Toxocariasis in Honduras: Seroprevalence and Risk of Infection in Schoolchildren from a
Coastal Community in Tela, Honduras.

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Dedication

To my parents, Maria Sofia and Guillermo and my sister Natalia

In loving memory of Luis Jorge Ortiz

A mis padres, María Sofía & Guillermo y a mi hermana Natalia

En memoria de Luis Jorge Ortiz

Abstract

Background: Human toxocariasis is a globally distributed zoonotic disease mainly caused by *Toxocara canis*, a common intestinal parasite of domestic dogs. In Honduras, favorable conditions for *T. canis* are widespread and dog infection is widely known. However, epidemiological data for animal and human infection are severely lacking.

Objectives: To determine the prevalence of anti-*Toxocara* antibodies in rural Honduran schoolchildren and to identify socioeconomic and biological factors associated with seropositivity. A secondary objective of comparing performance of the screening with the confirmatory test was also set.

Methods: Two cross-sectional studies conducted in 2015 and 2017 among schoolchildren living in rural Honduras. Demographic and socio-epidemiological data were collected though individual interviews. A blood sample was drawn to determine serum anti-*Toxocara* antibodies using a screening and a confirmatory test, TES-ELISA and Western blotting (WB), respectively. Eosinophilia was determined by complete haematological assay.

Results: Eighty-eight children completed the study. An overall prevalence of 90.9% was found by the TES-ELISA however, the confirmatory Western blot revealed a prevalence of 88.6% for anti-*Toxocara* antibodies. TES-ELISA and WB showed an agreement of $k = 0.87$, an indicator of an almost perfect agreement between the two diagnostic tools. Most WB-positive children were so for the lower molecular protein bands, suggesting specificity for *T. canis* infection. Higher levels of eosinophilia were observed in immunoreactive children and a statistically significant difference between the geometric means of circulating eosinophils in seropositive versus seronegative children was found. None of the socioeconomic factors analyzed were found statistically associated with antibody positivity.

Conclusions: The study confirms that *T. canis* transmission is present in Honduras and is affecting children in the rural countryside. The high exposure as determined by high antibody titres and eosinophilia levels among the studied children is a serious concern. Clinical examination of these children to assess their health status is warranted.

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List of Abbreviations

CDC: Centre for Disease Control and Prevention

CNS: Central nervous system

CSF: Cerebrospinal fluid

ECP: Eosinophil cationic protein

ELISA: Enzyme linked immunosorbent assay

EPG: Eggs per gram (of stool)

STH: Soil-transmitted helminths

NT: Neurotoxocariasis

OD: Optical density

OLM: Ocular larva migrans

OT: Ocular toxocariasis

TES: *Toxocara* excretory secretory (antigens)

VLM: Visceral larva migrans

WB: Western blot

WHO: World Health Organization

CHAPTER 1: INTRODUCTION AND RESEARCH OBJECTIVES

Humankind's consistent interaction with the environment and all other living things within it, specifically the human-animal relationship, has led to the zoonotic transmission of countless diseases originating from a multiplicity of animal hosts. Usually zoonoses are associated with wild or semi-domesticated animals. However, companion animals should also be thought of as a source for potential spillover. The helminth parasites known as *Toxocara* spp. and the disease they cause, toxocariasis, exemplify how people are susceptible to acquiring animal pathogens because of direct contact, and underscores the role of the environment in facilitating transmission. *Toxocara* spp. are cosmopolitan zoonotic parasites that utilize dogs, cats, foxes and other animal species as definitive hosts that extensively contaminate their surroundings with stools potentially containing parasite's eggs (Holland, 2017; Holland & Smith, 2006). Distribution of *Toxocara* is worldwide, with higher prevalence occurring in notoriously disadvantaged tropical countries where cohabitation with animals, poverty, lack of hygiene and insufficient health resources are rampant. Yet, toxocariasis differs from other parasitic infections in that it might become endemic in developed, high-income countries. The Centre for Disease Control and Prevention (CDC) of the U. S. identified it as one of the five neglected parasitic infections in the United States (CDC, 2013).

