Soil-Transmitted Helminth Infections, Nutrition and Growth in School-age Children from Rural Communities in Honduras

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Submitted in partial fulfilment of the requirements for the degree of

Master of Science in Applied Health Sciences
(Health Science)

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Dedication

To the Evergreen Memory of my Mum

Monica Uwazogie Obazee

RIP
Abstract

**Background:** Soil-transmitted helminth (STH) infections are endemic in Honduras but their impact on children’s health is not well studied.

**Objectives:** To evaluate the prevalence and intensity of STH infections and their association with nutrition and growth in a sample of Honduran children.

**Methodology:** A cross-sectional study was done among Honduran rural school-age children in 2011. Blood and stool samples and anthropometric measurements were obtained to determine nutritional status, STH infection and growth status, respectively.

**Results:** The STH prevalence among 320 studied children was 72.5%. Prevalence by species was 30%, 67% and 16% for *Ascaris*, *Trichuris* and 16% hookworms., respectively. High intensity infections were associated with decreased growth scores but regardless of intensity, co-infections negatively affected growth indicators.

**Conclusions:** The health burden of STH infections is related to high parasitic load but also to the presence of low-intensity concurrent infections. The synergistic effects of polyparasitism in underprivileged children warrants more attention.

**Keywords:** Soil-transmitted helminths, nutrition, growth, school-age children, Honduras
Acknowledgements

To my family - my husband Uyilawa and children, Riuyimen, Ede, Yuware, Osaze and Uwa, my Dad Gabriel and my sister Rosaria for all your wonderful love and support (both moral and financial), thanks too for your patience and understanding throughout my period of study.

To all my friends and colleagues – Lola and Tunde Fowler, Jose and Ivonne, Amidu Raifu, Phuc, Aysha, Dilani and Sabrina for all your wonderful input, your support and encouragement.

To Dr. M. Tammemagi and Dr. J. Liu for all your concern and expert input.

To all staff and students of the MEIZ programme of the National Autonomous University of Honduras as well as staff and students of the National University of Agriculture, Honduras, for your invaluable help with the implementation of the field work of this project.

To my committee – Dr. Ana Sanchez, Dr. Nota Klentrou and Dr. Eduardo Fernandez, for your time and valuable feedback.

To my supervisor – Dr. Ana Sanchez, an excellent mentor! Thanks for all the pains taken to ensure a successful work.

Omnes gloriam Deo

This project was made possible thanks to a scholarship and research grant by the Teasdale-Corti Honduras - Canada, 2007 to 2012 "Strengthening Capacities to Achieve the Millennium Goal No. 6 in Honduras: Combating Infectious Diseases".

This project was funded by the Teasdale-Corti program Partnerships for Global Health Research of the Canadian Initiative for Global Health Research (GHRI) (www.ghri.ca). This project also received partial funding from the Canadian Institutes for Health Research (CIHR) through funds granted to Dr. Theresa Gyorkos (McGill University) and subsequently subcontracted to Dr. Ana Sanchez (Brock University).
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List of Abbreviations

BMI: Body Mass Index
CDC: Center for Disease Control
DALY: Disability Adjusted Life Years
HAZ: Height for Age Z-score
Hb: Hemoglobin
Hct: Hematocrit
IDA: Iron Deficiency Anemia
LAC: Latin America and Caribbean countries
MEIZ: Maestría en Enfermedades Infecciosas y Zoonóticas (Master Program in Infectious and Zoonotic Diseases)
MoH: Ministry of Health
NHANES: National Health and Nutrition Examination Survey
NTD: Neglected Tropical Disease
PAHO: Pan American Health Organization
PEM: Protein Energy Malnutrition
QALY: Quality Adjusted Life Years
SPSS: Statistical Package for the Social Sciences
STH: Soil Transmitted Helminth
TDS: Trichuris Dysentery Syndrome
UNICEF: United Nations International Children's Emergency Fund
WAZ: Weight for age z-score
Glossary of Words:

**Malnutrition:** The WHO defines this as “malnutrition is the cellular imbalance between the supply of nutrients and energy and the body’s demand for them to ensure growth, maintenance, and specific functions” The term malnutrition is often used synonymously with undernutrition. Strictly speaking however, malnutrition includes extremes of underweight and overweight. For the purpose of this study however, malnutrition refers to undernutrition.

**Pre-school age children:** These refer to children aged 1 – 5 years old, not yet attending school

**School-age children:** Usually refers to children between the ages of 6 and 15 years old, regardless of whether they are attending school or not.
Soil-transmitted helminthes (STHs) are parasitic nematodes or round worms that are transmitted to humans through contact with or ingestion of faecally contaminated soil (Bethony et al., 2006; Hotez et al., 2008b). Although traditionally seen as rural diseases, they have become of great public health concerns in urban slums of developing countries (Crompton, 1999). They are most prevalent among impoverished populations particularly in developing countries characterized by low socio-economic status; poor housing and sanitation, lack of safe water supplies, inefficient or no health care, poor education and low earnings (Stephenson et al., 2000b). Climatic and environmental factors such as soil type are closely related with the distribution of STH infections in a country. For this reason, tropical and subtropical regions of the world where climatic and environmental conditions tend to be conducive for the development of infective stages are major endemic zones. These include countries of South and Central America, south and south-west China, India and south-east Asia as well sub-Saharan African countries (de Silva et al., 2003; Hotez et al., 2006). Figure 1 below shows the global distribution of STHs.
The common round worm known scientifically as *Ascaris lumbricoides*, the whipworm *or Trichuris trichiura* and the hookworms *Necator americanus* and *Ancylostoma duodenale* are regarded as the 3 most important STHs because they have the highest prevalence rates and they cause the greatest burden on health (Hotez, 2008). They are major public health problems with an estimated 135,000 deaths due to them annually. However, their major public health significance, lies in the chronic morbidities they cause in their hosts (WHO, 2002).

STH infections can have both short and long term effects on their hosts, where they impact on nutrition, growth, cognitive development and lifelong health of humans, especially children. Due to the chronic and often times asymptomatic nature of the diseases these impacts are often subtle and difficult to assess (Crompton and Nesheim,
as a result, health conditions such as anemia, growth stunting, protein-calorie malnutrition, fatigue, and poor cognitive development arising from the impacts of the infections, tend to persist in affected populations (Hotez et al., 2008b), even leading to the acceptance of helminth infections as normal part of life in such populations (Tanner et al., 2009).

STH infections have been found in several studies to be associated with malnutrition and anemia (Brooker et al., 2008; Correale and Farez, 2007; Ezeamama et al., 2005a; Ezeamama et al., 2005b; Sorensen et al., 2011). Malnutrition and STH infections often coexist in the same geographical locations with same individuals experiencing both conditions (Al-Mekhlafi et al., 2005; Hadju et al., 1995) and protein-energy malnutrition (PEM) followed by iron deficiency anemia (IDA) have been recognized as the most common forms of malnutrition in developing countries (Stephenson et al., 2000a). Growth stunting and anemia are indicators of chronic malnutrition (Allen, 2008). Infections including STH infections are common factors that can accelerate or exacerbate malnutrition. Hookworm infections are noted for their contribution to iron deficiency anemia which is probably the most significant cause of nutritional stress resulting from STH infections (Tanner et al., 2009). Through their feeding activities, the hookworms cause intestinal blood loss subsequently leading to loss of protein and iron. Chronic infections with whipworms can cause persistent blood loss, dysentery (bloody diarrhoea) leading to anemia, protein loss and malnutrition (Hotez, 2008).

Studies that have shown improvement in children’s growth after treatment provide indirect evidence of the relationship between STH and malnutrition (Al-Mekhlafi
et al., 2005). Deworming has been shown to result in improvement in appetite and growth of the children with an overall decrease in the prevalence of malnutrition. Physical fitness and psychological development of children have also been shown to improve with deworming (Adams et al., 1994; Latham, 1997; Northrop-Clewes et al., 2001). Deworming has been shown to prevent 82% of stunting, as well as cause an increase in weight gain by 35% in undernourished children and cause a 25% reduction in school absenteeism as well as improvement in school enrolment for the girl child (Ault et al., 2011).

In Honduras, STH infections and malnutrition have remained significant public health problems where they co-exist as in other endemic countries. Although, data on the prevalence of STH infections are not readily available, hospital and clinical records show varying rates of distribution in different regions of the country, and among different age groups (Kaminsky et al., 2004; PAHO, 2011). Based on a recent report, the overall prevalence of STH in Honduras is 62.5% with prevalences within departments ranging from 12.2 – 97% (Schneider et al., 2011). Malnutrition ranks as one of the top 20 causes of death in Honduras. Recent estimates also show that deaths due to malnutrition account for 1.96% of total deaths with the age-adjusted death rate being 10.46/100,000 (World Life Expectancy, 2012). Of the many causes of malnutrition in Honduras, STH infection remains a major contributing factor, with school-age children being the most vulnerable as obtains in other endemic populations. One in three children under 5 years of age is believed to suffer from growth stunting and anemia in Honduras (Allen, 2008) and as at 2006, 29.9% of children less than 5 years old were believed to be stunted in Honduras (WHO, 2010).
Improvements in sanitation and socio-economic status could provide effective control of the parasites (Awasthi et al., 2003; Ulukanligil and Seyrek, 2004b; WHO, 2002), but implementation of these strategies are often hampered by lack of resources and political will in endemic areas which are mostly resource poor populations (Egwunyenga and Ataikiru, 2005). As a form of short term measure, WHO recommends preventive chemotherapy, which is often implemented in mass drug administration programmes in endemic populations. In 2001, the 54th world health assembly (WHA) passed a resolution to increase awareness and to provide antihelminthic treatment to at-risk groups mostly school-age children. A target of providing regular deworming treatment to 75% of school-age children was set for 2010 (Brooker et al., 2006; Watson and Hickey, 2010; WHO, 2002). This target, as it is well known, has not been reached in many countries (Ault et al., 2011; Schneider et al., 2011). As in other developing areas, so also in Latin America and the Caribbean countries (LAC), STH infections pose a great danger to the health of millions of children. In 2009, the directing councils of PAHO and the Pan American Sanitary Bureau (PASB) pledged to eliminate or drastically reduce some 12 NTDs from LAC by 2015. Included in these 12 NTDs are STH infections (Ault et al., 2011; Schneider et al., 2011).

1.1: Statement of Purpose

In Honduras, the implementation of mass deworming through the Healthy School program began in 1998 and efforts to reach national coverage have intensified over the years (Ministry of Health Honduras, 2011). Despite these efforts, prevalence of STH infections remains high in Honduras. There are no available data on the evaluation of the
success of these interventions in terms of decreasing prevalences or intensities, improving health status, nutritional status and cognitive abilities of children that receive deworming treatment. Moreover, there is a scarcity of information on the association between STH, malnutrition and anemia (Dr. Ana Sanchez, personal communication). There is therefore an urgent need to investigate these situations especially in high risk areas of the country. Data generated from such studies will go a long way in assisting control efforts.

Within this context therefore, the aim of this study was twofold: 1) investigate the prevalence of STH infections, malnutrition and anemia among school-age children in rural communities of the Department of Olancho in Honduras and 2) examine the association of STH infections with malnutrition and anemia in this population.

1.1a: Study Objectives and hypotheses

- To determine the prevalence of STH among school children in rural Honduras.
  - Based on national reports and international literature it was hypothesized that prevalence for any STH will be above 50% and that, due to high endemicity, light infections will be predominant (Ministry of Health Honduras, 2001, 2006; Schneider et al., 2011; Smith et al., 2001)

- To assess the nutritional status/prevalence of malnutrition and anemia among school-age children in rural Honduras.
  - According to international literature, it was hypothesized that the children from the studied rural communities will suffer from a mild-to moderate degree of malnutrition and anemia due to the compounded effects of poverty and parasitism (Ahmed et al., 2012; Ministry of Health Honduras, 2011; Sorensen et al., 2011).
• To assess the prevalence of growth deficits among school-age children in rural Honduras
  - According to international literature, it was hypothesized that the prevalence of growth deficits among the children from the studied rural communities will be low to moderate due to the compounded effects of poverty and parasitism (Gray et al., 2006; Nichols et al., 2012; PAHO, 2004).

• To examine whether STH infections are associated with malnutrition and growth deficits among school-age children of rural Honduras.
  - It was hypothesized that among the studied children population, STH infections will be associated with negative health and growth outcomes of malnutrition (Ahmed et al., 2012; Casapia et al., 2006; Sorensen et al., 2011).
CHAPTER 2: LITERATURE REVIEW

2.1 The Etiologic Agents of STHs Infections

Soil transmitted helminths are a group of parasites acquired through contact with and or ingestion of soil contaminated with eggs or immature larval stages of the parasites, hence, the term “soil transmitted”. They are the most common neglected tropical diseases (NTDs). NTDs are a group of tropical diseases that are mostly endemic in poor populations of developing countries (Hotez, 2008). The common round worm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, the hookworms *Necator americanus* and *Ancylostoma duodenale*, and the thread worm *Strongyloides stercoralis* are the commonest human STHs. Globally however, *A. lumbricoides*, *T. trichiura* and the hookworms (*N. americanus* and *A. duodenale*) have the highest prevalence rates and they cause the greatest burden on health, hence they are regarded as the 3 most important STHs (Hotez, 2008).

Taxonomically, the three worms - *A. lumbricoides*, *T. trichiura*, and the hookworms – (*A. duodenale* and *N. americanus*) belong to the kingdom Animalia, subkingdom Metazoa and the Phylum Nematoda. They are however divided into different classes based on some morphological differences. *Ascaris* and the hookworms belong to the Class Secernentea, formally known as the class Phasmidia, while *Trichiura* belongs to the class Adenophorea formally known as Aphasmidia. The older classification into Phasmidia and Aphasmidia was based on the possession or non-possession of the phasmidial organs, which are a pair of sense organs positioned bilaterally close to the tail (Fagerholm et al., 2004). It turned out that Phasmidia had been earlier used to describe the walking stick insect (Orthoptera), hence the names Phasmidia and Aphasmidia were
later changed to Secernentea and Adenophorea respectively for the nematodes but still based on the presence or absence of the phasmidial organs (Olsen, 1974).

The above classification and further classification of the worms is shown in the table 1 below

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Animalia</th>
<th>Animalia</th>
<th>Animalia</th>
<th>Animalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Metazoa</td>
<td>Metazoa</td>
<td>Metazoa</td>
<td>Metazoa</td>
</tr>
<tr>
<td>Phylum</td>
<td>Nematoda</td>
<td>Nematoda</td>
<td>Nematoda</td>
<td>Nematoda</td>
</tr>
<tr>
<td>Class</td>
<td>Secernentea</td>
<td>Adenophorea</td>
<td>Secernentea</td>
<td>Secernentea</td>
</tr>
<tr>
<td>Order</td>
<td>Ascaridida</td>
<td>Trichocephalida</td>
<td>Strongylida</td>
<td>Strongylida</td>
</tr>
<tr>
<td>Family</td>
<td>Ascaridae</td>
<td>Trichurida</td>
<td>Ancylostomatidae</td>
<td>Ancylostomatidae</td>
</tr>
<tr>
<td>Superfamily</td>
<td>Ascaridoidea</td>
<td>Trichuroidea</td>
<td>Ancylostomatoidea</td>
<td>Ancylostomatoidea</td>
</tr>
<tr>
<td>Genus</td>
<td>Ascaris</td>
<td>Trichuris</td>
<td>Necator</td>
<td>Ancylostoma</td>
</tr>
<tr>
<td>Species</td>
<td>lumbricoides</td>
<td>trichiura</td>
<td>americanus</td>
<td>duodenale</td>
</tr>
</tbody>
</table>

As the adult stages live in the intestines of their hosts, STHs are also known as intestinal worms. They are known to cause nutritional and energetic stress in many populations (Tanner et al., 2009) where they have been associated with several health conditions notably impaired growth and stunting.

STHs infects all age groups, but it is more prevalent among children including pre-school children, school – age children and adolescents. However, the highest prevalence and intensities are found among school-age children (Hotez et al., 2008b)
where they are associated with several health and developmental conditions such as anemia, impairments in physical, intellectual and cognitive development as well as contributing to malnutrition and poor school performances (Ault et al., 2011; Casapia et al., 2006; Ezeamama et al., 2005a; Ezeamama et al., 2005b; Larocque et al., 2005; Mupfasoni et al., 2009; Northrop-Clewes et al., 2001; Sorensen et al., 2011).

Diseases caused by these worms are referred to as soil transmitted helminthiasis. *A. lumbricoides* causes ascariasis features of which include impaired childhood nutrition, surgical complications, allergic reactions and pneumonitis. *T. trichiura* causes trichiuriasis and its manifestations include impaired childhood nutrition, rectal prolapse and dysentery. Ancylostosomiasis is the disease caused by *A. duodenale* while *N. americanus* causes necatoriasis; both are commonly known as hookworm disease. The hallmark of hookworm disease is impaired iron status and iron deficiency anemia (Crompton and Nesheim, 2002).

### 2.1a Ascaris lumbricoides

*Ascaris lumbricoides* or the common round worm is the largest intestinal nematode worm known to infect humans. The females which are usually larger than the males can grow as long as 40cm and weigh as much as 9g, while a male worm may weigh 2-3g (Hall et al., 2008). *A. lumbricoides* is spread by faecal contamination of the soil and a person becomes infected by ingesting infective eggs in contaminated food or from hands that have become faecally contaminated. Following ingestion of the eggs, the larvae hatch in the small intestine and penetrate blood vessels in the intestinal wall. The larvae then undergo a heart – lung migration in the circulation, while developing in the process. After migrating up the trachea, they are swallowed and so get to the small
intestine where they develop into mature worms (Cheesbrough, 1992). Adult worms inhabit the entire small intestine (the jejunum being the preferred site) where they can live up to a year in the host feeding on intestinal contents with the head directed towards the intestinal flow (Bethony et al., 2006; Hotez et al., 2003). When present in large numbers, competition for space may set in and as such some worms may be found higher up or lower down the intestinal tract (Hall et al., 2008). They are able to maintain their positions in the intestine by swimming against the flow of food and when they die, they are passed out in feces (Hall et al., 2008).

Approximately 9 weeks post infection, the adult female worm begins egg production usually after mating (unfertilized eggs are produced in the absence of mating). Large quantities of eggs are laid and passed out in host’s feces (CDC, 2010; Cheesbrough, 1992). A female *Ascaris* produces about 100,000 - 200,000 fertilized or unfertilised eggs daily (Hotez et al., 2003; Sinniah, 1982). Under favourable conditions (shaded soil, a temperature of 20 – 40°C, and humidity of over 40%) fertilized eggs will develop with each egg having an infective larva about 30 – 40 days after being passed in faeces. The larvae will later hatch in the host after ingestion of the eggs (Cheesbrough, 1992; Hotez et al., 2003). *Ascaris* eggs can remain viable in soil for several years because of a strong protective coat that surrounds them thereby enabling them to withstand adverse weather conditions such as desiccation. This makes them infective even after several years especially among children who often play on open contaminated soil (Cheesbrough, 1992). Figure 2 below shows the life cycle pattern of *A. lumbricoides*. 
2.1b *Trichuris trichiura*

The adult *Trichuris trichiura* lives in the large intestine. *T. Trichiura* is also known as the whip worm because of the whip-like shape of the adult; the anterior portion is thin and contains a long pharynx while the posterior portion is wide and contains the intestines and reproductive system. The worm is spread by faecal contamination of the soil and a person becomes infected by ingesting infective eggs containing the L2 larva in contaminated food or fingers. Similar to *A. lumbricoides*, children are more often infected than adults as a result of their playing habits; they tend to play on faecally contaminated ground.
Larvae hatch in the small intestine from where they penetrate villi. The larvae then leave the small intestine for the large intestine after about a week. After approximately 12 weeks in the large intestine (cecum), they develop into mature worms with the thin anterior portion of the worm embedded in the intestinal mucosa (CDC, 2010; Cheesbrough, 1992). Although the cecum is the preferred site of invasion, in heavy infections, invasion may spread all over the colon and even as far as the rectum (Hotez et al., 2003). *T. trichiura* is both an intracellular and extracellular parasite. By means of novel pore forming proteins secreted by the parasite, holes or pores are made in the epithelial tissues. The thin anterior portion of the worm is embedded in these holes, while the wider posterior portion protrudes into the lumen (Bethony et al., 2006; Hotez et al., 2003). Adult worms can grow up to 30 - 50 mm in length with the female usually larger than the male. They are known to live up to 1.5 – 2 years in their hosts (Bethony et al., 2006).

Eggs laid by the female are passed out in faeces. A female worm will lay up to 3,000 to 5,000 eggs per day (Bethony et al., 2006). Larvae develop in the eggs in damp warm soil. After 2 - 3 weeks, each egg will have an infective larva. Eggs can survive and remain infective for several months in warm moist soil, they cannot however survive under dry conditions (Cheesbrough, 1992).

Figure 3 below shows the life cycle of *T. trichiura*. 

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13
2.1c *Ancylostoma duodenale* and *Necator americanus*

The upper part of the small intestine is home to the adult hookworms *Ancylostoma duodenale* and *Necator americanus* (Bethony et al., 2006). Through faecal contamination of the soil, infection is spread and this occurs when infective filariform larvae penetrate the skin of a person, very often as a result of walking barefooted on infected ground (Cheesbrough, 1992). After hatching from the egg in the soil, the larvae moult first into the first stage larvae (rhabditiform larvae). These undergo a second moult about a week later to become the infective filariform or third stage larvae. The third stage larvae do not feed, but they can move to get to the soil surface where contact with human skin will be easier to achieve (Bethony et al., 2006).
Other possible modes of infections for *A. duodenale* larvae are orally by ingesting infective larvae and from mother to child through breast milk (lactogenic transmission) (Bethony et al., 2006; Loukas and Prociv, 2001). The larvae undergo a heart – lung migration after skin penetration. They enter into the host’s afferent circulation through subcutaneous venules and lymphatic vessels and are then ultimately trapped in the pulmonary capillaries. They enter the lungs, pass over the epiglottis and are then swallowed after migrating up the trachea. They undergo further development in the small intestine; moulting twice while developing a buccal capsule in the process, after which they attain adulthood. The worms use the buccal cavity which contains teeth (*A. duodenale*) or cutting plates (*N. americanus*) for attachment to the intestinal wall (Loukas et al., 2005). By sucking part of the mucosa into their buccal cavity, they are then able to attach to the wall of the small intestine. They feed on mucous membrane and blood from their host with most of the blood passing through the worms undigested. The worms often move around in the intestine abandoning old feeding sites in search of new ones. *A. duodenale* is especially notorious for this behaviour. Abandoned sites may keep bleeding for some time (Cheesbrough, 1992; Watson and Hickey, 2010). It takes approximately 4–9 weeks for hookworms to develop into egg producing adults from the time of skin penetration, and they are known to live up to 5 – 7 years in their hosts (Bethony et al., 2006).

Figure 4 below, shows the life cycle pattern of the hookworms.
2.1d Clinical Features and Pathology of STH Infections

Most infections with *A. lumbricoides* are asymptomatic, however non-specific abdominal pains, nausea, anorexia, diarrhoea, vomiting and weight loss have been found associated with the parasite. Worms in the intestine may cause damages to the intestinal muscles leading to impairment of nutrient absorption (Cheesbrough, 1992; Valentine et al., 2001). During their heart-lung migration, *Ascaris* larvae may induce some inflammatory and hypersensitive reactions such as pneumonia-like symptoms, cough attacks and bronchial asthma (Cheesbrough, 1992). Loeffler’s syndrome, which is an immediate hypersensitivity type of immune reaction occurring in the lungs, is a type of pneumonitis associated with the larval migration phase of ascariasis due to *A. lumbricoides* (Hotez et al., 2003). Since the worms ingest proteins and vitamins from
their host, malnutrition may result especially in cases of heavy infections and in already malnourished children. *Ascaris* worms are large and form worm masses in heavy infections. These masses may result in the most severe manifestation of ascariasis which is obstruction of the intestinal tract (Hotez et al., 2003). Other acute complications of ascariasis include perforations of the intestine and occasional obstruction of the bile duct and pancreatic duct especially in children. These acute complications can be fatal. Other complications include liver abscesses, biliary colic or choledocholithiasis, periappendiceal abscess and appendicitis caused by migrating worms (Cheesbrough, 1992; Hotez et al., 2003; Valentine et al., 2001).

There are usually little or no problems associated with light infections with *Trichuris*. But abdominal pain and diarrhoea (often bloody) may be seen in cases of heavy infections. Severe infections can lead to chronic diarrhoea, intestinal ulceration, anemia, poor developmental rate, weight loss and rectal prolapse in young children. Massive infections can be fatal (Cheesbrough, 1992). *Trichuris* dysentery syndrome (TDS) is the worst manifestation of trichiuriasis. It usually occurs in children with very heavy infections and it is associated with chronic dysentery, rectal prolapse, anemia and growth stunting (Hotez et al., 2003).

A skin rash known as the ground itch which develops at the site of larval penetration is often the first sign of hookworm infection and some mild respiratory symptoms may develop during the heart-lung migration process. Chronic blood loss is a common feature of hookworm infections. Hookworms cause blood loss by puncturing the blood vessels and releasing anticoagulants that maintain continuous blood flow (Hotez et al., 2003). The worms then ingest host haemoglobin, which ultimately leads to iron and
protein deficiencies (Hotez et al., 2003). Iron deficiency anemia may develop in chronic high intensity infections, a condition that may become severe, and even fatal in vulnerable persons, such as infected pregnant women and persons with insufficient iron stores or low iron intake. Oedema (swellings as a result of fluid retention) may result due to loss of protein (Cheesbrough, 1992; Watson and Hickey, 2010). Diseases associated with infections with STHs are listed in table 2 below;

Table 2: Illnesses associated with STH infections (Sabin Vaccine Institute et al., 2011)

<table>
<thead>
<tr>
<th>Type of illness</th>
<th>Signs of illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional impairment</td>
<td>Intestinal bleeding, anemia</td>
</tr>
<tr>
<td></td>
<td>Malabsorption of nutrients</td>
</tr>
<tr>
<td></td>
<td>Competition for micronutrients</td>
</tr>
<tr>
<td></td>
<td>Impaired growth</td>
</tr>
<tr>
<td></td>
<td>Loss of appetite, reduced food intake</td>
</tr>
<tr>
<td></td>
<td>Diarrhoea or dysentery</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>Reduced verbal fluency and memory</td>
</tr>
<tr>
<td>Conditions requiring surgical intervention</td>
<td>Intestinal biliary obstructions</td>
</tr>
<tr>
<td></td>
<td>Rectal prolapse</td>
</tr>
</tbody>
</table>

2.1e Diagnosis of STH Infections

Diagnosis of STH infections has two main objectives

1. Primary diagnosis, to find out if an individual has an infection

2. To check the effectiveness of some interventions such as deworming in a population.

The usual diagnostic stages for STHs are the eggs or ova (WHO, 1991), but occasionally, adult stages of Ascaris may be passed in faeces or from the mouth (Cheesbrough, 1992;
Garcia and Bruckner, 1993; WHO, 1991). Eggs of the different species have distinct morphological features that make it easier to identify them (WHO, 1994).

The direct wet smears and concentration techniques are the two techniques that are basically used for the clinical and epidemiological diagnosis of these parasites (Garcia and Bruckner, 1993). The direct wet smear involves the detection of parasite eggs by direct parasitological examination of fresh faecal specimens. It assesses worm burdens and provides a quick diagnosis of heavily infected specimens (Garcia and Bruckner, 1993). The Kato-Katz technique which is very useful in epidemiological field surveys is a modification of the direct wet smear technique (WHO, 1994). The direct wet smear is however less efficient in cases with low intensities and watery stools, therefore, concentration techniques become useful in such cases (Garcia and Bruckner, 1993; Knopp et al., 2011a; WHO, 1994).

Concentration techniques can be categorized into two – sedimentation and floatation methods (Garcia and Bruckner, 1993). These techniques are designed in such a way that parasitic organisms are separated from fecal debris by centrifugation and/or differences in specific gravity (Garcia and Bruckner, 1993; Mahon et al., 2007). Concentration techniques are especially useful in the detection of parasites in cases of low intensities (Cheesbrough, 1992; Garcia and Bruckner, 1993; Knopp et al., 2011a; WHO, 1994). In sedimentation methods, parasites or parasite materials such as eggs are concentrated in sediments at the bottom of the tube whereas parasites and parasite materials are suspended at the top of a high density fluid in floatation methods (Mahon et al., 2007).
There are several concentration techniques used for the diagnosis of intestinal parasites, however, the most commonly used concentration techniques in epidemiological and clinical diagnosis are

1. **The Formol-Ether Concentration Technique** is a sedimentation technique that is very useful in routine clinical laboratory. It is usually used as a confirmatory method for negative direct smear method. It is believed to have a sensitivity that is 15 to 50 times greater than the direct method (Allen and Ridley, 1970). This method is however not widely used in epidemiological studies as it does not allow for the quantitative counting of parasite eggs per gram faeces which is necessary for the assessment of intensity of infections and the monitoring of drug efficacy.

