, butter, nuts
Added Sugars	Candy, lollipop, ice cream, sugar, honey, catsup
Beverages	Soft drinks, juices, artificial flavored water, fruit water, atole

Data Analysis

T-test for independent samples or a chi-squared test were performed to determine differences between boys and girls in anthropometric variables, total energy, macronutrient, food groups and the prevalence of parasitic infection. We performed a multivariate analysis of covariance (MANCOVA) adjusted by % of body fat, gender, child's age, and caretaker's educational level. To determine if there are differences between children infected with each parasite (i.e. *A. Lumbricoides, E. coli*, *Endolimax nana, Balantidium coli, Entamoeba histolytica, Iodamoeba bütschlii, Giardia lamblia)* and non-infected children in energy, carbohydrates, protein, fiber and fat intake and the frequencies of food group consumption. The same set of analysis was performed considering the intensity of infection with each parasite.

To avoid spurious significance due to multiple testing in the parasite-specific models, parasites that were associated with energy macronutrients or food group intake were included in a single MANCOVA model. In this model, differences in energy, macronutrient and the frequencies of food group consumption were tested between children infected only with the parasites of interest (i.e. *E. coli* and *A. lumbricoides*) and parasite-free children. The statistical analyses were performed by SPSS 21.0 (SPSS, Chicago IL).

Results

A total of 284 school-age children (6-10 years) were included in the study; 54% were girls. A low prevalence of stunting (2%) and underweight (6%) was found; 19% of children were overweight and 11% were obese. The general characteristics of the children and the daily intake of energy, macronutrients and food groups are summarized in Table 2. No difference in age, height for age z-score, BMI for age z-score, energy, macronutrient intake or servings per day of food group was found between boys and girls.

Table 2. General characteristics and daily intake of energy macronutrients and food group of the population studied (n=284)

	Mean	95% C.I.
Age (years)	8.0	(7.8 - 8.2)
Height for Age (z-score)	-0.6	(-0.7 - -0.5)
BMI for Age (z-score)	0.3	(0.2 - 0.5)
Waist Circunference (cm)	59.9	(58.9 - 61.0)
Energy (Kcal/day)	1634	(1579 - 1690)
Carbohydrates (g/day)	229.1	(220.8 - 236.3)
Protein (g/day)	55.5	(53.0 - 57.9)
Lipids (g/day)	57.2	(54.6 - 59.9)
Fiber (g/day)	13.8	(12.9 - 14.6)
Fruits (spd)	1.4	(1.3 - 1.5)
Vegetables (spd)	2.6	(2.4 - 2.8)
Dairy (spd)	1.5	(1.4 - 1.6)
Legumes (spd)	1.1	(1.0 - 1.2)
Meat (spd)	1.9	(1.8 - 2.0)
Cereal (spd)	4.5	(4.3 - 4.6)
Fat (spd)	1.3	(1.2 - 1.4)
Sugar (spd)	2.0	(1.9 - 2.2)
Beverages(spd)	3.1	(2.9 - 3.3)

spd: servings per day. Daily intake of energy macronutrients and food group is reported as the mean of three 24 hour recalls.

Sixty percent of the children that participated in the study were positive for one or more parasites. The most prevalent helminth was *A. lumbricoides* (16%), followed by hookworm (3%); no infection with *T. trichiura* was found in these children. There were no children with moderate or heavy infection with these helminths. Six different protozoa parasites were found: *Entamoeba coli* was the most commonly found protozoa, with a prevalence of 20%, followed by *Endolimax nana* (15%) and *Balantidium Coli* (11%). *Entamoeba histolytica, Iodamoeba Bütschlii* and *Giardia lamblia* had a prevalence below 6%. Table 3 shows the prevalence of children with mono-infections (i.e children infected only with one parasite), and separately the prevalence of children infected with more than one parasite (12%).

Table 3. Parasitic infection prevalence (n=284)

Parasitic Infection			n
Overall Prevalence	59.9	%	170
Hookworm [1]	1.4	%	4
Ascaris lumbricoides [1]	10.6	%	30
Entamoeba coli [1]	12.7	%	36
Entamoeba histolytica [1]	2.5	%	7
Endolimax nana [1]	11.3	%	32
Balantidium coli [1]	6.3	%	18
Giardia lamblia [1]	2.8	%	8
More than one parasitic infection	12.3	%	35

[1] Mono-infection prevalence (infected only with the specified parasite).

The results of the 24hR show that children infected with *E. coli* had a higher intake of energy and fat, and a higher consumption of the food groups fruit, legumes, and added sugar than non-infected children Table 4 Similarly, results of the FFQ show that children infected with *E. coli* reported a more frequent consumption of dairy and meat food groups compared to non-infected children. There were no differences in reported energy, macronutrient or food group intake between the children with light moderate or heavy *E. coli* infection. In contrast to *E. coli* infection, the results of the 24hR show that children infected with *A. lumbricoides* had a lower intake of energy, carbohydrates, fiber, and the food groups fruit, cereals, legumes, dairy, meat, fat and sugar that non-infected children. In addition, children infected with *A. lumbricoides* had a lower frequency of consumption of dairy and meat food groups according to the FFQ. No differences in energy, macronutrient or food group intake were found between children infected with *Endolimax nana, Balantidium coli, Entamoeba histolytica, Iodamoeba bütschlii* and *Giardia lamblia* and their non-infected counterparts even after taking in consideration the intensity of infection (data not-shown).

Table 4. Daily intakes of children infected with *E. coli* and *A. lumbricoides vs. non-infected* reported by 24 hour recall and food frequency questionnaire

	Entamoeba coli						*Ascaris lumbricoides*					
	Non-Infected (n=227)			Infected (n=57)			Non-Infected (n=239)			Infected (n=45)		
24 hour recall	Mean[1]	95% C.I.		Mean[1]	95% C.I.		Mean[1]	95% C.I.		Mean[1]	95% C.I.	
Energy (Kcal/day)	1575	(1517	- 1633)	1713	(1593	- 1826)*	1631	(1575	- 1687)	1440	(1308	- 1573)**
Carbohydrates(g/day)	221.2	(213.0	- 229.4)	232.1	(215.6	- 248.6)	227.7	(219.8	- 235.6)	200.3	(180.7	- 217.9)**
Protein (g/day)	53.6	(51.0	- 56.2)	57.6	(52.4	- 62.9)	55.3	(52.7	- 57.8)	49.8	(43.7	- 55.7)
Fat (g/day)	54.4	(51.5	- 57.4)	63.1	(57.1	- 69.1)**	57.1	(54.2	- 60.0)	50.2	(44.0	- 57.7)
Fiber (g/day)	13.0	(12.1	- 13.9)	14.8	(12.9	- 16.6)	13.7	(12.8	- 14.6)	11.5	(9.2	- 13.3)*
Fruits (spd)	1.7	(1.5	- 1.8)	2.0	(1.7	- 2.6)*	1.8	(1.7	- 1.9)	1.3	(1.0	- 1.6)**
Vegetables (spd)	2.9	(2.6	- 3.2)	3.0	(2.5	- 3.5)	3.0	(2.7	- 3.3)	2.4	(1.9	- 3.1)
Cereals (spd)	4.4	(4.3	- 4.6)	4.6	(4.3	- 4.9)	4.5	(4.4	- 4.7)	4.1	(3.8	- 4.2)*
Legumes (spd)	1.0	(0.9	- 1.1)	1.4	(1.2	- 1.6)**	1.1	(1.0	- 1.2)	0.8	(0.6	- 1.1)*
Dairy (spd)	1.5	(1.4	- 1.6)	1.7	(1.5	- 1.9)	1.6	(1.5	1.7)	1.3	(1.1	- 1.5)**
Meat (spd)	2.0	(1.9	- 2.1)	2.0	(1.8	- 2.2)	2.0	(1.9	2.1)	1.7	(1.5	- 1.9)*
Fat (spd)	1.4	(1.3	- 1.5)	1.4	(1.2	- 1.6)	1.4	(1.3	1.5)	1.1	(0.9	- 1.4)
Sugar (spd)	2.0	(1.9	- 2.1)	2.3	(2.0	- 2.6)*	2.1	(2.0	2.3)	1.7	(1.4	- 2.0)**
Food Frequency [2]												
Fruits	5.25	(4.97	- 5.54)	5.4	(4.83	- 5.97)	5.33	(5.05	- 5.6)	5.03	(4.36	- 5.69)
Vegetables	5.24	(4.94	- 5.53)	5.73	(5.14	- 6.32)	5.36	(5.07	- 5.64)	5.2	(4.52	- 5.89)
Cereal	6.07	(5.82	- 6.32)	6.68	(6.19	- 7.18)*	6.18	(5.94	- 6.42)	6.27	(5.68	- 6.85)
Legumes	1.48	(1.36	- 1.60)	1.9	(1.66	- 2.14)**	1.56	(1.44	- 1.67)	1.62	(1.33	- 1.9)
Dairy	2.27	(2.12	- 2.42)	2.78	(2.47	- 3.08)**	2.42	(2.28	- 2.57)	2.04	(1.68	- 2.39)*
Meat	2.26	(2.16	- 2.36)	2.53	(2.33	- 2.73)*	2.36	(2.26	- 2.46)	2.06	(1.82	- 2.29)*
Fat	2.72	(2.59	- 2.85)	2.99	(2.72	- 3.25)	2.77	(2.64	- 2.9)	2.78	(2.48	- 3.09)
Sugar	2.43	(2.29	- 2.57)	2.41	(2.13	- 2.68)	2.47	(2.33	- 2.6)	2.19	(1.88	- 2.51)
Beverages	2.47	(2.27	- 2.67)	2.8	(2.4	- 3.21)	2.57	(2.37	- 2.76)	2.36	(1.89	- 2.84)

spd: Servings per day. MANCOVA adjusted by age, gender and mother´s educational level. [1]Estimated marginal mean [2]Frequency of consumption per day. Statistically different from non-infected *(p<0,05), **(p<0,01)

Table 5 shows the estimated marginal means of energy, macronutrient and food group intake of children infected only with *E. coli,* only with *A. lumbricoides* and parasite-free children together in the same MANCOVA model. Similarly with the previous model, children infected only with *E. coli* had a higher intake of energy fat and fiber, and the fiber and dairy groups than parasite-free children according to the 24hR and FFQ (Table 5). In contrast children infected only with *A. lumbricoides* had a lower intake of energy, carbohydrates and of the fruit, dairy, fat and sugar food groups than parasite-free children according to both the 24hR and the FFQ (Table 5).