The significance of human toxocariasis as a disease remains enigmatic, partly due to the multifaceted, nonspecific, and cryptic nature of symptoms making this an insidious disease more closely related to disability and infirmity than mortality (Holland, 2017). Existing literature seems to suggest that awareness to this disease is rising within the scientific community, but toxocariasis remains relatively unknown to the general public (Rubinsky-Elefant, Hirata, Yamamoto, & Ferreira, 2010). Despite this increased awareness, significant gaps remain in the knowledge of fundamental aspects, including the epidemiology and public health significance of *Toxocara* spp.

infection. Deficient information in these areas hinder the ability to establish a comprehensive understanding and hence, prevents development of control of this parasitic disease and its transmission (Holland, 2017; Ma et al., 2017). Notably, toxocariasis can lead to significant and irreversible damage such as blindness and fibrotic lesions in visceral organs (Arevalo, Espinoza, & Arevalo, 2013; Hartleb & Januszewski, 2001; Magnaval, Glickman, Dorchies, & Morassin, 2001). Moreover, research is now indicating that infection may partially account for cognitive deficits leading to school achievement gap observed in socioeconomically disadvantaged students (Hotez, 2014; Walsh & Haseeb, 2012). More recently, Fan et al. have also highlighted the possible link between toxocariasis and debilitating neurological manifestations including epilepsy, idiopathic Parkinson's disease, schizophrenia, and Alzheimer's disease (Fan, Holland, Loxton, & Barghouth, 2015).

While the amount of available seroprevalence data is increasing, it continues to be scarce and fragmented. One of the regions sternly lacking published peer-reviewed literature investigating seroprevalence and risk factors of *Toxocara* spp. is that of Central America and the Caribbean. Due to climate and high levels of poverty, nations in this region are typically endemic for other parasitic infections with similar transmission requirements [*e.g.,* soil-transmitted helminths (STH)]. Hence, the presence of toxocariasis in humans and animals should not be overlooked.

Among Central American countries, Honduras reports high endemicity for STH (Sanchez et al., 2013). Officially classified as a medium-development country, approximately 60% of the Honduran population lives in poverty as defined by earning less than $2 a day. Moreover, almost half of these 60% live in extreme poverty, as defined by earning less than $1.25 a day (WHO, 2012). Dog population in Honduras is large but no census could be found. Dogs are allowed to

wander free outside their homes for the purposed of defecation and recreation. Personal communications were obtained asserting the commonality of *T. canis* infections in dogs (M Canales, UNAH. Personal communication 2018).

High proportions of disenfranchisement in conjunction with the country's climate, largely insufficient sanitary infrastructure, and inadequate domestic dog ownership, suggest that *Toxocara* spp. infections are prevalent in dogs. Humans, especially children, are likely to have higher exposed to infection. However, the level of endemicity, exposure and impact on human health are unknown for the country. Studies assessing human exposure and associated risk factors for *Toxocara* spp. within Honduras are necessary. Such investigations would also contribute to toxocariasis epidemiologic data for Central America and would provide another piece to the global puzzle of *Toxocara* spp. infections.

1.1: Research Goal

The main goal of the present study is to determine whether exposure to *Toxocara* spp. infection is occurring among children, and if so, whether risk factors for exposure can be identified.

1.1a: Research Objectives

1. To measure the seroprevalence of anti-*Toxocara* specific antibodies in the study sample
   a. A secondary objective of comparing performance of the screening with the confirmatory test was also set.
2. To assess epidemiological factors associated with exposure to infection by *Toxocara* spp.
   a. Comparison of eosinophil numbers in seropositive vs seronegative children

CHAPTER 2: LITERATURE REVIEW

2.1: *Toxocara* spp.

The genus *Toxocara* includes more than 30 different species of helminths categorized under the class Nematoda, order Ascaroidea and superfamily Ascaridoidea (Fan et al., 2015). Within the *Toxocara* genus, two species are of major public health importance, namely *Toxocara canis* and *Toxocara cati*, which are gastrointestinal parasites of mainly domestic canids and felids, respectively. Other species have been identified as having zoonotic potential such as *T. malaysiensis* and *T. lyncis* in wild felines, *T. vitulorum,* an endoparasite of bovines and ruminants, and even *T. pteropodis*, which infects bat populations (Davila, Irsik, & Greiner, 2010; Gibbons, Jacobs, & Sani, 2001; Macchioni, 1999). *T. canis* and *T. cati* have numerous similarities in their life cycle and clinical presentations in human infections, yet *T. canis* is generally believed to play a more significant role in zoonotic transmission (Despommier, 2003; Macpherson, 2013). While *T. cati's* etiology of human clinical disease, however, should not be underestimated (Fisher, 2003), the current thesis will focus on describing toxocariasis from the perspective of *T. canis* only.