2. **McMaster Counting Technique** is a quantitative technique used in the determination of the number of eggs present per gram of faeces. It uses a counting chamber that allows the microscopical examination of a known volume (0.15 ml) of faecal suspension. A known weight of feaces and a known volume of floatation fluid is used to prepare the suspension. Usually, 2g of fresh faecal sample is mixed in 30 ml of a saturated salt solution (density of 1.2). The resultant slurry is then passed three times through a wire mesh in order to filter off large debris. 0.15 ml of the filtrate is then placed in the McMaster counting chamber and examined microscopically using the x100 magnification. The number of eggs counted for each helminth species is then multiplied by a factor of 50. This technique will detect $\geq 50$ epg of feaces (Vercruysse et al., 2011).
3. **The FLOTAC Technique** is a recently developed floatation technique gaining wide acceptance and usage in both human and veterinary medicine (Becker et al., 2011). The technique has been found to be a more sensitive technique than Kato-Katz technique in the diagnosis of STH especially in low infection intensity cases. A single FLOTAC preparation was found to be more sensitive than a triplicate preparation of Kato-Katz (Knopp et al., 2009). It has also been demonstrated to be better both qualitatively and quantitatively than the McMaster technique in the diagnosis of hookworm infections (Rinaldi et al., 2009). FLOTAC technique was also demonstrated to show the most sensitivity (88.2%) in the diagnosis of hookworm infections in a study that compared three diagnostic techniques (Kato-Katz, ether concentration technique and FLOTAC technique). Kato-Katz had a sensitivity of 68.4% while the ether concentration technique showed a sensitivity of 38.2% (Utzinger et al., 2008). FLOTAC technique is however quite complex and requires the use of special instruments (FLOTAC apparatus); this is a limiting factor in its usage.

4. **The Kato-Katz Technique** was first introduced in 1954 by Kato and Miura (Kato and Miura, 1954). It has since undergone several modifications with the most popular modification being that of Katz et al in 1972 (Katz et al., 1972). Hence the method has been known as Kato-Katz technique ever since.

   The Kato-Katz technique is a modification of the direct wet smear technique and is widely used in epidemiological field surveys (WHO, 1994). It is the gold standard technique recommended by the WHO for the qualitative and quantitative diagnosis of human intestinal helminth infections (Montresor et al., 1998; WHO, 2002). This
recommendation is mainly based on its been more sensitive than the traditional direct smear method as it uses more samples (41.7 mg) compared to 2 mg needed for direct smear. The Kato-Katz technique is also simple, cheap, accurate and very fit for use on a large scale, allowing for the classification of infection intensity into categories of light, moderate and heavy as shown in table 3 (Glinz et al., 2010; WHO, 2002).

Kato-Katz technique is an excellent tool for the estimation of the prevalence and infection intensities in populations and the assessment of drug efficacy and in the monitoring and evaluation of control programs (Knopp et al., 2011a). The technique however loses sensitivity in cases of low infection intensities as occurs after treatment due to the small amount of fecal sample use (41.7 mg). In such situations, the sensitivity can be increased by making several smears from one stool sample or by having several stool samples examined from the same individual (Becker et al., 2011; Glinz et al., 2010; Knopp et al., 2011a).

2.2: Soil Transmitted Helminths, Malnutrition and Anemia

Children, especially school – age children as well as adolescents and pre – school children tend to harbour the greatest numbers of intestinal helminths (Hotez et al., 2008b). They are usually parasitized with at least one, and very often, with all 3 STHs, negatively impacting on the health and wellbeing of these children. The association or relationship of STH infections with these health and developmental conditions have been demonstrated by several studies (Casapia et al., 2006; Ezeamama et al., 2005a; Ezeamama et al., 2005b; Larocque et al., 2005; Mupfasoni et al., 2009; Northrop-Clewes et al., 2001; Sorensen et al., 2011).
2.2a: *STH Infections and Malnutrition*

According to the World Health Organization (WHO) “malnutrition is the cellular imbalance between the supply of nutrients and energy and the body’s demand for them to ensure growth, maintenance, and specific functions” (WHO, 2000). The term malnutrition is often used synonymously with undernutrition. Strictly speaking however, malnutrition includes extremes of underweight and overweight (Halsted, 2001). According to the UNICEF definition “People are malnourished if their diet does not provide adequate calories and protein for growth and maintenance or they are unable to fully utilize the food they eat due to illness (undernutrition). They are also malnourished if they consume too many calories (overnutrition)” (UNICEF). For the purpose of this study however, malnutrition refers to undernutrition.

Malnutrition affects all age groups and the four most important forms worldwide are protein-energy malnutrition, iron deficiency anemia, iodine deficiency disorders, and vitamin A deficiency (Stephenson et al., 2000a). Protein energy malnutrition (PEM) is the most common form of malnutrition in many developing countries, followed by iron deficiency anemia (IDA). Severe forms of protein energy malnutrition manifest as kwashiorkor, nutritional marasmus and marasmic kwashiorkor. They are less prevalent in developing countries than the mild to moderate forms of PEM that cause growth impairment (Stephenson et al., 2000a). Infants and pre-schoolers are mostly susceptible to severe malnutrition, mild to moderate forms are found more in school-age children (Pollitt, 1990).
Malnutrition results from many and often times complex factors. Often a combination of factors such as insufficient intake of food, abnormal assimilation of food, stress response to acute injury or chronic inflammation and abnormal metabolism of nutrients will lead to malnutrition (Halsted, 2001). Infection is a common factor that can accelerate or exacerbate malnutrition. In many developing countries where infections due to several and varied agents such as viruses, bacteria and parasite abound, a negative impact on the nutritional status of both children and adults is often observed (Latham, 1997; Saunders and Smith, 2010).

Malnutrition contributes to poor school enrolment, absenteeism, school drop-outs, and weaknesses in physical and intellectual performance in primary school children as well as poor mental and physical growth which impedes national socio-economic growth (Brooker et al., 2006; WHO, 2000). Adverse consequences include disability and death. It is estimated that of the 10.8 million deaths among children less than 5 years of age in developing countries, about 54% are related to malnutrition (Schaible and Kaufmann, 2007; WHO, 2000).

Several parasites have been associated with malnutrition, however, intestinal parasites such as STH infections, *Giardia duodenales*, coccidian parasites, *Schistosoma* sp have been noted to be the most important ones (Al-Mekhlafi et al., 2005). The severity of the effects of an infection on the nutritional status of an individual is determined by factors such as the previous nutritional status of the host as well as the type and duration of the infection (Scrimshaw and SanGiovanni, 1997). Malnutrition is an important part of acute and chronic illness (Halsted, 2001) and the relationship between malnutrition and infection has been found to be synergistic (Schaible and Kaufmann, 2007; Scrimshaw
and SanGiovanni, 1997). The combined effects observed when both malnutrition and infection are present at the same time are far more serious than the additive effects resulting from when they occur separately. In other words, infections worsen cases of malnutrition and malnutrition worsen the severity of infectious diseases (Latham, 1997; Macallan, 2009; Schaible and Kaufmann, 2007).

There are several ways by which infections can affect nutritional status, notably by leading to an increased loss of nitrogen from the body. This is mainly manifested through the increased breakdown of tissue protein and the mobilization of amino acids mostly from the muscles. Nitrogen is subsequently excreted in urine indicative of the loss of body proteins from the muscles (Latham, 1997; Scheinfeld et al., 2011). Also, infections especially those accompanied by fever and those that cause vomiting affect nutritional statuses by leading to anorexia or loss of appetite. With loss of appetite, there is often a reduction in food intake (Latham, 1997; Saunders and Smith, 2010). Growth retardation is often observed during and after infection especially in children with minimal protein diet, so that to gain full recovery after an infection, a child will have to have an increased protein intake (usually above maintenance level) in order to restore lost amino acids back to the tissues (Latham, 1997).

With the high prevalence of STH infections, more studies continue to reveal their adverse effects on nutritional status especially in heavily infected individuals. Although these diseases are rarely fatal, however, they are known to cause nutritional and energetic stress in many populations (Tanner et al., 2009). They contribute to malnutrition by impairing growth and development especially in persons with marginal nutrition (Weller and Nutman, 2001).
Adult worms are able to interfere with their hosts’ nutrition because they live in their intestines. And host’s capacity to extract and utilize nutrients from food may be diminished as a result of damages caused to the host’s intestinal mucosa by the worms (Tanner et al., 2009). These damages may also lead to reduced appetite or total loss of appetite. Impairment of nutrient absorption is recognized as one of the mechanisms utilized by intestinal helminths to induce or worsen cases of malnutrition (Crompton and Nesheim, 2002). *A. lumbricoides* worms are relatively large, about 15 to 30 cm in length, it is expected that they will have some significant metabolic needs. The parasites compete for nutrients with their hosts especially children and as a result, malnutrition sets in leading to poor growth. Deworming has been shown to improve child growth in many cases of malnutrition (Latham, 1997). Other conditions resulting from STH infections like vomiting, diarrhoea, anorexia, abdominal pain and nausea may also lead to reduced food intake, further reducing availability of nutrient to the host (Stephenson et al., 2000b).

Although the effects of intestinal helminths infection on growth is manifested more in heavily infected children those with light infections may also manifest some growth faltering especially if the nutritional status of the community is poor (Stephenson et al., 2000b). Most infections in endemic settings are light infections (Ezeamama et al., 2005b) and polyparasitism is not uncommon; many children harbour more than one parasite at a time, which often leads to malnutrition. Studies have shown that STH infections also exhibit negative effects such as growth faltering on children with light infections. For example, in their study among 507 children from Leyte, The Philippines, Ezeamama and coworkers (2005) observed that malnutrition and reduced physical fitness
were associated with mild to moderate intensity childhood helminth infections (Ezeamama et al., 2005b). A significant improvement in growth, anemia and appetite was also observed after antihelminthic treatment in Zanzibari infants < 30 months old that had very light infections (Stoltzfus et al., 2004).

Apart from the direct effects of the worms on the gut such as mucosal damage that may lead to disturbances with nutrition such as malabsorption and loss of nutrients, other indirect effects such as the immune responses they elicit may also cause problems with nutrition (Wright et al., 2009). Local inflammation at the site of infection is known to induce a systemic inflammatory response characterized by high levels of acute phase proteins and cytokines in the plasma (Cooper et al., 1992; Kung'u et al., 2009). The release of these proinflammatory Th2 cytokines in response to STH infections have been shown to be associated with the impacts of STH infections on nutrition (Crompton and Nesheim, 2002). In children, proinflammatory cytokines and acute phase proteins may cause anorexia or loss of appetites, which may lead to growth faltering [reviewed by (Northrop-Clewes et al., 2001)]. They can also cause protein loss, elevated levels of resting energy expenditure and in chronic cases, may also affect anemia [reviewed by (Wright et al., 2009)].

A Th2 dominated cytokine response to intestinal helminths may lead to disruption in nutrition by affecting gut function. An altered mucosa has been demonstrated in murine models to lead to increased mucosal permeability, reduced glucose absorption, increased ion secretion and intra-luminal fluid accumulation (McDermott et al., 2003). Similar effects have been found in A. suum (pig Ascaris) which is a good model of A. lumbricoides of humans (Dawson et al., 2005).
As earlier stated, the causes of malnutrition are many; however, any disturbance in the health and nutrition of children no matter the cause will subsequently affect their growth. Therefore, assessment of growth tends to be the best way to evaluate the health and nutritional statuses of children (de Onis et al., 1993). The determination of the nutritional status of an individual can be done in several ways. Dietary intake documentation, anthropometry, laboratory analyses of blood and urine, clinical examination and taking the health history of the person are some of the ways this can be done (Halsted, 2001; NHANES, 1988). Laboratory analyses of blood and urine will assess deficiencies in macro and micro nutrients such as proteins, iron, zinc, folate, magnesium, copper and vitamins A and C (Scrimshaw et al., 1968; Shamah-Levy et al., 2012).

Anthropometry is the most widely used method for the assessment of nutritional status. It provides information on the size of bones, muscles and fat (adipose tissues) of the human body (NHANES, 1988, Jan. 2007). In the assessment of nutritional statuses of children, the best information is got from the following indices; height-for-age or stunting, this indicates past growth failure or chronic malnutrition; weight-for-height or wasting which indicates current or acute malnutrition and weight-for-age or underweight which is an overall indicator for malnutrition, it assesses both present (acute) and chronic malnutrition or mixed malnutrition (de Onis et al., 1993).

2.2b: STH Infections and Anemia

Anemia is a clinical condition in which haemoglobin (the molecule in blood that carries oxygen to the cells of the body) concentration is below certain criteria established by the WHO. In a normal population and at a given altitude, an individual is considered
anaemic, if his/her haemoglobin (Hb) concentration is below two standard deviations (-2SD) of the mean of the population of the same sex and age (WHO, 2001). The WHO has set certain criteria below which anemia is considered in a population (table 6 below). For children 5 - 11 years of age, an Hb of less than 11.5 g/dL is considered anaemic while a Hb less than 12 g/dL will be considered anaemic for children 12 – 14 years of age (WHO, 2001).

Over 2 million people are estimated to be anaemic worldwide (Casey et al., 2009; WHO, 2001) with children and pregnant women suffering the brunt of the problem (Hotez, 2008; Tsuyuoka et al., 1999). Anemia is thought to affect about half of school-aged children and adolescents in developing countries (Crompton, 2000; Tsuyuoka et al., 1999). Anemia in infants and children has been found to be associated with increased mortality, impairment of growth, delayed motor development, impaired cognitive abilities, reduced school performance and a compromised immune system (Crompton, 2000; Pollitt, 1990; Stoltzfus et al., 1997). In pregnant women, it is associated with low birth weight, as well as high incidence of morbidity and mortality (McDermott et al., 1996). It is estimated that about 16 – 20 % of the around 500,000 maternal death that occur annually is due to iron deficiency anemia (Crompton, 2000; Viteri, 1994).

Causes of anemia vary from place to place but includes deficiencies in essential micronutrients such as iron, vitamin A, vitamin C, folate, riboflavin, and vitamin B12; physical conditions such as haemorrhages, abnormal menstrual bleeding; genetic abnormalities like thalassemia, sickle-cell anemia; diseases such as parasitic infections (hookworms and malaria) and other infections like HIV and tuberculosis and cancer (Pollitt, 1990; Tsuyuoka et al., 1999). However, iron deficiency stands out as the most
frequent cause of anemia in the developing world (WHO, 2001). Iron deficiency anemia is probably the most common health condition resulting from nutritional deficits (Scrimshaw and SanGiovanni, 1997; WHO, 2001).

Hookworm infections are noted for their contribution to iron deficiency anemia which is probably the most significant cause of nutritional stress resulting from STH infections (Tanner et al., 2009). Through their feeding activities, the hookworms cause intestinal blood loss subsequently leading to loss of protein and iron. However, there is evidence that most of the protein is absorbed back lower down the intestinal tract, but there is a significant loss of iron, and hookworms have been recognized to be the major cause of iron deficiency anemia in many parts of the world (Latham, 1997; Watson and Hickey, 2010).

A light hookworm infection of 20 -50 adult worms can result in significant iron losses. An estimated blood loss of about 1 ml/day is associated with a light infection of 25 hookworms. This is equivalent to a loss of about 0.5 mg of iron which is roughly a child’s daily iron needs (Hotez, 2008). In their 1966 study in Venezuela, Roche and Layrisse reported that the daily faecal blood loss caused by one *N. americanus* hookworm was 0.031 ± 0.015 ml. An estimate of 10ml of blood or 2 mg of iron was lost daily with an infection intensity of 350 hookworms. Higher infection intensities leading to a loss of greater than 3 mg of iron per day were not uncommon in this study population. Anemia was found in adult males that lost more than 3 mg of iron per day and in young children and women of child bearing age losing about half that amount (Roche and Layrisse, 1966). Chronic infections with whipworms can cause persistent blood loss, dysentery (bloody diarrhoea) leading to anemia, protein loss and malnutrition (Hotez, 2008).
As earlier stated most infections in endemic places are of low intensities and it is very common for one child to harbour more than one parasite at a time. It is also a fact that the effects of STH infections are manifested more in high intensity infections, however, low-intensity polyparasite infections have been observed to have significant effects on children’s nutritional status. A study among East Guatemalan school children with low intensity STH infections, showed a significant decrease in haemoglobin level in children infected with more than one STH (Sorensen et al., 2011). In the Philippines, children with low intensity polyparasite STH infections were observed to have an almost 5 fold higher odds of having anemia than children that were uninfected or that had low intensity infection with only one parasite (Ezeamama et al., 2005b). However, in a study in Rwanda, the investigators found no significant impact on anemia and malnutrition in children with low intensity polyparasite infections (Mupfasoni et al., 2009).

School-aged children are the most vulnerable group to STH infections and they often harbour the highest intensities of the infections. Deworming has been shown to result in improvement in appetite and growth of the children with an overall decrease in the prevalence of malnutrition. Significant improvements in appetites, conditions such as anemia, wasting malnutrition, physical fitness and development of the children as well as the children’s psychological development have been associated with deworming in many studies (Adams et al., 1994; Latham, 1997; Northrop-Clewes et al., 2001; Stephenson et al., 1989; Stephenson et al., 2000b; Stoltzfus et al., 1997). There are however other studies that demonstrated no significant improvement in these conditions (Watkins and Pollitt, 1996). An explanation for this trend is difficult as differences in growth, nutrition and prevalence and intensities of infections in the different study populations complicate
understanding. Investigating the mechanisms by which helminths affect growth, nutrition and physiology of their host is necessary as this will help in the understanding of the impact of helminth infections on child health (Northrop-Clewes et al., 2001).

STH infections, malnutrition and anemia are serious public health issues especially in developing countries. An assessment of the gravity of these maladies and their associations or relationships in different populations will help to generate data that can guide control efforts. The use of geographic information systems (GIS) and remote sensing (RS) devices in recent years have greatly helped in the mapping of the distribution of STH infections consequently helping to locate target treatment communities or populations (Brooker et al., 2006; PAHO, 2011). Baseline surveys among school children to ascertain prevalence and levels of intensities are also highly recommended. These will assist in guiding control and prevention efforts at different levels of governmental administration in these countries (Montresor et al., 1998).

2.3 Burden of STH Infections

Intestinal helminths are known to infect humans in almost all geographic and climatic regions of the world excepting areas of extreme weather conditions (heat or cold) which do not favour the survival of the infectious stages. Between 2 - 3 billion people worldwide are estimated to be infected with intestinal helminths and many billions more are at risk especially in the warm and moist climates of the tropics and sub tropics where the infections are often associated with poverty, poor hygiene (environmental and personal) and inefficient health services (Cooper et al., 2008; Olliaro et al., 2011).
With 2 to 3 billion people being infected with one or more STHs worldwide, the disease accounts for almost 40% of the global morbidity from infectious diseases (Hotez et al., 2003). Presently it is estimated that approximately 800 million – 1.4 billion people are infected with *A. lumbricoides*, approximately 600 million – 1 billion people are infected with *T. Trichiura* and approximately 500 million – 1.2 billion people are infected with the hookworms (CDC, 2010; Shoff et al., 2010). Estimation of worm burden or intensities for the STHs is usually done indirectly following the WHO recommendation of calculating the mean number of eggs per gram (epg) of feces. In line with this recommendation, the WHO has set certain thresholds by which these intensities can be categorized into light, moderate and heavy infections as shown below in table 3. Likewise, within a population, a prevalence of ≥ 70% is regarded as high, a moderate prevalence is ≥ 50% but < 70% while a low prevalence is < 50% (WHO, 2002).

**Table 3: Classes of intensity for soil-transmitted helminths according to the number of eggs per gram (epg) in stool examination by the KK technique (WHO 2002)**

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Light-intensity infections</th>
<th>Moderate-intensity infections</th>
<th>Heavy-intensity infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>1-4,999 epg</td>
<td>5,000-49,999 epg</td>
<td>≥ 50,000 epg</td>
</tr>
<tr>
<td><em>Trichurus trichiura</em></td>
<td>1-999 epg</td>
<td>1,000-9,999 epg</td>
<td>≥ 10,000 epg</td>
</tr>
<tr>
<td>Hookworms</td>
<td>1-1999 epg</td>
<td>2,000-3,999 epg</td>
<td>≥ 4,000 epg</td>
</tr>
</tbody>
</table>
Table 4 below shows the estimated number of persons infected with the three commonest STHs in the world. It also shows their positions in the intestinal tract as well as the major diseases they cause and their global distribution. STH infections occur mostly in the Americas, China and East Asia as well as in sub-Saharan Africa (Hotez et al., 2006).

Table 4: The Soil Transmitted Helminths A. lumbricoides, T. trichiura, N. americanus and A. duodenale {Adapted from (Hotez, 2008)}

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Length as adult male or female</th>
<th>Major location in the intestine</th>
<th>No. Of cases worldwide</th>
<th>Major diseases other than impairment of child growth and development</th>
<th>Global distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. lumbricoides</td>
<td>Roundworm</td>
<td>15 - 35 cm</td>
<td>Small intestine</td>
<td>800 million - 1.4 billion</td>
<td>Intestinal obstruction</td>
<td>Asia, Africa, Americas</td>
</tr>
<tr>
<td>T. trichiura</td>
<td>Whipworm</td>
<td>3 - 5 cm</td>
<td>Large intestine (colon)</td>
<td>600 million – 1 billion</td>
<td>Colitis, dysentery</td>
<td>Asia, Africa, Americas</td>
</tr>
<tr>
<td>N. americanus and A. duodenale</td>
<td>Hookworm</td>
<td>7 - 13 mm</td>
<td>Small intestine</td>
<td>500 million – 1.2 billion</td>
<td>Iron deficiency anemia</td>
<td>Asia, Africa, Americas</td>
</tr>
</tbody>
</table>

Tightly tied to poverty, the highest prevalence of STH infections is seen mostly in the poorest populations (rural and urban slum areas) of developing countries in tropical
and subtropical regions of the Americas, China and East Asia, and Sub-Saharan Africa (Hotez et al., 2006). For *Ascaris*, highest prevalence rates are found in China and Southeast Asia, as well as in coastal regions of West Africa and in Central Africa while prevalence rates are highest for *Trichuris* infections in Central Africa, southern India as well as Southeast Asia, and for hookworms highest prevalence rates can be found in most of sub-Sahara Africa, southern India and Southeast Asia (de Silva et al., 2003). These infections are perpetuated by poverty and poor health resulting from the infections has long term effects on educational and economic productivity. Capacities for learning, productivity and income earning can be significantly reduced in such populations (Schneider et al., 2011). For example, anemia resulting from STH infections has been found to be associated with decreased worker productivity and children’s cognitive capacity as it affects school attendance (de Silva et al., 2003). In other words, these infections cause poverty and are also consequences of poverty (Schneider et al., 2011). Affected populations continue to have high prevalences of STHs and poor economic growth, trapped in a cycle of poverty as it were (de Silva et al., 2003; Hotez, 2008; Hotez et al., 2006; Sabin Vaccine Institute et al., 2011).

Death, resulting from STH infections is not very common; therefore, the burden of disease is manifested more in the morbidity caused by these parasites. These morbidities are often times chronic, having subtle but harmful effects or impacts on the health and nutritional status of the host (Stephenson et al., 2000b).

A good understanding of the impact of these infections in endemic areas is important in order to really appreciate the burden of these infections on people who live in these areas (King, 2010). As a result of the chronic and asymptomatic nature of the
diseases, their impact on nutrition, growth, cognitive development and lifelong health of humans are often subtle and difficult to assess (Crompton and Nesheim, 2002). Moreover, health conditions arising from their impact such as anemia, growth stunting, protein-calorie malnutrition, fatigue, and poor cognitive development tend to persist in affected populations (Hotez et al., 2008b) with the consequent acceptance of helminth infections as normal and unavoidable part of life in endemic populations (Tanner et al., 2009).

Natural infections with STHs usually show an over dispersed pattern, with the tendency for majority of hosts to harbour a few parasites, while only a few hosts become heavily infected (Moreau and Chauvin, 2010). Although morbidities, due to STH infections are often associated with high intensities infections, nevertheless, morbidities such as growth deficits have also been shown to be associated with low intensity infections especially in children living in communities with poor nutritional status (Ezeamama et al., 2005b; Stephenson et al., 2000b). Pathology and disability caused by the worms usually occur concurrently with the active infection in an established infection. Impacts of these diseases such as anemia, growth stunting, malnutrition, poor school and work performance etc. are attributable to the chronic inflammations produced as a consequence of the chronic nature of the infections (King, 2010).

2.3a: Assessment of the Impact/Burden of STH Infections

In endemic areas, STH infections are often long lasting and chronic. Moreover, termination of these infections may take a long time due to problems of superinfections and reinfection which are common occurrences in these populations. Parasite-associated
inflammation and tissue damage may also persist for the rest of a person’s life even after the infection ends (King, 2010).

STH infections cause more disability than death, and as such, the burden of disease is measured in disability-adjusted life years (DALYs) or the number of years of healthy life lost due to chronic illness or disability (Bethony et al., 2006). The DALY for the three STHs combined is an estimated 39 million life years. Hookworms alone have a DALY of 22.1 million years, that for *A. lumbricoides* is 10.6 million, while *T. trichiura* contributes 6.4 million years (Stephenson et al., 2000b).

Recently however, the use of quality-adjusted life years (QALY) has been proposed as a better method of estimating the health impact or burden of chronic parasitic diseases. It is posited to give a better assessment of patient function as well as the impact on family and community health and productivity (Brooker, 2010; King, 2010).

### 2.3b: Intervention Effort

Prevention and control of STH infections are mainly done by the use of antihelminthic treatment. Several studies have shown significant improvements in children’s health and development after deworming treatment (Adams et al., 1994; Cooper et al., 2006; de Silva, 2003; Northrop-Clewes et al., 2001; Stephenson et al., 1989; Stoltzfus et al., 1997; Watkins and Pollitt, 1996), as well as in pregnant women (Casey et al., 2009; Larocque et al., 2006).

In order to reduce morbidity, mortality as well as disease transmission, the 54th world health assembly of 2001 passed a resolution to increase awareness (by health education) and to provide antihelminthic treatment to at-risk groups mostly school-age
children. A target of providing regular deworming treatment to 75% of school-age children was set for 2010 (Brooker et al., 2006; Watson and Hickey, 2010; WHO, 2002).

The four WHO recommended drugs are the two Benzimidazole carbamates Albendazole and mebendazole as well as levamisole and pyrantel pamoate (WHO, 2002). Albendazole and mebendazole are usually the drugs of choice as they seem to be more effective than levamisole and pyrantel (Olliaro et al., 2011). These drugs have been in use for several decades and are relatively generally safe and cost effective. They are usually given in single dose format in mass drug administration programs to all persons in target populations usually without prior diagnosis or testing for possible drug reactions (Olliaro et al., 2011).

Concerns about the sustainability of the use of these drugs are on the increase especially with suboptimal efficacy of the Benzimidazole against *T. trichiura* when administered in a single dose, some contraindications in early pregnancy and the fear of drug resistance in humans, a phenomenon that is already very wide spread in veterinary medicine (Harris, 2011; Olliaro et al., 2011; Vercruysse et al., 2011). As a result of these concerns, alternative interventions are being sought. There are encouraging advances being made in the development of new chemotherapeutic agents and an effective vaccine (Watson and Hickey, 2010).

Improvements in personal and environmental hygiene also provide effective prevention of the parasites as well as the provision of portable water, improvement in socio-economic statuses and health education (Awasthi et al., 2003; Ulukanligil and Seyrek, 2004a; WHO, 2002). However, a combination of improvement in human behaviour and drug treatment gives better results (Awasthi et al., 2003).
2.4 STH in Latin America and the Caribbean

About 580 million people are estimated to live in Latin America and the Caribbean. Of these, about 195 million are believed to live in poverty (earning less than 2 dollars per day) while another 71 million live in extreme poverty (earning less than a dollar per day). These people, mainly made up of indigenous populations, the poor in rural areas and urban slums, migrant workers, the elderly, women and children bear the brunt of infectious diseases including STH infections (Schneider et al., 2011). An estimated 46 million children are found to be at risk of these infections in these areas with millions of them and pregnant women being infected often with resultant negative effects on the mental and physical growth of the children as well as complications in pregnancies and birth outcomes (Sabin Vaccine Institute et al., 2011).