Table 5. Daily intakes of children infected with *E. coli* or *A. lumbricoides* versus parasite-free, reported by 24 hour recall and food frequency questionnaire

	Parasite-free (n=113)		*Entamoeba coli* infection (n=36)		*Ascaris lumbricoides* infection (n=30)	
	Mean[1]	95% C.I.	Mean[1]	95% C.I.	Mean[1]	95% C.I.
24 hour recall						
Energy (Kcal/day)	1604	(1540 - 1667)	1744	(1621 - 1867)*	1443	(1300 - 1586)*
Carbohydrates(g/day)	225.4	(216.5 - 234.3)	237.6	(220.3 - 255.0)	201.3	(181.2 - 221.4)*
Protein (g/day)	54.2	(51.3 - 57.1)	59.6	(54.0 - 65.1)	51.0	(44.5 - 57.5)
Fat (g/day)	55.5	(52.2 - 58.8)	63.6	(57.3 - 70.0)*	49.7	(42.3 - 57.0)
Fiber (g/day)	13.2	(12.3 - 14.2)	15.4	(13.5 - 17.4)*	11.6	(9.4 - 13.8)
Fruits (spd)	1.72	(1.57 - 1.87)	2.09	(1.80 - 2.38)*	1,33*	(1.00 - 1.67)
Vegetables (spd)	2.96	(2.67 - 3.25)	3.16	(2.60 - 3.73)	2.61	(1.96 - 3.27)
Cereals (spd)	4.50	(4.32 - 4.68)	4.68	(4.33 - 5.02)	4.14	(3.73 - 4.54)
Legumes (spd)	1.03	(0.93 - 1.14)	1.41	(1.23 - 1.65)**	0.88	(0.63 - 1.12)
Dairy (spd)	1.57	(1.46 - 1.67)	1.70	(1.50 - 1.90)*	1.21	(0.98 - 1.45)**
Meat (spd)	2.01	(1.90 - 2.13)	2.12	(1.89 - 2.35)	1.80	(1.53 - 2.06)
Fat (spd)	1.45	(1.33 - 1.56)	1.45	(1.22 - 1.68)	1,13*	(0.88 - 1.40)
Sugar (spd)	2.07	(1.92 - 2.22)	2.35	(2.07 - 2.64)	1,66*	(1.34 - 2.00)
Food Frequency [2]						
Fruits	5.33	(5.01 - 5.64)	5.37	(4.76 - 5.99)	4.98	(4.24 - 5.72)
Vegetables	5.29	(4.96 - 5.62)	5.82	(5.19 - 6.45)	5.30	(4.54 - 6.06)
Cereal	6.05	(5.78 - 6.33)	6.73	(6.20 - 7.27)*	6.33	(5.69 - 6.97)
Legumes	1.48	(1.34 - 1.61)	1.90	(1.65 - 2.16)**	1.59	(1.28 - 1.90)
Dairy	2.35	(2.19 - 2.52)	2.75	(2.43 - 3.07)*	1.88	(1.50 - 2.27)*
Meat	2.31	(2.20 - 2.42)	2.55	(2.34 - 2.76)*	2.01	(1.76 - 2.27)*
Fat	2.71	(2.57 - 2.86)	2.96	(2.69 - 3.24)	2.76	(2.43 - 3.09)
Sugar	2.48	(2.33 - 2.64)	2.45	(2.15 - 2.74)	2.23	(1.88 - 2.58)
Beverages	2.50	(2.27 - 2.73)	2.89	(2.45 - 3.33)	2.41	(1.88 - 2.94)

spd: Servings per day. MANCOVA adjusted by age, gender and mother´s educational level. [1]Estimated marginal mean [2]Frequency of consumption per day . Statistically different from non-infected *(p<0,05), **(p<0,01)

Discussion

The findings of the present study show that both *E. coli* and *A. lumbricoides* infection are associated with energy and food intake. We observed that energy and food group intake is higher in children with *E. coli* infections and lower in children with *A. lumbricoides* infections as compared to their non-infected peers. In a previous study *E. coli* infection intensity was found to be associated with increased body fat[7]. Increased food intake may thus be a possible explanation for this observation. Similarly, for *A. lumbricoides* infection, decreased reported food intake could explain the morbidities that have been related to this parasite in both animal and human studies, such as stunting, impaired nutritional status and anorexia[4, 11, 15]. The changes in food intake of the infected children appear not to be related to low socioeconomic-status (SES) as measured by caretaker's educational level. If the

associations would have been explained purely by SES, the same trend and direction on the associations would have been observed for both parasites, which was not the case.

The underlying mechanisms of this association between intestinal parasites and energy or food group intake are still unclear, and should be further investigated. For example, intestinal parasites may induce changes in gut microbiota profiles, resulting in specific effects on food intake, appetite, food preferences and metabolic functions[30]. Gut microbiota have been associated with appetite regulation by microbiota toll like receptors and can increase the synthesis of free fatty acids (FFA) and change leptin concentrations[17, 18, 31]. Both FFA and leptin are mediators of energy sensing in hypothalamic feeding pathways and therefore may increase or decrease appetite and thereby influence food intake. The observed differences in reported energy and food group intake between *E. coli* and *A. lumbricoides* suggest that the effects may be parasite group and species-specific. Indeed, helminth infections have shown to cause anorexia in and to increase the diversity of the gut microbiota in animal models[16, 32], while infection with the protozoan *Blastocystis hominis* showed a reduction in the protective bacteria of the gut[33]. In addition, *E.coli* colonizes the large intestine whereas *A. lumbricoides* resides in the small intestine, thus it is plausible that they would lead to different gut microbiota profiles[16].

No differences in energy or food group intake were found between infected and non-infected children for *Endolimax nana, Balantidium coli, Entamoeba histolytica, Iodamoeba bütschlii* and *Giardia lamblia*. Due to the low prevalence of these parasites in this population, it is not possible to determine if these parasites are associated with food intake.

Our study has strengths and limitations that require consideration. This is a cross-sectional study, which means that causality cannot be inferred between parasitic infection and diet. An additional limitation is that the results of parasitic infection were based on the parasitological examination of just one stool sample per individual[34]. In combination with low infection intensities, this may have led to a low sensitivity of the diagnostic test, resulting in a possible under-estimation of the infection prevalence[35]. However, the prevalence of *A. lumbricoides and E. coli* infection found was high and similar to that reported previously in rural Mexico[36]. Another limitation relates to systematic bias in the use of reported intake data. Individuals tend to omit or forget foods in the 24hR, whereas with the FFQ they tend to over-report consumption of certain items[37]. In spite of these limitations, it is a strength of the study that the results were consistent using both independent methods, the validated FFQ and the average of three 24 hour recalls (i.e. the gold standard method for diet assessment)[38].

Conclusion

Results of this study show that intestinal parasitic infections were associated with reported food intake; infection with *E. coli* was associated with an increased while *A. lumbricoides* was associated with a decreased energy and food group intake. This finding suggests that alterations in food intake may represent an important link in the chain connecting intestinal parasite infection and nutritional status outcomes such as higher BMI z-score, lower height for age and micronutrient deficiencies. The observed differences between *E. coli* and *A. lumbricoides* suggest that the effects on energy and food intake, may be species-specific. However, the underlying mechanisms are still unclear, and should be further investigated.

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CHAPTER

Specific micronutrient concentrations are associated differently with intestinal parasitic infections and this relationship is affected by body fat in school-aged children

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Abstract

Background: Parasitic infections and micronutrient deficiencies in school aged children are public health problems in several developing countries. The aim of this study was to determine the relationship between specific intestinal parasitic infections and the concentration of micronutrients and how this relationship is affected by individual´s body fat. **Methods:** 269 school aged children (8.2 ± 1.5 y) from a rural community in Queretaro, Mexico, were included in a cross-sectional study. Anthropometric and body fat (DXA) measurements were taken. Concentrations of zinc, iron, ferritin, vitamins A, E, C, D, and B12 were determined in a fasting blood sample. We screened for infection of both soil transmitted helminths (STHs) and intestinal protozoa in a fecal sample. **Results:** There was a high prevalence of overall intestinal parasitic infection (61%). The main micronutrient deficiencies observed were vitamin D (19.3%) and zinc (15.2%). Children infected with any STH or *A. lumbricoides* had significantly lower concentrations of zinc and vitamin C than parasite-free children ($p<0.05$). Children infected with any intestinal protozoa, *Endolimax nana* or *Entamoeba coli*, had significantly higher concentrations of iron and vitamin B12 than parasite-free children ($p<0.05$). Among the children with high body fat, those infected with STH had lower zinc concentrations than parasite-free children, and those infected with intestinal protozoa had lower vitamin A concentrations than parasite-free children ($p<0.05$). **Conclusion:** STHs and intestinal protozoa have different associations with micronutrient concentrations, and these associations may differ according to the body fat content of the individuals.