2.1a: *Toxocara canis* in Dogs

T. canis, commonly known as the dog roundworm, is a nematode responsible for one of the most common parasitic infections in canid species (Traversa, 2012). As a member of the superfamily Ascaridoidea, adult worms highly resemble those of *Ascaris* spp., possessing a filiform, bilaterally symmetrical body, separate sexes and a complete digestive tract which includes the three lips characteristic of ascarids (Dubey, 1978). Unlike *Ascaris* spp. however, *T. canis* has prominent cervical alae that stretch from the anterior end down the lateral margins of the body (Figure 1A). These alae are present in both female and male worms. The sexes can be differentiated morphologically through the observation of their posterior ends, as males possess a curved

posterior end while the female posterior end is straight and rounded (Roberts & Janovy, 2009). Microscopic examination of the worms can also aid in differentiation of sexual dimorphism, as observation of reproductive organs such as testis, vas deferens, a seminal vesicle, ejaculatory duct and cloaca indicates male gender. In contrast, presence of ovaries, uteri or oviducts signifies the worm is a female (Holland & Smith, 2006). Further, secondary characteristics such as size can also help in differentiation. Adult females tend to be longer and larger than males, reaching sizes anywhere between 6-15 cm in length and 3 mm in diameter. The adult male can grow 4-6 cm and has a smaller diameter of approximately 2.5 mm. The morphological differences between the two genders are depicted in Figure 1C.

Figure 1. **A)** Anterior end of *T. canis* adult worm, displaying cervical alae. **B)** Frontal view of adult *T. canis* worm where the three lips are visible. **C)** *T. canis* adult female (left, larger) and male (right, curled end) (Bojanich & Lopez, 2009).

These nematodes reach sexual maturity and reside in the dog's small intestine, where through chemosensory mechanisms males can locate females and mating can occur. Once a mate is found, males then stabilize the female and inseminate them through the vulva, where sperm then travels through the oviduct into the uterus and fertilization occurs (Gaugler & Bilgrami, 2004). Females are known to be highly fecund and have the capacity to produce up to 200,000 eggs per day, easily producing millions of eggs throughout the parasite's lifetime, all of which are deposited in the small intestine of the definitive host (Berrocal, 1980). *T. canis* eggs are then expelled along with

the definitive host's feces, have a rounded shape, and measure approximately 85 – 95 µm by 75 – 90 µm. The eggs possess a thick, chitinous shell that contains small depressions giving it a rugged appearance. The shell protects the globular egg cell on the interior, which has a uniform brown colouration and occupies essentially the entire internal cavity. The shell aids in the resistance of these eggs, as they can survive in a wide variety of chemical and physical conditions and even thermic stress (Berrocal, 1980; Soulsby, 1982). At fertilization, the egg shell forms and is made up of four layers; three made by the egg itself, and the fourth is added by uterine secretions. These secretions give the shell its adhesive nature and distinctive yellow-brown colour after coming into contact with bile present in the flow of intestinal contents within the dog (Bouchet et al., 2003). An illustration of a *T. canis* egg is presented in Figure 2. It is also important to note that females can lay eggs in the absence of fertilization. Infertile eggs present a more irregular form and generally do not have well-defined shell layers, as this differentiation is stimulated by oocyte fertilization, as previously mentioned (Prociv, 1990).

Figure 2: Unembryonated *T. canis* egg with its irregular pitted surface and well-defined shell. Scale bar is of 50 µm (Lee, Schantz, Kazacos, Montgomery, & Bowman, 2010).

Eggs excreted in feces are not immediately infective, but instead require a period of embryonation in the environment, which is influenced by soil composition, temperature and humidity. At temperatures between 10-20°C this period typically takes between 2-6 weeks (Macpherson, 2013). Higher temperatures can accelerate the evolution of larvae with development to the infective stage having been documented in as little as 9-15 days in conditions of 25-30°C and a relative humidity of 85-95%. This short maturation period has led to the identification of these ranges of temperature and humidity as ideal conditions for egg embryonation (Okoshi & Usui, 1968; Schacher, 1957; Schnieder, Laabs, & Welz, 2011). Nevertheless, temperatures lower than 10°C or warmer than 38°C can have detrimental effects on egg survival and can hinder larval development inside the egg to up to several months (Azam, Ukpai, Said, Abd-Allah, & Morgan, 2012; Gamboa, 2005; Trejo, Romero Nunez, Contreras, & Mendoza Barrera, 2012). Other conditions such as soil type and pH levels can also negatively affect *Toxocara* spp. ova viability. Literature suggests that the content of clay in soil as well as soils of a sandy composition result in reduced egg recovery and viability in comparison to loam soils (Nunes, Sinhorini, & Ogassawara, 1994; Stromberg, 1997; Trejo et al., 2012). Experiments have demonstrated that pH is important for egg viability, as soils with more alkaline pH levels did not have the same level of recovery of infective eggs as soils within the normal pH range of 5.5-7.0 (Stromberg, 1997; Trejo et al., 2012). Temperature is still the main determinant of development and embryonation is therefore a seasonal occurrence in regions with temperate climates but occurs year-round in tropical areas. Additionally, reports show that eggs can survive winters in these temperate regions with the larval infective stages remaining viable for at least 1 year or up to 4 years under appropriate moisture conditions (Azam et al., 2012; Overgaauw, 1997). The infectious stage of larvae is not the second or L2 stage. Instead, the third or L3 stage that emerges following a second moult inside the egg is