To many authors, STH infections are the most common infections among the poor in the Americas because of their high prevalence. Even with the declaration of WHA 54.19 in 2001, PAHO records still showed that as at 2007, only 32.7% of the estimated 398 million at-risk school-age children have been reached with treatment in Latin American countries leaving 63.7% unreached (Schneider et al., 2011). In a recent study of 14 countries in Latin America and the Caribbean, a prevalence of at least 20% was found in 12 countries, with prevalence reaching as high as 90% in some remote communities (Sabin Vaccine Institute et al., 2011). Any prevalence of 20% or more is regarded as a generalized public health problem by the WHO. Table 5 and figure 5 below show the prevalence of STH infections in the Americas and Caribbean.
Table 5: Prevalence of STH infections in highly affected countries in the Americas (Sabin Vaccine Institute, Global Network for Neglected Tropical Diseases et al. 2011)

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence</th>
<th>Country</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolivia</td>
<td>34.9%</td>
<td>Haiti</td>
<td>51.0%</td>
</tr>
<tr>
<td>Brazil</td>
<td>19.0%</td>
<td>Honduras</td>
<td>62.5%</td>
</tr>
<tr>
<td>Colombia</td>
<td>30.0%</td>
<td>Mexico</td>
<td>8.2%</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>30.3%</td>
<td>Nicaragua</td>
<td>53.5%</td>
</tr>
<tr>
<td>Ecuador</td>
<td>49.8%</td>
<td>St. Lucia</td>
<td>40.0%</td>
</tr>
<tr>
<td>Guatemala</td>
<td>40.4%</td>
<td>Suriname</td>
<td>39.5%</td>
</tr>
<tr>
<td>Guyana</td>
<td>25.2%</td>
<td>Venezuela</td>
<td>11.0%</td>
</tr>
</tbody>
</table>

Figure 5: Prevalence of STH infections in Latin America and the Caribbean, 1998 – 2007 (Schneider et al., 2011)
Prevalence of STH in Honduras, where this study is to be done is 62.5% as shown in table 5 above. Prevalences at the first sub national administrative levels (the departments), ranges from 12.2 – 97% (Schneider et al., 2011). Several studies have demonstrated the high prevalence of STH infections in Honduras, although very few papers have been published in this regards. Many of these studies have been done on hospital populations or in specific populations with data that cannot be extrapolated to the general population (Kaminsky, 1999, 2000; Kaminsky and Lupiac, 2011). Hospital and clinical records show varying rates of distribution in different regions and among different age groups (Kaminsky et al., 2004; PAHO, 2011).

Some studies from Honduras have been found in international databases. Although they are majorly concerned with taeniasis-cysticercosis studies, some do actually make reference to STH infections in their study populations. For example, a study done in 562 people in a rural population showed a prevalence of 85% for *T. trichiura*, 67% for *A. lumbricoides*, 12% for hookworms and 4% for Strongyloides (Sanchez et al., 1997). Another study among 72 children from the island of Tigre, Ampala in 1996 showed a 19.4% prevalence for *T. trichiura*, 18% for *A. lumbricoides* and 0% for hookworm (Kaminsky and Retes, 2000). Another study done in 1998 among 2 – 12 year olds in four rural communities of the Department of Francisco Morazán, showed a prevalence rate of 45% and 38% for *A. lumbricoides* and *T. trichiura* respectively (Smith et al., 2001). In 2006, Wilfredo Sosa for his undergraduate thesis for the degree in Microbiology conducted a prevalence study for STH infections among 340 children from 9 rural communities in Macuelizo valley, Santa Barbara and found a prevalence of 24% for *A. lumbricoides*, 61% for *T. trichiura* and 5% for hookworms.
(Sosa, 2007). And in a 2001 report from the Honduran Ministry of Health report, a prevalence of 36% for *A. lumbricoides*, 52% for *T. trichiura* and 16% for hookworms was given for a study done among 1,197 study participants aged 9 and 10 years old from 5 different geographical areas of Honduras (Zuniga et al., 2003).

Government efforts to control STH infections in Honduras began in 1998 with the establishment of the Healthy School program, where antihelminthic drugs are distributed to children through the school lunch program (Ministry of Health Honduras, 2011). With the declaration of WHA 54.19, efforts have been made for specific national STH control. To this end, there have been mass campaigns and deworming of school children since 2003. The government also utilizes the primary health care facilities to reach preschool children, children out of school, women of child bearing age and pregnant women. This is usually done during immunization days and distribution of vitamin A (PAHO, 2007).

Despite these efforts, prevalence of STH infections remains high in Honduras. Moreover evaluations of the programmes are not been done or monitored so that success of these interventions in terms of decreasing prevalences or intensities, improving health status, nutritional status and cognitive abilities of children that receive deworming treatment are not known.

STH infections constitute one of the 12 NTDs to be eliminated or drastically reduced from LAC by 2015 according to the pledge made by the directing councils of PAHO and the Pan American Sanitary Bureau (PASB) in 2009. (Ault et al., 2011; Schneider et al., 2011). To achieve this target by 2015, a good knowledge of the actual situation of things in regards to STH infections in LAC will be beneficial. Mapping the disease in order to target communities that are in most need of interventions is desirable.
With little or no information on the association between STH, malnutrition and anemia in Honduras, it will be worthwhile to investigate these situations especially in high risk areas of the country as data generated from such studies will assist control efforts. With these thoughts in mind, therefore, this study seeks to investigate the prevalence of STH infections, malnutrition and growth deficits as well as seek the association of STH infections with malnutrition and growth deficits among school-age children in rural communities of the Department of Olancho, Honduras.
CHAPTER 3: METHODOLOGY

The present study was part of a larger study titled ‘Gender and parasitic diseases: Integrating gender analysis in epidemiological research on parasitic diseases to optimize the impact of prevention and control measures’. This larger study is henceforth referred to as ‘The gender study’ while this thesis’ study is referred to as ‘The present study’.

3.1: Study Area

3.1a Study Community

The gender study was conducted in selected rural communities of the municipality of Catacamas in the Department of Olancho, Honduras. Catacamas is a semi-humid tropical forest agricultural zone. It has an annual precipitation of 1300 mm and an average maximum temperature of 26°C with a relative humidity of 7 (Murphy et al., 1999). The geographical coordinates of Catacamas is 14° 51' 0" North, 85° 50' 0" West. It is about 210Km north-east of the capital of Honduras, Tegucigalpa and it is located in a valley 450m above sea level. It has a total area of 7,173.89Km² and as at 2010, the population stood at 44,198 (Wikipedia, 2011).

The selected rural communities where this study was done are within 2 hours drive from Catacamas; they are, Colonia de Poncaya, Las Lomas de Poncaya, Las Parcelas, Corosito de Poncaya, El Cerro del Vigia, El Hormiguero, Santa Clara and Campamento Viejo. Colonia de Poncaya was the largest community having geographical coordinates of 14°29'89.85" North, 85°47'36.33".

Inhabitants of these rural communities engage in mixed agricultural farming, rearing animals such as cattle, goats, pigs and poultry (chickens and ducks) and growing
crops such as maize and other vegetables as means of livelihood. Others work as traders, labourers while a few work as public service workers. Sources of water for drinking and other household activities for these communities include taps (communal and private), water tankers, wells, pools, streams and rivers.

Figure 6: Study Community
3.1b: Study Population

The target population were children enrolled in rural schools of the area (grades 3-5).

This population was selected because:

1. The gender study focused on the potential effect of gender on deworming that is done through school-based programs, therefore, primary school children enrolled in rural schools in the target communities were invited to participate.

2. Children enrolled in grades 3-5 were invited and interviewed to collect demographical and epidemiological data. At this age, they are old enough to understand the questions and provide reliable answers.

3. STH infections tend to disproportionately affect children of ages 4 to 15 on average more than adults (Hotez, 2008). The peak of infections is reported in school-age children especially for Ascaris and Trichuris (WHO, 2002).

3.2: Study Design

Both the gender and present studies were school-based, cross-sectional studies, designed as explorative and hypothesis generating studies.

3.2a Ethical Approvals

The gender study received ethical approval from all participating institutions, namely;

1) Brock University, St. Catharines, ON. File number - BU 10-161 – Sanchez/Gyorkos Jan 13th 2011;
2) McGill University Health Centre, Montreal, QC File number MUHC 10 -175 – PED Nov. 23rd 2010;

3) Ethics officer, Master Program in Infectious and Zoonotic Diseases of the School of Microbiology National Autonomous University of Honduras, Dr. Vilma Espinoza. File number OF-MEIZ-Dictamen-001-2011

As the study was undertaken during class time at participating schools, authorizations from Schools’ Principals were sought in advance. Also, as the study population comprised minors, both parental consent and children’s assent were required prior to enrolment, as described below in section 3.2c.

**3.2b Sample Size Determination**

Inferences for sample size estimation were performed by Dr. Theresa Gyorkos of McGill University, QC, Canada in the PS software (version 3.0, January 2009, by William D. DuPont and Walton D. Plummer. Jr.) for performing power and sample size determination. This was based on a two-sided chi-square test. Using previous studies in Peru as a reference (Casapia et al., 2006) it was assumed that the prevalence of STH is a conservative 50% and that half of the children in this school-age group will be male and half female. An estimated design effect of 2.7 was used with a significance level of 0.05. A total of 314 participants are therefore needed to detect a minimum risk ratio of 1.5 with 80% power. Accounting for a response rate of at least 80%, based on past school surveys in Peru (Casapia et al., 2006) and Honduras (Dr. Ana Sanchez, personal communication), a target sample of 377 children (314+63) was targeted for enrolment.
3.2c Recruitment of Research Participants

This study was done with the collaboration of the staff of the University of Agriculture - Universidad Nacional de Agricultura (UNA) in Catacamas who has worked with these rural communities for years. UNA partners provided a list of eligible schools, location, enrollment, deworming program status and schools’ principal’s expression of interest in participating. With this information the research team selected the schools to be approached based on two additional inclusion criteria:

a) Size (larger schools were approached first and then researchers proceeded with the smaller ones until the target sample was reached), and

b) No deworming treatment in the last 3 months.

Enrolment of Schools: there were preliminary visits to meet with school principals and provide them with detailed explanations and print material about the study. They were given invitation letters to participate in the study (Appendix 1A1 –Spanish version and 1A2 – English version). Authorizations of their schools to participate in the study were obtained in writing by means of a standardized form provided by the researchers (Appendix 2A1 –Spanish version and 2A2 – English version). Schools with principal authorizations were selected for the study.

Enrollment of Children

Parental Consent: Parents and guardians of children in grades 3-5 were invited to an information session where the objectives of the study were fully explained, making clear the risks and benefits of participating in the study. Parents and guardians who gave oral consent for their children to enrol in the study were provided with an information/invitation/acceptance package (appendices 2B1- Spanish version and 2B2 –
English version). The package contained information on what the study was all about, an invitation to participate and a consent form to which they appended their signature. This was issued in print but was also explained verbally. The research team made sure all questions or concerns were clarified before asking parents and guardians if they consented.

**Children’s Assent:** children, whose parents consented, were invited to participate in the study. For this, an information session was held at the schools, in each grade to be invited. Children who expressed assent in responding to a questionnaire, providing a stool sample and allowing for the collection of a venous blood sample and for the collection of anthropometric measurements were then enrolled in the study. Children assents were obtained verbally and documented through a child assent form (Appendix 2C1 – Spanish version and 2C2 – English version).

### 3.3 Data Collection

#### 3.3a Structured Questionnaires

Demographic and epidemiological data as well as children’s knowledge regarding STH infections were collected using a pre-tested, 30-minute, face-to-face standardized questionnaire - Child’s questionnaire (Appendix 3A). This questionnaire which has both Spanish and English translations was administered in Spanish and was conducted in such a way as not to interfere with school activities. Children’s cleanliness (dirty or clean finger nails) and other risk factors for STH (wearing of shoes) were also observed and recorded. Briefly, the questionnaire was structured into categories as follows: Questions were grouped into the following categories
• **Basic information** such as name, date of birth, age, gender, height (cm), weight (kg). (Questions 1 -7 on the questionnaire).

• **Questions on the children’s perception and knowledge of intestinal parasites** such as - if they knew what worms are, how they could get worms, if they thought worms were good for them and if they knew how to prevent getting them. (Questions 8 – 12 on the questionnaire).

• **Information on factors related to community and household characteristics.** This included but was not exclusive to information on the type of material the floor of the home was made of, source of water, whether they drank treated or untreated water, presence or absence of toilet and latrine facilities, number of children and adults per household, type of household chores the children do, possession of animals and the type of animal and the type of interactions they had with the animals. (Questions 13 – 24 on the questionnaire).

• **Information on factors related to hygiene** such as practice and frequency of open defecation, number of times hands were washed on a daily basis and when this was done (after bathroom use, before eating) and whether this was done with or without soap. They were also asked how often they bathed on a weekly basis and if this was done with or without soap. Other questions included if they bit their nails and if they sucked their fingers and if they ate with their hands. (Questions 25– 32 on the questionnaire).
• **Information on factors related to the type of activities they engage in** were also collected. These included the type of work done if the child worked other than schooling, and how many hours were spent on that job weekly. The type of game they played, and how long per day they engaged in it and if they played in dirt or water. (Questions 33 – 34 on the questionnaire).

• **Information on factors related to the wearing of shoes in and out of the house** was also collected. (Questions 35 – 37 on the questionnaire).

• **Information on factors related to the use of health services** was also obtained. Questions in this category included distance of the health center from the homes, number of times they were sick in the last year, number of times they visited the doctor, health center, or healer, due to sickness or for routine checkup/vaccination and if they have had treatment for intestinal worms and when they were last treated. (Questions 38 – 43 on the questionnaire).

To maintain anonymity, a unique identifier (numerical code) was assigned to each participant. This code was used to label all materials from the child including all specimens (stool and blood) collected from the child, the questionnaire and measurements sheets. The schools’ environmental and sanitary conditions and the status of deworming program were also recorded on a standardized form – School questionnaire (Appendix 3B) through both an interview with the Principal and direct observation. Interview guides to help the interviewers were made available to the interviewers (Appendices 3C1, 3C2 and 3D).
3.3b Anthropometry

Body weight and height measurements were taken for each child to assess growth and nutritional status. Weights were taken using a digital electronic balance to the nearest 0.1 kilogram (kg). The children were measured wearing their school uniforms (light clothing) and without shoes. Height was taken to the nearest 0.1 centimetres (cm) using a height pole mounted on the wall. Each child was measured in a standing and relaxed position while breathing normally. A ruler was placed on the head of the child perpendicular to the measuring pole. Readings were taken at eye level from above the ruler. In order to minimize intra-individual errors, all measurements were taken twice by different members of the research team and the average value calculated and used thereof.

Certain indices are used as indicators of malnutrition in comparison with a healthy reference population. These include stunting (low height for age z-score - HAZ), underweight (low weight for age z-score - WAZ) and thinness (low body-mass-index for age z-score – BMI-for-age z-score). HAZ for stunting indicates past growth failure or chronic malnutrition, while WAZ for underweight is an overall indicator for malnutrition as it assesses both present (acute) and chronic malnutrition or mixed malnutrition (de Onis et al., 1993). WAZ is not recommended for the assessment of growth beyond childhood (> 10 years of age) because of its inability to differentiate between relative height and body mass. Therefore, BMI-for-age z-score is used as a complement to HAZ in the assessment of thinness (low BMI-for age); overweight and obesity (high BMI-for-age) as well as stunting (low HAZ) (de Onis et al., 2007).
The z-score or standard deviation system of comparing a child or group of children to a reference population has been recognized as the best system for analysing and presenting anthropometric data in population based assessments (WHO, 1997). The system expresses an individual anthropometric value as a number of standard deviations (Z-scores) below or above the mean or median of the reference value. It is calculated as the difference between the observed value for an individual and the mean/median value of the reference population divided by the standard deviation (SD) of the reference population (WHO, 1997). Therefore, the WHO uses z-score cut-offs of < -1 SD, < -2 SD and < -3 SD for HAZ, WAZ and BMI-for-age z-score to classify undernutrition into mild, moderate and severe undernutrition respectively (Cogill, 2003). The most commonly used z-score cut-off is < -2 SD for all growth indicators, so that a HAZ, WAZ or BMI-for-age Z score < -2 SD is considered as stunting, underweight or thinness respectively (de Onis et al., 2007; WHO, 1997).

Stunting, underweight (for children ≤ 10 years of age) as well thinness in the study population were determined using Anthroplus 2007 software (version 1.0.4.), and were defined as < -2 standard deviations (SD) height-for-age, weight-for-age, and BMI-for-age z-score respectively of the 2007 WHO growth reference for school-aged children and adolescents (Appendices 4A1 to 4C2).

3.3c Stool Collection and Parasite Determination

After obtaining the children’s assent, each child in the study was given a ‘stool collection kit’ to take home after instructions on how to collect the samples had been explained to them. Each kit, properly labelled with the child’s numeric code, contained

- one disposable ‘chamber pot’ for defecation
- one non sterile wide-mouthed leak-proof container with cover in a plastic bag – for bringing faecal specimen to the investigators

- One wooden spatula for scooping faecal specimen into the container.

A single faecal sample was collected from each child, after which they were taken to the UNA laboratory for analysis using the Kato-Katz technique (Kato and Miura, 1954; Katz et al., 1972) as recommended by the WHO (WHO, 2008). The technique is summarized in appendix 5A. Briefly, with a clean wooden spatula, a portion of the fecal specimen was taken and placed on the lid of the cup. A nylon screen was then placed and pressed over it and scraped with the wooden spatula so that some faecal material could sieve through. This was to help to separate fecal materials from the large debris. The sieved faecal material was then placed into the hole of a standard 41.7 mg Kato-Katz template placed centrally on a 2 X 3 microscope slide. The template hole was completely filled and levelled in order to deliver an approximate 41.7 mg of faecal specimen. The template was taken off the slide leaving behind the 41.7 mg of feces. A strip of cellophane soaked in malachite green-glycerine solution was then placed over the fecal specimen. The slide was flipped over and pressed against the cellophane strip. This was to ensure an even spread of the faecal specimen. The slide was then flipped up again so that the cellophane strip faced up and then a second slide was then used to spread the faecal specimen some more by pressing it over the set up. Eventually the smear resulting was placed over a newspaper in order to check if the prints could be read through the smear. This ensured that the thickness of the smear was alright.

Within 30 mins to 1 hour of preparation, the Kato–Katz slides were examined microscopically in a systematic manner by members of the research team for STH eggs.
Eggs were identified by their characteristic features as the eggs are different for each parasite type (WHO, 1994). Helminth eggs were counted for each species of STHs and multiplied by a factor of 24 in order to get the number of eggs per gram of stool (epg).

Infection intensities were classified as light, moderate, or high according to the WHO criteria as shown in table 3 above. Briefly, for *A. lumbricoides*, an infection of 1-4999 epg is categorized as a light infection; an infection of 5,000 – 49,999 epg is moderate, while a heavy infection will have a ≥ 50,000 epg. For *T. trichiura*, an infection of 1-999 epg is a light infection; an infection of 1,000 – 9,999 epg is moderate, while a heavy infection will have an epg of ≥ 10,000. For hookworms, an infection of 1-1,999 epg is a light infection; an infection of 2,000 – 3,999 epg is moderate, while a heavy infection will have a ≥ 4,000 epg (see table 3 above).

The Kato-Katz method as mentioned earlier is the internationally accepted method for epidemiological field surveys. Although its sensitivity has been questioned, a recent study by Tarafder et al., 2010 using a Bayesian approach in the absence of a gold standard, estimated the sensitivity of the Kato-Katz technique to be 96.9% for *Ascaris*; 91.4% for *Trichuris*; 62.5% for Hookworm and the specificity was estimated to be 96.1% for *Ascaris*; 94.4% for *Trichuris*; 93.8% for Hookworm (Tarafder et al., 2010). The lower sensitivity observed for hookworms was attributed to over clarification (Tarafder et al., 2010).

3.3d: Venous Blood Collection for Haematological and Protein Analysis

Two 3-mL tubes of blood were collected from each child that was willing to give blood. This was done by certified phlebotomists in the research team. The first tube contained no anticoagulant and was used to extract serum for protein analysis. The
second tube containing EDTA (Ethylenediaminetetraacetic acid) anticoagulant was used for haematological analysis.

The tubes without anticoagulant were spun in a centrifuge for 15 mins at 3,000 rpm. After spinning, the sera were carefully separated with plastic transfer pipettes and put into appropriately labelled 2 mL eppendorf vials in aliquots of 1.5 ml. These were properly sealed and taped with scotch tape to prevent leakages. They were then packed in styrofoam boxes and frozen in preparation for shipment to Canada where they were later analyzed for total protein estimation. The EDTA tubes were sent to a private clinical laboratory for analyses of haemoglobin and hematocrit values.

**Haemoglobin and Haematocrit**

Prior arrangements were made with Laboratorio Clinicas El Carmen, a private medical diagnostic laboratory outfit in Catacamas. The EDTA tubes were taken here for the analyses of hemoglobin (Hb) and hematocrit (Hct) values. The BC – 3000Plus Auto Hematology Analyzer by Mindray Medical Instrumentation, was used for the analyses.

Haemoglobin (Hb) concentration is expressed in grams per decilitre (g/dL) while haematocrit levels are expressed in millimoles per litre (mmol/L). Children aged 6 – 11 years old with Hb values lower than 11.5g/dl or Hct values lower than 34% and children aged 12 – 14 years old with Hb values lower than 12g/dL or Hct values lower than 36% were considered anaemic. Table 6 below shows the haemoglobin and haematocrit levels below which anaemia is considered.
Table 6: Haemoglobin and Haematocrit levels for children below which anemia is present in a population. Adapted from (WHO, 2001)

<table>
<thead>
<tr>
<th>Age/gender group</th>
<th>Haemoglobin (g/dL)</th>
<th>Haematocrit (mmol/L)</th>
<th>Haematocrit (l/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children 6 months to 59 months</td>
<td>11.0</td>
<td>6.83</td>
<td>0.33</td>
</tr>
<tr>
<td>Children 5 – 11 years</td>
<td>11.5</td>
<td>7.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Children 12 – 14 years</td>
<td>12.0</td>
<td>7.45</td>
<td>0.36</td>
</tr>
</tbody>
</table>

**Total Protein Determination**

Total protein concentrations were determined by an Atago automatic compensation clinical refractometer (Master-Sur/Nα model). Refractometers work with the principle of light refraction through liquids. Light slows down as it travels from air into a liquid solution. The more concentrated a liquid solution is, the slower light passes through it indicating that the concentration of a liquid solution is related to its refractive index. With this principle, the amount of dissolved solids in liquid solutions such as blood plasma and sera can be determined by a refractometer when light passes through a sample, showing the refracted angle on a scale. Since proteins are the major constituents of blood serum and plasma, their refractive indices will depend mainly on their protein concentration. Appendix 5B shows the standard operating procedure (SOP) for this technique.

A second analysis of the children’s samples was done by the biuret test for total proteins. This was to confirm results obtained by refractometry as the biuret test is the
gold standard test for total proteins. A one-way ANOVA comparing the two methods found no statistically significant difference between the two methods \( F(1,596) = 3.480, p = 0.063 \).

Total protein analysis is one way of assessing nutritional status as it decreases in cases of malnutrition. It is expressed in grams per decilitre (g/dl) and the normal range is 6 – 8.3 g/dl.

### 3.4 Data Analysis

All data were entered into Microsoft office Excel spreadsheet 2007, where they were cleaned by checking for accuracy, errors and missing values. Statistical analyses were done using SPSS for windows ver.20 statistical package.

#### 3.4a Statistical Analyses

Descriptive statistics including means (SD) for continuous variables and frequency (proportion) for categorical variables were used to describe the characteristics of the study population (objectives 1, 2 and 3).

Weight and height measurements were subjected to a reliability test and the inter observer technical error of measurements was done using the Mueller and Martorell method (Mueller and Martorell, 1988). Similarly, total proteins estimation by both the Biuret method and refractometry were compared by a one way ANOVA.

Chi square test of independence was done to show the association between age and gender with infection status (infected vs. non-infected) by all three STHs and by each STH as well as between the dependent variables - stunting, thinness, underweight and anemia with infection status (infected vs. non-infected) by all three STHs and by each
STH. Chi square test of independence was also done to show the association between age (>10 y/o) and gender (male) with growth and nutritional indicators stunting, thinness, underweight and anemia.

The student t test analysis was done in order to compare children mean scores on nutritional indices (HAZ, WAZ, BMIAZ and low total proteins) and parameters for anemia (Hb and Hct) in relation to their STH infection statuses (infected vs. non-infected) by all three STHs and by each STH.

A one way ANOVA was done in order to test differences in means between the 3 different infection statuses (STH negative, mono-parasitism and poly-parasitism) on various dependent variables - HAZ score, BMIAZ score, WAZ score, Hb, Hct and total proteins. In addition, a one way ANOVA was used to test differences in mean HAZ score, BMIAZ score, WAZ score, Hb, Hct and total protein values across the different levels of infection intensity (negative, light, moderate and heavy) for each STH species. And in order to check the trend of the effect of STH infections on the mean values of HAZ, BMIAZ, and WAZ, means plots for HAZ, BMIAZ, and WAZ against intensity of infections for each parasite and against infection status (negative, mono-parasitism and poly-parasitism) were plotted. These are further described in the data analysis plan below.
Table 7: Data Analysis Plan

<table>
<thead>
<tr>
<th>Specific Objective</th>
<th>Source of Data</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective 1:</strong></td>
<td>Determination of infection by Kato-Katz technique. Egg count by Kato-Katz technique. Number of eggs counted multiplied by a factor of 24</td>
<td><strong>Descriptive Statistics:</strong> Prevalence of STH infection, Prevalence by species, Eggs per gram (epg) in stool, Arithmetic mean epg for each parasite</td>
</tr>
<tr>
<td>To determine the prevalence of STH among school children in rural Honduras</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Objective 2:</strong></td>
<td>Total proteins values, Hemoglobin (Hb) values, Hematocrit (Hct) values</td>
<td><strong>Descriptive Statistics:</strong> Prevalence of low total protein levels (&lt; 6 g/dL), Prevalence of low Hb values (&lt; 11.5 g/dL), Prevalence of low Hct values (&lt; 7.13 mmol/L)</td>
</tr>
<tr>
<td>To assess the nutritional status/prevalence of malnutrition including anemia among school-age children in rural Honduras</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Objective 3:</strong></td>
<td>Z-scores of anthropometric measures (HAZ, WAZ, BMI-for-age)</td>
<td><strong>Descriptive Statistics:</strong> Prevalence of stunting (HAZ &lt; -2 SD), Prevalence of thinness (BMI-for-age z-score &lt; -2 SD), Prevalence of underweight (WAZ &lt; -2 SD)</td>
</tr>
<tr>
<td>To assess the prevalence of growth deficits among school-age children in rural Honduras</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Objective 4:</strong></td>
<td><strong>Independent-Variables</strong> Arithmetic mean epg, STH infection intensities and categories</td>
<td><strong>Chi squared tests</strong>, <strong>Student’s t test</strong>, <strong>One way ANOVA</strong>, <strong>Means plots</strong></td>
</tr>
<tr>
<td>To examine whether STH infections are associated with malnutrition and growth deficits among school-age children of rural Honduras</td>
<td><strong>Dependent Variables</strong> Total protein concentration, Hb and Hct values, HAZ, WAZ, BMI-for-age z-score</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 4: RESULTS

4.1 Study Participation

The field implementation of the study took place during February and March 2011. The schools of the following communities in the Department of Olancho were visited: Colonia de Poncaya, Las Lomas de Poncaya, Las Parcelas, Corosito de Poncaya, El Cerro del Vigia, El Hormiguero and Campamento Viejo. According to school records, the number of potential participants in grades 3 to 5 among these schools was 445 children. The parents of 387 children provided written informed consent for their children to participate in the study but only 357 (92%) children assented to participate. The final sample for the study was 320 children as 37 were dropped due to insufficient or no stool sample provided (n=20), or unreliable Kato-Katz results (n=17). Five children declined blood collection but they were kept among the final sample since their remaining data was complete. Figure 7 shows a flow chart of participants’ enrollment in the study.

An overall response rate of 89% and 92% was obtained from parents and children respectively. It is important to note that the number of participants that dropped out from the study was not voluntary drop-outs, but rather due to technical reasons or insufficient samples (see box 6 in figure 7). It is good to note as well that, according to sample size calculations, a total of 314 participants were needed to detect a minimum risk ratio of 1.5 with 80% power, therefore, with 320 participants, a 100% enrolment of the target sample was achieved.

In total, 320 children (aged 7-14 years, mean 9.76 ± 1.4) completed the study, 166 (52%) were boys and 154 (48%) were girls. The children were grouped into two age
groups (7 – 10 y/o and >10 y/o) because older children are believed to have higher levels of hygiene than younger children and STH infections are believed to decrease with age.