Introduction

Intestinal parasites (helminth and protozoa) cause one fourth of the known human infectious diseases and are a worldwide public health problem[1]. Socioeconomic status, health conditions, education, the presence of domestic animals and poor water and food hygiene, are factors associated with the presence of intestinal parasites[2-4].Both malnutrition and intestinal parasitic infections have been associated with food intake, and inflammation[5-10]. Additionally, intestinal parasitic infection (5.7%) and malnutrition; i.e. obesity (3.9 %), and micronutrient deficiencies (6.1%) account for more than 10% of the global disability-adjusted-life-years (DALYs)[11-13]

Intestinal parasitic infections are known to affect the nutritional status of the host through several mechanisms: lower food intake (anorexia), reduced nutrient absorption, higher nutrient loss, leading to malnutrition and low micronutrient concentrations[14]. Micronutrients are necessary for an adequate immune response[15]. Multiple micronutrient deficiencies impact both disease progression and infection[14, 16], and infection in turn may exacerbate malnutrition resulting in a vicious cycle[17]. Both, micronutrient deficiencies and intestinal parasitic infections in children, affect growth and cognitive development[18]. To the best of our knowledge, most of the studies that have evaluated the association of low micronutrient concentrations with intestinal parasitic infection in children have focused on zinc, iron and vitamin A[19]. The relationship between intestinal parasitic infections and other micronutrients that are essential for an adequate immune response such as vitamin C, D, E and B12 has not yet been studied[20].

Studies have focused for many years on the relationship between intestinal parasites and micronutrients in undernourished populations; no studies have been done in pediatric populations with high rates of overweight and obesity. This is particularly important in countries where childhood obesity, parasitic infections and micronutrient deficiencies coexist and are public health problems, such as in Mexico. The combined prevalence of overweight and obesity in Mexican children is among the highest in the world (33%)[21]. In addition, the prevalence of micronutrient deficiencies is high, especially among children[22]. The most prevalent deficiencies in school-age children (5 to 11 years) are iron (13%) and zinc (23.6%)[23]. Also, intestinal parasitic infections in children have been reported in a range from 30% to approximately 70%, depending on the geographical area, being the highest in the southern states of the country[24]. It has been observed that infections with intestinal parasites, in particular *G. lamblia* and STH, are associated with children living in dual burden households (coexistence of overweight/obesity and stunting)[25]. Also, our group observed that in Mexican children, moderate to heavy infection with *E. coli* was associated with higher body fat and higher reported food intake as compared to no infection[26]. Also, obese children living in urban areas in Argentina had less parasite infections compared with undernourished children living in periurban areas[27].

The aim of this study was to determine the relationship between specific intestinal parasitic infections and the concentration of micronutrients in a rural population of Mexican children, and to assess how this relationship is affected by children´s percentage of body fat.

Methods

Subjects and experimental design

A cross-sectional study was conducted in a total of 269 school-aged children (6-10 years of age) recruited from two rural communities (Santa Cruz and Santa María Begoña) of Quéretaro, Mexico. Parents and caretakers received oral and written information about the study and were asked to sign an informed consent form. The study was conducted according to the guidelines of the Declaration of Helsinki[28], and the study protocol was approved by the Bioethics Committee of the School of Natural Sciences at the Universidad Autónoma de Querétaro (UAQ).

Children's parents or legal guardians were asked to attend their local health clinic to answer a medical history and a socioeconomic status questionnaire validated in previous studies [29]. Children that received any deworming treatment in the last 4 months or had any physical or mental disability were excluded from the study.

Anthropometry and body composition

Children were transported from their local communities to the Nutrition Clinic at UAQ. Weight and height were measured by trained personnel following World Health Organization (WHO) procedures[30]. Weight was determined using a calibrated digital scale (SECA Mod. 813, Hamburg, Germany) and height was measured with a stadiometer (SECA Mod 206, Hamburg, Germany). Nutritional status was calculated based on the WHO criteria of BMI-for-age for children aged 5-19 years. Underweight children were defined as two z-scores below the WHO reference median, overweight as one standard deviation and obese as two standard deviations above the reference median of the BMI-for-age z-score. Stunting was defined as two z-scores below the WHO reference median for height-for-age[31].

Whole body composition was measured to determine body fat percent, using dual-energy X-ray absorptiometry (DXA) (Hologic Mod Explorer, Bedford, MA, USA). High body fat for girls was considered above 30% and above 25% for boys[32].

Stool samples

A single stool sample was collected from each participant and analyzed the same day of collection. A direct copro-parasitological test consisting of a wet mount with iodine staining of slides was performed to determine the presence of protozoan intestinal parasites, as described by WHO[33]. These protozoa included *Entamoeba coli* (*E.coli*), *Endolimax nana (E. nana), Balantidium coli (B. coli), Entamoeba histolytica, Iodamoeba Bütschlii* and *Giardia lamblia.* Two Kato-Katz smears (2 x 41.7g) were made according to standard procedures to determine the presence and quantify the number of eggs of soil transmitted helminths (STHs)[34]. These parasites included *Ascaris lumbricoides (A. lumbricoides), Trichuris trichiura* (*T. trichiura*) and hookworm present. The intensity of infection was characterized following WHO recommendations[35]: individuals with 1-4999 of *A. lumbricoides* eggs per gram of feces (epg) were categorized as light-intensity infection, and samples with 5000 or more epg were categorized as moderate-heavy intensity infection. Children with 1–999 epg of *T. trichiura* were categorized with light intensity infection and with 1000 or more as moderate-heavy intensity infection. For hookworm, 1–1999 epg were categorized with light intensity infection and with 2000 or more were considered with moderate-heavy intensity infection. Absence of intestinal protozoa and STH eggs were categorized as parasite-free. Children infected with more than ones species of intestinal parasites were combined and categorized as "multiple infection" group

Blood samples

A fasting blood sample was collected by arm venipuncture from each subject. Children were instructed to fast at least 12 hours before the blood sample was collected early in the morning. Plasma and serum were separated in blood samples by centrifugation at 1800–2000 rpm for 15 min, and aliquots were stored at −70 °C for later analysis. Blood analysis included iron, zinc and vitamins A, C, E, D and B12. All laboratory analyses were done in duplicate.

Vitamins A and E were measured in serum using the modified technique by Bieri et al[36]. These vitamins were measured simultaneously by reverse phase high pressure liquid chromatography (HPLC) (Mod 2996, Waters Associates, Milford, MA, USA). Vitamin A deficiency was considered with retinol concentrations <10 µg/dL and low concentrations <20 µg/dL[37]. Vitamin E deficiency was defined with a concentration of alpha-tocopherol <3 µg/mL

and low concentrations <5 µg/mL[38]. When using the vitamin E:lipids ratio, vitamin E deficiency was considered with <0.8 mg/g[39]. Serum vitamin C was determined by reverse phase HPLC, as previously reported[40]. Vitamin C deficiency was considered with concentrations of ascorbic acid <2 µg/mL and low concentrations with levels of <4 µg/mL[37]. Serum vitamin D was determined by a commercial 25(OH)-Vitamin D direct ELISA kit (Immundiagnostik AG, Bensheim, Germany) and a microplate spectrophotometer (Multiskcan Ascent, Thermo Electron Corporationn, Ma, EUA) . Vitamin D deficiency was defined with a concentration D <50 nmol/L and low concentrations <75 nmol/L[41]. Serum B12 was assessed by the Simul TRAC SNB radioassay kit (57Co/Folate1251) (MP Diagnostics, Orangeburg, NY) at the Western Human Nutrition Research Center, Davis. Low and marginal B12 concentrations were considered with values <148pmol/L and between 148 and 221 pmol/L, respectively[42].

Ferritin concentrations were determined by a commercial ELISA kit (ab108837, Abcam, UK) and a microplate spectrophotometer (Multiskcan Asecnt, Thermo Electron Corporationn, Ma, EUA). Ferritin deficiency was considered < 15 ng/mL[43]. Total iron concentration in serum was measured using a commercial kit (Iron Ferrozine, Elitech, Sées, France) and a spectrophotometer (Perkin Elmer, Mod Zeeman 5100). Low iron concentrations were considered <60 µg/dL and iron deficiency with concentrations <45 µg/dL[44]. Zinc concentrations were measured in serum by atomic absorption spectrometry (AAnalyst 7000, Perkin Elmer Instruments, Norwalk, CT, USA). Zinc deficiency was defined with zinc plasma concentrations <65 mg/L[45].

Statistical Analysis

A chi square test was performed to determine differences in micronutrient status (deficient vs. non deficient, low vs sufficient concentrations) between specific parasitic infections and parasite free children. In addition, a multivariate analysis of covariance (MANCOVA) was used to evaluate differences in the concentration of micronutrients concentrations between children infected with any STH, any intestinal protozoa or specific parasites with a prevalence over 10% (i.e. *A. lumbricoides, E. coli, B. coli and E. nana*) with parasite-free children. Age (months), gender (male/female) and mother's education level (years of education) were included as co-variables. A linear regression model was performed to test the interaction between parasitic infection and high body fat percent on micronutrient concentrations. Interaction terms with a p value below 0.05 were then analyzed separately and graphed. All statistical analyses were performed by SPSS 21.0 (SPSS, Chicago IL).

Results

General characteristics of the children are summarized in Table 1. A total of 269 children (7.99 ± 1.55 y) participated in the study; 126 were boys and 143 were girls. A total of 18.4% of the children were overweight, 9.6% obese, 2% underweight and 4% were stunted. The prevalence of any intestinal parasitic infection (being infected with at least one intestinal parasite) was 60% and the prevalence of any intestinal protozoa infection was 46%. Among the intestinal protozoan infections, *E. coli (*19 %), *E. nana* (16%) and *B. coli* (12%) were the most common. The prevalence of any STH infection was 19%; *A. lumbricoides* was the most prevalent one (16.4%). Additionally, 14.5% had multiple-intestinal parasitic infections. The most common micronutrient deficiencies were vitamin D (19.3%) and zinc (15.2%). Prevalence of B12 and vitamin E:lipids deficiencies were very low (1.1% and 0.4%, respectively). No deficiencies were observed for vitamin A, vitamin C or iron.