a phenomenon that was elucidated when several research groups observed strong evidence of two moults occurring inside the embryonating egg between 11 and 15 days (Araujo, 1972; Brunaska, Dubinsky, & Reiterova, 1995; Schnieder et al., 2011). Eggs can be physically dispersed by animal activity such as: dogs, birds, beetles, flies, slugs, earthworms and even rainfall. Water-borne transmission of *T. canis* eggs—both through recreational water and of drinking use, has been posited but currently requires further investigation (Beer, Novosil'tsev, & Mel'nikova, 1999). Of note, Fan *et al.* preserved embryonated *T. canis* eggs in 2% formalin and 14 months later used these same eggs to inoculate ICR outbred mice. Infection developed and larvae isolated from the liver and brain of these mice 469 days post-infection were alive and causing inflammatory organ injury, a result indicative that both infectivity and pathogenicity could be retained even after long-term exposure to 2% formalin (Fan, Lin, Du, & Su, 2003).

2.2: Life Cycle in Canids: The Definitive Hosts

Ingestion of the previously described eggs in their infective stage by any canid species will result in the animal becoming infected. Since humans are in close proximity to and have heavy contact with dogs (*Canis familiaris)*, the life cycle of *T. canis* within this species of canid will be described. Following consumption of eggs, these will hatch within the duodenum of the animal between 2 and 4 hours later. The infective L3 larvae are released and can penetrate the mucosa of the intestine through molecular mechanisms that are not yet fully understood (Fan et al., 2015; Macpherson, 2013; Schnieder et al., 2011). Robertson and colleagues were able to detect an elastase-like protease in excretory-secretory larval products. It is believed that this protease can play a role in the penetration of tissues such as the intestinal mucosa, parenchyma of the kidney and liver and even blood vessel walls (Robertson, Bianco, McKerrow, & Maizels, 1989). After piercing the

intestinal wall, larvae migrate to the mesenteric lymph nodes through invasion of the lymph vessels. Once there, they manage to reach the liver via portal circulation and venous capillaries all within the first 24 hours post infection (Webster, 1958b). Those larvae that become trapped within capillaries remain in the liver and will be encapsulated, causing the mottled hepatic appearance characteristic of canine infection (Webster, 1958b). For those not encapsulated, larval migration continues and exodus from the liver occurs through the vena cava, where larvae will pass through the heart and gain access to the lung via the pulmonary artery. Here they will remain for anywhere between 24 – 36 hours. Interestingly, Webster was able to detect larvae in the pulmonary artery and cardiac blood up to 72 hours post infection (Webster, 1958b). From their arrival in the lung, larvae can follow two different migratory routes, depending on the dog's age (which increases immunity, as explained below). First, larvae can penetrate the alveolar wall, continue through the bronchioles and trachea all the way until reaching the pharynx where they will be swallowed and will grow to adulthood in the intestine, establishing a patent infection (Schnieder et al., 2011). Alternatively, larvae may re-enter circulation ensuing alveoli penetration but, in this case, will begin distribution to somatic tissue and as such, will never mature into adult worms. These somatic larvae will never be eliminated by the immune system once arrested in the tissues and will become *larva migrans* (Schnieder et al., 2011; Webster, 1958a). It has been concluded that the route of migration to somatic tissues is progressively more likely in dogs aged 6 months or older, while the majority of larvae will undergo tracheal migration into the intestine in puppies younger than 6 months of age (Claerebout et al., 2009; Greve, 1971). This phenomenon, known as age resistance is based on the principle that dogs > 6 months of age, will have a better developed immune system (Barriga, 1988). However, this conflicts with the observation that adult dogs inoculated with low numbers of embryonated eggs can also develop patent infections while inoculation with large

numbers of eggs seems to be less likely to produce these patent infections even in non-immune dogs (Fahrion, Staebler, & Deplazes, 2008; Overgaauw & van Knapen, 2013).