The household conditions of the children were typical of rural areas in Honduras: 32% had no electricity, 14% had no access to tap water, 31% had total or partial earthen floor and 12% had no sanitary facilities for stool disposal with 15.6% reporting open defecation always, and some other 12.8% open defecated sometimes. And as for history of worms, 78.8% reported having had worms in the past, with 85.9% reporting past treatment for worms. As well, 62% of children reported more than 5 persons living in their homes.

Interviews with the schools’ principals yielded some interesting observations. Five of the seven schools enrolled in the study had on going deworming programs some starting as far back as 2007. Frequency of deworming was as frequent as twice a year for two schools and once a year for three schools. The last deworming treatment had been within the last 4 – 6 months for four schools; only one school reported a last deworming treatment of 2 years prior to the study.
Visits to rural communities in the Department of Olancho, Honduras
Eligible participants in grades 3 – 5 [n = 445]

Written informed parental consents received for children’s participation in the study [n = 387]

Children’s assents received and enrolled in the study [n = 357]

Children with complete data: Written informed consents, child assents, complete questionnaires, parasitological and anthropometric measurement data [n = 320]

- Dropped: Insufficient or no stool sample n = 20
- Unreliable Kato-Katz results n = 17
  Total dropped [n = 37]

Blood work declined, (Still included in the study) [n = 5]

Final study population [n = 320]

With blood work [n = 315]

Figure 7: Flow chart of study participation of children from 7 rural communities of the Department of Olancho, Honduras, 2011
4.2 Descriptive Statistics

4.2a Prevalence of STHs among study participants

A total of 232 children were infected with one or more STH for an overall point prevalence for any STH infection of 72.5% (95% CI 67.6 - 77.4). Specifically, the prevalence for *Trichuris trichiura*, *Ascaris lumbricoides*, and hookworms was 66.9%, 30.3% and 15.9%, respectively (Figure 8).

![Figure 8: Point prevalence of Soil-transmitted-helminth infections among 320 school-age children in rural communities of the Department of Olancho, Honduras, 2011.](image)

Among the 166 boys in the study, 122 were infected with any of the three parasites giving a prevalence of 73.5% (95% CI 66.8 – 80.2) in boys, while 110 girls of 154 in the study were infected with any of the three parasites, giving a prevalence of 71.4% (95% CI 64.3 – 78.6). Prevalence of STH by age and gender as presented in Table 8, shows that
of the 122 infected boys, 112 (91.8%) had *T. trichiura*, 56 (45.9%) had *A. lumbricoides* and 34 (27.9%) had hookworms. On the other hand, of the 110 infected girls, 102 (97.7%) had *T. trichiura*, 41 (37.3%) had *A. lumbricoides* while 17 (15.5%) had hookworms. Infections and age were statistically associated: in terms of infection with any of the three STHs, 161 (68.8%) of the 7-10 y/o were infected while 71 (82.6%) of the >10 y/o were infected. The difference in proportions was statistically significant ($\chi^2(1, N=320) = 5.97, p = 0.015$). The same trend of infection increasing with age was observed with specific infection with *T. trichiura* and hookworms. However, the reverse was the case for *A. lumbricoides* infection in which case, 28.7% of the younger girls were infected as compared to 20.5% of the older girls.

Table 8: Prevalence of Soil-transmitted-helminth infections by age and gender in 320 school-aged children from rural communities of the Department of Olancho, Honduras, 2011

<table>
<thead>
<tr>
<th></th>
<th>Any STH†</th>
<th><em>T. trichiura</em></th>
<th><em>A. lumbricoides</em></th>
<th>Hookworm¶</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys (n = 166)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–10 y/o (n = 119)</td>
<td>82 (68.9%)</td>
<td>77 (64%)</td>
<td>39 (32.8%)</td>
<td>21 (17.6%)</td>
</tr>
<tr>
<td>&gt;10 y/o (n=47)</td>
<td>40 (85.1%)</td>
<td>35 (74.5%)</td>
<td>17 (36.2%)</td>
<td>13 (27.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>122 (73.5%)</td>
<td>112/122 (91.8%)</td>
<td>56/122 (45.9%)</td>
<td>34/122 (27.9%)</td>
</tr>
<tr>
<td><strong>Girls (n = 154)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–10 y/o (n = 115)</td>
<td>79 (68.7%)</td>
<td>73 (63.5%)</td>
<td>33 (28.7%)</td>
<td>11 (9.6%)</td>
</tr>
<tr>
<td>&gt;10 y/o (n = 39)</td>
<td>31 (79.5%)</td>
<td>29 (74.4%)</td>
<td>8 (20.5%)</td>
<td>6 (15.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>110 (71.4%)</td>
<td>102/110 (97.7%)</td>
<td>41/110 (37.3%)</td>
<td>17/110 (15.5%)</td>
</tr>
</tbody>
</table>

† Children older than 10 y/o were more likely to be infected with any STH [$\chi^2(1, N=320) = 5.97, p = 0.015$]
¶ Boys of any age were more likely to be infected by hookworms than girls [$\chi^2(1, N=320) = 5.32, p = 0.021$]
Infection and gender were not found to be statistically associated in terms of overall positivity for any STH ($p = 0.679$). However, a closer look of infection by species revealed that boys of any age were more likely to be infected by hookworms than girls ($\chi^2(1, N = 320) = 5.32, p = 0.021$).

As shown in Table 3 (page 34, Chapter 2), intensity of infection can be categorized in 3 levels as light, moderate and heavy depending on the number of eggs counted in the Kato-Katz preparation. In this study, *T. trichiura* and hookworm infections were mostly light (73.4% and 94.1%, respectively) while, *A. lumbricoides* infections were more evenly distributed between light and moderate infections among the children: the proportion of moderate infections (53.6%) was a little higher than that of light infections (40.2%). Proportions of light, moderate and heavy infections for the three STHs are shown in figure 9.

![Figure 9: Intensities of Soil-transmitted-helminth infections among 320 school-age children in rural communities of the department of Olancho, Honduras, 2011](image)
Intensity of infection for each STH species is further explained in Table 9 that shows the actual values of the mean eggs per gram (epg) of faeces at each level of infection intensity, in relation to age and gender.

The mean epgs for light (249.33 ± 231.60) and moderate (2057.21 ± 1125.28) infections of *T. trichiura* were very close to the lower end of the range (1 – 999 epg and 1,000 – 9,999 epg respectively). Since majority (73.4%) of the children with *T. trichiura* infections had light infections, the mean epg suggests that parasite load for *T. trichiura* was mostly very light in the study population. However, the mean epg for heavy infections (27,522 ± 29,921.96) shows a very heavy load of the parasite as this value far exceeds the minimum value in the range (10,000 epg).

The pattern is somewhat different for *A. lumbricoides* infections, in which the mean epg for light infections (1,355.08 ± 1435.85) was on the lower end of the range (1 – 4,999), but the mean epgs for moderate and heavy infections (19,171.15 ± 12,100 and 69,988 ± 15,224.88 respectively) are more on the mid-point to the upper end for their respective ranges (5,000 – 49,999 and > 50,000 respectively). Since more than half of the children with *A. lumbricoides* infection had moderate infection, the mean epg reflects a situation where most of the children (considering carriers of moderate and heavy infections) carry quite a substantial load of *A. lumbricoides*. It was also interesting to note that the younger children were the ones with heavy *A. lumbricoides* infection.

Almost the same pattern as for *A. lumbricoides* was observed for hookworm infections, in which the mean epg for light infections (350 ± 450.36) was very much on the lower end of the range (1 – 1,999) while the mean epgs for moderate and heavy infections (3,240 and 6,444 ± 2,596.50 respectively) were closer to the mid-point to
upper end of the range (2,000 – 3,999 and >4,000 respectively). Finally, just as was found with *T. trichiura* infections, most children with hookworm infection had light infections (94.1%); the mean epg therefore indicates that parasite load for hookworm in the study population was very light.

Table 9: Prevalence and Intensity of each Soil-transmitted-helminth Species in 320 School-age Children from Rural Communities of the Department of Olancho, Honduras, 2011

<table>
<thead>
<tr>
<th></th>
<th>7 – 10 y/o</th>
<th>&gt;10 y/o</th>
<th>Mean epg</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
<td>Girls</td>
<td></td>
</tr>
<tr>
<td><em>T. trichiura</em> (n = 214)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>53</td>
<td>55</td>
<td>29</td>
<td>20</td>
<td>249.33 ± 231.60</td>
</tr>
<tr>
<td>Moderate</td>
<td>23</td>
<td>17</td>
<td>6</td>
<td>7</td>
<td>2057.21 ± 1125.28</td>
</tr>
<tr>
<td>Heavy</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>27,522 ± 29,921.96</td>
</tr>
</tbody>
</table>

| *A. lumbricoides* (n = 97) |     |       |     |       |              |       |
| Light            | 14   | 15    | 6  | 4     | 1,355.08 ± 1435.85 | 39  | 40.2 |
| Moderate         | 21   | 16    | 11 | 4     | 19,171.15 ± 12,100 | 52  | 53.6 |
| Heavy            | 4    | 2     | 0  | 0     | 69,988 ± 15,224.88 | 6   | 6.2  |

| Hookworms (n = 51) |     |       |     |       |              |       |
| Light            | 21   | 9     | 12 | 6     | 350 ± 450.36 | 48  | 94.1 |
| Moderate         | 0    | 1     | 0  | 0     | 3,240    | 1   | 2.0  |
| Heavy            | 0    | 1     | 1  | 0     | 6,444 ± 2,596.50 | 2   | 3.9  |

The data presented so far, shows the prevalence of infection by helminth species, however, almost half of the children were infected by more than one species simultaneously: of the 232 children infected by any STH, 55.6% (95% CI = 49.61 - 62.39)
had monoparasitism (i.e., caused by a single species) whereas 44.4% (95% CI = 37.61-50.39) had polyparasitism (i.e., infections with two or three STH). More details about the frequency and type of STH infections are provided in Table 10.

### Table 10: Cases of Monoparasitism and Polyparasitism among School-age Children in Rural Communities of the Department of Olancho, Honduras (n = 232)

<table>
<thead>
<tr>
<th>Children with monoparasitism [129/232 (55.6%)]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. trichiura</em></td>
<td>113/129</td>
</tr>
<tr>
<td></td>
<td>(87.6%)</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>9/129 (7.0%)</td>
</tr>
<tr>
<td>Hookworms</td>
<td>7/129 (5.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Children with polyparasitism [103/232 (44.4%)]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with double infections</td>
<td>76/103 (73.8%)</td>
</tr>
<tr>
<td>- <em>T. trichiura</em> and <em>A. lumbricoides</em>: 59/76 (57.3%)</td>
<td></td>
</tr>
<tr>
<td>- <em>T. trichiura</em> and Hookworm: 15/76 (19.7%)</td>
<td></td>
</tr>
<tr>
<td>- <em>A. lumbricoides</em> and Hookworm: 2/76 (2.6%)</td>
<td></td>
</tr>
<tr>
<td>Children with triple infections</td>
<td>27/103 (26.2%)</td>
</tr>
</tbody>
</table>

### 4.2b: Assessment of Children’s Growth and Nutritional Status

Anthropometric indicators and total protein estimates were used for the assessment of growth and nutritional status of the studied children; these data are presented in Table 11.
The mean height for the study population was 134.4 cm ± 8.75 and the mean weight was 30.4 kg ± 6.5. The mean height and weight for both boys and girls are shown below;

**Boys:**
- Mean weight = 30.1 kg ± 5.5
- Mean height = 134.4 cm ± 8.7

**Girls:**
- Mean weight = 30.8 kg ± 7.4
- Mean height = 134.4 cm ± 8.8

The growth and nutritional status of most of the studied children were within healthy parameters. Moderate forms of stunting (estimated by HAZ), thinness (estimated by BMI-for-age z score), and underweight (estimated by WAZ), were observed among the children. Eighteen (5.6%) children were stunted and 7 (2.2%) were thin. As earlier explained in chapter 3, WAZ was not calculated for children > 10 as it is not recommended for the assessment of growth beyond childhood (> 10 y/o), therefore WAZ was only calculated for the 7 – 10 y/o children (n = 234) in this study population. Only 3 (1.3%) of them were underweight. In addition 19 (5.9%) children were overweight with a BMI-for-age z score of > +1 SD but < +2 SD and 15 (4.7%) of them were obese (BMI-for-age > +2 SD). The mean HAZ score in the study population was -0.44 ± 0.96 and of the 18 stunted children, there were 9 boys and 9 girls. The mean BMI-for-age z score was -0.04 ± 1.00 and of the 7 thin children, there were 4 boys and 3 girls. The mean WAZ score was -0.09 ± 0.92 and of the 234 children with WAZ scores, 3 (1.3%, 1 boy, 2 girls) were underweight.
Data presented above, is merely descriptive. In section 4.2c, an analysis of nutritional status and growth in relation to STH infection is presented.

Of the 320 children in the study, 315 gave blood samples, but one sample was found to be haemolysed and another sample was not sufficient for the total protein estimation. Therefore, 313 samples were analyzed for total proteins. The normal range for total proteins is 6 – 8.3 g/dl. Any value below 6 g/dl is considered malnourished. For the study population, the mean total protein was 7.5 g/dl ± 0.5. No child had any total protein value below the normal range.

All 315 blood samples collected were analyzed for Hb and Hct values. The mean Hb value for the study population was 12.94 g/dl ± 0.76 and the mean Hct value was 38.99% ± 2.12. Of the 315 children examined, 7 (2.2%) were anemic – 3 boys and 4 girls.

Since anthropometric measures for height and weight were double blind, the inter observer technical error of measurement (TEM) was assessed and a reliability coefficient of 0.97 and 0.96 were obtained for height and weight respectively. A one-way ANOVA comparing the Biuret and refractometry methods for total proteins found no statistically significant difference between the two methods [\( F_{(1,596)} = 3.480, p = 0.063 \)].
Table 11: Anthropometric and Nutritional Statuses of School-age Children in Rural Communities of the Department of Olancho, Honduras, 2011

<table>
<thead>
<tr>
<th></th>
<th>7 – 10 years old</th>
<th>&gt;10 years old</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
</tr>
<tr>
<td>Mean Height (cm)</td>
<td>131.5 ± 7.2</td>
<td>131.3 ± 6.7</td>
<td>141.8 ± 7.9</td>
</tr>
<tr>
<td>Mean Weight (kg)</td>
<td>28.8 ± 4.7</td>
<td>28.7 ± 6.1</td>
<td>33.2 ± 6.0</td>
</tr>
<tr>
<td>Mean HAZ</td>
<td>-0.28 ± 0.9</td>
<td>-0.36 ± 0.9</td>
<td>-0.80 ± 09</td>
</tr>
<tr>
<td>Percent stunted</td>
<td>4/119 (3.4%)</td>
<td>4/115 (3.5%)</td>
<td>5/47 (10.6%)</td>
</tr>
<tr>
<td>Mean BMI-for-age z score</td>
<td>0.17 ± 0.9</td>
<td>-0.02 ± 1.0</td>
<td>-0.64 ± 1.0</td>
</tr>
<tr>
<td>Percent thin</td>
<td>0/119 (0.0%)</td>
<td>3/115 (2.6%)</td>
<td>4/47 (8.5%)</td>
</tr>
<tr>
<td>Mean WAZ</td>
<td>-0.02 ± 0.9</td>
<td>-0.16 ± 1.0</td>
<td>NA</td>
</tr>
<tr>
<td>Percent underweight</td>
<td>1/119 (0.84%)</td>
<td>2/115 (1.74%)</td>
<td>NA</td>
</tr>
<tr>
<td>Mean Total protein (g/dl)</td>
<td>(n = 118) 7.4 ± 0.3</td>
<td>(n = 110) 7.5 ± 0.6</td>
<td>(n = 46) 7.5 ± 0.4</td>
</tr>
<tr>
<td>Percent low Total protein</td>
<td>0/118 (0%)</td>
<td>0/46 (0%)</td>
<td>0/110 (0%)</td>
</tr>
<tr>
<td>Mean Hb (g/dl)</td>
<td>(n = 118) 13 ± 0.8</td>
<td>(n = 111) 12.86 ± 0.7</td>
<td>(n = 47) 13.1 ± 0.7</td>
</tr>
<tr>
<td>Mean Hct (%)</td>
<td>(n = 118) 39 ± 2.3</td>
<td>(n = 111) 38.83 ± 1.9</td>
<td>(n = 47) 39.3 ± 2.0</td>
</tr>
<tr>
<td>Percent anemic</td>
<td>3/118 (2.54%)</td>
<td>3/111 (2.7%)</td>
<td>0/47 (0%)</td>
</tr>
</tbody>
</table>

A total of 33 (10.3%) children had one form of nutritional or growth deficits. Of these, 5 (15.2%) were negative for any STH, while 28 (84.8%) were infected with one or more STH. Fifteen (45.5%) children were monoparasitized, while 13 (39.4%) were polyparasitized. Moderate to heavy infections were observed in 10 (30.3%) of the children. Two children were both stunted and thin. One was negative for any STH, while the other had light infections of Trichuris. The characteristics of these children are shown in table 12 below.
<table>
<thead>
<tr>
<th>ID</th>
<th>Stunted</th>
<th>Anemic</th>
<th>Thin</th>
<th>Underweight</th>
<th>Infection Status</th>
<th>Ascaris</th>
<th>Trichuris</th>
<th>Hookworms</th>
</tr>
</thead>
<tbody>
<tr>
<td>01-29</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
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<td>08-16</td>
<td>Yes</td>
<td>No</td>
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<td>No</td>
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<td>Negative</td>
<td>Negative</td>
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<td>08-52</td>
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<td>No</td>
<td>No</td>
<td>No</td>
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<td>Negative</td>
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<td>No</td>
<td>No</td>
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<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<td>No</td>
<td>No</td>
<td>No</td>
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<td>Light</td>
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<td>Negative</td>
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<tr>
<td>03-83</td>
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<td>No</td>
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<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
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<td>Negative</td>
</tr>
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<td>04-03</td>
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<td>Yes</td>
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<td>Monoparasitized</td>
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</tr>
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<td>No</td>
<td>No</td>
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<td>Negative</td>
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<td>No</td>
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<td>Light</td>
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<td>01-49</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
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<td>Moderate</td>
<td>Light</td>
</tr>
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<td>03-36</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Light</td>
<td>Light</td>
<td>Negative</td>
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<tr>
<td>03-37</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Moderate</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>03-42</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Moderate</td>
<td>Light</td>
<td>Light</td>
</tr>
<tr>
<td>03-67</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
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<td>Light</td>
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<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
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<td>Heavy</td>
<td>Light</td>
</tr>
<tr>
<td>08-20</td>
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<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Moderate</td>
<td>Heavy</td>
<td>Light</td>
</tr>
<tr>
<td>08-60</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Moderate</td>
<td>Negative</td>
<td>Heavy</td>
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<td>05-01</td>
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<td>No</td>
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<tr>
<td>01-63</td>
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<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>03-47</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>05-10</td>
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<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>05-17</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Moderate</td>
<td>Negative</td>
</tr>
<tr>
<td>08-06</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>08-76</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Light</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>04-07</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
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<tr>
<td>08-77</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>02-04</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Light</td>
</tr>
<tr>
<td>03-58</td>
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<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Polyparasitized</td>
<td>Moderate</td>
<td>Light</td>
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<td>04-05</td>
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<td>No</td>
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<td>Negative</td>
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<tr>
<td>01-42</td>
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<td>Yes</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>03-87</td>
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<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
<tr>
<td>08-03</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Monoparasitized</td>
<td>Negative</td>
<td>Light</td>
<td>Negative</td>
</tr>
</tbody>
</table>
4.3: Finding Associations between STH infections and Nutritional and Growth Status.

4.3a: Associations between STH Infections and Children’s Nutritional Status

Chi square test of independence was done to show the association between the age (> 10 y/o) and gender (male) with infection status (infected vs. non-infected) by all three STHs and by each STH. Significant associations were found between the following:

- Age (>10y/o) and infection with any of the three parasites \( \chi^2 (1, N = 320) = 5.97, p = 0.015 \); children >10 y/o were twice more likely to be infected with any of the three parasites than children 7 – 10 y/o (OR = 2.146, 95% CI = 1.2 - 4.0).

- Gender (boys) and hookworm infection \( \chi^2 (1, N = 320) = 5.32, p = 0.021 \); boys in general were twice more likely to have hookworms than girls (OR = 2.076, 95% CI = 1.1 - 3.9).

A marginally significant association was found between the following:

- Age (>10 y/o) and \textit{T. trichiura} infection \( \chi^2 (1, N = 320) = 3.02, p = 0.082 \); children >10 y/o were about 1.5 times more likely to be infected with \textit{T. trichiura} than children 7 – 10 y/o (OR = 1.63, 95% CI = 0.94 – 2.83).

- Age (>10 y/o) and hookworm infection \( \chi^2 (1, N = 320) = 3.33, p = 0.068 \); again, children >10 y/o were almost twice more likely to be infected with hookworms than 7 – 10 y/o children (OR = 1.79, 95% CI = 0.95 – 3.37).
Chi square test of independence was also done to show the association between the dependent variables: stunting, thinness, underweight and anemia with infection status (infected vs. non-infected) by all three STHs and by each STH. Significant associations were found between the following:

- Stunting and hookworm infection \( \chi^2 (1, N = 320) = 4.31, p = 0.049 \); stunted children were almost 3 times more likely to be infected with hookworms than non-stunted children (OR=2.856, 95% CI = 1.02-8.00).

- Underweight and *A. lumbricoides* infection \( \chi^2 (1, N = 234) = 6.84, p = 0.028 \). An odds ratio could not be calculated for this association as all 3 underweight children were infected with *A. lumbricoides*, so the uninfected cell had zero entry.

Independent sample *t* test analysis showed that children with *T. trichiura* infection had statistically lower mean WAZ score (\( M = -0.18, SD = 0.91 \)) than children without *T. trichiura* infection (\( M = 0.08, SD = 0.93 \)), \( t_{(232)} = 2.071, p = 0.040 \). The same observation was made for children with hookworm infection, who had a significantly lower mean WAZ score (\( M = -0.43, SD = 0.71 \)) than children without hookworm infection (\( M = -0.03, SD = 0.94 \)), \( t_{(232)} = 2.310, p = 0.022 \).

A one way ANOVA was done in order to test differences in means between the 3 different infection status (STH negative, mono-parasitism and poly-parasitism) on various dependent variables - HAZ score, BMIAZ score, WAZ score, Hb, Hct and total proteins.
The mean WAZ scores differed significantly across the various infection status, $F_{(2,231)} = 4.15, p = 0.017$. The mean WAZ (0.05 ± 0.89) of STH negative children was significantly the highest followed by that of mono-parasitized children (0.01 ± 0.93), and then that of poly-parasitized children (-0.34± 9.91). A marginal significant difference in the mean HAZ score across the different infection status was also observed, $F_{(2,317)} = 2.30, p = 0.100$. The mean HAZ of STH negative children was significantly the highest (-0.03 ± 0.94), followed by that of mono-parasitized children (-0.42 ± 0.96), while poly-parasitized children had the least mean HAZ score (-0.60± 0.98). No other significant difference was observed in the means of the other tested variables across the 3 different infection status. Statistical analyses and $p$ values associated with STH infection status (infected vs. non-infected) is shown in table 13 below.
Table 13: Statistical Analysis and $p$ Values Associated with STH Infection Status (Infected vs. Non-Infected, Mono-parasitized and Poly-parasitized) Among School-Age Children from Rural Communities of the Department of Olancho, Honduras, 2011

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistical Analysis</th>
<th>Any STH</th>
<th>Trichiura</th>
<th>Ascaris</th>
<th>Hookworm</th>
<th>Infection Statuses$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&gt;10y/o)</td>
<td>Chi Square</td>
<td>0.015*</td>
<td>0.082**</td>
<td>0.769</td>
<td>0.068**</td>
<td>0.123</td>
</tr>
<tr>
<td>Gender (Boys)</td>
<td>Chi Square</td>
<td>0.679</td>
<td>0.814</td>
<td>0.167</td>
<td>0.021*</td>
<td>NA</td>
</tr>
<tr>
<td>HAZ</td>
<td>Ind. t-test</td>
<td>0.097**</td>
<td>0.091**</td>
<td>1.920</td>
<td>0.053**</td>
<td>0.100**</td>
</tr>
<tr>
<td>Stunting</td>
<td>Chi Square</td>
<td>0.788*</td>
<td>0.620</td>
<td>0.415</td>
<td>0.049**</td>
<td>NA</td>
</tr>
<tr>
<td>BMIAZ</td>
<td>Ind. t-test</td>
<td>0.492</td>
<td>0.260</td>
<td>0.167</td>
<td>0.287</td>
<td>0.145</td>
</tr>
<tr>
<td>Thinness</td>
<td>Fisher’s exact test</td>
<td>0.678</td>
<td>0.432</td>
<td>0.437</td>
<td>1.000</td>
<td>NA</td>
</tr>
<tr>
<td>WAZ</td>
<td>Ind. t-test</td>
<td>0.131</td>
<td>0.040*</td>
<td>0.075**</td>
<td>0.022*</td>
<td>0.017*</td>
</tr>
<tr>
<td>Underweight</td>
<td>Chi square</td>
<td>0.554</td>
<td>0.555</td>
<td>0.028**</td>
<td>0.358</td>
<td>NA</td>
</tr>
<tr>
<td>Hb</td>
<td>Ind. t-test</td>
<td>0.607</td>
<td>0.801</td>
<td>0.155</td>
<td>0.209</td>
<td>0.785</td>
</tr>
<tr>
<td>Hct</td>
<td>Ind. t-test</td>
<td>0.255</td>
<td>0.821</td>
<td>0.013*</td>
<td>0.922</td>
<td>0.212</td>
</tr>
<tr>
<td>Anemia</td>
<td>Fisher’s exact test</td>
<td>0.434</td>
<td>0.287</td>
<td>0.339</td>
<td>0.358</td>
<td>NA</td>
</tr>
<tr>
<td>Total proteins</td>
<td>Ind. t-test</td>
<td>0.482</td>
<td>0.775</td>
<td>0.300</td>
<td>0.237</td>
<td>0.207</td>
</tr>
</tbody>
</table>

* $p < 0.05$  ** $p < 0.15$  $^a$ = ANOVA  $^\psi$ = Fisher’s exact test

Chi square test of independence was also done to determine whether or not an association existed between age (>10 y/o) and gender (male) with growth and nutritional indicators: stunting, thinness, underweight and anemia with infection status as shown in Table 14. The only significant association found was between age (>10 y/o) and stunting
$\chi^2_{(1, N = 320)} = 7.98, p = 0.011$; children >10 y/o were almost four times more likely to be stunted than 7 – 10 y/o children (OR = 3.72, 95% CI = 1.42 – 9.76)

Table 14: Chi square Analysis of Growth and Nutritional Indicators in Relation to Age and Gender among School-age Children from Rural Communities of the Department of Olancho, Honduras, 2011

<table>
<thead>
<tr>
<th></th>
<th>Stunting</th>
<th>Underweight</th>
<th>Thinness</th>
<th>Anemia</th>
<th>Low total proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi Square value</td>
<td>0.027</td>
<td>0.373</td>
<td>0.080</td>
<td>0.260</td>
<td>NA</td>
</tr>
<tr>
<td>p value</td>
<td>0.870</td>
<td>0.617</td>
<td>1.00</td>
<td>0.713</td>
<td>NA</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi Square value</td>
<td>7.98</td>
<td>NA</td>
<td>3.34</td>
<td>0.611</td>
<td>NA</td>
</tr>
<tr>
<td>p value</td>
<td>0.011*</td>
<td>NA</td>
<td>0.087**</td>
<td>0.678</td>
<td>NA</td>
</tr>
</tbody>
</table>

*p = < 0.05  NA – values here are a constant, chi square test not done

4.3b: Relationship between Intensity of Infection with Growth and Nutritional Indicators

Additionally, a one way ANOVA was used to test differences in mean HAZ score, BMIAZ score, WAZ score, Hb, Hct and total protein values across the different levels of infection intensity (negative, light, moderate and heavy) for each STH species - associated $F$ and $p$ values are shown in table 15. The mean HAZ and WAZ scores differed significantly across the different infection intensities for $T. trichiura \ [(F_{(3,316)} = 5.31, p = 0.001) \text{ and } (F_{(3,230)} = 3.19, p = 0.025), \text{ respectively}]. \text{ A marginally significant difference in the mean WAZ score across the different infection intensities for } A.$
lumbricoides, $F_{(2,231)} = 2.392, p = 0.069$ was also obtained. No statistically significant difference was demonstrated with the other dependent variables.