Table 1. Main characteristics of the population studied (n=269)

	Mean		S.D.
Age (years)	7.99	±	1.55
Anthropometry and body composition			
Weight (Kg)	27.56	±	8.22
Height (cm)	126.13	±	9.88
Height-for-age (Z-Score)	-0.61	±	0.97
BMI-for-age (Z-Score)	0.33	±	1.25
Total body fat (Kg)	4.01	±	2.64
Micronutrient concentrations			
Ferritin (ng/mL)	99.03	±	51.32
Iron (µg/dl)	92.10	±	24.45
Zinc (µg/dl)	74.24	±	11.21
Vitamin A (µg/dl)	32.50	±	6.70
Vitamin D (nmol/l)	57.00	±	9.80
Vitamin E (µg/mL)	2.71	±	1.01
Vitamin E lipids ratio (mg/g)	2.72	±	1.00
Vitamin C (mg/ml)	7.61	±	1.80
Vitamin B12 (pmol/l)	486.62	±	197.26

S.D. : Standard deviation

Children infected with any intestinal parasite had lower vitamin D and higher vitamin B12 concentrations compared with parasite-free children (Table 2). Children infected with *A. lumbricoides* or any STH had lower concentrations of zinc and vitamin C, whereas children infected with any intestinal protozoa, *E. coli* or *E. nana* had higher concentrations of iron and vitamin B12 compared with parasite-free children. In addition, children that had infection with *E.coli* had marginally higher concentrations of vitamin A. Finally, children infected with STHs were more likely to be zinc deficient (25%) than parasite free-children (13%) ($p<0.05$).

Table 2. Association between micronutrient and intestinal parasitic infection

	Parasite-free (107)	Infected[2] (162)	Soil transmitted helminths (52)	*A. lumbricoides* (44)	Intestinal protozoa (123)	*E. coli* (52)	*B coli* (33)	*E. nana* (44)	Multiple-infections[3] (39)
Ferritin (µg/L)	99.95 ± 5.12	98.43 ± 4.10	90.42 ± 7.09	90.81 ± 7.94	100.31 ± 4.79	100.39 ± 7.48	89.39 ± 8.98	108.20 ± 8.80	91.93 ± 8.73
Iron (µg/dl)	88.49 ± 28.57	94.67 ± 31.61	91.27 ± 28.99	93.07 ± 29.11	96.17 ± 31,89*	98.71 ± 26,17*	93.25 ± 37.10	100.78 ± 32,58*	100.27 ± 27.56
Zinc (mg/l)	0.75 ± 0.09	0.74 ± 0.11	0.70 ± 0,11*	0.70 ± 0,11*	0.75 ± 0.11	0.77 ± 0.10	0.72 ± 0.14	0.75 ± 0.11	0.75 ± 0.11
Vitamin A (µg/dl)	32.37 ± 7.62	32.82 ± 6.05	32.62 ± 5.51	32.73 ± 5.58	32.76 ± 6.07	32.84 ± 5,89*	32.52 ± 6.58	33.15 ± 5.54	32.46 ± 4.21
Vitamin D (nmol/l)	58.64 ± 9.14	55.90 ± 10,37*	56.15 ± 11.06	56.00 ± 11.24	55.95 ± 10.04	55.50 ± 11.06	55.63 ± 8.21	58.23 ± 5.75	56.34 ± 7.91
Vitamin E (µg/mL)	5.28 ± 1.68	5.35 ± 1.64	5.12 ± 1.50	5.25 ± 1.49	5.48 ± 1.67	5.27 ± 1.59	5.35 ± 1.69	5.62 ± 1.76	5.65 ± 1.57
Vitamin E lipids (µg/mL)	2.68 0.97	0.39 0.16	2.75 1.04	2.72 1.05	2.75 1.05	2.65 1.25	2.68 1.12	2.85 1.05	2.74 1.02
Vitamin C (mg/ml)	7.77 ± 1.90	7.52 ± 1.82	7.11 ± 1,85*	7.05 ± 1,88*	7.69 ± 1.75	7.72 ± 1.64	7.24 ± 2.31	7.78 ± 1.82	7.89 ± 2.01
Vitamin B12 (pmol/l)	471.9 ± 154.0	498.8 ± 210,62*	474.9 ± 176.0	471.1 ± 172.7	515.1 ± 220,6*	530.6 ± 239,6*	450.4 ± 123.8	536.0 ± 241,6*	477.9 ± 192.1

[1] MANCOVA, Mean ± S.D. adjusted by age, sex and mother's educational level [2] Infected with any intestinal parasite [3]Infection with 2 or more species of intestinal parasites

* Significantly different from parasite-free with MANCOVA ($p<0.05$)

To determine if the interaction of body fat and parasite infection has an effect on micronutrient concentration, we performed a linear regression analysis (data not shown), and results of interaction terms with a p value below 0.05 are shown in Figure 1. Body fat was found to interact with STHs infection in relation to zinc concentration and with intestinal protozoa in relation to vitamin A concentration. Among the children with high body fat, those infected with STH had lower zinc concentrations than parasite-free children, and those infected with intestinal protozoa had lower vitamin A concentrations than parasite-free children ($p<0.05$). In addition, among the children with normal body fat, those infected with STH has similar zinc concentrations compared with

parasite-free children and those infected with intestinal protozoa had higher vitamin A concentrations than parasite-free children ($p<0.05$).

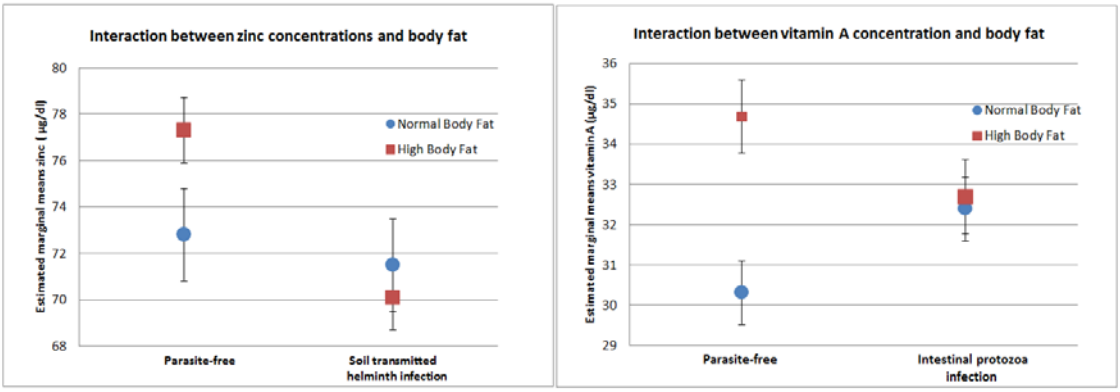

Figure 1. Interaction between high/normal body fat and parasitic infection (helminth or protozoan) on zinc and vitamin A concentrations, the lines represent 95% confidence interval.

Discussion

In the present study, infection with intestinal protozoa and STHs was associated differently with the concentration of specific micronutrients in Mexican children. In addition, we found that the association between zinc and vitamin A with intestinal parasites is different depending on the child's body fat content.

Intestinal parasites were associated with some of the studied micronutrients. The lower concentrations of zinc and vitamin C found in children infected with *A. lumbricoides* may be explained by different physiological and immunological pathways. For instance, impaired micronutrient absorption has been related to weakened gastrointestinal function, damage to the gut mucosa and competition for available micronutrients when infected by STHs[46]. In addition, lower micronutrient concentrations and food intake has been associated with STH infection, due to symptoms such as abdominal pain and loss of appetite[17, 47-50]. However, the possibility that children with lower zinc and vitamin C concentrations are more likely to be infected should also be considered. Zinc and vitamin C are important immuno-modulators[15]. They are vital for an adequate mucosal and innate immune response[51]. Additionally, vitamin C is among the most important exogenous antioxidants, necessary for collagen synthesis required for an adequate epithelial barrier function[52, 53]. The association found between STH infection and lower zinc concentrations is in line with several studies in different age-groups and populations that have found similar associations[54, 55]. In contrast, there are no available studies evaluating the relationship between vitamin C and STHs[56].

The higher concentrations of iron and vitamin B12 found in children infected with *E. coli* and *E. nana*, may be linked to higher food intake. It has been observed that children infected with *E.coli* have higher intake of meat, poultry, fish and eggs which are the main sources of vitamin B12 and bioavailable iron in the diet[50]. To the best of our knowledge, only one study has evaluated the association between intestinal protozoa and micronutrient concentrations. In this study no association was observed between *E. coli* infection with iron, retinol, zinc or vitamin B12 concentration in children[57]. Since it has been shown that that *E. coli* is associated with higher food intake, discrepancy among studies may be attributed to differences in food group intake (i.e. sea food, meat, egg and legumes), food quality, availability and affordability. No association between any of the studied infections and the concentrations of vitamin E was found. According to a systematic review, few available studies have addressed this and are in line with our findings[58].

The concentrations of zinc were associated to STH infections in children with high body fat. The lower zinc concentrations in the STH infected children that had high body fat content might be caused by zinc malabsorption associated with both, obesity and STH infection[59, 60]. In addition, as zinc is necessary to have adequate immune function, children with lower zinc concentrations are more likely to become infected with intestinal parasites[61, 62]. Thus, obesity together with low zinc concentrations may have a larger weakening effect on the immune response and defense against STHs[63, 64].

The interaction between vitamin A and intestinal protozoa infection was altered by body fat content. In the children with high body fat, the lower vitamin A concentrations in the intestinal protozoa infected children might be attributed to an inflammation process, specifically a T-helper type 1 (Th1) proinflammatory response, caused by both, infection and obesity[60, 65]. A Th1 response has been associated with lower vitamin A concentrations[66-68]. In contrast, in the children with normal body fat, the higher vitamin A concentration observed in the protozoa infected children might be attributed to a higher food intake[50].

To our knowledge, this is the first study to evaluate the relationship between micronutrients and intestinal parasites while considering the interaction with body fat. This finding and possible underlying mechanisms that may lead to the observed differences in zinc and vitamin A concentration need to be further explored.