In infected dogs, the gastrointestinal tract is colonized 7-15 days after egg ingestion although the process and occurrence of full maturation is still under investigation, as previous research described three moulting steps within the canine. However, knowledge that is more recent has proven that only two moults occur inside the host (Araujo, 1972; Brunaska et al., 1995; Schnieder et al., 2011). Concrete studies are missing, yet previous literature states that the first moult within the dog (now known to be the L4 stage), happens sometime between bronchiole migration and passing into the stomach (Schacher, 1957; Webster, 1958b). Less doubt surrounds the last moult into the L5 pre-adult stage, as original observations concluding that this final moult takes place in the small intestine have been corroborated (Fahrion et al., 2008; Webster, 1958a). Final maturation ensues and the first eggs begin to shed, approximately 4-5 weeks after initial infection but prepatency can last up to 56 days. Adult *T. canis* worms are believed to have an average life span of 4-6 months (Fahrion et al., 2008; Parsons, 1987). Once dead, worms will be passed passively with stools.

As dogs age, their propensity for patent infections decreases and as previously mentioned, upon infection, most larvae will enter circulation and travel to somatic tissue. This migratory ability leads to the appearance of larvae in kidneys, liver, skeletal muscle and even the central nervous system within 24 – 72 hours (Schnieder et al., 2011). Larval tissue migration is arrested shortly thereafter. Larvae remain in tissues, as they cannot be completely cleared. In female dogs these somatic larvae can be reactivated during pregnancy, cross the placental barrier and can thereby infect newborns prenatally, as explained below (Overgaauw & van Knapen, 2013). Most puppies are born with intestinal toxocariasis.

2.2a: Transplacental Transmission

Also known as intrauterine or prenatal transmission, transplacental infection is considered the biggest contributor towards infection by *T. canis* in puppies, as they are commonly found to be infected postpartum. Transplacental transmission seems to occur because of both infection of the bitch while pregnant and reactivation of arrested somatic larvae from previous infections (Koutz, Groves, & Scothorn, 1966; Webster, 1958a). However, it has not been established how long these somatic larvae can remain in the tissue to be passed on to the offspring. It has been observed that an infected bitch can transmit *T. canis* larvae during three consecutive pregnancies (Soulsby, 1983). The exact mechanism as to how larvae are reactivated in the tissues of the pregnant dog is also a matter of debate, but it is strongly believed to be linked to fluctuating hormonal levels, as a study in mice demonstrated that migration of larvae to the mammary gland was triggered by prolactin (Jin, Akao, & Ohta, 2008; Schnieder et al., 2011). Although vertical transmission in mice occurs only lactogenically and not transplacentally, a similar path for reactivation is thought to apply to pregnant dogs. Conversely, an occurrence referred to as periparturient immunosuppression may also influence the reactivation and migration of the larvae (Lloyd, Amerasinghe, & Soulsby, 1983). It has been demonstrated that both pregnancy and initiation of lactation in dogs leads to diminished immunological responsiveness. This immunosuppression might allow tissue-arrested larvae as well as those of recently acquired infections to undergo tracheal migration and eventually develop in the intestine, as well as mobilization to the uterus resulting in fetal infection (Lloyd et al., 1983).

If not spayed or neutered, female dogs tend to go into estrus for approximately three weeks, twice a year. If fertilization occurs, the canine gestation period lasts between 60 to 65 days from conception until birth, with the variation depending on the breed and dog size. Breed and size of