Table 15: One-way ANOVA Analysis of STH Levels of Infection (Negative, Light, Moderate and Heavy) Among School-Age Children from Rural Communities of the Department of Olancho, Honduras, 2011

<table>
<thead>
<tr>
<th></th>
<th>T. trichiura</th>
<th>A. lumbricoides</th>
<th>Hookworms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$p$</td>
<td>$F$</td>
</tr>
<tr>
<td>HAZ</td>
<td>5.314</td>
<td>0.001*</td>
<td>1.814</td>
</tr>
<tr>
<td>WAZ</td>
<td>3.185</td>
<td>0.025*</td>
<td>2.392</td>
</tr>
<tr>
<td>BMIAZ</td>
<td>0.793</td>
<td>0.499</td>
<td>0.720</td>
</tr>
<tr>
<td>Hb</td>
<td>0.329</td>
<td>0.805</td>
<td>0.794</td>
</tr>
<tr>
<td>Hct</td>
<td>0.104</td>
<td>0.958</td>
<td>2.137</td>
</tr>
<tr>
<td>Total protein</td>
<td>0.415</td>
<td>0.742</td>
<td>3.383</td>
</tr>
</tbody>
</table>

* $p < 0.05$  ** $p < 0.15$

4.3c Means Plots: HAZ, WAZ and BMIAZ against STH Levels of Infection and Infection Status

In addition to exploring potential associations between intensity of infections with the means for HAZ, BMIAZ, and WAZ, a comparison of these means against intensity of infections for each parasite and against infection status (negative, mono-parasitism and poly-parasitism) was also done by means of plot diagrams as shown in figure 10. Some interesting trends were observed; as parasite load increased (intensity of infection) – plots A, B and C, and the more concurrent infection a child had (poly-parasitism) – plot D, it was observed that the means for these three nutritional indices decreased.
Figure 10: Means Plots of HAZ, WAZ and BMIAZ vs. STH Levels of Infection and Infection Status

As shown, this observation provides evidence that concurrent infections (though light) may be having as much effect on the children’s growth and nutrition as much as any heavy single infection.
CHAPTER 5: DISCUSSION

This is to our knowledge, the first Honduran attempt to undertake a systematic assessment of the relationship between STH infections and children’s nutritional status and growth. The findings obtained in the study add valuable insight into the potential clinical significance of low–to-moderate intensity infections and begins to reveal the subtle effects of concurrent infections in children’s health. Moreover, this study fills a critical gap in the body of knowledge of STH infections’ prevalence in Latin America and the Caribbean (LAC) in general, and Honduras in particular. The paucity of information is a serious challenge for LAC countries to plan, deploy and monitor STH control initiatives and it is hoped that the results of the present study will be particularly useful in informing policy makers and other stakeholders in Honduras.

Below, the findings of the present study are analyzed in detail taking into consideration the Honduran context and in light of recent relevant literature.

5.1: Prevalence and intensity of STH infections

5.1a: Prevalence

The data obtained in this study revealed a very high prevalence (72.5%) of STH infections in the studied communities. As mentioned before, Honduras is considered endemic for soil-transmitted helminthiasis with active transmission across the country (PAHO, 2009). The main technical health organization for the Americas, PAHO, reports that most Honduran school-age children live in areas where the prevalence is > 20% and that around 550,000 school-age children are at risk of infection (PAHO, 2009).
Additional reports based on a few studies and a small national survey led to the estimation of a prevalence ranging between 12.2% to 97% and a national average of 62% (Schneider et al., 2011). Endorsed by PAHO, the Honduras Ministry of Health set out to decrease its STH prevalence following the WHA 2001 recommendation of implementing a national deworming program that, as presented earlier, have yet to achieve the desired steady 75% coverage (Ault et al., 2011; Schneider et al., 2011). In fact, since its implementation, Honduras has reported an upward trend of deworming coverage increasing from an initial 36% in 2003 to an absolute 100% in 2009, followed by a decrease to 71.23% in 2010 (the last year reported) (WHO, 2012a). Even with its fluctuations -mainly related to the availability of treatment and administrative issues- these coverage data are encouraging; however, the success of the preventive STH chemotherapy in Honduras remains to be proven as consistent monitoring is not performed (Ault et al., 2011; Ministry of Health Honduras, 2011). In the present study, 5 of the 7 participating schools reported some form of mass-deworming during the past year and yet, the prevalence figures obtained are far above the target of reducing prevalence among school-age children in high risk areas where prevalence is >50% to 20% or less, figure above which STH infections are regarded as a public health problem (Schneider et al., 2011; WHO, 2006).

Analyzing the overall prevalence found in the present study with data from 3 national surveys conducted between 2000 and 2006 by the Honduran Ministry of Health to determine STH prevalence in school-age children, yields interesting information.

The first survey was carried out in 2000-2001 and sampled children from 11 of the 18 departments; the second one, carried out in 2005-2006 sampled children from 14
departments. The third one sampled children from all 18 departments in the country. The overall national averages found estimated by these surveys show an interesting downward trend: 70.4% and 51.3%, and 43.5%, respectively (Ministry of Health Honduras, 2001, 2006, 2011). Such decrease is in fact consistent with global estimates that suggest that since 1994 there has been a marked decline in prevalence and in absolute numbers for all 3 species of STH in both the Americas and Asia (de Silva, 2003). In contrast, the present study found a much higher overall prevalence but the difference may be explained by the small geographic area covered, as there are localized ecological areas where endemicity may be higher than the national averages (Schneider et al., 2011). Of interest, however, is the fact that the 2011 survey reports a much lower STH prevalence (43.8%) for the Department of Olancho, the same political division in which the present study took place (Ministry of Health Honduras, 2011). It is possible that the studied communities are indeed a “hot spot” for STH transmission in Olancho and this is why higher general and specific prevalences were obtained. However, an additional factor may also be at play: a small research study is likely to have more laboratory capacity and personnel and its findings may be more accurate thus reflecting prevalence estimates closer to the real values.

In regards to the frequency by parasite species, the national surveys also demonstrate a consistent reduction of all 3 STH showing, respectively, the following point-prevalences: for *T. trichiura*, 51.7%, 38.3%, and 34%; for *A. lumbricoides*, 36.5%, 35%, and 22.3%; and for hookworms, 15.7%, 5.2%, and 0.86%. The findings of the present study are considerably higher for *T. trichiura* (66.9%), somewhat similar for *A. lumbricoides* (29.4%) and remarkably different for hookworms (15.9%). The reasons
mentioned above can also account for the latter discrepancy but a more technical explanation might be responsible. It is well known that over-clarification of the sample can lead to under-estimation of hookworm eggs (Zamen and Cheong, 1967) and in the present study, some Kato-Katz preparations had to be repeated upon suspicion of over clarification of the sample. Indeed, a number of samples became positive for hookworms in the second preparation (data not shown). In national surveys where hundreds, if not thousands, of samples must be analyzed in a short period of time under field conditions hookworm eggs detection may be compromised. If this were the case, an underestimation of hookworm infections may lead to equivocal conclusions when monitoring deworming strategies and all efforts should be made to verify the data.

In terms of the different proportions found in the present study for trichuriasis versus ascariasis (66.9% vs. 29.4%), the two most common STH infections in the Americas (Saboyá et al., 2011), it is noteworthy to mention that the predominance of *T. trichiura* is a phenomenon recorded in previous Honduran studies (Kaminsky and Retes, 2000; Sanchez et al., 1997; Sosa, 2007) as well as in the national surveys mentioned above. Whereas in past decades, both species were thought to be distributed somewhat similarly (Cooper and Bundy, 1988), more recent estimates point to an increasing predominance of *T. trichiura*. For example, de Silva et al (2003) in their important update of the STH global prevalence provide an updated estimate of 100 million cases of trichuriasis and 84 million cases of ascariasis for the LAC region (de Silva et al., 2003; Hotez et al., 2008a). The reasons for the current preponderance of *T. trichiura* infections are not clear. One plausible explanation is that most of the latest data is based on laboratory results obtained with the Kato-Katz method, which shows higher sensitivity
for light infections than the direct method widely used prior the 1990s (WHO, 1994). Also, the widespread use of single-dose Albendazole for the deworming campaigns in endemic countries might be exerting a positive selective pressure upon *T. trichiura* since the drug is less efficacious for this parasite than for *A. lumbricoides* (Vercruysse et al., 2011). More research is needed to elucidate the reasons for *T. trichiura* predominance and if this is the case, what are the epidemiological and health implications?

5.1b: Intensity of infection

According to the number of eggs per gram counted in the Kato-Katz preparation, the majority of *Trichuris trichiura* and hookworm infections were categorized as light (73.4% and 94.1% respectively). For *A. lumbricoides* this was not the case as the proportions for light and moderate infections was less disparate (40.2% and 53.6%, respectively). Whether or not, the fact that most infections could be categorized as light or moderate is encouraging depending on the point of view. While low-parasitic burden in a community is an indication of endemicity and chronicity (Crompton et al., 2003; Moreau and Chauvin, 2010), from the public health point of view it is often interpreted as low health impact and therefore low priority (Ezeamama et al., 2005b; Pullan and Brooker, 2008). Moreover, in communities were deworming programs are implemented, low intensity infections might be interpreted as a success indicator (Anees et al., 2003; Knopp et al., 2011b). Nevertheless, light to moderate intensity infections (especially in cases of co-infection) have been demonstrated to have as much detrimental effects on health as high-intensity-single infections in some studies (Ezeamama et al., 2008; Mupfasoni et al., 2009; Pullan and Brooker, 2008). In already malnourished children,
relatively light infections can contribute to growth deficits (Crompton and Nesheim, 2002; Stephenson et al., 2000b).

5.1c: Polyparasitism

Almost half (44.4%) of the infected children were parasitized with multiple STHs, of which 73.8% were double and 26.2% triple infections. Since the 3 STH studied in this thesis share similar transmission mechanisms and are distributed in similar ecological areas (although with greater variations for hookworms), polyparasitism seems to be the usual pattern in endemic areas (Ezeamama et al., 2005b; Mupfasoni et al., 2009; Tchuem Tchuente et al., 2003). Considering the effect that single STH infections can have on their host, it is likely that multiple infections may have an additive or multiplicative effect that can cause negative effects when certain thresholds are exceeded (Pullan and Brooker, 2008; Steinmann et al., 2010). However, recent findings by some researchers suggest a synergistic effect as their findings demonstrate that polyparasitism, even of light intensity have adverse health effect on their hosts (Ezeamama et al., 2005a; Ezeamama et al., 2005b; Mupfasoni et al., 2009; Sorensen et al., 2011).

The Th2 dominated cytokine response to intestinal helminths is one way through which malnutrition associated with polyparasitism is believed to be mediated, leading to disruption in nutrition by affecting gut function (McDermott et al., 2003). In children, the proinflammatory cytokines and acute phase proteins may cause anorexia or loss of appetites, which may lead to growth faltering (Northrop-Clewes et al., 2001). They can also cause protein loss, elevated levels of resting energy expenditure and may also affect anaemia in chronic cases (Wright et al., 2009). As well, this immune response to STH has
been implicated in the modulation of the human immune response to common coinfections such as malaria, TB and HIV/AIDS (Wiria et al., 2010). Some human studies have associated polyparasitic infections with higher frequency of malarial attacks (Spiegel et al., 2003).

5.2: STH and Children’s Nutrition and Growth

5.2a: Parasitism and Children’s’ Age and Gender

Overall parasitism and sex of the children was not found to be statistically associated in this study. However, a closer look at parasites’ species distribution by sex revealed that boys were more likely than girls to be infected with hookworms \( (p = 0.021) \). Given that in rural communities of endemic countries all children may be similarly exposed and experience comparable risks of infection, looking for an association between biological sex and parasitism may not be useful. However, there used to be a time when innate biological predisposition was proposed to explain higher hookworm prevalence in boys than in girls (Bundy, 1988). This hypothesis was not substantiated and later fell into oblivion but more recently, the possibility of gender -and the roles associated with being a boy or a girl - as a differential factor for such infections has been proposed (Behnke et al., 2000; Brooker et al., 2004).

In terms of age, older children (>10 y/o) were found to be more infected with any STH than younger children (7-10 y/o) \( (p = 0.015) \). A similar trend was observed for this age group for trichuriasis and hookworm infections but this difference was marginally significant \( (p = 0.082 \text{ and } p = 0.068, \text{ respectively}) \). Although these 2 age groups are very
close to each other, both are still in primary school and share the same spaces at school and at home, there may be more subtle differences that lead to differential exposures. While it might be true that older children exhibit better hygiene practices, it may also be true that older children may be more independent and engage in more risky behaviours such as wearing their shoes less often or are required to do more chores that bring them closer to contaminated soil. By the same token, it would be worth exploring if age and gender are factors associated with deworming treatment access. For example, a mother might be more inclined to deworm a younger child, or conversely, an older child might refuse the treatment more easily than a younger child. These are all variables worthy of exploring in future studies (Bath et al., 2010).

5.2b: Parasitism and Children’s’ Growth and Nutritional Status

It was encouraging to find that the growth status of the studied children was generally within healthy parameters. Of 320, only 18 children (5.63%) were deemed as suffering of stunted growth [low HAZ (height-for-age z-score]. It is difficult to compare this figure with Honduran data as most of the nutritional and growth indicators are given for children under 5 years of age. In 2006, the national institute of health, Honduras reported that 24.7% of these children (under five years) were stunted [cited in (Allen, 2008)] and according to a recent WHO statistics 29.9% of them are believed to be stunted (WHO, 2012b). Stunting is recognized as the most common type of malnutrition in the Americas [cited in (PAHO, 2004). A recent study among school children in neighbouring Guatemala revealed a moderate prevalence rate of stunting among the children (18% in boys and 16% in girls) (Sorensen et al., 2011), and a high rate of 34.5%
was found among grade 5 children in Peru (Casapia et al., 2006) and a recent review of published literature on the nutritional status of school-age children from different regions of the world showed that 16% of children are stunted in Latin America (Best et al., 2010). Stunting is an indicator of chronic malnutrition which has its origin in infancy. It results from poor nutrition and is aggravated by infectious diseases (Walker et al., 2007).

No significant association between gender and stunting was found in this study but older children (>10 y/o) were found to be significantly more stunted than the younger ones ($p = 0.011$). This finding is consistent with findings from other cross sectional studies, (Casapia et al., 2006; Khuwaja et al., 2005; Mupfasoni et al., 2009) as well as longitudinal studies (Friedman et al., 2005), where older children were found to be significantly more stunted than younger children. This finding indicates that the risk of stunting increased with age thereby necessitating the need to target school-age children for stunting (Casapia et al., 2006; Shang et al., 2010) as stunted children are more likely to remain stunted into adulthood (Grantham-McGregor et al., 2007) because, this growth failure, once established is difficult to compensate for, even after treatment and subsequent adequate diet (Andrade et al., 2001).

In terms of parasitism, this study found a significant association between stunting and hookworm infection ($p = 0.049$). Similar results have been found in the neighboring country of Guatemala where Sorensen and colleagues surveyed 1,001 East Guatemalan school children (Sorensen et al., 2011). In the case of trichuriasis, the mean HAZ scores were found to be significantly different across the different levels of infection intensity for $T.~trichiura$ ($p = 0.025$), and marginally significantly different across the different infection status ($p = 0.100$). In other words, the mean HAZ decreased with a higher
intensity of *T. trichiura* infection and with the more co-infections the child had. This is corroborated by other studies where stunting was found to be significantly associated with moderate to heavy *T. trichiura, A. lumbricoides* infections or co-infections with both STHs (Casapia et al., 2006; Shang et al., 2010). STH infections are known to disturb normal nutrient uptake from food in infected persons especially in those with heavy infections, disrupting normal growth especially in children such as those undergoing rapid growth phases of prepuberty and puberty (Andrade et al., 2001).

Three 3 (1.3%) of the 234 children for whom WAZ scores could be calculated (i.e., from 7 to 10 years of age) were underweight. Underweight is assessed by the weight-for-age z score (WAZ) and it is an overall indicator for malnutrition, as it assesses both present (acute) and chronic malnutrition or mixed malnutrition (de Onis et al., 1993). No significant associations were found between age or gender and underweight status. However, all 3 underweight children were infected with *A. lumbricoides* (*p* = 0.028).

Since there were only a few children in the underweight category, a different approach to the analysis was taken: WAZ numerical scores were investigated for an association with infections and interesting findings were obtained. Children with either *T. trichiura* or hookworm infections were found to have statistically significantly lower mean WAZ scores (-0.18 and -0.43 respectively) than children without such infections (0.08 and -0.03 respectively). The mean WAZ was also found to be significantly different across the different levels of infection intensity for *T. trichiura* (*p* = 0.025) and marginally significantly different across the different levels of infection intensity for *A. lumbricoides* (0.069). Moreover, WAZ means differed significantly across the different
infection status ($p = 0.017$) with children with no infection having the highest mean WAZ score (0.05) followed by that of mono-parasitized children (0.01) while poly-parasitized children had the least mean WAZ (-0.34) – figure 10, page 74. All these indicate that the presence of these worms is undermining the health of these children however subtly. The higher the intensity of the infections and with more concurrent infections, the more underweight the children were as reflected in the decreasing mean WAZ. Due to the damages done to the intestines by *A. lumbricoides* and *T. trichiura*, childhood nutrition is impaired resulting in symptoms such as nausea, anorexia, diarrhoea, vomiting and subsequently weight loss (Cheesbrough, 1992; Crompton and Nesheim, 2002).

Similar findings have also been found in other studies (Adefioye et al., 2011; Egwunyenga and Ataikiru, 2005; Phathammavong et al., 2007). Risk factors for underweight may indicate chronic or acute malnutrition as underweight is an indicator of both (Casapia et al., 2006)

The analysis of BMI-for age z-score revealed that 7 children (2.2%) in this study population fell within the “thin” category and none were at levels of severe thinness. There was no significant association found between age or gender and thinness or between thinness and parasitism (either with any STH, a specific species or any combination of the three), or with infection intensity. The relative small sample size utilized in this study perhaps prevented finding any meaningful association with these variables. However, as observed for WAZ and HAZ scores, BMI-for-age z-scores showed a decreasing trend when plotted against intensity of infections or mono- and polyparasitism. In other words, the more intense the infections or the more concurrent infections, the lower the BMI scores - Figure 10, page 78 in other words, the more
infected (increased intensity and more co-infections) a child was, the thinner the child was. As explained earlier, thinness indicates acute malnutrition, normally due to inadequate nutrient intake or diseases such as infectious diseases, and it has been judged a better assessment of malnutrition than wasting which is low weight for height z score – WHZ (Cole et al., 2007). It may be said that malnutrition in this study population is more chronic than acute, since stunting was the commonest form of malnutrition found. However, it will be difficult to draw such conclusions due to the cross sectional nature of the study design. Acute malnutrition is easier to reverse than chronic malnutrition with the provision of adequate nutrients.

Finally, in agreement with the fact that most children fell into the healthy categories for growth and nutrition, only very few children were identified with anemia (7 cases, for a 2.2%) and none was identified with low total protein concentration. In contrast, during the last STH survey in Honduras anemia was found in 19.4% of the children and a significant ($p = 0.039$) association was found between anemia and gender (boys). Boys were found to be more likely to be anemic than girls (OR = 1.23, 95% CI = 1.011 – 1.487) (Ministry of Health Honduras, 2011). As information relating STH to anemia is scarce in Honduras, we have limited basis for comparison, however, a national survey in 2005, showed a prevalence of moderate anemia among pre-school children to be 29.9% (WHO, 2005) and in 2006 37.3% of under 5 children were reported to be anemic in Honduras by the National Institute of Statistics [cited in (Allen, 2008)].

Probably because of the few cases of anemia, no significant associations or relationship was found between anemia and infection categories or status and none was found too with the growth indices. Similar studies have indeed found these associations.
especially with hookworm and *T. trichiura* infections (Casapia et al., 2006; Ezeamama et al., 2005b; Gyorkos et al., 2011; Mupfasoni et al., 2009; Sorensen et al., 2011). However, Legesse and Erko (2004) did not find an association between STH infection and hematocrit values in their study among school age children in Ethiopia; they attributed this finding to factors such as good nutrition and low hookworm intensity in their study population (Legesse and Erko, 2004).

Although dietary intake was not taken into consideration in this study, the low prevalences of stunting, undernutrition, thinness as well as anemia observed in this study may be suggestive of adequate food consumption. This may also be an explanation for the absence of low total proteins among the children. Nevertheless, more subtle effects were found; instead of using categorical values, mean scores were analyzed against infection. This provides evidence that STH infections even when light may be causing health effects that are not clinically obvious. There is evidence in literature showing the deleterious effect of light intensity poly-parasitic infections with STH on children’s health (Ezeamama et al., 2005b; Pullan and Brooker, 2008; Sorensen et al., 2011).

### 5.3: Study Strengths and Limitations

The strengths of this study include the fact that the final study population of 320 children was over and above the target sample size of 314 children.

Also, methodologies used in this study were all reliable as standard protocols were followed and all necessary precautions were taken to obtain accurate and reproducible data.
• Kato-Katz method for the assessment of prevalence and intensity of STH has been found to have an estimated sensitivity of 96.9% for *Ascaris*, 91.4% for *Trichuris* and 62.5% for Hookworm and a specificity of 96.1% for *Ascaris*, 94.4% for *Trichuris* and 93.8% for Hookworm (Tarafder et al., 2010).

• Anthropometric measurements (weight and height) were subjected to a reliability test by the Mueller and Martorell method for calculating inter observer technical error of measurement (TEM), and a reliability coefficient of 0.97 was obtained for height while that of 0.96 was obtained for weight.

• Total serum proteins were analyzed by both refractometry and Biuret method (gold standard) and there was no statistically significant difference between the values obtained by both methods ($p = 0.63$).

• Haemoglobin and haematocrit (for anaemia) were analyzed with an auto analyzer – The BC – 3000Plus Auto Haematology Analyzer.

• As well, the study was cross sectional, which was appropriate in meeting the research objectives of this study.

Limitations of this study include the fact that nutritional dietary intake and energy expenditure as possible confounding factors were not considered for the relationship between nutrition and STH infections. Also, due to budgetary reasons, assessment of micronutrients such as vitamin A, iron, folate, vitamin B12, zinc, magnesium, copper etc. (Shamah-Levy et al., 2012) could not be done. It is therefore recommended that such analyses should be taken into consideration in future studies.
The area of study is endemic for malaria (Bell et al., 2009; PAHO, 2000), but malaria as a potential cause of anemia was not assessed (clinically or microscopically). However, the children in the study did not show any physical sign of malaria. Moreover, in the last national survey where 2,554 children were surveyed for STH, malaria was also assessed, but no case was found (Ministry of Health Honduras, 2011).

The study children sample does not capture all children of that age range (7 – 14 y/o) in the communities, because only children enrolled in school participated in the study. However, the most recent Millennium development goal report shows that at least 90% of primary school age children are enrolled in schools in LAC (United Nations, 2012) which indicates that, the study participants were representative sample of the age group.

Clustering effect that may have been present in this study were not measured. However, it was beyond the scope of this thesis to do an in-depth risk factor analysis.
CHAPTER 6: CONCLUSIONS, RECOMMENDATIONS AND FUTURE DIRECTIONS

6.1: Conclusions

The prevalence data obtained in this study contributes with accurate and updated information to map out the situation of STH infections in Honduras. As recently reported in the compilation of a decade of peer reviewed publications and technical documents from the Latin American region, Honduran data is scarce: out of 120 bibliographic sources analyzed, only four originated in the country, 2 journal articles, one parasitology manual and one government report (Saboyá et al., 2011). In light of such paucity of research and publications on the topic in the country, the prevalence data provided here is in itself a valuable contribution.

Further, this study provides unique insight into the growth and nutrition status of a cohort of Honduran children living in rural Honduras. The children were found to be in better nutrition and growth than the Honduran average for a similar cohort (PAHO, 2004). Although a low prevalence of stunting (5.6%) was found among the studied children, this finding still warrants actions taken in this community as this prevalence is still higher than the 2.3% prevalence that can be expected in a normal population (WHO, 1997), moreover, stunting is a sign of long term nutritional deficits. As well, it is important to remember that in Honduras, there may be an important segment of the children population that do not attend school and, as argued by Gray et al (2006), might be in worse health condition and nutritional status than their counterparts who do attend school (Gray et al., 2006).

Most children were infected with STH and a very high prevalence of STH infections was observed in this study. In fact, the prevalence found (72.5%) is
significantly higher than the 50% threshold at which WHO recommends continued actions at the community level, such as regular deworming twice a year (WHO, 2007). In addition, the high prevalence found is contrary of what would be expected since the majority of study participants were enrolled in schools within the network of the Honduran Ministry of Health’s deworming program. This raises questions in respect of the efficacy of this intervention.

Most children harboured light infections and almost half were polyparasitized; both findings are consistent with endemic areas. To prioritize action measures, health authorities tend to focus on infection intensity as their clinical impact is not apparent. However, as shown in this study, concurrent light infections may indeed have a synergistic detrimental effect on children’s’ health. Our findings suggest that, in the context of growth and nutrition, looking at polyparasitism is as important as determining parasitic burden in children. The threshold at which STH can cause morbidity in children certainly depends on a combination of factors including host and parasitic factors.

6.2: Recommendations and Future Directions

In light of the high prevalence of STH infections in the study population, it is recommended that integrated control efforts be intensified in these communities; efforts including preventive therapy as well as improvements in sanitary infrastructure and health education. In the long term, elimination of transmission could be attainable as suggested by some advocates (Knopp et al., 2011b).

A closer examination into the delivery, uptake and efficacy of anti-parasitic treatment is warranted in Honduras. Monitoring for drug resistance could be also beneficial as this has become a concern in recent times. Central to this monitoring is the
development of more sensitive diagnostic procedures that can be implemented in the field (Harhay et al., 2010).

Future studies are needed to determine the degree of stunting and under nutrition in the studied communities and particular attention should be given to children of pre-school age as they are in the most sensitive stage of development. Similarly, STH studies in pre-school children are also necessary to investigate the most sensitive age for intervention strategies to reduce the prevalence of soil transmitted helminths.

Finally, as clinical studies do not provide a full understanding of the impact of STH infections (in particularly light and multiple infections), immunological and physiological studies are necessary to help clarify the consequences of STH infections on children’s health.
REFERENCES


CDC 2010. Parasites – Soil Transmitted Helminths STHs (Atlanta, CDC).

Cheesbrough, M., 1992. Medical Laboratory Manual for Tropical Countries


Evans, D.B., Jha, P., Mills, A., Musgrove, P. (Eds.) Disease Control Priorities in Developing Countries
World Bank, Washington, DC, p. 1440.


association to anaemia and undernutrition in Northern Rwanda. PLoS Negl Trop Dis 3, e517.


PAHO 2007. Workshop on STH control in Central America, Mexico and Dominican Republic. (Copán Ruinas, Honduras, PAHO), p. 25.

PAHO 2009. Epidemiological profiles of neglected diseases and other infections related to poverty in Latin America and the Caribbean (Washington, DC).


Sabin Vaccine Institute, Global Network for Neglected Tropical Diseases, Inter-American Development Bank, Organization, P.A.H. 2011. A Call to Action:
Addressing Soil-Transmitted Helminths in Latin America and the Caribbean (Washington DC).


Sosa, W., 2007. Relationship between infections of soil-transmitted helminths and anemia in school children during pre-and post-treatment with anthelmintic and supplement with vitamin supplements, in the Valley of Macuelizo, Department of


UNICEF. Malnutrition In Malnutrition - Popup Definition.


WHO 1994. Bench Aids for the diagnosis of intestinal parasites (Geneva,).


World Life Expectancy 2012. World health rankings (LeDuc Media).


Asunto: Invitación para que su Escuela participe en un estudio de investigación

Estimado(a) Sr(a) Director(a),

Por este medio quisiéramos hacerle una cordial invitación para que su Escuela participe en un estudio de investigación acerca de parásitos intestinales (lombrices). Este estudio es un esfuerzo conjunto de diversas instituciones de Honduras y Canadá.

En Honduras, los investigadores principales son la Dra. Maritza Canales (catedrática) y María Mercedes Rueda (estudiante de maestría) de la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH).

En Canadá, los investigadores principales son la Dra. Ana L. Sánchez (catedrática de la Universidad de Brock) y la Dra. Theresa Gyorkos (catedrática de la Universidad de McGill), así como también Mary-Theresa Usuanlele y José Antonio Gabrie (estudiantes de maestría de la Universidad de Brock).

Propósito del estudio
El propósito principal del estudio es explorar si existen algunos determinantes de género que-condicionen las infecciones de lombrices intestinales en los niños de 4\textsuperscript{to} y 5\textsuperscript{to} grado que atienden las escuelas en su área. Adicionalmente, trataremos de identificar qué otros factores podrían estar influenciando la transmisión de dichos parásitos en los niños participantes del estudio, así como también, evaluar el impacto que estas infecciones tienen en la salud de ellos.