Some methodological limitations of our study need to be addressed. Due to the cross-sectional design of the study, we were unable to determine the causal relationship between intestinal parasitic infections and micronutrient concentrations. In this population the most prevalent STH was *A. lumbricoides* with 44 cases, followed by hookworm with only 6 cases, thus the association between STH and micronutrients in this study are mostly driven by *A. lumbricoides* infection, and should be taken in consideration while interpreting the results. Parasitic infection prevalence was determined by parasitological examination of one stool sample per individual that may lead to an underestimation of the infected children[69]. However, the prevalence of the studied intestinal parasites was high and similar to that reported previously in rural Mexico[70].

Conclusions

According to our results STH and intestinal protozoa have different and opposite associations with micronutrient concentrations. STH infection is associated with lower concentrations and intestinal protozoa infection with higher concentrations of some micronutrients. The associations between zinc and vitamin A with intestinal parasites are modified by the percentage of body fat of the individuals.

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CHAPTER

Intestinal parasites: associations with intestinal and systemic inflammation

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Provisionally accepted: Parasite immunology

Abstract

Background: Evaluate associations between intestinal parasitic infection with intestinal and systemic inflammatory markers in school-aged children with high rates of obesity. **Methods:** Plasma concentrations of CRP, leptin, TNF-α, IL-6 and IL-10 were measured as systemic inflammation markers and count of stool leukocytes as marker of intestinal inflammation in 291 children (6-10y). Intestinal parasitic infection was measured by stool examination. Logistic regression analyses were performed to determine the odds of having high inflammatory markers for each parasite or group of parasites as compared to parasite-free children. **Results:** The prevalence of soil transmitted helminths and intestinal protozoa infections was 12% and 36%, respectively. Parasitic infection was not associated with CRP, IL-6, IL-10 or TNF-α. Children infected with *Ascaris lumbricoides* (aOR: 5.91, 95%CI: 1.97-17.70) and *Entamoeba coli* (aOR: 8.46, 95%CI: 2.85-25.14) were more likely to have higher stool leucocytes than parasite-free children. Children with multiple-infections (aOR: 10.60, 95%CI: 2.85-25.14) were more likely to have higher leptin concentrations than parasite-free children. **Conclusion:** Intestinal parasitic infection was not associated with systemic inflammation, but was associated with intestinal inflammation. Having multiple-infections were associated with higher leptin concentrations.

Introduction

Mexico is undergoing a nutritional transition, with rising rates of obesity and related chronic diseases[1, 2]. Childhood obesity in particular, is considered to be a challenging health problem. High body fat stimulates systemic inflammation via an increased secretion of inflammatory molecules, most importantly IL-6 and Tumor Necrosis Factor alpha (TNF-α), which is the mechanism by which obesity relates to chronic disease[3]. Regulatory markers such as IL-10 dampen the obesity related inflammatory response[4]. In addition to the obesity challenge, parasitic infections are also an important health problem in Mexico; it is estimated that half of the pediatric population is infected with at least 1 species of intestinal parasite[5, 6]. Interestingly, these parasites have been associated with the same molecules involved in the systemic inflammatory process[7, 8]. For instance, soil transmitted helminths (STHs) can regulate systemic inflammation via a response dominated by anti-inflammatory cytokines such as IL-10, regulating the concentration of the inflammatory cytokines secreted by adipose tissue such as TNF-α, IL-6 and leptin[9-11]. In addition to its role in inflammation processes, leptin is a hormone with other roles in human metabolism such as regulation of energy intake/expenditure, hematopoiesis, and gut permeability[12-15].

In contrast to STHs, less is known about the association between intestinal protozoa with systemic inflammation. *In vitro* and animal studies have shown that intestinal protozoa such as *Blastocystis* and *Entamoeba histolytica* trigger TNF-α promoting an inflammatory response, but the mechanisms are not known. STHs have shown to regulate intestinal inflammation by the secretion of regulatory molecules such as IL-10 by white blood cells[16]. On the other hand, pathogenic protozoa have shown to cause intestinal inflammation and tissue damage[17, 18], which have shown to exacerbate the systemic inflammatory process[19].

Given the high rates of childhood obesity and parasitic infection in Mexico and the effects both have on inflammatory reactions, intestinal parasites may be associated with systemic and intestinal inflammation[12-15]. The aim of this study is to evaluate the associations between intestinal parasites with intestinal and systemic inflammation in a population of Mexican school children with high prevalence of obesity.

Methods

Subjects and experimental design

A total of 291 children (6 -10 years of age) participated in this cross-sectional study from February till May of 2013. The children were randomly selected from the local school of the rural community of "Santa Cruz" in Queretaro, Mexico. The children's legal guardians received oral and written information about the study, and were asked to sign an informed consent letter. Children who had received any treatment against intestinal parasites in the last 4 months or with any physical or mental disability were excluded from the study. The study was approved by the Bioethics Committee of the Universidad Autonoma de Querétaro (UAQ).

A sample size of 284 children was calculated in order to find differences in terms of body fat (main outcome) as described in a previous study[20]. This sample size also allows to find differences in TNF-α concentration between infected and parasite-free children, with an estimated prevalence of infection of 20% and an estimated standard deviation of 3 pg/ml for TNF-α, assuming a type I error of 5% and a statistical power of 80%[21]. Once the children were selected for inclusion, legal guardians were asked to attend the community health clinic to answer a socioeconomic and medical history questionnaire, including queries of mother's educational level, sex and age of the children.

Systemic inflammation markers

A fasting blood sample (7 ml) was taken from each participant in the morning (7:00 – 8:30 a.m.) and collected in vacuum tubes (Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged at 1500 rpm for 15 min (Beckman Allegra 21R, Palo Alto CA), and plasma was separated. Concentrations of leptin and C-Reactive Protein (CRP) in plasma were measured in duplicate using commercial ELISA kits (Human Leptin Elisa Kit, Linco Research; High Sensitivity C-Reactive Protein ELISA Kit, Bioquant). The concentration of the inflammatory cytokines TNF-α, IL-6 and the regulatory cytokine IL-10 were measured using high-sensitivity commercial ELISA kits (Millipore CRP ELISA, Mo, USA). All ELISA kits were analyzed in a Multiskan Ascent microplate photometer (Thermo Electron Corporationn, Ma, EUA). All the biochemical analysis were done by trained personnel at the Human Nutrition Laboratory, UAQ.

Parasitology and intestinal inflammation

A stool sample was collected from each participant in the morning (7:00 – 8:30 a.m.). A coproparasitological test consisting of a wet mount with iodine staining of slides was performed to screen for the presence of protozoa parasites, as described by WHO. Samples with one or more protozoa trophozoites or cysts were considered as infected. In addition, two Kato-Katz smears (2 x 41.7 mg) was performed according to standard procedures to screen, determine the presence and quantify the number of eggs of STHs. Infection was defined as the presence of species-specific eggs or trophozoites or cysts detected by either of the two methods. Children with no protozoa trophozoites or cysts, and no STH eggs were classified as parasite-free. All children diagnosed with intestinal parasites were referred to the local health clinic for treatment.

Intestinal inflammation was measured by the count of stool leukocytes[22, 23]. The samples were examined for the presence of fecal leukocytes on direct wet smears. Each sample was stained with methylene blue and the number of leukocytes per field was recorded[22, 24]. All microscopy tests were performed by a trained technician.

Body Composition

Children and legal guardians were transported from their local communities to the Nutrition Clinic at UAQ for anthropometry and body composition measurements. Weight and height were measured in duplicate by trained and standardized personnel with a precision of 0.1 g or 0.1 cm, respectively, following World Health Organization (WHO) procedures[25]. Weight was measured in all participants using light clothing and barefoot using a calibrated digital scale (SECA, mod 813 Hamburg, Germany); height was measured using a stadiometer (SECA, mod 206 Hamburg, Germany). Body Mass Index (BMI)-for-age z-score and Height-for-age z-score (HAZ) were calculated using the AnthroPlus software (Geneva: WHO, 2009) based on the WHO criteria of BMI-for-age for children aged 5-19 years. Children were considered to be underweight if they had 2 z-scores below, overweight if they had 1 z-score above and obese if they had 2 z-scores above the reference median of the BMI-for-age z-score and were considered to be stunted if they had 2 z-scores below the WHO reference median of height for age z-score[26].

Whole body composition was measured by a certified technician using Dual-energy X-ray absorptiometry (DXA) (Hologic Mod Explorer, 4500 C/W QDR, INC 35 Crosby Drive, Bedford, MA 01730, USA). Body fat percent and body fat content in Kg were determined directly from DXA. Elevated body fat was considered above 30% for girls and above 25% for boys[27].

Data Analysis

A t-test for independent samples was performed to study differences between children infected with any species of intestinal parasite and parasite-free children for the variables with a normal distribution (i.e. age, weight, height BMI for age z-score and height for age z-score). In principle, all parasites were analyzed separately (i.e. *Ascaris lumbricoides, Entamoeba coli, Endolimax nana*), unless the prevalence was below 10%, then they were grouped. Parasites with a prevalence below 10% were analyzed only as part of the group with STHs infection (*Ascaris lumbricoides* and hookworm) or protozoa infection (*Entamoeba coli, Entamoeba histolytica/dispar, Endolimax nana, Balantidium coli, Giardia lamblia*). Children with more than one species of intestinal parasite were categorized and analyzed as a different group called multiple-infections.

The inflammatory markers were not normally distributed and therefore they were categorized as low and high concentration (below and above the median). Logistic regression analyses were carried out and expressed as adjusted odds ratios (aOR) to determine the association between a high concentration of inflammatory markers and each parasite or parasite group separately (STHs, *Ascaris lumbricoides*, protozoa, *Entamoeba coli, Endolimax nana and* multiple-infection) comparing them to parasite-free children. To decrease the occurrence of type 1 errors due to multiple comparisons, the Bonferroni-adjusted test of significance was used[28]. Body fat (%), sex (m/f), age (y), mother's education level (y) and malnutrition (stunted or under-weight) were included in the model as confounders, since these factors are associated with both elevated inflammatory markers and intestinal parasitic infection[29].