Metodología del estudio
Si Ud. acepta que su escuela participe en este estudio, los niños de 4\textsuperscript{to} y 5\textsuperscript{to} grado se visitarán para explicarles el estudio; también se invitarán los padres de familia o tutores de los niños a una reunión informacional para poder explicarles a ellos la importancia de este tipo de parasitosis así como los beneficios y riesgos de participar en este estudio.

El estudio se desarrollará en dos etapas diferentes:

\textit{Etapa 1}.
Tendrá lugar en su escuela. Utilizando un cuestionario, le preguntaremos al niño o niña acerca de su conocimiento sobre los parásitos, su salud, sus hábitos y costumbres relacionadas con la salud, etc. Se le solicitará a cada niño que nos provea de una muestra de heces y de sangre para investigar la presencia de parásitos intestinales, anemia y otros estudios relacionados con parásitos. También, a cada participante le tomaremos la altura, el peso y otras medidas relacionadas.
**Etapa 2.**
Un pequeño subgrupo de niños será aleatoriamente seleccionado para esta etapa, en la cual se llevará a cabo una entrevista más profunda. En este caso, nos reuniremos con cada niño(a) en sus casas y discutiremos temas relacionados a su salud y las infecciones por parásitos intestinales.

**Aspectos éticos del estudio**
Queremos asegurarle que este estudio ha sido minuciosamente revisado y ha recibido la aprobación de los Comités de Revisión Ética de la Universidad de Brock en St. Catharines, y el Centro de Salud de la Universidad de McGill en Montreal, ambas instituciones en Canadá.

**Riesgos y molestias**
Podría existir cierta vergüenza entorno a brindar las muestras de heces y un temor natural al dolor asociado a la toma de la muestra de sangre; sin embargo, haremos los esfuerzos necesarios para lograr que los padres y los niños superen esta ansiedad, explicándoles el beneficio del estudio y la importancia de conocer el estado de salud de los niños.

**Beneficios potenciales**
Debido a que la escuela está localizada en un área donde los parásitos son comunes, los niños están expuestos a infectarse con lombrices intestinales. Estos parásitos pueden ocasionar retraso y deterioro en el desarrollo y crecimiento físico e intelectual de los niños. El beneficio directo a cada niño(a) participante del estudio será el tratamiento para eliminar los parásitos intestinales, si estuvieran infectados, y la anemia, si esta condición fuera encontrada en el(la) niño(a) en ese momento. Esto mejorará su estado de salud. Adicionalmente, los niños pueden tomar medidas preventivas (mejorar higiene, modificar conductas, etc.) y así evitar futuras infecciones y enfermedades potenciales.

Además, el sistema educativo podría utilizar los resultados de este estudio para apoyar los programas continuados de desparasitación en Honduras.

Igualmente, una vez que los datos se hayan obtenido, se desarrollará un taller para enseñar a los niños cómo lavarse sus manos apropiadamente y otros conceptos básicos de higiene.

**Confidencialidad**
Toda la información obtenida durante este estudio será mantenida estrictamente confidencial. Los nombres de los niños y/o sus padres no serán provistos a nadie fuera del equipo de investigación y la información será guardada en un archivo con candado en la oficina del investigador. Solamente el equipo investigador tendrá acceso a la misma, previa aprobación del investigador principal. Los resultados de este estudio serán publicados pero en ningún momento los nombres serán utilizados, manteniendo los resultados completamente anónimos. En algún momento posterior, para poder verificar los datos del estudio, los Oficiales de Aseguramiento de la Calidad de la Universidad de Brock y la Universidad de McGill podrían revisar dichos datos.

**Participación voluntaria y retiro de este estudio**
La participación de los niños en este estudio es estrictamente voluntaria. Un niño(a) podrá rehusar participar e incluso, retirarse en cualquier momento, sin necesidad de explicación alguna y sin ningún perjuicio o represalia. Los padres serán informados de cualquier decisión del niño(a) al respecto.
Costo y compensación

No hay costos para la escuela o los participantes asociados a este estudio. Si el niño está infectado, se le ofrecerá gratuitamente una dosis única de Albendazol 400 mg (según la recomendación de la Organización Mundial de la Salud). Si se detectara anemia, el tratamiento apropiado se dispensará gratuitamente a través de las autoridades de salud de su comunidad.

Ni los niños ni sus padres o tutores recibirán dinero por su participación en este estudio. Sin embargo, un pequeño presente les será otorgado como signo de apreciación.

Por favor, no dude en contactar a la Coordinadora del estudio si tiene alguna pregunta al respecto. Agradeciéndole la atención brindada a la presente y esperando poder tener una pronta respuesta a la misma.

Muy atentamente

Maritza Canales, MSP
Coordinadora del Estudio en Honduras
Coordinadora Académica del Programa de Maestría en Infecciones y Enfermedades Zoonóticas
Catedrática
Escuela de Microbiología, Tegucigalpa
Universidad Nacional Autónoma de Honduras (UNAH)
Tel.: 2252-8089
Cel.: 9487-0165
E-mail: <marygchn@yahoo.com>
Research Project: Soil-Transmitted Helminthes Infections in Honduran School Children

Date:

To:

Re: Invitation to enroll your school in a research study

Dear School Principal,

We would like to invite your school to participate in a study about intestinal parasitic worms. This study is a collaborative effort between various institutions in Honduras and Canada.

In Honduras the main investigators are Maritza Canales (Professor) and Maria Mercedes Rueda (Grad student) of the School of Microbiology of the National Autonomous University of Honduras.

In Canada the main investigators are Dr. Ana L. Sanchez (Professor of Brock University) and Dr. Theresa W. Gyorkos (Professor of McGill University) and Mary-Theresa Usuanlele and Jose Antonio Gabrie (Grad students, Brock University).

Purpose of the study
The main purpose of this study is to explore gender determinants of worm infections in Grade 4 and 5 children attending primary schools in your areas. Additionally, we will try to determine what other factors are influencing the transmission of parasites among the participating children as well as assess the health impact of these infections.

Study procedures
If you agree that your school participates in this study, 4th and 5th grades will be visited to explain the children about the study and extend an invitation for the parents or guardians to attend an informational meeting with the purpose of explaining the importance of intestinal parasites as well as the benefits and risks of participating in this study.

The study will be conducted in two different stages as follows:

- **Stage 1.** This stage will take place at your school. Utilizing a questionnaire, we will ask the child some questions about their knowledge about parasites, health and health-related behaviours, etc. We will also ask the child to provide us with a stool specimen and a blood sample to check for intestinal parasites, anemia and other studies related to parasites. We will also take the child’s weight and height measurements.

- **Stage 2.** A small subsample of children will be randomly selected for this stage in which an in-depth interview will take place. In this case, we will meet every child at his/her home and discuss topics related to his/her health and worm infections.
Ethical issues
We would like to assure you that this study has been reviewed and has received ethics approval from the Ethics Review Board at Brock University in St. Catharines, and the McGill University Health Centre in Montreal, both in Canada.

Risks and discomfort
There could be some embarrassment attached to providing stool samples and a natural fear of the pain associated with venipuncture for blood collection, but we will make all efforts to help parents and children to overcome any anxiety by stressing out the benefit of the study and the importance of knowing the children’s health status.

Potential benefits
Because the school is located in an area where parasites are common, children are at risk of developing worm infections. The worm parasites can cause delays and impairment in the physical and intellectual growth and development of children. The direct benefit to every child from participating in this study is that he/she will be treated for these intestinal parasites if they are found to be infected, and will be treated for the anemia if that condition is found in the child at that time. This will improve their health. Additionally, they can implement prevention measures (improve hygiene, modify behaviour, etc.) and avoid future infections and potential illness.

Furthermore, the school system could use the information from this study to support continued deworming programs in Honduras.

Additionally, a workshop will be held once data collected is completed to teach the children how to wash their hands properly and other hygiene concepts.

Confidentiality
All information obtained during this study will be kept strictly confidential. Parents and children’s names will not be given to anyone outside of the research team and the information will be locked in a filing cabinet in the investigator’s office. Only the research team will have access to the information and only after first receiving the approval of the principal investigator. The results of this study will be published but no names will be used at any time. Participants’ identity will not be revealed in the combined results. In order to verify the research data, the Quality Assurance Officers from Brock University and MUHC Research Ethics Boards may review these records.

Voluntary participation and withdrawal from this study
The participation of every child in this study is strictly voluntary. A child may refuse to participate and also may discontinue their participation at any time without explanation, and without penalty or loss of benefits to which they are otherwise entitled. Doing that, the child will suffer no prejudice regarding the medical care or participation in any other research study. Parents will be informed of any new findings that may affect their child willingness to continue his/her participation.

Cost and compensation
There are no costs associated to either school or participants in this study. If the child is infected they will be offered a single dose of Albendazole 400 mg, free of charge, (as recommended by the World Health
Organization). If anemia is detected, the proper treatment will be dispensed through the health authorities in your community, free of charge too.

Neither children nor their parents will be given any money to participate in this study. However, a small token will be given as a sign of appreciation.

We are looking forward to your response. Please do not hesitate to contact the Coordinator of the study if you have any questions regarding.

Sincerely,

Maritza Canales, MSP
Coordinator of the Study in Honduras
Academic Coordinator of the Master Program in Infectious and Zoonotic Diseases
Associate Professor
School of Microbiology, Tegucigalpa
National Autonomous University of Honduras (UNAH)
Office phone: 2252-8089
Cell phone: 9487-0165
E-mail: <marygchn@yahoo.com>
Aceptación de Participación de la Escuela en el Estudio de Investigación

Yo ______________________________________________________________________ en calidad de director(a) de la Escuela ______________________________________________________________________ en la Comunidad ______________________________________________________________________, perteneciente al municipio de ______________________________________________________________________ en el departamento de __________ ______________________________________________________________________, doy fe que he sido informado(a) detalladamente acerca del estudio de investigación denominado “Parásitos intestinales en niños escolares de Honduras”, que será desarrollado en distintas escuelas de varias comunidades de nuestro país, por la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH) y las Universidades de Brock y McGill de Canadá.

Habiendo conocido y evaluado los potenciales beneficios y riesgos que dicho estudio conlleva, y luego de haber tenido la oportunidad de realizar las preguntas pertinentes y de obtener respuesta satisfactoria a todas ellas, por este medio acepto que nuestra Escuela participe en el estudio ya mencionado y que los investigadores puedan acercarse a nuestros alumnos y sus padres para invitarlos a participar en el estudio.

Queda entendido, sin embargo, que esta aceptación a nivel de la escuela no conlleva obligación alguna hacia los padres de familia ni hacia los niños que asisten a nuestra escuela. La participación de los niños en este estudio será absolutamente voluntaria y sólo podrá darse una vez que se haya obtenido el consentimiento previo de sus padres o encargados y que el niño(a) acepte participar. Asimismo, el niño(a) será capaz de retirarse del estudio, si así lo desea, en cualquier momento, sin ninguna explicación ni represalia de ningún tipo.

_________________________________________________________________________  ______________________________________________________________________
Firma del director(a)                                                         Fecha (día/mes, 2011)
CONSENTIMIENTO DE PADRES O ENCARGADOS

Fecha:

Introducción:
Hola, nos gustaría invitar a sus hijos a participar en un estudio de investigación sobre parásitos y lombrices intestinales en niños escolares. Para poder invitar e inscribir a sus niños, necesitamos primero hablar con ustedes y obtener su permiso para hacerlo. Quisiéramos contarles un poco más acerca de nosotros y de este estudio.

¿Quiénes somos?
Somos un grupo de profesores y estudiantes de Honduras y Canadá interesados en realizar este estudio. Nuestro equipo está integrado de esta forma:

En Honduras, los investigadores principales son la Dra. Maritza Canales (catedrática) y María Mercedes Rueda (estudiante de maestría) de la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH). La Dra. Canales es la Coordinadora del Proyecto.

En Canadá, los investigadores principales son la Dra. Ana L. Sánchez (catedrática de la Universidad de Brock) y la Dra. Theresa Gyorkos (catedrática de la Universidad de McGill), así como también Mary-Theresa Usuanlele y José Antonio Gabrie (estudiantes de maestría de la Universidad de Brock).

Invitación:
Les agradecemos que estén presentes acá hoy. La razón por la que les llamamos es invitarle a usted y a su hijo(a) que está en el 3\textsuperscript{er}, 4\textsuperscript{to} ó 5\textsuperscript{to} grado de escuela a participar en un estudio sobre parásitos y lombrices intestinales.

Antes que decidan participar, es importante que ustedes entiendan el estudio, incluyendo los riesgos y beneficios. En cualquier momento que no entiendan algo o tengan alguna duda, por favor pregúntenos con confianza que con gusto le contestaremos. Este documento que estamos leyendo en voz alta es una Hoja de Consentimiento y se llama Consentimiento de Padres o Encargados y es por medio de él que ustedes darán permiso para que sus hijos participen en el estudio.

A cada uno de ustedes les entregaremos una copia de este documento para que la conserven, pero también queremos leérselas en voz alta para estar seguros que lo hayan entendido. Si deciden permitir que sus hijos participen en este estudio, queremos que sea sólo si lo han entendido completamente y desean libremente hacerlo.
Propósito del estudio
Deseamos conocer más sobre las infecciones de parásitos y lombrices intestinales en niños escolares. Queremos entender mejor la frecuencia de los parásitos en los niños, cómo se transmiten y cómo están afectando su salud. Deseamos realizar el estudio con niños del 3\textsuperscript{er}, 4\textsuperscript{to} y 5\textsuperscript{to} grados pues ellos están más expuestos a contraer estas infecciones y también porque tienen la edad suficiente para entender el estudio y contestar las preguntas que deseamos hacerles.

¿Qué es lo que implica este estudio?
Si deciden participar, se les pedirá su permiso para poder reunirnos con los niños en una o dos ocasiones dentro del siguiente mes. Necesitamos aproximadamente 30 minutos con cada niño(a) para la primera reunión y 45-60 minutos para la segunda visita. La primera reunión se realizará en la escuela y la segunda sería en sus casas, si así nos lo permiten ustedes.

Ahora deseamos darles más detalles del estudio. El estudio tiene 2 etapas:

- **Etapa 1.** Esta etapa se hará en la escuela. Primero le tomaremos algunas medidas a los niños como el peso, la altura, la cintura y alguna otra relacionada. Luego, les haremos algunas preguntas sobre su salud, sus hábitos, tipo de juegos y labores que realizan, el conocimiento sobre parásitos, etc. Estas preguntas duran unos 20 minutos aproximadamente. También les pediremos una muestra de heces para investigar si tienen parásitos intestinales y una muestra de sangre para conocer si hay anemia y realizar otros estudios relacionados con los parásitos. En total necesitamos aproximadamente 30 minutos con ellos. Esto se coordinará con los maestros para no afectar los estudios de los niños.

- **Etapa 2.** Solamente unos pocos niños serán seleccionados para la segunda etapa. Sus hijos pueden ser seleccionados para una entrevista un poco más profunda. En este caso, nos reuniremos con sus hijos en sus casas y platicaremos sobre diversos asuntos de salud, hábitos, actividades y otros temas relacionados con los parásitos y lombrices intestinales. Como lo mencionamos antes se necesitarán de 45 a 60 minutos para realizarlo.

Aspectos éticos
Queremos asegurarles que este estudio ha sido minuciosamente revisado y ha recibido la aprobación de los Comités de Revisión Ética de la Universidad de Brock en St. Catharines ( Expediente # BU 10-171), y el Centro de Salud de la Universidad de McGill en Montreal ( Expediente # MUHC 10-175-PED), ambas instituciones en Canadá. En Honduras, el estudio ha sido revisado y aprobado por el Comité de Ética del Programa de Maestría en Infecciones Enfermedades Zoonóticas (MEIZ) de la Escuela de Microbiología de la UNAH o el oficial de Investigación de la MEIZ, Dra. Vilma Espinoza.

Riesgos y molestias
Este estudio no tiene riesgos serios para sus hijos. Los niños pueden sentir cierta pena de dar una muestra de heces; sin embargo, quisiéramos decirles que nosotros estamos totalmente familiarizados con este tipo de muestras y lo único que nos interesa es buscar parásitos en ellas.

También, ustedes quizás puedan estar preocupados por la muestra de sangre pues el piquete de la aguja causa un pequeño dolor. Deseamos garantizarles que ese dolor es sólo temporal y en unos pocos minutos se irá; adicionalmente, tenemos bastante experiencia en tomar muestras de sangre y los niños no recibirán ningún daño sino sólo la molestia de un pequeño piquete. Les aseguramos que ninguna de esas muestras será entregada a alguien más, por ningún motivo. Una parte de las muestras serán enviadas a Canadá para examinarlas en los laboratorios allá.
Beneficios potenciales

Debido a que ustedes viven en áreas con todas las condiciones para la transmisión de parásitos y lombrices intestinales, sus hijos están en riesgo de infectarse con ellos. Los parásitos pueden ocasionar que los niños no crezcan con la rapidez que deberían debido a que les roban sangre y nutrientes. Igualmente, los parásitos pueden ocasionar que los niños tengan bajo rendimiento en la escuela pues los niños con parásitos pasan cansados y no aprenden tanto como los niños sin parásitos.

El beneficio directo para sus hijos que participen en este estudio es que si ellos están infectados, les ofreceremos gratuitamente el tratamiento; además, si tienen anemia u otros parásitos diferentes a las lombrices, nosotros les proveeremos el tratamiento al Centro de Salud de su comunidad para que ellos puedan darles el tratamiento a los niños. Estos tratamientos les mejorarán sus condiciones de salud.

El resumen de los resultados de este estudio les será presentado a ustedes en una reunión similar a esta. Les daremos recomendaciones para reducir el riesgo de infecciones parasitarias en los niños y los miembros de la comunidad.

Además, la información de este estudio será utilizada para apoyar los programas de desparasitación regulares en sus áreas. Esto significa que hablaremos con las autoridades de Salud Pública y Educación acerca del establecimiento de este tipo de programas.

Confidencialidad

Toda la información obtenida durante este estudio será mantenida estrictamente confidencial. Los nombres de los niños y/o ustedes no serán provistos a nadie fuera del equipo de investigación y la información será guardada en un archivo con candado en la oficina del investigador. Solamente el equipo investigador tendrá acceso a la misma, previa aprobación del investigador principal. Los resultados de este estudio serán publicados pero en ningún momento los nombres serán utilizados, manteniendo los resultados completamente anónimos. En algún momento posterior, para poder verificar los datos del estudio, los Oficiales de Aseguramiento de la Calidad de la Universidad de Brock y la Universidad de McGill podrían revisar dichos datos. Al firmar este consentimiento ustedes nos autorizan a brindarles a estos oficiales la información del estudio.

Participación voluntaria y retiro de este estudio

La participación de sus hijos en este estudio es estrictamente voluntaria. Ustedes pueden rehusar que sus hijos participen e incluso, retirarlos en cualquier momento, sin necesidad de explicación alguna y sin ningún perjuicio o represalia. Ustedes serán informados de cualquier condición que motive la decisión del niño(a) a retirarse o a rehusarse a participar.

Si sus hijos se retiran durante el estudio, nosotros quisiéramos conservar la información que hasta ese momento se haya obtenido, si eso les parece bien. En este caso, les daremos los resultados de laboratorio y si los niños resultan infectados, igualmente les proveeremos gratuitamente el tratamiento al Centro de Salud de la comunidad para que lo puedan obtener allí.

Costo y compensación

La participación de sus hijos en el estudio es gratuita. Si sus hijos están infectados, se les ofrecerá gratuitamente una dosis única de Albendazol 400 mg (según la recomendación de la Organización Mundial de la Salud). Si se detectara anemia, el tratamiento apropiado se dispensará gratuitamente a través de las autoridades de salud de su comunidad.
Ni ustedes ni los niños recibirán dinero por su participación en este estudio, pero les daremos un pequeño presente a los niños como signo de apreciación.

**Persona de contacto**

Si ustedes tienen alguna pregunta respecto a este estudio pueden llamar a la Coordinadora del Proyecto en Honduras, la Dra. Maritza Canales. Ella trabaja en la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH) en Tegucigalpa. Sus teléfonos son: Oficina 2252-8089, Cel. 9487-0165. Si ustedes o algún conocido tienen acceso a internet, pueden enviarle a ella un correo electrónico a <marygchn@yahoo.com>.

**Declaración de consentimiento**

Yo entiendo el contenido de esta Hoja de Consentimiento, y estoy de acuerdo que mi hijo(a) participe en este estudio de investigación. Yo entiendo que no todos los niños que participen en este estudio serán elegidos para la Etapa 2 y estoy de acuerdo que mi hijo(a) participe en la Etapa 2 si fuera seleccionado(a) para esto. He tenido la oportunidad de preguntar en la sesión de información y todas mis preguntas han sido contestadas satisfactoriamente. He tenido suficiente tiempo para considerar la información arriba descrita y buscar consejo de otras personas si he decidido hacerlo. Con la firma de esta Hoja de Consentimiento, no estoy renunciando a ninguno de mis derechos legales, sino solamente accediendo a que mi hijo(a) participe en este estudio de investigación.

**Yo acepto que mi hijo(a) participe en**

Etapa 1 del estudio  [ ] Sí  [ ] No
Etapa 2 del estudio  [ ] Sí  [ ] No

**Yo acepto que mi hijo(a) brinde**

Muestra de heces  [ ] Sí  [ ] No
Muestra de sangre  [ ] Sí  [ ] No

_____________ Escuela_______________ CÓDIGO: ______________________
Nombre del niño(a)

_____________ Nombre del padre/madre/tutor  _____________ Firma del padre/madre/tutor  _____________ Fecha (día/mes, 2011)

_____________ Nombre del testigo  _____________ Firma del testigo  _____________ Fecha (día/mes, 2011)
Date:

Introduction:
Hello, we would like to invite your children to participate in a research study about parasites and intestinal worms in school children. In order for us to invite and enroll your children in this study, we first need to talk to you and obtain your permission. So we would like to tell you more about us and what is involved in the study.

Who are we?
We are a group of professors and students from Canada and Honduras interested in conducting this study. Our names and institutions are as follows:
In Honduras the main investigators are Dr. Maritza Canales (Professor) and Maria Mercedes Rueda (Grad student) of the School of Microbiology of the National Autonomous University of Honduras. Dr. Canales is the Honduran Project Coordinator.
In Canada the main investigators are Dr. Ana Sanchez (Professor at Brock University) and Dr. Theresa W. Gyorkos (Professor at McGill University) and Mary-Theresa Usuanlele and Jose Antonio Gabrie (Grad students, at Brock University).

Invitation:
We thank you for your presence here today. The reason why we are here is because we would like to invite you and your child who attends 4th or 5th grade at the school to participate in a study about parasites and intestinal worms.

Before you decide to participate, it is important that you understand the study including its risk and benefits to make an informed decision. We invite you to ask questions if there is anything that you do not understand. This document we are reading aloud is called a consent form. Because it is actually for you to give permission for your child, the form is called Parental/3rd Party Consent form.

We will give a copy of this form to each and everyone of you for you to keep but we also want to read it aloud today to make sure everybody understands it. If you decide to allow your child to participate in the study we want to propose, it should be because you fully understand the study and have come to an informed decision.

Purpose of the study
Like we said, we would like to know more about parasites and intestinal worms in school children. We would like to know why and how children are getting intestinal worms and if being a boy or a girl has anything to do with being infected. We would like to do the study with children in Grades 4th and 5th because they are at high risk of having worms and also because they are old enough to understand the study and answer some questions we would like to ask them.
What is involved in the study?
If you agree to participate, you will be asked to grant us permission to meet your child on two occasions within the next month. We will need approximately 30 minutes of his/her time in our first meeting and about 45-60 minutes for the second visit. The first meeting will be at the school and the second at your house, if you give us permission.

Now we would like to give you more details about the study:

The study will be conducted in two different stages as follows:

- **Stage 1.** This stage will take place at your child’s school. First we will take your child’s weight and height measurements, including a measurement of their waist and hip circumference. Then, we will ask your child some questions about his/her health and health habits and other questions about parasites, the games they play, if they help around the house, etc. This period of questions will last about 20 minutes. We will also ask your child to provide us with a stool specimen and a blood sample to check for intestinal parasites, anemia and other studies related to parasites. In total we would need about 30 minutes of your child’s time. Of course we will ask the teacher for a moment when we don’t interfere with your child’s activities at school.

- **Stage 2.** Only a few children will be selected for the second stage. Your child may be selected for this stage in which an in-depth interview will take place. In this case, we will meet your child at your home and discuss topics related to his/her health, beliefs, activities, and other things that may be related to worm infections. This visit will require 45-60 minutes of your child’s time.

Ethical issues
We would like to assure you that this study has been reviewed and has received ethics approval from impartial professionals in Honduras and in Canada. More specifically, the Ethics Review Board at Brock University in St. Catharines, and the McGill University Health Centre in Montreal, both in Canada have approved the study. Additionally, it has been approved by the principal at your child’s school. These approvals mean that we will do nothing harmful to you or your children. Not only we want to do good things but also it is our obligation to conduct ourselves in a way that we don’t cause any problems as a result of the study.

Risks and discomfort
This study does not pose serious risks to your child. Your child may feel a little embarrassed of providing a stool samples but we would like to say that we are very familiar with this kind of samples and we only are interested in finding parasites in them.

Also, you may have concerns about your child giving a blood sample because getting a needle is a little painful. But we want to reassure you that the pain is temporary and will go away in a few minutes. We are very experience in taking blood samples so we will not cause any more pain than a little prick.

We want to assure you that we will not give any of the stool or blood sample to anybody else, for any purpose. We may send a little bit of the samples to Canada so they can examine them in their laboratories.

Potential benefits
Because you live in an area with all the conditions for worm transmission, your child is at risk of having worm and other parasitic infections. Parasites can cause children not to grow as fast as they should, because they still nutrients and blood. Parasites can also cause poor school performance because children with worms are tired and don’t learn as much as children without parasites.
The direct benefit to your child from participating in this study is that if they are found to be infected, we will offer you treatment for free. And if they have anemia or other parasites, we will provide treatments to the health care centre so you can get treatment there. These treatments will improve his/her health.

The aggregated results from the study will be presented to all of you in a meeting similar to this one. We will present recommendations as to how reduce this risk of worm infections in children in particular and community members in general.

Furthermore, we will use the information from this study to support continued deworming programs in your area. This means that we will speak with health and education authorities about developing school-based deworming programs.

Confidentiality
All information obtained during this study will be kept strictly confidential. Your name and the name of your child will not be given to anyone outside of the research team and the information will be locked in a filing cabinet in the investigator’s office. Only the research team will have access to the information and only after first receiving the approval of the principal investigator. The results of this study will be published but no names will be used at any time. Your identity will not be revealed in the combined results. In order to verify the research data, the Quality Assurance Officers from Brock University and MUHC Research Ethics Boards may review these records. By signing this consent form, you give us permission to release information regarding to your participation in this study to these individuals.

Voluntary participation and withdrawal from this study
The participation of your child in this study is strictly voluntary. You may refuse your child’s participation and you may discontinue his/her participation at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you discontinue your child’s participation, your child will suffer no prejudice regarding the medical care or participation in any other research study. You will be informed of any new findings that may affect your child willingness to continue your participation. If your child’s participation is discontinued at any point in the study, we may want to keep the information you already gave us, if that’s ok with you. In that case, we will still provide laboratory results if your child were to be infected and make treatment available for you at the health centre.

Cost and compensation
There are no costs associated with your participation in this study. Your child will receive one dose of Albendazole at school if he/she is found to be infected, free of charge. In case of your child be found with anemia, the proper treatment will be dispensed trough the health authorities in your community, free of charge too. Neither you nor your child will be given any money to participate in this study. However, a small token will be given to your child as a sign of appreciation.

Contact persons
If you have any questions regarding the study, you can contact the Project Coordinator in Honduras, Dr. Maritza Canales. She works at the School of Microbiology of the National Autonomous University of Honduras (UNAH) in Tegucigalpa. Her phone numbers are: office Tel. 2252-8089; Cell. 9487-0165. If you or anybody you know have access to email, you could e-mail her at <marygchn@yahoo.com>.
Declaration of consent

I understand the contents of this Consent Form, and I agree that my child participates in this research study. I understand that not all the children who participate in this study will be selected for the Stage 2 and I agree that my child participates in Stage 2 if he/she is selected to do so. I have had the opportunity to ask questions in an information session and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. By signing this consent form, I am not given up any of my legal rights, just agreeing that my child participates in the research study.

I agree that my child participates in
First stage of the study  [ ] Yes  [ ] No
Second stage of the study  [ ] Yes  [ ] No

I agree that my child provides
Stool sample  [ ] Yes  [ ] No
Blood sample  [ ] Yes  [ ] No

_______________________________________ School_______________ CODE: __________________
Child’s name

___________________________ ___________________________ ___________________
Parent’s/guardian’s name  Parent’s/guardian’s signature  Date (day/month, 2011)

___________________________ ___________________________ ___________________
Witness’s name    Witness’s signature   Date (day/month, 2011)
ASENTIMIENTO INFORMADO DE LOS NIÑOS

Fecha:

Introducción:
Hola, estamos acá porque tus padres nos han dado permiso de hablar contigo e invitarte a participar en un estudio de investigación acerca de parásitos y lombrices intestinales en niños que vienen a la escuela. Aunque tus padres nos han dado permiso, necesitamos saber si TÚ quieres participar en este estudio, así que nos gustaría contarte más acerca de nosotros y de este estudio.

¿Quiénes somos?
Somos un grupo de profesores y estudiantes de Honduras y Canadá interesados en realizar este estudio. Nuestro equipo está integrado de esta forma:

En Honduras, los investigadores principales son la Dra. Maritza Canales (catedrática) y María Mercedes Rueda (estudiante de maestría) de la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH). La Dra. Canales es la Coordinadora del Proyecto.
En Canadá, los investigadores principales son la Dra. Ana L. Sánchez (catedrática de la Universidad de Brock) y la Dra. Theresa Gyorkos (catedrática de la Universidad de McGill), así como también Mary-Theresa Usuanlele y José Antonio Gabrié (estudiantes de maestría de la Universidad de Brock).

Invitación:
Queremos agradecerte por estar aquí hoy. Como lo mencionamos hace un rato, la razón por la que estamos hablando contigo es porque queremos invitarte a participar en un estudio sobre parásitos y lombrices intestinales. Primero te contaré más sobre nuestro proyecto y luego te preguntaré si estás interesado en participar en él.

Este estudio se va a realizar con niños como tú que asisten a las escuelas en estas comunidades. Queremos encontrar la manera de eliminar los parásitos intestinales que pueden causar daño a tu salud.

¿Qué pasará en este estudio?
Si decides participar en este estudio hay algunas cosas que queremos preguntarte sobre tu salud y tus hábitos. Te entrevistarás con uno de nosotros acá en la escuela. Te mediremos tu altura, cintura y cadera, y te pesaremos. También te pediremos que nos traigas una muestra de heces (pupú) y que nos des una muestra de sangre. Podría ser que fueras seleccionado para la segunda etapa de nuestro estudio, en ese caso nos gustaría platicar contigo un poco más, en tu casa, acerca de tu salud y los parásitos intestinales.

Mientras todo esto pasa, sólo te pediremos que hagas tu mejor esfuerzo. Más o menos durará unos 30 minutos todo el proceso de la primera etapa, esto es, incluyendo las preguntas, las medidas y la toma de una muestra
de tu sangre. Ese día tú deberás traer la muestra de heces a la escuela, en un recipiente especial que te daremos. Si nos reuniéramos una segunda vez, será en tu casa y la charla debería durar aproximadamente una hora.

¿Hay cosas buenas y malas en este estudio?
Una de las cosas buenas es que tú eliminarás los parásitos de tu cuerpo y si tienes anemia (¿sabes lo que es eso?) también recibirás tratamiento. Esto significa que probablemente te sientas mejor, con más ganas y seas capaz de prestar más atención en tus clases. Otra cosa muy buena de este estudio es que nos permitirá ayudar a niños de escuela alrededor del mundo que también tengan parásitos.

Y ¿cosas malas?... en realidad no hay cosas malas en este estudio, pero entendemos que puedas sentir algo de pena de traer una muestra de heces y, quizás, algo de miedo a que te tomemos una muestra de sangre. Es muy importante que sepas que los científicos pueden examinar estos tipos de muestras para entender cómo estás de salud. Las heces son productos de desecho, algo natural a todos nosotros, y su estudio es algo de rutina que se hace para saber si una persona tiene parásitos o no.

Cuando te tomemos la muestra de sangre sentirás un dolorcito como cuando una hormiga te pica; pero podría ser que ya te hayan sacado sangre antes. Algunas veces, podría quedar un pequeño morete en el sitio donde entró la aguja; pero si sucediera esto, no debes preocuparte pues desaparecerá en pocos días. Es importante que entiendas que algunos parásitos pueden causarte anemia y la única manera de saber si tienes anemia es examinándote la sangre. Tú puedes decidir si quieres que te examinemos la sangre o no. O sea, tú puedes decidir si nos quieres dar únicamente la muestra de heces o ambos. Lo que decidas está bien para nosotros.

Te queremos asegurar que ninguna de tus muestras de heces o sangre se las daremos a nadie más. Sin embargo, enviaremos una pequeña parte de tus muestras a Canadá para que se le realicen más pruebas allá.

¿Tendrás que responder todas las preguntas y hacer todo lo que se te pida?
Las preguntas que te haremos son fáciles de responder, pero si tú no quieres responder alguna de ellas, no tienes que hacerlo, sólo dínoslo ese día. Igualmente, si no quieres hacer algo de lo que te pedimos, no lo hagas, está bien y sólo dínoslo. Nada malo te pasará si no quieres contestar o hacer algo que te pidamos. Las preguntas que te haremos no son un examen por lo que no hay respuestas correctas o incorrectas.

¿Quiénes sabrán que estás en el estudio?
Las cosas que nos digas o la información tuya que obtengamos de los exámenes de heces y sangre se conservarán como un secreto y se guardarán bajo llave. Solamente los investigadores tendrán derecho a verla. Tus maestros no la verán, tampoco tus padres o el director de la escuela. Cada vez que nosotros hablemos de este estudio, nunca usaremos tu nombre o el de ninguno de los demás niños participantes.

Participación voluntaria y retiro del estudio
Tu participación es estrictamente voluntaria. Puedes decir que sí ahora y retirarte después. Si así lo decides, no necesitas dar ninguna explicación sino sólo comunicarnos tu deseo de retirarte. Si te sales del estudio nada malo va a pasarte y tampoco nadie te castigará (ni tus padres ni tus maestros).

Si decides retirarte después de haber comenzado, quisiéramos conservar la información que nos hayas dado, si te parece bien a ti permitirlo. En este caso, siempre les daremos tus resultados de laboratorio a tus padres, especialmente si tuvieras parásitos y tu tratamiento estará disponible en el centro de salud de tu comunidad.
¿Tienes alguna pregunta?
Puedes preguntar en cualquier momento, ahora o después. Puedes hablar conmigo o con alguien más en cualquier momento durante el estudio.

Si tienes alguna pregunta después que nos hayamos ido, puedes decirles a tus padres o tus maestros que te ayuden a contactar a la Coordinadora del Proyecto acá en Honduras, la Dra. Maritza Canales. Ella trabaja en la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH) en Tegucigalpa. Sus números telefónicos son: Oficina 2252-8089; Cel. 9487-0165. Si tú o alguien que conozcas tiene acceso a internet puedes enviarle un correo electrónico a <marygchn@yahoo.com>.

Declaración de Asentimiento
Me han explicado todos los detalles de este estudio de investigación y lo he entendido. He tenido la oportunidad de hacer preguntas y todas ellas fueron contestadas satisfactoriamente. Mi participación en este estudio es libre y voluntaria. Yo sé que puedo retirarme de este estudio en cualquier momento si así lo quiero y que esto no me causará ninguna represalia o consecuencia mala en contra mía. Yo acepto participar.

El(la) niño(a) brinda asentimiento verbal a:
Ser entrevistado en la escuela para responder al cuestionario
☑ Sí ☐ No
Ser entrevistado en la casa si fuera seleccionado para esto
☐ Sí ☐ No
Proveer una muestra de heces
☐ Sí ☐ No
Proveer una muestra de sangre
☐ Sí ☐ No
Autorizar a los investigadores a conservar los datos aún si se retira del estudio
☐ Sí ☐ No

______________________________________ Escuela______________________ CÓDIGO: ______________________
Nombre del niño(a)

______________________________________ Nombre del investigador   Firma del investigador   Fecha (día/mes, 2011)

______________________________________ Nombre del testigo   Firma del testigo   Fecha (día/mes, 2011)
INFORMED ASSENT FOR CHILDREN

Date:

Introduction:
Hello, we are here because your parents gave us permission to talk to you and invite you to participate in a research study about parasites and intestinal worms in school children. Eventhough your parents gave us permission, we need to know if YOU agree to participate in the study. So we would like to tell you more about us and what is involved in the study.

Who are we?
We are a group of professors and students from Canada and Honduras interested in conducted this study. Our names and institutions are as follows:
In Honduras the main investigators are Dr. Maritza Canales (Professor) and Maria Mercedes Rueda (Grad student) of the School of Microbiology of the National Autonomous University of Honduras. Dr. Canales is the Honduran Project Coordinator.
In Canada the main investigators are Dr. Ana Sanchez (Professor at Brock University) and Dr. Theresa W. Gyorkos (Professor at McGill University) and Mary-Theresa Usuanlele and Jose Antonio Gabrie (Grad students, at Brock University).

Invitation:
We thank you for your presence here today. The reason why we are here is because we would like to invite you to participate in a study about parasites and intestinal worms. I am going to spend a few minutes to telling you about our project, and then I am going to ask you if you are interested in taking part in the project.

Why we are meeting with you?
We want to tell you about a study that involves children like yourself in schools of your area. We want to see if you would like to be in this study too. We want to find out ways to get rid of the intestinal parasites that can cause harm to your health.

What will happen to you if you are in the study?
If you decide to take part of this study there are some different things we will ask you about your health and habits. You will meet with one of our interviewers. We will measure your weight and height, your waist and your hip circumference. We will also ask you to give us a small stool specimen and blood sample. We might also visit you at your home at a later time to have a chat about your health and intestinal worms. While doing these things all you have to do is try your best. It will take you about 30 minutes to answer our questions and to give us your blood sample on our first visit to your school. At that day you will have to bring the stool sample
at school in a special container that we will provide to you. If we meet a second time, it will be at your home, and the chat should last about an hour.

Are there good things and bad things about the study?

One of the good things is that you will get rid of your parasites and if you have anemia you will get treatment too. This means that you will probably feel better and be able to pay more attention in school. Another good thing is that we will use what we find out from this study to help school children around the world who also have parasites.

There are not really bad things in this study, but we understand that you can feel anxious about providing the stool and getting a needle to give a blood sample. It is important that you know that scientists can examine these types of samples in order to understand your health status. Stools are a natural byproduct of our bodies and their examination is a routine procedure if we want to know if the person has parasites.

When we take your blood sample you will feel some pain, but maybe you had your blood sample taken before? In this case you know that this pain will go away in a few minutes. Sometimes you can get a small bruise on the site where the needle was applied but this will also go away in a few days. It is important you understand that some parasites can give anemia and the only way to know if you have anemia is by examining your blood. So you can decide if you want to have your blood examined or not. You can decide if you only want to give us the stool sample or both. Either way, it’s ok with us.

We want to assure you that we will not give any of the stool or blood sample to anybody else, for any purpose. We may send a little bit of the samples to Canada so they can examine them in their laboratories.

Will you have to answer all of the questions and do everything you are asked to do?
The questions we will ask you will be easy to answer but if you don’t want to answer, then you don’t have to. Just tell us. Also, if you don’t want to do something else we ask you to do, it’s all right; just tell us that you don’t want to do it. Nothing bad will happen if you don’t want to tell us something or if you don’t want to do something we have asked you to do. There is no right or wrong answer to any of the questions.

Who will know that you are in the study?
The things you tell us and any information that we have about you will be kept secret in a locked place. Only the researchers will be allowed to see it. Your teachers will not see it and your parents won’t see it. When we talk about this study, we will never use your name.

Voluntary participation and withdrawal from this study
Your participation in this study is strictly voluntary. You may enroll now and refuse to continue later. If that is the case, you don’t have to give us too many explanations, just tell us you don’t want to continue. If you drop out from the study, nothing bad will happen to you. Nobody will reprimand you or anything like that (including your parents or teachers).

If you no longer wanted to be part of the study, we may want to keep the information you already gave us, if that’s ok with you. In that case, we will still give your parents your laboratory results, especially if you had parasites. If you did, we will make treatment available for you at the health centre.
Do you have any questions?
You can ask questions at any time. You can ask now or you can ask later. You can talk to me or you can talk to someone else at any time during the study.

If you have questions after we are gone, you can tell your parents or your teacher to help you contact the Project Coordinator in Honduras. Her name is Dr. Maritza Canales. She works at the School of Microbiology of the National Autonomous University of Honduras (UNAH) in Tegucigalpa. Her phone numbers are: office Tel. 2252-8089; Cell. 9487-0165. If you or anybody you know has access to email, you could e-mail her at <marygchn@yahoo.com>.

Declaration of Assent
I have been explained the details of this research study and I understand it. I have had the opportunity to ask questions and all of them were answered satisfactorily. My participation in this study is free and voluntary. I know that I can withdraw from this study at any time if I want to, without any bad consequences to me. I agree to participate.

The child provides verbal assent to:
- Being interviewed at school for the questionnaire
  - [ ] Yes  [ ] No
- Being interviewed at home if selected
  - [ ] Yes  [ ] No
- Providing a stool sample
  - [ ] Yes  [ ] No
- Providing a blood sample
  - [ ] Yes  [ ] No
- Authorizing researchers to keep their data even if withdraw from the study
  - [ ] Yes  [ ] No

_______________________________________ School________________________ CODE: __________________
Child’s name

_______________________________________ ___________________________ ___________________
Researcher’s name        Researcher’s signature     Date (day/month, 2011)

_______________________________________ ___________________________ ___________________
Witness’s name           Witness’s signature       Date (day/month, 2011)
# QUESTIONNAIRE – Children

ENCUESTA – Niños

Project title: Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures

## IDENTIFICATION OF SCHOOL, CHILD, INTERVIEWER

### IDENTIFICACIÓN DE LA ESCUELA, NIÑO(A), ENTREVISTADOR

<table>
<thead>
<tr>
<th>SCHOOL: ESCUELA</th>
<th>LAST, FIRST NAME OF CHILD APELLIDO, NOMBRE DEL NIÑO(A):</th>
<th>INTERVIEWER ENTREVISTADOR:</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>LAST, FIRST NAME OF TEACHER APELLIDO, NOMBRE DEL MAESTRO(A):</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>GRADE/CLASS GRADO/AULA:</th>
<th>IDENTIFICATION CODE CÓDIGO DE IDENTIFICACIÓN:</th>
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<tbody>
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</table>

(SCHOOL INITIALS - CLASS – CHILD’S CODE) (INICIALES ESCUELA - AULA - CÓDIGO NIÑO)

<table>
<thead>
<tr>
<th>DATE OF INTERVIEW (dd/mm/yyyy) FECHA DE ENTREVISTA (dd/mm/aaaa):</th>
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</table>

## ELIGIBILITY CRITERIA

### CRITERIOS DE ELEGIBILIDAD

### A

- Obtained parent/guardian consent? ¿Se obtuvo el consentimiento de sus padres o encargados?
- Yes Sí Continue to question B Pase a la pregunta B
- No Excluya su participación

### B

- Obtained child assent? ¿Se obtuvo el asentimiento del niño?
- Yes Sí Continue to question 1 Pase a la pregunta 1
- No Excluya su participación

## BASIC INFORMATION

### INFORMACIÓN BÁSICA

<table>
<thead>
<tr>
<th>1</th>
<th>Indicate in which shift the child attends school: Indique el horario en el cual el niño(a) atiende a la escuela:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning Mañana (1) Afternoon Tarde (2) Night Noche (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2</th>
<th>What is the child’s sex? ¿Cuál es el sexo del niño?</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Male Masculino (1) Female Femenino (0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3</th>
<th>When is the child’s birthday? ¿Cuándo cumple años el niño? (dd/mm/yyyy)</th>
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<tbody>
<tr>
<td></td>
<td>Date / Age / Don’t know No sabe</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>4</th>
<th>Child weight Peso del niño</th>
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<tbody>
<tr>
<td></td>
<td>kg Scale # Don’t know No sabe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5</th>
<th>Child height Estatura del niño</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>cm Don’t know No sabe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6</th>
<th>Does the child have dirty finger nails? ¿El niño(a) tiene las uñas sucias?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes Sí (1) No No (0)</td>
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</table>

# ID - -
### Is the child wearing shoes/sandals?

<p>| | | |</p>
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<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Sí</td>
<td>No</td>
</tr>
</tbody>
</table>

**¿El niño lleva puestos zapatos o sandalias?**

### PERCEPTION/KNOWLEDGE OF PARASITES

#### PERCEPCIÓN/NIVEL DE CONOCIMIENTO SOBRE LOS PARÁSITOS

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<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
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<tr>
<td>8</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
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</table>

#### Do you know what (parasitic) worms are?

¿Conoces qué son los parásitos intestinales (lombrices)?

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<tr>
<td></td>
<td>Sí</td>
<td>No</td>
<td>Don’t know</td>
</tr>
<tr>
<td>9</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
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</table>

#### Do you think that you could get worms?

¿Crees que podrías infectarte con lombrices o parásitos intestinales?

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<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
</tr>
<tr>
<td>A</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
</tr>
<tr>
<td>B</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
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</tbody>
</table>

#### A- Do you think that worms are good for you?

¿Crees que estos parásitos son buenos para tu salud?

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<td></td>
<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
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<tr>
<td>10</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
</tr>
</tbody>
</table>

#### B- If yes or no, why? En caso de sí o no, ¿por qué?

Not applicable (NA)

#### Do you try to prevent getting worms?

¿Tratas de evitar infectarte con estos parásitos?

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<td></td>
<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
</tr>
<tr>
<td>11</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
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</table>

#### A- Do you try to prevent getting worms?

<p>| | | | |</p>
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<td></td>
<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
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<tr>
<td>B</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
</tr>
</tbody>
</table>

#### B- If yes, how? En caso de sí ¿cómo?

Not applicable (NA)

#### Do you know of any other way(s) to prevent getting worms?

¿Conoces alguna otra forma de evitar infectarte con estos parásitos?

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<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
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<td>C</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
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#### D- If yes, how? En caso de sí ¿cómo?

Not applicable (NA)

#### Do you know how you get worms?

¿Sabes cómo pueden transmitirse estos parásitos?

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<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
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<tr>
<td>12</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
</tr>
</tbody>
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#### A- Do you know how you get worms?

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<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
</tr>
<tr>
<td>B</td>
<td>Sí</td>
<td>No</td>
<td>No sabe</td>
</tr>
</tbody>
</table>

#### B- If yes, how? En caso de sí ¿cómo?

Not applicable (NA)
<table>
<thead>
<tr>
<th>ID</th>
<th>Question</th>
<th>Options</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>A- What community do you live in?</td>
<td>Community: ………………………</td>
<td>□ Don’t know No sabe (99)</td>
</tr>
<tr>
<td></td>
<td>B- On what street/sector/lot is your house?</td>
<td>Address: ………………………</td>
<td>□ Don’t know No sabe (99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>□ Walk (0) □ Camina (0)</td>
<td>□ Carro (□)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>□ Bus (1) □ Autobús (1)</td>
<td>□ Otro :</td>
</tr>
<tr>
<td></td>
<td>C- How do you get to school?</td>
<td>□ Walk (0) □ Camina (0)</td>
<td>□ Carro (□)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>□ Bus (1) □ Autobús (1)</td>
<td>□ Otro :</td>
</tr>
<tr>
<td></td>
<td>D- How long does it take you to get to school (in minutes)?</td>
<td>□ Walk (0) □ Camina (0)</td>
<td>□ Carro (□)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>□ Bus (1) □ Autobús (1)</td>
<td>□ Otro :</td>
</tr>
<tr>
<td>14</td>
<td>What is the floor of your home made of?</td>
<td>□ Tiles (0) □ Granito (0)</td>
<td>□ Earth (2) □ Tierra (2)</td>
</tr>
<tr>
<td></td>
<td>In your house, do you cook with gas, kerosene, carbon or firewood?</td>
<td>□ Gas (0) □ Kerosen (1)</td>
<td>□ Carbon (2) □ Carbón (2)</td>
</tr>
<tr>
<td></td>
<td>Does your house have</td>
<td>□ Gas (0) □ Kerosen (1)</td>
<td>□ Carbon (2) □ Carbón (2)</td>
</tr>
<tr>
<td></td>
<td>A- Electrical energy?</td>
<td>Yes (1) □ Sí (1)</td>
<td>No (0) □ No (0)</td>
</tr>
<tr>
<td></td>
<td>B- A radio?</td>
<td>Yes (1) □ Sí (1)</td>
<td>No (0) □ No (0)</td>
</tr>
<tr>
<td></td>
<td>C- A television?</td>
<td>Yes (1) □ Sí (1)</td>
<td>No (0) □ No (0)</td>
</tr>
<tr>
<td></td>
<td>D- A refrigerator?</td>
<td>Yes (1) □ Sí (1)</td>
<td>No (0) □ No (0)</td>
</tr>
<tr>
<td>15</td>
<td>A- Do you have potable water in your house (tap water)?</td>
<td>Yes (1) □ Sí (1)</td>
<td>No (0) □ No (0)</td>
</tr>
<tr>
<td></td>
<td>B- If no, where do you get water for your house?</td>
<td>Neighbor’s tap (0)</td>
<td>Tank Tanque (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Llave donde un vecino (0)</td>
<td>Public pool Pila o llave pública (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>River (1) □ Río (1)</td>
<td>Other Otro (6) Don’t know No sabe (99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Well (2) □ Pozo (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water truck Cisterna (3)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>In your house, do you drink water directly or treated (boiled/chemicals/filtered)?</td>
<td>Directly (1) □ Directo (1)</td>
<td>Treated (0) □ Tratada (0)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
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<td>---</td>
</tr>
<tr>
<td><strong>19</strong></td>
<td>A- Do you have a flushable toilet? ¿Tienes servicio sanitario (inodoro)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Sí (1)</td>
<td>No</td>
</tr>
<tr>
<td>B- Do you have a latrine? ¿Tienes letrina?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Sí (1)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>20</strong></td>
<td>How many other children (&lt; 20 years of age; boys/girls; older/younger than you) live in your house? ¿Cuántas personas &lt; 20 años de edad (mayor/menor que ti) viven en tu casa?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boys: Niños: older mayor(es) younger menor(es)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girls: Niñas: older mayor(es) younger menor(es)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>21</strong></td>
<td>How many adults (≥20 years of age; men/women) live in your house? ¿Cuántas personas (≥ 20 años de edad) viven en tu casa?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>22</strong></td>
<td>How many children (boys/girls) under 5 years old live in your house? ¿Cuántos niños o niñas menor(es) de 5 años de edad viven en tu casa?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>23</strong></td>
<td>Do you do any of the following chores on a regular basis? ¿Haces regularmente algunas de las siguientes tareas?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No chores (0) Ninguna tarea</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cooking Cocinar (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Changing diapers Cambiar pañales (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Taking care of younger children Cuidar niños menores (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Taking care of people who are sick Cuidar personas enfermas (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other: Otro:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>24</strong></td>
<td>A- Do you have any animals? ¿Tienes algunos animales?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B- If yes, what kind of animal(s)? ¿En caso que sí, qué tipo de animal?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable No aplica (NA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dog Perro (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cat Gato (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C- If yes, do you do any of the following tasks to take care of your animal? ¿En caso que sí, haces algunas de las siguientes cosas para cuidar de tu animal?:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable No aplica (NA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feed Darles comida (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Provide water Darles agua (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brush Cepillarlos (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D- If yes, do you play with your animal(s) – always, sometimes or never? ¿En caso que sí, juegos con los animales – siempre, a veces o nunca?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable No aplica (NA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Always Siempre (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sometimes A veces (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never Nunca (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Version: January 25, 2011
Versión: 25 de Enero, 2011

# ID - -
<table>
<thead>
<tr>
<th></th>
<th><strong>RISK FACTOR CATEGORY 2: HYGIENE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Do you defecate in open air (eg. in the bush) - always, sometimes, or never? ¿Haces pupú en campo abierto (huerta o patio) - siempre, A veces, o nunca?</td>
</tr>
<tr>
<td>26</td>
<td>Do you wipe with toilet paper after defecating - always, sometimes, or never? ¿Te limpias con papel higiénico cuando haces pupú - siempre, A veces, o nunca?</td>
</tr>
<tr>
<td>27</td>
<td>A- How many times per day, approximately, do you wash your hands? ¿Cuánto veces por día, aproximadamente, te lavas las manos?</td>
</tr>
<tr>
<td>28</td>
<td>B- Do you use soap when washing your hands – always, sometimes or never? ¿Usas jabón cuando te lavas las manos – siempre, A veces o nunca?</td>
</tr>
<tr>
<td>29</td>
<td>A- Do you wash your hands after going to the bathroom – always, sometimes, or never? ¿Te lavas las manos después de ir al baño – siempre, A veces, o nunca?</td>
</tr>
<tr>
<td>30</td>
<td>B- Do you use soap when washing your hands after going to the bathroom- always, sometimes or never? ¿Usas jabón cuando te lavas las manos después de ir al baño – siempre, A veces o nunca?</td>
</tr>
<tr>
<td></td>
<td>A- How many times per week do you bathe? ¿Cuánto veces por semana te bañas?</td>
</tr>
</tbody>
</table>
### Risk Factor 3: Activities

**Factor de Riesgo Categoría 3: Actividades**

<table>
<thead>
<tr>
<th>A- Do you bite your nails – always, sometimes or never? ¿Te muerdes las uñas – siempre, A veces o nunca?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Always  (2) □ Never (0) □ Don’t know (99)</td>
</tr>
<tr>
<td>□ Siempre  (2) □ Nunca (0) □ No sabe (99)</td>
</tr>
<tr>
<td>□ Sometimes (1) □ A veces (1) □ Don’t know (99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B- Do you suck on any of your fingers (eg. suck your thumb) – always, sometimes or never? ¿Te chupas los dedos (ej. pulgar) – siempre, A veces o nunca?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Always  (2) □ Never (0) □ Don’t know (99)</td>
</tr>
<tr>
<td>□ Siempre  (2) □ Nunca (0) □ No sabe (99)</td>
</tr>
<tr>
<td>□ Sometimes (1) □ A veces (1) □ Don’t know (99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For the main meal of the day, do you use your fingers to eat? ¿Usas tus dedos para comer durante la comida principal del día?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Always  (2) □ Never (0) □ Don’t know (99)</td>
</tr>
<tr>
<td>□ Siempre  (2) □ Nunca (0) □ No sabe (99)</td>
</tr>
<tr>
<td>□ Sometimes (1) □ A veces (1) □ Don’t know (99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A- Do you presently work (other than at school or home)? ¿Trabajas actualmente (diferente que en escuela o en la casa)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Yes  (1) □ No (0) □ Don’t know (99)</td>
</tr>
<tr>
<td>□ Sí (1) □ No (0) □ No sabe (99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B- If yes, what do you do? ¿En caso que sí, que haces?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Not applicable</td>
</tr>
<tr>
<td>No aplica (NA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C- If yes, how many hours/week, approximately, do you work? ¿En caso que sí, cuánto horas por semana, aproximadamente, trabajas?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Not applicable</td>
</tr>
<tr>
<td>No aplica (NA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A- How often do you play outside per day, on average? ¿Cuánto horas por día, en promedio, juegos afuera?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 0 hours/day  (3) □ 1-3 hours/day (1) □ &gt; 3 hours/day (0) □ Don’t know No sabe (99)</td>
</tr>
<tr>
<td>□ 0 horas/día (3) □ 1-3 horas/día (1) □ &gt; 3 horas/día (0) □ No sabe (99)</td>
</tr>
<tr>
<td>□ &lt; 1 hour/day (2) □ &gt; 3 horas/día (0) □ Don’t know No sabe (99)</td>
</tr>
<tr>
<td>□ &lt; 1 hora/día (2) □ No sabe (99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B- What games do you play outside? ¿Qué juegos juegas afuera?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ None</td>
</tr>
<tr>
<td>Ninguno (NA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C- Do you play in dirt- always, sometimes or never? ¿Juegas en la tierra – siempre, A veces o nunca?</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Always  (2) □ Never (0) □ Don’t know (99)</td>
</tr>
<tr>
<td>□ Siempre  (2) □ Nunca (0) □ No sabe (99)</td>
</tr>
<tr>
<td>□ Sometimes (1) □ A veces (1) □ Don’t know (99)</td>
</tr>
</tbody>
</table>
### Risk Factor Category 4: Wearing Shoes

**Factor de riesgo categoría 4: Llevar zapatos**

<table>
<thead>
<tr>
<th>35</th>
<th>Do you go outside without shoes on—always, sometimes, or never? ¿Caminas descalzo(a) — siempre, A veces, o nunca?</th>
<th>Always</th>
<th>Siempre (2)</th>
<th>Never</th>
<th>Nunca (0)</th>
<th>Don’t know</th>
<th>No sabe (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Siempre (1)</td>
<td>A veces</td>
<td>Never</td>
<td>Nunca</td>
<td>Don’t know</td>
<td>No sabe (99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A veces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 36 | When you are at home, do you wear shoes or sandals? ¿Cuándo estás en casa, llevas zapatos o sandalias? | Shoes | Zapatos (2) | Neither | Ningunas (0) | Don’t know | No sabe (99) |
|    |                                                                                                                                                      | Sandals | Sandalias (1) |        |          |             |             |

| 37 | In what activities do you not wear shoes? ¿En qué actividades del día estás descalzo(a)? | A- Activity | Actividad: .......................................................... |
|    |                                                                                                                                                      | B- Activity | Actividad: ..........................................................
|    |                                                                                                                                                      | C- Activity | Actividad: ..........................................................