Results

The prevalence of parasitic infection in this population was 60% (Table 1). STH mono-infections were detected in 12.1% of the population while protozoa mono-infections were present in 35.6% of the population and 12.7% had multiple-infections. The most common STH mono-infection was *Ascaris lumbricoides (A. lumbricoides)* with a prevalence of 10.7%. The most prevalent intestinal protozoa mono-infections were *Entamoeba coli (E.coli)* with a prevalence of 12.7%, followed by *Endolimax nana* (*E. nana*) with a prevalence of 11.3%. All other studied protozoa had a prevalence below 7%. We did not find any children infected with *Trichuris trichiura* or *Blastocystis hominis.* There were no differences between infected and parasite-free children in terms of age, sex, mother's educational level and percentage of body fat.

Table 1. Prevalence of parasitic infection in the studied children (n=291)

	n	
Overall Infection	176	60.5%
Soil transmitted helminths	35	12.1%
Ascaris lumbricoides [1]	31	10.7%
Hookworm[1]	4	1.4%
Protozoa	104	35.6%
Entamoeba coli [1]	37	12.7%
Entamoeba histolytica/dispar [1]	8	2.7%
Endolimax nana [1]	33	11.3%
Balantidium coli [1]	18	6.2%
Giardia lamblia [1]	8	2.7%
Multiple-infection	37	12.7%

[1] Infected only with the specified species (mono-infection)

Among the studied children, 54% were girls. A low prevalence of underweight (1.7%) and stunting (5.5%) and a high prevalence of overweight (18.6%), obesity (9.6%) and elevated body fat (53.3%) were found. Table 2 summarizes the general characteristics of the children that participated in the study.

Table 2. Main characteristics of the study population (n=291)

	Mean		S.D.
Age (years)	7.99	±	1.55
Mother's educational level (years)	4.51	±	1.43
Weight (Kg)	27.63	±	8.26
Height (cm)	126.28	±	9.93
BMI-for-age (Z-Score)	0.31	±	1.31
Height-for-age (Z-Score)	-1.23	±	1.22
% body fat	29.12	±	6.68
Stool leukocytes (CPF)	2.36	±	1.52
C-Reactive Protein (mg/L)	0.97	±	1.60
Interleukin 6 (pg/ml)	3.12	±	3.95
Interleukin 10 (pg/ml)	4.33	±	6.82
Tumor necrosis factor-α (pg/ml)	4.25	±	2.87

S.D: Standard Deviation, CPF: Cells per observation field

After adjusting by sex age and mother's educational level, overweight/obese children were more likely to have higher concentrations of IL-6 (aOR: 2.31 95%CI: 1.35-3.93), TNF- α (aOR: 6.58 95%CI: 3.68-11.76) and leptin (aOR: 119.71 95%CI: 32.331-443.305) than normal weight children. In contrast, no association was found between overweight/obesity with CRP, IL-10 and stool leukocytes.

Children with multiple-infections (aOR: 10.69 95%CI: 3.62-31.54) were more likely to have higher leptin concentrations as compared to parasite-free children. IL-6, lL-10, TNF-α, or CRP were not associated with the presence of any of the studied parasites (Table 3). Children infected with intestinal protozoa, STH, *A. lumbricoides, E. coli*, and multi-infections were more likely to have a higher level of stool leukocytes as compared with parasite-free children (Table 3).

Table 3. Logistic regression between parasite (specific) infection with systemic (acute and chronic) and local inflammation markers

	Soil transmitted helminths (35)		*A. lumbricoides (31)*		Protozoa (104)		*E. coli (37)*		*E. nana (33)*		Multiple-infections (37)	
	aOR	95% C.I.	aOR	95% C.I.	aOR	95% C.I.	aOR	95% C.I.	aOR	95% C.I.	aOR	95% C.I.
Systemic												
C-Reactive Protein	0.76	(0.34 - 1.69)	0.50	(0.34 - 1.88)	1.48	(0.83 - 2.61)	1.26	(0.57 - 2.77)	1.28	(0.56 - 2.91)	1.53	(0.73 - 3.20)
Interleukin-6	1.27	(0.58 - 2.76)	1.11	(0.48 - 2.55)	0.82	(0.47 - 1.44)	0.53	(0.24 - 1.18)	0.84	(0.37 - 1.89)	0.99	(0.49 - 2.01)
Interleukin-10	1.01	(0.48 - 2.15)	1.12	(0.50 - 2.53)	1.10	(0.64 - 1.90)	1.09	(0.51 - 2.32)	0.94	(0.42 - 2.07)	0.73	(0.35 - 1.52)
Tumor necrosis factor-α	0.75	(0.33 - 1.71)	0.88	(0.36 - 2.11)	1.03	(0.58 - 1.83)	1.23	(0.55 - 2.75)	0.73	(0.31 - 1.72)	0.71	(0.33 - 1.53)
Leptin	3.33	(1.06 - 10.49)	2.43	(0.74 - 7.97)	1.55	(0.70 - 3.43)	1.67	(0.63 - 4.42)	1.68	(0.60 - 4.71)	10.68	(3.62 - 31.54)*
Intestinal												
Stool leukocytes	6.16	(2.28 - 16.68)*	5.91	(1.97 - 17.70)*	3.21	(1.83 - 5.60)*	8.46	(2.85 - 25.14)*	1.10	(0.51 - 2.36)	4.63	(2.16 9.92)*

Adjusted Odds ratio (aOR) (95% Confidence Interval), adjusted by sex, age, mother educational level, stunting and % of body fat.

* Significative association using Bonferroni-adjusted test of significance. Considering the number of comparisons made: 6 comparisons (0.05/6, α=.0.008)

Cut off values: CRP: 0.366 mg/l; IL-6:1.81pg/ml; IL-10: 3.16 pg/ml; TNF-α: 3.50 pg/ml; stool leukocytes: 3 cells per field

Discussion

In the present study, specific intestinal parasites were associated with higher stool leukocytes and leptin concentrations, but not with the other systemic inflammation markers measured. These results provide new evidence concerning the relationship between intestinal parasitic infection with systemic and intestinal inflammation in a population with a high prevalence of overweight and obesity.

In this study CRP, IL-6, IL-10 and TNF-α were not associated with any of the studied intestinal parasites, and these results are in line with other studies[30]. For instance, Sanchez *et. al.*, found no association between STH and IL-10 in a study in Honduran children. Also, de Gier *et. al.*, did not find differences in acute phase protein or CRP in Cuban or Cambodian children between infected and non-infected children with STH[31]. Our results confirm the lack of association between systemic inflammatory markers and intestinal parasitic infection. The lack of association observed in these studies could be attributed to the strategies intestinal parasites have developed to remain unnoticed by the systemic immune response, such as immunological modulation and evasion[32].

To our knowledge, this is the first study to evaluate the relationship between inflammation and intestinal parasites in a population where overweight and obesity are highly prevalent. This is relevant since excess body fat and weight promote systemic inflammation[33]. In the studied population, children with overweight and obesity had higher concentrations of TNF- α, IL-6 and leptin which may lead to an increased risk of other diseases such as hypertension and type II diabetes[34]. However, the inflammation observed in the children that participated in the study is apparently not related to intestinal parasitic infection.

Children with multiple-infections were more likely to have higher leptin concentrations when compared to parasite free-children, even after adjusting for body fat content[35]. Similarly, recent studies in animal models and *in vitro* have shown that intestinal parasitic infection may have an effect on blood leptin concentrations[36]. In contrast, Karul, *et. al.* in a case-control study evaluating 40 patients, found no association between intestinal parasites and leptin concentration. However, Karul, *et. al.* did not adjust results for socioeconomic status, adiposity, and age of the participants, which are well known factors affecting leptin concentrations and parasitic infection[37]. The findings of the present study might be related to role of leptin as a hormone in the gut[38]. Leptin has shown to prevent epithelial apoptosis and promoting tissue repair, which are required for mucosal defense against pathogens[39]. Due to the study design, the complexity and the multiple roles of leptin in human metabolism, it is not possible to unravel the mechanisms or determine the causality of the association. To fully understand the influence of intestinal parasites on leptin concentrations, multiple leptin measurements should be taken throughout the day, as well as before and after anti-parasitic treatment, while taking in consideration sex, age and body fat-related differences[40-43].

All the studied intestinal parasites were associated with higher stool leukocytes. Intestinal parasites are invasive, they require living space and in many cases they physically harm the intestine. For instance, *A. lumbricoides* penetrates through the gut and migrates to the bloodstream causing tissue injury that might trigger an immune response in the gut[44]. In contrast, the association of *E. coli* with fecal leucocytes is unexpected, since *E. coli* is considered a "non-pathogenic" protozoa. Yet, children infected with this parasite were more likely to have a higher number of stool leukocytes than other pathogenic parasites such as *A. lumbricoides* or children with multiple-infections. Thus, even though *E.coli* is non-pathogenic, infection with this parasite may have implications related to immunological and inflammatory pathways that may have a long-term effect on human health, particularly in obese/overweight individuals. While literature addressing the effect of STH and intestinal protozoa parasites on stool leukocytes is scarce, similarly to our results, stool leukocytes have been associated with pathogens, such as *Salmonella* and *Shigella*[45]. Our results indicate than intestinal parasites might have the ability to evade systemic inflammatory reactions, but they fail to do so in the gut.

The present study has strengths and limitations that are worth mentioning. The cross sectional design of the study does not allow to distinguish between causal and non-causal relationships between local and systemic inflammation markers with intestinal parasitic infection. We performed multiple comparisons that increase the probably of type 1 errors; still, the magnitude of the associations and the consistency of the results together with the Bonferroni test for multiple comparisons suggest this is not the case. The results of the parasitological examination were based on one stool sample per child, thus the number of infected children may be underestimated. However, a single Kato-katz has been widely used in epidemiological studies such as this one[46, 47]. The count of stool leukocytes measures the number of cells which may lead to non-systematic misclassification and does not differentiate between the different immune cell sub-types. Further research that includes techniques that provide information on the linage of the cells could give more insight on the specific inflammatory reaction associated with each parasite.

Conclusion

According to our results, intestinal parasitic infection was not associated with IL-6, CRP or TNF-α, markers related to obesity and chronic disease, but were associated with intestinal inflammation. In addition, STH infection and having multiple-infections were associated with higher leptin concentrations. Further research is needed to evaluate the effect of different intestinal parasites in inflammatory pathways and chronic disease over time.

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CHAPTER 

General Discussion

Intestinal parasites and malnutrition are two major public health problems in most low-income and middle lower-income countries[1, 2]. In Mexico, children living in poverty have access to low quality food at relatively low prices making them not only at a higher risk of obesity but also of micronutrient deficiencies[3]. The same children are at the highest risk of infection with intestinal parasites (i.e. soil transmitted helminths and intestinal protozoa) due to behavioural risks, poor sanitation and education[4]. Intestinal parasitic infection has been associated with poor nutritional status in different populations[5]. Yet, information on the associations between intestinal parasitic infection and nutritional status in populations with high food availability and high rates of overweight and obesity is still scarce. This thesis analyses for the first time the association between intestinal parasites with nutritional status and inflammation in a population of children with a high prevalence of overweight and obesity. In this chapter, the results of the previous chapters are integrated, their public health significance discussed and directions for further research are proposed.

Mexico is the country with the highest combined rates of overweight and obesity in children woldwide[6]. The results from chapters 2, 3 and 5 confirm that malnutrition is a major public health problem in Mexican children, and demonstrate that intestinal parasitic infections are still highly endemic. As shown in figure 1, we found a high prevalence of intestinal parasitic infection (60%) and malnutrition; high body fat (53.8%) and micronutrient deficiencies (37.2%).

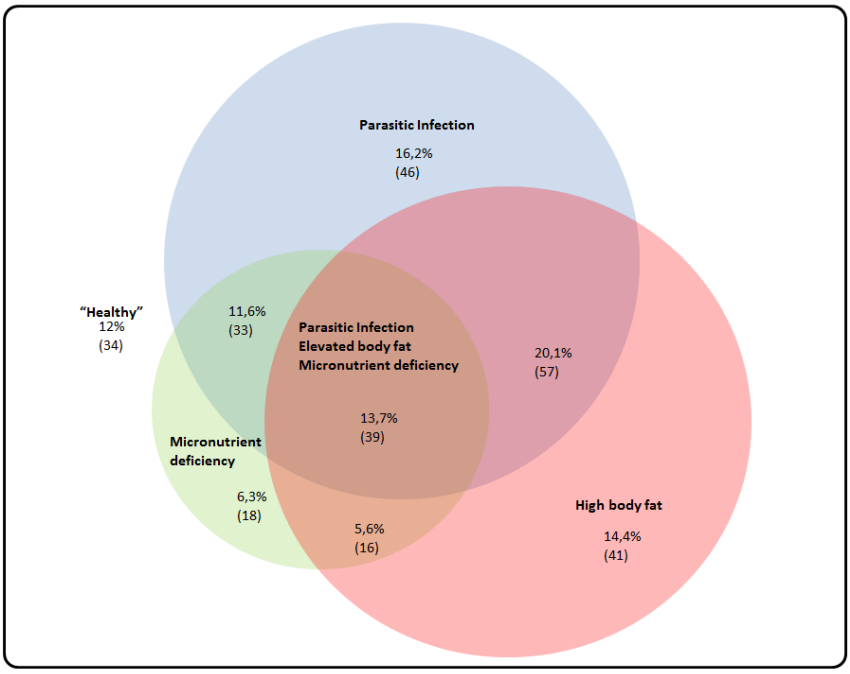

Figure 1. Distribution of children with parasitic infection, micronutrient deficiencies and high body fat in a rural community of Mexico (n=284)

We investigated the associations between intestinal parasitic infection and nutritional status in a population with high rates of overweight and obesity. This thesis included two main research questions: 1) are intestinal parasitic infections associated with body composition?, and 2) are intestinal parasitic infections associated with micronutrient status? In addition we included two research questions addressing the possible mechanisms underlying these associations: 1) are intestinal parasites associated with food intake?, and 2) are

intestinal parasites associated with systemic and intestinal inflammation? These questions are going to be addressed in the following sections separately for each parasite group.

Intestinal Protozoa

The results of chapter 2 and 3 suggest that the "non-pathogenic" protozoa *Entamoeba coli (E. coli)* is associated with obesity and might contribute to fat deposition over time (figure 2). While there is no information on the mechanisms that could explain these results, we propose a possible biological mechanism. *E.coli* lives and reproduces in the gut, and it might change the composition of the gut microbiota as reported for other intestinal protozoa[7]. Changes in gut microbiota are linked with an increased caloric uptake from the diet and thereby increase the risk of diet-induced obesity[8-10]. Still, the metabolic and immune consequences of being infected with *E.coli* need to be further investigated.

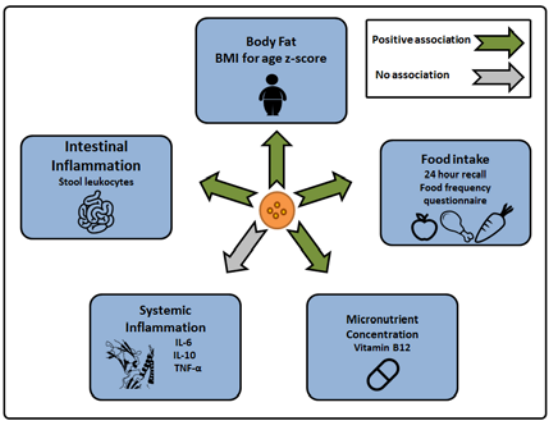

Figure 2. Association between nutritional status and inflammation with *Entamoeba coli* (chapters 2-6)

In chapter 4 we showed how *E. coli* is associated with higher food intake (figure 2), which might contribute to fat deposition. To our knowledge, no other studies have evaluated this association or the mechanisms that could explain the role of *E. coli* in food intake. Similarly to other intestinal protozoa, *E. coli* might reduce the diversity of bacteria in the gut and cause intestinal inflammation (chapter6)[7]. Both intestinal inflammation and reduced diversity of the gut have shown to have an effect on appetite and therefore food intake[11].

Food is the only source of micronutrients for the organism (except for vitamin D that is also synthetized by the body). In chapter 4 we found that *E. coli* and *Endolimax nana (E. nana)* infected children had a higher concentration of iron and vitamin B12 than parasite-free children (figure 2). The higher concentrations of micronutrients found in children infected with *E. coli* and *E. nana* may be associated with the higher reported intake of meat and egg, which are the principal sources of vitamin B12 and bioavailable iron in the diet (see chapter 4). It is important to highlight that these children also had a higher consumption of energy (Kcal) and the fat and sugar food groups. In contrast with our findings, a study in a Malaysian population reported no association between *E. coli* infection and iron, retinol, zinc or vitamin B12[12]. The discrepancy between studies may be attributed to diet, economic and food environment differences. Thus studies evaluating the association between *E. coli* with micronutrient concentrations should also adjust for supplementation programs, food intake, food quality, availability and affordability in each population.

Systemic and intestinal inflammation are relevant to body composition and micronutrient status[13-15]. Additionally, excess body fat and micronutrient deficiencies (i.e. vitamin A and zinc) are associated with higher

concentration of inflammatory markers such as TNF- α, IL-6 and leptin which lead to an increased risk of diseases such as hypertension and type II diabetes[3, 16]. Interestingly pathogenic intestinal protozoa are associated with the same inflammatory markers[17, 18]. The systemic inflammation observed in the children of this study is apparently not related to intestinal parasitic infection; none of the intestinal protozoa were associated with the measured systemic inflammation markers CRP, IL-6, IL-10 or TNF-α (chapter 6, figure 1). It appears that intestinal protozoa only have an effect on intestinal inflammation; children (from our study) infected with *E. coli* were more likely to have higher stool leucocyte counts than parasite-free children, but also than children infected with *A. lumbricoides* or with multiple infections (chapter 6). Thus, even though *E.coli* is a non-pathogenic parasite, infection with this protozoa may have implications related to immunological and inflammatory pathways that may have a long-term effect on human health, particularly in obese/overweight individuals.

Soil transmitted helminths

In line with most available studies, the results of chapter 3 showed that *A. lumbricoides* is associated with a lower BMIz (see fig 3)[19, 20]. Many mechanisms have been proposed to explain this association, including: 1) higher energy requirements; 2) lower available micronutrients; 3) anorexia due to abdominal pain, nausea or discomfort; and, 4) anorexia caused by inflammatory reactions[21]. The lower BMIz found in chapter 3 and in some other epidemiological studies might be related to mechanisms involving anorexia, that are going to be discussed in the section below[19, 20, 22].

In chapter 4 we found for the first time that *A. lumbricoides* infected children reported a lower food intake than parasite-free children using two objective measurements (i.e. 24 hour recall and food frequency questionnaire) (Figure 3). There are some biological pathways that might be playing a role in this association: 1) *A. lumbricoides* infection might cause abdominal pain, nausea and discomfort, common reasons for anorexia in children[19, 20, 23]; 2) *A. lumbricoides* infections has been associated with an increased diversity of the gut microbiota [24], that might result in specific effects on food intake, appetite and food preferences[8]. To the best of our knowledge there are no available studies evaluating this association. The only available studies have shown that anthelminthic treatment increases appetite, measured with an appetite questionnaire and a single meal (*at libitum)* intake in the morning. However they did not study the direct association of specific intestinal parasitic infection and food intake[22].