### Risk Factor Category 5: Use of Health Services

**Factor de riesgo categoría 5: Uso de servicios de salud**

<table>
<thead>
<tr>
<th>38</th>
<th>Can you go to the health centre and return to your home in the same day? ¿Puedes ir a un centro de salud y regresar a tu casa en el mismo día?</th>
<th>Yes</th>
<th>Sí (1)</th>
<th>No</th>
<th>No (0)</th>
<th>Don’t know</th>
<th>No sabe (99)</th>
</tr>
</thead>
</table>

| 39 | How many times have you been sick in the last year? ¿Cuántas veces estuviste enfermo(a) en el último año? | | times in last year | veces en el último año |

| 40 | How many times have you been to the doctor/health centre/healer in the last year? ¿Cuántas veces fuiste al doctor/centro de salud/curador porque estabas enfermo(a) en el último año? | | times in last year | veces en el último año |

| 41 | How many times have you been to the doctor/health centre/healer in the last year for a routine check-up or vaccinations? ¿Cuánto veces fuiste al doctor/centro de salud/curador para un visita rutina o para vacunas en el último año? | | times in last year | veces en el último año |

| 42 | Who takes care of you when you are sick? ¿Quién te cuida cuando estás enfermo(a)? | Mother | Madre (4) | Other family member: | Otro familiar: | Other: | Otro: | Don’t know | No sabe (99) |
|    |                                                                                                                                                     | Father | Padre (3) |               |             |        |         |             |             |
|    |                                                                                                                                                     | Nobody | Nadie (0) |               |             |        |         |             |             |

| # ID | - | - |
### Risk Factor Category 6: History of Deworming

**Factor de riesgo categoría 6: Historia de desparasitación**

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> - Have your parent(s) or teacher(s) ever given you treatment (traditional or modern medicine) for intestinal worms?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¿Tu(s) padre(s) o tu(s) profesor(es) te dieron tratamiento o remedio para los parásitos intestinales?</td>
<td>□ Yes (1)</td>
<td>□ No (0)</td>
<td>□ Don’t know No sabe (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **B** - If yes, what did they give you? |   |   |   |   |   |   |
| ¿En caso que sí, qué te ha(n) dado? | □ Not applicable No aplica (NA) |

| **C** - If yes, when was the last time you were dewormed? |   |   |   |   |   |   |
| ¿En caso que sí, cuándo fue la última vez que te dieron este tratamiento? | □ Not applicable No aplica (NA) |

| Months ago | Over a year ago | □ Don’t know No sabe (99) |
| Meses | Más de un año |

**Comments**:________________________________________________________
________________________________________________________________________________________

**Checked for completeness**: □ : by: __________________ ; date: __________________

**Verificó que estaba completo**: __________________ ; fecha: __________________

(name) ; (dd-mm-yyyy)

(nombre) ; (dd-mm-aaaa)

**Thank you for participating! ¡Gracias por tu participación!**
### ADDITIONAL QUESTIONS

#### PREGUNTAS ADICIONALES

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>44.</strong> Have you ever had worms? ¿Has tenido alguna vez parásitos intestinales (lombrices)?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-</strong> If yes, when was the last time? En caso de sí, ¿Cuándo fue la última vez?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 6 months</td>
<td>≥ 6 months</td>
<td>Don’t know</td>
</tr>
<tr>
<td><strong>45.</strong> Do you use the same type of water for drinking and wash your hands? ¿Usas el mismo tipo de agua para tomar y para lavarte las manos?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-</strong> If no, where do you get water for washing your hands? ¿En caso que no, de dónde se abastecen de agua para lavarse las manos?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neighbor’s tap</td>
<td>Tank</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>Neighbors tap</td>
<td>Tank</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>River</td>
<td>Water truck</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>Public pool</td>
<td>Other</td>
</tr>
<tr>
<td><strong>46.</strong> Where is your latrine located? ¿Dónde está ubicada la letrina?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 10 m</td>
<td>≥ 10 m</td>
<td></td>
</tr>
<tr>
<td><strong>47.</strong> Do you have pigs? ¿Tienes cerdos?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-</strong> If yes, How many? En caso de sí, ¿Cuántos?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C-</strong> Do you eat pork meat? ¿Comes carne de cerdo?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excellent (feel very well / plenty of energy)</td>
<td>Regular (tired and sleepy sometimes)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excelente (me siento muy bien / con mucha energía)</td>
<td>Regular (A veces cansado y con sueño)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>Bad (sick or tired and sleepy often/affecting my studies)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bien</td>
<td>Mal (me enfermo mucho o paso cansado y con sueño, me afecta en los estudios)</td>
<td></td>
</tr>
<tr>
<td><strong>48.</strong> How would you describe your health status? ¿Cómo describirías tu estado de salud?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excellent (feel very well / plenty of energy)</td>
<td>Regular (tired and sleepy sometimes)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excelente (me siento muy bien / con mucha energía)</td>
<td>Regular (A veces cansado y con sueño)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>Bad (sick or tired and sleepy often/affecting my studies)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bien</td>
<td>Mal (me enfermo mucho o paso cansado y con sueño, me afecta en los estudios)</td>
<td></td>
</tr>
<tr>
<td><strong>49.</strong> Did you miss more than 3 days from school last year? ¿Has faltado a clases por más de tres días en el último año?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-</strong> If yes, why? En caso de sí, ¿por qué?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sickness</td>
<td>Other</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enfermedad</td>
<td>Otro</td>
<td></td>
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</table>
# CHILD'S MEASUREMENTS

**MEDIDAS DEL NIÑO(A)**

<table>
<thead>
<tr>
<th>Date</th>
<th>School’s name</th>
<th>Child’s name</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CODE #</th>
<th>CÓDIGO #</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

## STATION #1 / ESTACIÓN #1

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Scale # / Balanza #</th>
<th>Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight / Peso</strong></td>
<td>lb</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (standing) / Altura (de pie)</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (sitting) / Altura (sentado)</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hips circumference / Cadera</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Waist circumference / Cintura</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## AVERAGE / PROMEDIO

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Scale # / Balanza #</th>
<th>Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight / Peso</strong></td>
<td>lb</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (standing) / Altura (de pie)</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (sitting) / Altura (sentado)</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hips circumference / Cadera</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Waist circumference / Cintura</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## STATION #2 / ESTACIÓN #2

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<th>Scale # / Balanza #</th>
<th>Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight / Peso</strong></td>
<td>lb</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (standing) / Altura (de pie)</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (sitting) / Altura (sentado)</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hips circumference / Cadera</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Waist circumference / Cintura</strong></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# QUESTIONNAIRE – School

Project title: Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures

## IDENTIFICATION

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Name of school</td>
</tr>
<tr>
<td>2</td>
<td>Address of school</td>
</tr>
<tr>
<td>3</td>
<td>Date of interview (dd/mm/yyyy)</td>
</tr>
<tr>
<td>4</td>
<td>Name of school director or principal (interviewee)</td>
</tr>
<tr>
<td>5</td>
<td>Name of interviewer</td>
</tr>
</tbody>
</table>

### A – Does the school have a deworming program?
- Yes (1)
- No (0)
- Don’t know (99)

### B – If yes, in what year did the deworming program begin?
- Not applicable (NA)
- Year program began (yyyy): __________
- Don’t know (99)

### C - If yes, when was deworming last given to all children (in months and/or days)?
- Not applicable (NA)
- ______ months, ______ days
- Don’t know (99)

### D - If yes, how often is deworming given?
- Not applicable (NA)
- ______ time(s) every ______ year(s)
- Don’t know (99)

### 7 What are the classroom floors made of?
- Tiles (0)
- Earth (2)
- Don’t know (99)
- Cement (1)
- Other: ______________________ (3)

## SCHOOL COMPOSITION

### 8 How many education levels are there in the school?
- Only primary (1)
- Pre-school and primary (2)
- Primary and secondary (3)
- Don’t know (99)

### A- How many grade 1 classes are there in the school?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1 classes</td>
</tr>
<tr>
<td></td>
<td>Don’t know (99)</td>
</tr>
</tbody>
</table>

### B- How many grade 1 students (boys and girls) are there in the school?
- Grade 1 boys: ______
- Grade 1 girls: ______
- Don’t know (99)
<table>
<thead>
<tr>
<th>10</th>
<th>A- How many grade 2 classes are there in the school?</th>
<th>Grade 2 classes</th>
<th>Don't know (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B- How many grade 2 students (boys and girls) are there in the school?</td>
<td>Grade 2 boys; Grade 2 girls</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td>11</td>
<td>A- How many grade 3 classes are there in the school?</td>
<td>Grade 3 classes</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td></td>
<td>B- How many grade 3 students (boys and girls) are there in the school?</td>
<td>Grade 3 boys; Grade 3 girls</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td>12</td>
<td>A- How many grade 4 classes are there in the school?</td>
<td>Grade 4 classes</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td></td>
<td>B- How many grade 4 students (boys and girls) are there in the school?</td>
<td>Grade 4 boys; Grade 4 girls</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td>13</td>
<td>A- How many grade 5 classes are there in the school?</td>
<td>Grade 5 classes</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td></td>
<td>B- How many grade 5 students (boys and girls) are there in the school?</td>
<td>Grade 5 boys; Grade 5 girls</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td>14</td>
<td>A- How many grade 6 classes are there in the school?</td>
<td>Grade 6 classes</td>
<td>Don't know (99)</td>
</tr>
<tr>
<td></td>
<td>B- How many grade 6 students (boys and girls) are there in the school?</td>
<td>Grade 6 boys; Grade 6 girls</td>
<td>Don't know (99)</td>
</tr>
</tbody>
</table>

**QUESTIONNAIRE - WATER**

| 15 | A- Is there water in the school? | Yes (1) | No (0) | Don’t know (99) |
|    | B- If yes (to A), do the students drink this water? | Yes (1) | No (0) | Don’t know (99) |
|    | Not applicable (NA) | |
|    | C- If yes (to A), where does the school’s water come from? | Well with pump (0) | Potable (tap) water (4) |
|    | | Well without pump (1) | Rain water (5) |
|    | | Water truck (2) | Other:______________ (6) |
|    | | Tank (3) | Don’t know (99) |
|    | D- If yes (to A), does the school use chlorine to treat the water? | Yes (1) | No (0) | Don’t know (99) |
|    | Not applicable (NA) | |
| 16 | A- Does the school have water available all day? | Yes (1) | No (0) | Don’t know (99) |
|    | B- If no, when is water available? | If no, water is available: | |
|    | Not applicable (NA) | | |
### QUESTIONNAIRE - SANITATION

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>A- Are there toilet facilities for the students of the school?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B- If yes (to A), are there separate toilet facilities for boys and girls?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C- If yes (to A), are the toilet facilities in a separate building from the school?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>A- Are there functioning, flushable toilets for the students in the school?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B- If yes, what type of toilet?</td>
<td>□ Other:_________ (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□ Not applicable (NA)</td>
<td>□ Structure above ground (1) □ Hole (2) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Is there a functioning latrine for the students of the school?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>A- Are there sinks for the students to wash their hands in the school?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B- If yes, do the sinks have potable (tap) water?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>A- Is there soap available in the school for students to wash their hands?</td>
<td>□ Yes (1) □ No (0) □ Don’t know (99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B- If no, why is it not available?</td>
<td>If no, reason:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□ Not applicable (NA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes (1)</th>
<th>No (0)</th>
<th>NA</th>
<th>In General</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td>-The toilet or latrine is functional</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-The toilet or latrine is clean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-There is toilet paper available</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-There is a clean cloth or paper towel to dry the hands</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-The garbage is covered or emptied often</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-There is no dirty toilet paper on the floor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-Has a good smell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-The doors are on the hinges</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-The floor is frequently cleaned</td>
</tr>
</tbody>
</table>
### How are the conditions of the boys' toilet or latrine?

<table>
<thead>
<tr>
<th></th>
<th>Yes (1)</th>
<th>No (0)</th>
<th>NA</th>
<th>Boys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The toilet or latrine is functional</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The toilet or latrine is clean</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>There is toilet paper available</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>There is a clean cloth or paper towel to dry the hands</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The garbage is covered or emptied often</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>There is no dirty toilet paper on the floor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Has a good smell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The doors are on the hinges</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The floor is frequently cleaned</td>
</tr>
</tbody>
</table>

**Not applicable (NA)**

### How are the conditions of the girls' toilet or latrine?

<table>
<thead>
<tr>
<th></th>
<th>Yes (1)</th>
<th>No (0)</th>
<th>NA</th>
<th>Girls</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The toilet or latrine is functional</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The toilet or latrine is clean</td>
</tr>
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<td></td>
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<td>There is a clean cloth or paper towel to dry the hands</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>The garbage is covered or emptied often</td>
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<tr>
<td></td>
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<td>There is no dirty toilet paper on the floor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Has a good smell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The doors are on the hinges</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The floor is frequently cleaned</td>
</tr>
</tbody>
</table>

**Not applicable (NA)**

**Comments or observations:**
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

**THANK YOU FOR PARTICIPATING!**
CUESTIONARIO PARA LOS NIÑOS
GUÍA PARA LA ENTREVISTA
PARA ALGUNAS PREGUNTAS SELECCIONADAS

Estudio de Investigación: Parásitos Intestinales en niños escolares de Honduras

Este cuestionario es para ser aplicado a los niños de las escuelas

A-B Asegurarse de que los formularios de Consentimiento / Asentimiento fueron obtenidos (todas las casillas apropiadamente marcadas, firmado, fechado y la firma del testigo obtenida).

1-2 Observación.

3 Preguntar al niño su fecha de nacimiento y su edad.

4-5 Dejar en blanco en este momento. Luego se colocarán las medidas promedio del niño, obtenidas de la hoja de medidas. NOTA: ¡Las balanzas deberán ser calibradas diariamente! Si se usara el promedio de las 2 balanzas (en el caso que ambas estuvieran en el sitio), en la casilla de balanza se colocará N/A, de lo contrario se anotará el número de la balanza utilizada.

6-7 Observación.

8 Se debe explicar a los niños el concepto de parásitos intestinales, utilizando las palabras más apropiadas de acuerdo a la comunidad visitada (p.ej. lombrices). De ser necesario, dar una breve descripción de estos parásitos. Si se dispone de ejemplares preservados, mostrarlos a los niños.

10B Deberá ser contestada sólo si la respuesta en la 10A es “Sí” o “No”. La opción “NA” será utilizada sólo si la respuesta en 10A es “No sabe”. Referirse a la pregunta 44 (preguntas adicionales).

11 Se debe distinguir entre lo que el niño(a) hacen para prevenir la infección (11A-B) de lo que saben que debe hacerse (pero que no lo hacen) para prevenirlo (11C-D)

12 Describir lo que ellos consideran que es la forma de transmisión de los parásitos intestinales.

14 Esta pregunta debe adecuarse a las condiciones socio-económicas de la comunidad. Marcar una casilla únicamente (si es de varios tipos, utilizar “otro” y describir)

15 Marcar una casilla únicamente.

17B Marcar todas las que apliquen. Referirse a la pregunta 45 (preguntas adicionales).

18 Marcar una casilla únicamente. Tratado se refiere a ebullición (hervir), adición de químicos (cloro, yodo) o filtrado antes de consumir el agua.

19 Una vez realizada esta pregunta, referirse a la pregunta 46 (preguntas adicionales)

20 Se refiere a cualquier persona menor de 20 años que viva en la misma casa del niño(a), puede ser hermano, hermana o cualquier otro.

21 Se refiere a cualquier persona mayor de 20 años que viva en la misma casa del niño(a).

23 Marcar todas las que apliquen

24B Marcar todas las que apliquen. Referirse a la pregunta 47 (preguntas adicionales).

28A-B Es muy importante explicar que “después de ir al baño” significa después de defecar. Conocer el entorno cultural de la comunidad para utilizar las palabras apropiadas a fin de que este concepto quede muy claro al niño(a).

32 Se refiere a la comida principal, podría ser almuerzo, cena o ambos.

33 Se refiere a trabajo, ya sea pagado o no.

34B Anotar los 4 juegos más importantes (si existen más de 4).

37 Anotar las 3 actividades más importantes (si existen más de 3).

40-41 Se refiere a cualquier servicio de salud disponible (CESAR, CESAMO, Hospital de área, centro de medicina natural, etc.).

42 Una vez realizada esta pregunta, referirse a las pregunta 48 y 49 (preguntas adicionales).

43 Se refiere a cualquier tipo de tratamiento (p.ej. antihelmínticos, hierbas tradicionales, etc.).

Completado Alguien distinto al entrevistador deberá revisar el cuestionario para asegurar que ha sido completado de manera clara y apropiada. Esta persona deberá firmar y colocar la fecha en los espacios respectivos.
INTERVIEW GUIDE - CHILD QUESTIONNAIRE
FOR SELECTED QUESTIONS

Project title: Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures

This questionnaire is to be administered to the schoolchildren

A-B Ensure that consent/assent forms are complete (all appropriate boxes checked, signed, dated, witnessed)

1-2 Interviewer observation

3 Ask child their birthday and age

4-5 Measurement (NOTE: if more than 1 scale is being used in the study, each scale should be identified with a number, and the scale # used should be indicated in the questionnaire) Remember that each scale needs to be calibrated daily!

6-7 Interviewer observation

8 This question will need to be translated carefully. It is important that the culturally-appropriate words to describe parasitic worms are used. If necessary, a brief description may need to be given so that the concept of worms is adequately explained.

10B Note that this should be answered if answer to 10A is “yes” OR “no”. The NA answer is to be used only if “don’t know” was the answer to 10A.

11 Note distinction between things that the child actually does her/himself to prevent worms (11A-B) and things that the child knows he or she can do (but doesn’t actually do) to prevent worms (11C-D)

12 Hypothetically

14 This question could be changed to a different socio-economic status indicator of home, if a better one exists (eg. # of rooms), depending on each site. Check one box only (if mixed, include in “other”)

15 Check one

17B Check all that apply

18 Check one; Treated refers to all options of boiling, adding chemicals or filtering water before consuming it.

20 This refers to any other child living in the same home (could be brothers/sisters, but not necessarily); Child refers to being younger than 20 years of age.

21 Any adults; Adult refers to being 20 years of age or older

23 Check all that apply

24B Check all that apply

28A-B Cultural translation of “going to bathroom”? – this is important.

32 Refers to biggest meal of the day (may be culturally dependent – this could be changed to specify what this meal is, if obvious, in each site)

33 Paid or unpaid work

34B List top 4 games (if more than 4 exist), or, as many games as child can think of

37 List top 3 activities if more than 3 exist

40-41 Refers to any health services (traditional or modern)

43 This refers to any type of treatment (eg. anthelmintics, traditional herbs, etc.)

Completeness Have someone other than the interviewer review the entire questionnaire to ensure clarity and completion. This person should sign and date the form.

Version: January 25, 2011
INTERVIEW GUIDE – SCHOOL QUESTIONNAIRE
FOR SELECTED QUESTIONS

Project Title: Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures

This questionnaire is to be administered to school principal/director

4 Name of person who is answering questions (interviewee).
6C Indicate how many months AND/OR days ago the LAST deworming was given to all students.
6D (eg. 1 time every 2 years)
8 This question will likely need to be adapted depending on local school system.
9-14 Again, this may need to be adapted (add or remove grades) depending on school system and how many grades can exist in a primary school.
15B-D Ask questions 15 B,C,D if the answer to 15A is “yes”; NA to 15 B,C,D if answer to 15A is “no”.
17 B-C Ask both questions if the answer to 17A is “yes”; NA to B-C if answer to A is “no”.
22 - 24 Q 22: if the toilet/latrine facilities are NOT SEPARATE for boys and girls.
Q 23/24: if the toilet/latrine facilities ARE SEPARATE for boys and girls . (23 = boys’ facilities; 24 = girls’ facilities)
Comments Any relevant comment/observations about the school that could have an effect on STHs (eg. Cleaning, deworming, additional programs, etc).
BMI-for-age GIRLS
5 to 19 years (z-scores)

Age (completed months and years)

BMI (kg/m²)

2007 WHO Reference
# Height-for-age GIRLS

5 to 19 years (z-scores)

<table>
<thead>
<tr>
<th>Height (cm)</th>
<th>Age (completed months and years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td></td>
</tr>
<tr>
<td>170</td>
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<td>100</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>5 6 7 8 9 10 11 12 13 14 15 16 17 18 19</td>
</tr>
</tbody>
</table>

Appendix 4B2
Weight-for-age BOYS

5 to 10 years (z-scores)

Weight (kg)

Age (completed months and years)

-3
-2
-1
0
1
2
3

369 Months
Years 5 6 7 8 9 10

Appendix 4C1

World Health Organization

2007 WHO Reference
Weight-for-age GIRLS
5 to 10 years (z-scores)
STANDARD OPERATING PROCEDURE

Title: The Kato-Katz technique for the diagnosis of soil-transmitted helminthiasis

Objective:
The aim of this SOP is to describe the steps involved in the diagnosis of Soil-transmitted helminthiasis using the Kato-Katz Technique (Kato and Miura 1954)

Principle:
The adult female worms of the soil-transmitted helminths (STHs) (*Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms *Ancylostoma duodenale* and *Necator americanus*) lay eggs that are passed in the feaces of infected persons. Each species lay characteristic eggs which can be seen and counted when stool samples are examined under a microscope. The WHO recommends the use of the Kato-Katz technique for community diagnosis of STH infections in areas where there is moderate to high intensity infections (WHO 2008)

Materials:
Materials needed for the Kato-Katz technique include

1. Kato-Katz set containing a plastic template with hole (Standard = 41.7 mg), nylon screen or sieve and spatula (wooden or plastic)
2. Malachite green + glycerol solution (1 mL of 3% aqueous Malachite green in 100 mL of glycerol + 100 mL of distilled water)
3. Cellophane strip - acts as a cover slip (this should be soaked in the malachite green + glycerol mixture for at least 24 hours prior to use)
4. Two 2’X 3’ microscope slides
5. Plastic template (Standard = 41.7 mg)
6. Fresh feacal sample in a wide mouthed container (plastic cup), properly covered
7. A pair of forceps
8. Some newspaper
9. Lab coat and gloves


**Equipments:**

1. Binocular microscope

**Steps or procedure:**

1. Wear your lab coat and gloves
2. Spread a sheet of newspaper over your working surface
3. Label a microscope slide with the patients information (code number etc.) and place the plastic template in the middle, place this on the newspaper
4. Using the spatula, place a small amount of feacal specimen onto the inside of the cover of the stool container
5. Place and press the nylon screen/sieve over the feacal specimen so that some feacal material can filter through
6. Scrape the sieved material with the spatula - this helps to minimize the amount of debris picked up
7. Place the collected feaces into the hole in the template on the slide, fill the hole, avoiding air bubbles and level the feaces off in order to avoid having excess feaces
8. Lift the template carefully so that the feacal specimen is left on the slide. The template is then put into the bucket of detergent/disinfectant mixture (it can be washed and reused). The template helps to measure an approximate 41.7 mg of feaces
9. Using the pair of forceps, pick a cellophane strip, previously soaked in malachite green-glycerol mixture and place it over the feacal specimen on the slide
10. Flip the slide and press the feacal material against the cellophane slip on the work bench. The feacal material will spread evenly making a smear
11. Lift up the slide carefully so that the cellophane side is up, and the placing the second slide over the cellophane press down to spread the feacal specimen some more. Carefully remove the second slide
12. Place the slide on the newspaper, one should be able to read the prints through the resulting feacal smear
13. The slide is the placed on the microscope and examined systematically in a zigzag manner starting with the X10 objective lens. Any egg found is properly indentified with the X40 objective lens
14. The number and types of eggs found are recorded and later multiplied by a factor of 24 when using the standard 41.7 mg template

CAUTION!
The slide should be read within 30 – 60 minutes when hookworms are suspected, otherwise, hookworm eggs tend to disappear after this time (WHO 2008), this is due to over clarification by glycerin (WHO 1991)

Other sources of information for this write up include (Endriss, Escher et al. 2005)

More information

Classes of intensity for soil-transmitted helminths according to the number of eggs per gram (epg) in stool examination by the KK technique (WHO 2002)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Light-intensity infections</th>
<th>Moderate-intensity infections</th>
<th>Heavy-intensity infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>1-4,999 epg</td>
<td>5,000-49,999 epg</td>
<td>≥ 50,000 epg</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>1-999 epg</td>
<td>1,000-9,999 epg</td>
<td>≥ 10,000 epg</td>
</tr>
<tr>
<td>Hookworms</td>
<td>1-1999 epg</td>
<td>2,000-3,999 epg</td>
<td>≥ 4,000 epg</td>
</tr>
</tbody>
</table>
STH eggs

Source: http://public.health.oregon.gov/LaboratoryServices/ImageLibrary/Pages/round2.aspx

References

Samples: Serum or plasma, fresh or frozen

Instruments and equipment: Refractometer MASTER-SUR/Nα, Vortex, Micropipette 300 µL capacity

Materials: Total proteins control, tips 300 µL capacity, Kimwipes or dry soft tissues, distilled water in a squeeze water bottle, rack for vials, absorbent underpad, biohazard bags, bucket or beaker with 5% bleach solution to discard liquids, permanent markers and pen, laboratory book.

PPE: Laboratory coat, gloves.

Normal values: 6.0 – 8.3 gm/dL

Procedure:

1. - If starting with frozen samples, thaw samples ahead of time to reach ambient temperature. Allow any reagents, materials or equipment reach room temperature

2. - Place an absorbent underpad on to work area and place all materials on it.

3. - Verify the calibration of the refractometer by following the next steps:

   3a) Confirm that the prism is clean and free of scratches; Wipe it with a lint free wipe as necessary
3b) Verify that distilled water gives a reading of zero on the centre scale of the refractometer’s reading scale. The centre scale indicates the concentration of protein in g/100mL.

To place the sample

Place 200 µL of distilled water on the rear end of the sample stage without lifting the daylight plate and allow it to spread by capillarity. To do this, position the instrument flat on the counter for better stability.

Reading: (Perform the reading under a direct overhead white light source) View the scale through the eye piece. To focus, turn eye piece in either direction until scale is clear (sharply in focus). Read the measurement value where boundary line intersects the scale. Distilled water measurement should read zero (Wt. line).

4. - Once you have verified the calibration (Distilled water measurement), proceed with your samples as detailed below.

4a) Gently stir the specimen with a vortex 3 times. Using a micropipette, slowly add 200 µL of specimen as described above. Since serum/plasma samples are more viscous than water, take extra precautions to avoid air bubbles. Discard the micropipettes tip into a biohazard bag.

4b) Take the measurement: View the scale (center) through the eyepiece and under the white light; you can get a better focus by turning the eyepiece in either direction until clear; you will see a boundary line intersecting the scale that is your sample’s measurement.
4c) If a reading is above or below of the normal range, repeat the procedure once more. If the reading is the same or +/- 0.2, the first reading can be taken as a true value; but if the second reading differs from the first by more than 0.2 then perform a third measurement and accept the average of the two closest readings as your value. If all three readings differ for more than 0.4, start over by rinsing and cleaning the instrument and making sure you follow the standard operative procedures properly.

4d) Rinse the prism and sample stage with distilled water, carefully support the daylight plate with your thumb to avoid breaking it, and wipe off any remaining water with a dry soft tissue (Kimwipes) to avoid any scratch in the prism, discard the wipes into a biohazard bag, do this after each sample and be sure to change the tissue every time. Let the water run into a beaker containing 5% bleach.

5. - For quality assurance, run a control sample every 20 samples

At the end of your session

6. - Sanitize the instruments with ethyl alcohol and put away materials. Dispose of biohazards properly (tips, wipes, etc) and collect underpad, gloves and other items not yet decontaminated into a biohazard bag, seal the bag and label it for autoclaving. Place the beaker in a safe place (to prevent leakage of liquids) containing bleach and serum recollected during the process, stand for 24 hours and discard into the sink.

7. - Return unused samples to the freezer making sure to indicate in your database the samples you processed, the ones that were used up and the ones with remaining samples. Record all your readings in the laboratory book including the controls and don’t forget
the date, sample identification, results, technician name and any change or incident during the process.