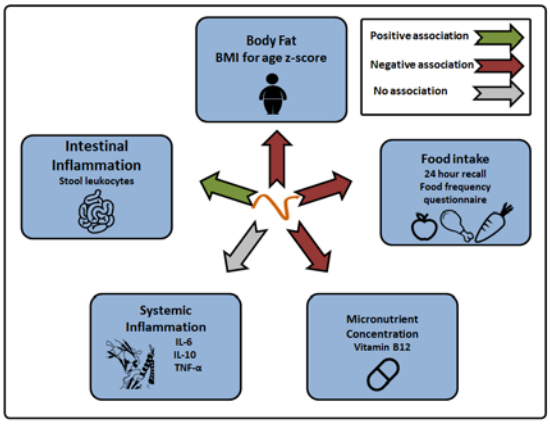

Figure 3. Association between nutritional status, diet and inflammation with Ascaris lumbricoides (chapters 2-6).

The lower food intake found in chapter 4 might be one of the causes of the lower concentrations of zinc and vitamins C and E found in *A. lumbricoides* infected children in chapter 5 (figure 3). Still, more than one biological mechanism might be taking place simultaneously, for instance: 1) an impaired micronutrient absorption,

resulting from a weakened gastrointestinal function and/or damage to the gut mucosa; 2) lower micronutrient concentration in blood due to inflammatory reactions; 3) competition for available micronutrients[22, 25-27]. The results of chapter 5 regarding zinc are in line with previous studies[28, 29]. Other studies addressing the relationship or causality between vitamins C or E and STH infections are still lacking[30]. Zinc and vitamins C and E are necessary for an adequate mucosal and epithelial barrier function and an efficient immune reponse[31, 32 33, 34]. Thus, there is a possibility that children with lower concentrations of these micronutrients are more likely to be infected.

We found that in addition to a lower food intake *A. lumbricoides* may be affecting micronutrient absorption by damaging the gut integrity and promoting gut inflammation. In chapter 6 we found that *A. lumbricoides* infected children were more likely to have higher stool leukocytes that parasite-free children. Additionally we ruled out the possibility of a lower micronutrient concentration in blood due to systemic inflammation, since we found no association between *A. lumbricoides* infection and the systemic inflammatory markers CRP IL-10, Il-6 or TNF-α. Our findings are in line with other studies in children. For instance, Sanchez et al. found no association between STHs and IL-10 in a cohort of Honduran children[35]. Similarly, de Gier et al. found no differences between infected and non-infected children in the concentration of acute phase protein (CRP)[28].

Additionally in chapter 6 we found that *A. lumbricoides* infected children were more likely to have higher leptin concentrations as compared to parasite-free children. This is in line with recent animal and *in vitro* studies that have shown that intestinal parasitic infection may have an effect on leptin concentrations[36, 37]. However, due to the study design, the complexity and the multiple roles of leptin in human metabolism, it has not been possible to unravel the mechanisms or determine the causality of the association. To fully understand the influence of intestinal parasites on leptin concentrations, multiple leptin measurements should be taken throughout the day, as well as before and after anti-parasitic treatment, while taking in consideration sex, age and body fat-related differences[38-41].

Methodological considerations

The above findings should take in consideration some methodological limitations that are intrinsic of the study design and the measuring tools, which are going to be discussed in the section below. Due to the cross-sectional design of the studies we could not determine the causality of the identified associations (chapters 2-6). Additionally, the prevalence of parasitic infection reported in chapters 2, 4, 5 and 6 were based on the parasitological examination of just one stool sample per individual[42]. This may have led to an under-estimation of the infection prevalence[43]. Still, the prevalence of infection found in this thesis is similar to that previously reported from different studies in Mexico, where parasitological examination were performed on 3 consecutive days[44, 45]. Another limitation is the use of reported food intake in chapter 4 which has the natural limitations of a reported outcome and is prone to systematic bias. Individuals tend to omit or forget foods in the 24hR, whereas with the FFQ questionnaire they tend to over-report consumption of certain items[46]. Nevertheless, the results using two independent methods to asses food intake, the validated FFQ and the average of three 24 hour recalls (i.e. reference method for diet assessment), were consistent (chapter 4). The count of stool leukocytes in chapter 6 measures the number of cells which may lead to non-systematic misclassification and does not differentiate between the different immune cell sub-types.

The results of chapter 3 should be interpreted in the context of it being an ecological analysis and not an estimate on any causal effect of parasitic infection on obesity at individual level (ecological fallacy). We intended to minimize this issue using individual level data on BMIz and covariates. In chapter 3, we used incidence data of the studied parasitic infections as a measure of the probability of infection, and the true prevalence of parasitic infection is most likely underestimated[47]. A major strength of our study was the use of ENSANUT and INEGI surveys as these are representative of the Mexican population at national and state level. The parasite infection data of

the SINAVE has been collected following the same procedures nationwide and is therefore very suitable for comparison purposes and generalizable conclusions.

Public health implications

In spite of these limitations, according to our findings, more than 60% of the children in our study population were infected with at least one species of intestinal parasites, and more than 73% had some type of malnutrition. Public programs to improve education and living conditions of the population at highest risk of infection and malnutrition are urgently needed in the country.

To discuss the public health implications, it is necessary to consider the negative impact the changing food environment or "nutritional transition" is having in Mexico. Low income populations undergoing a nutritional transition have access to energy dense food with low nutritional quality at relatively low prices, which leads to a rapid increase in the rates of overweight and obesity, as observed in Mexico from 1990 to 2016 [6]. We found *E. coli* (the most common intestinal parasite in Mexico) to be associated with a higher BMIz, reported food intake and intestinal inflammation (chapter 2 - 4). Therefore, in the context of Mexico (where low quality food is accessible and affordable) children infected with this parasite might be at greater risk of becoming obese and suffer of its co-morbidities (Figure 4). Reduction of the burden of *E. coli* might contribute as part of a multidisciplinary strategy to reduce the rates of overweight and obesity in these unprivileged populations. As suggested by other authors, improvement on food environment (accessibility and availability of "healthy food"), education and living conditions are needed for any strategy to be efficient at a community level[48-50].

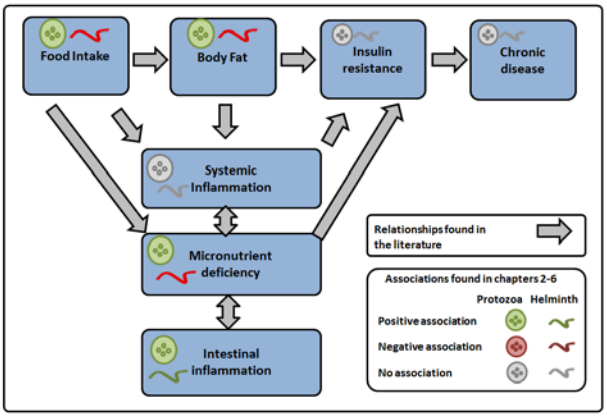

Figure 4. Relationship between nutritional status, parasitic infection and inflammation.

In contrast to *E. coli*, *A. lumbricoides* infection was associated with lower BMIz and food intake. In addition, *A. lumbricoides* infection was also associated with lower concentrations of zinc and vitamins E and C and higher intestinal inflammation. These micronutrients are essential for growth, and adequate immune function, among others. Thus, efforts for the elimination of *A. lumbricoides* infection should continue through the ongoing deworming national program in Mexico (every 6 months).

Directions for further research

Further studies are needed not only to understand the mechanisms behind the associations found in the thesis, but also to study the burden associated with coexisting malnutrition and intestinal parasitic infections (figure 1). It is known that at least 30% of Mexican children are overweight or obese (more than 12 million children) and at least 50% are estimated to be infected with at least 1 species of intestinal parasite (more than 21 million children)[51]. Given the high probability of the co-existence of malnutrition and intestinal parasitic infection,

the DALYs associated with each condition and the possibility of an interaction between them (chapter 6), more epidemiological studies are needed. 1) To evaluate if the high prevalence of malnutrition and intestinal parasitic infection seen in this community is similar in communities and cities in different regions of Mexico; 2) To understand the determinants of the association between intestinal parasitic infection and malnutrition; and, 3) To study if the reduction of the burden of intestinal parasite infections has an effect on the rates of obesity, micronutrient deficiencies, inflammation and chronic disease over time.

In addition to epidemiological studies, more research is needed to elucidate the mechanisms in which each intestinal parasite is associated with nutritional status and inflammation. For instance, in chapter 5 we observed that intestinal parasite infections were associated with intestinal inflammation. However, we used stool leukocytes as a measurement of inflammation. This measurement gives no information on the type of inflammatory reactions (Th1, Th2 or Th17) taking place in the intestine. Therefore, studying the effect of different intestinal parasites on the polarization (Th1/Th2) of the immune reactions in the intestine, might help explain the pathways of the associations found in the thesis.

Conclusions

We observed a high prevalence of malnutrition and intestinal parasitic infection in this study population. *E. coli* infection was associated with a higher body fat %, BMIz, food intake and micronutrient concentrations, whereas *A. lumbricoides* infection was associated with lower BMIz, food intake and micronutrient concentrations. None of the studied parasite infections was associated with inflammatory markers (CRP, Il-6, Il-10 and TNF-α) while all of them were associated with higher intestinal inflammation. *E. coli* was the intestinal parasite infection with the highest association with intestinal inflammation. Considering the high rates of malnutrition and intestinal parasitic infections in Mexico and the fact that malnutrition and intestinal parasitic infections are linked through several mechanisms (figure 4 and chapters 2-6), improvement on food environment (i.e. accessibility and availability of “healthy food”), education and living conditions (i.e. access to health and sanitation services) are needed for any strategy reducing the burden of intestinal parasitic infection and malnutrition to be effective.

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