the canine will also determine the number of puppies birthed per litter. Miniature and small breeds average 3 to 4 puppies per litter, while large and giant breeds have an average of 7 in each litter but can have up to 15 (Borge, Tonnessen, Nodtvedt, & Indrebo, 2011). The number of canines born per bitch is crucial in the propagation of disease since as previously mentioned, the probability of a puppy being born infected from a parasitized mother is high. Furthermore, unlike humans, female dogs do not experience menopause and can remain fertile well into seniority (Verstegen-Onclin & Verstegen, 2008). The timing of larval reactivation during pregnancy and subsequent fetal transmission is currently known to occur in the latter stages of pregnancy after approximately 40 days of gestation. Scothorn and colleagues were unable to find larvae in fetuses of an infected bitch at 35 days of pregnancy but succeeded in doing so on the 56th day of pregnancy (Scothorn, Koutz, & Groves, 1965). Similarly, another study which aimed to detect the timing of larval reactivation and transplacental movement found the first larva in one of six fetuses after 43 days of gestation and by the 47th day, all fetuses were infected (Koutz et al., 1966). In regards to introduction of *T. canis* larvae to the fetus, Webster proposed that larvae travelled through the circulatory system of the pregnant bitch and upon encountering the placenta, they could penetrate it and cross into the fetal circulation (Webster, 1958a). The umbilical cord is also thought to be an essential transfer point between fetus and mother, as larvae have previously been found in umbilical cord tissue (Koutz et al., 1966; Scothorn et al., 1965). After transplacental infection, larvae migrate to the fetuses' liver and will remain here until birth. Migration to the lungs will begin immediately postpartum, with larvae being detectable in the lung just 30 minutes after birth in some cases (Koutz et al., 1966; Scothorn et al., 1965). Once in the lung, larvae will then make their way towards the trachea and can reach the intestine two days postpartum. In others, larvae can remain in the liver for up to two days before travelling towards the lungs. In all cases, larval

presence can be detected in the intestine after seven days postpartum and the prepatent period following transplacental transmission is between 21-28 days (Koutz et al., 1966; Schnieder et al., 2011).

2.2b: Transmammary Transmission

Transmammary or lactogenic transmission has been identified as another infective pathway of *T. canis* in dogs. Larval shedding with milk appears within the first days following parturition and increases consistently thereafter with a maximum being reached at 7 – 14 days postpartum. Larvae can also be recovered lactogenically as late as 28 days postpartum (Schnieder et al., 2011). It appears however that lactogenic transmission is not as significant as transplacental, based on the studies of Burke and Roberson that compared the number of infections from either transplacental or transmammary origin. One of their studies involved infecting bitches before pregnancy and exchanging the newborn puppies before nursing began with those from a litter whose mother had not been inoculated with *T. canis*. Following 4 weeks of nursing, the researchers were able to recover 2% of the original infectious dose, with only 1.5% of this recovered amount having been transmitted lactogenically to the previously naïve puppies (Burke & Roberson, 1985b). They then conducted a similar study but instead infected the bitch mid-pregnancy and found that only 4.5% of the original infectious dose that was recoverable had been transmitted through nursing (Burke & Roberson, 1985a). Therefore, it appears that the lactogenic route is subordinate to the prenatal route for offspring infection. Little is known about the development of larvae in puppies following acquisition through ingested milk, although a prepatent period varying between 27 – 35 days has been reported and seems indicative of a lack of direct intestinal development following ingestion (Schnieder et al., 2011). Due to lactogenic transmission being coupled with transplacental

infection, an exceedingly high number of young dogs are infected with *T. canis,* making infection of offspring almost certain if present in the mother (Traversa, 2012).

2.2c: Paratenic Hosts

While dogs and cats act as definitive hosts for *Toxocara* spp., larvae can also persist and even lead to severe disease in a variety of other species that serve as paratenic hosts. The term paratenic describes a host, which a parasite has the capacity to infect, but is not necessary to complete the parasite's life cycle since no significant development takes place within this host. In the case of *Toxocara* spp., when comparing these to definitive hosts, the main difference is the absence of complete development into the adult stage in paratenic hosts as third stage (L3) larvae persist in tissues in an arrested state (Brunaska et al., 1995). These hosts may be preyed upon by definitive hosts, at which stage the arrested larvae are reactivated and will continue to develop in their new host into egg-producing adult worms. If predation occurs, larvae may exhibit aberrations in their migration patterns such as a lack of tracheal migration (Overgaauw, 1997). Within tissues of the paratenic host, larval distribution largely depends on the infected species but persistence as encapsulated larvae up to 10 years has been demonstrated (Strube, Heuer, & Janecek, 2013). This provides a method of life cycle continuation even a long-time post infection, if the paratenic host is consumed by the definitive host or by another paratenic host, such as humans (Oryan, Sadjjadi, & Azizi, 2010; Taira, Saitoh, & Kapel, 2011). Although organ migration and predilection sites vary, nearly all organs may be affected with varying degrees of larval density, with *T. canis* demonstrating specific affinity to the central nervous system (CNS), with the brain and the ocular organ being commonly infected (Alba-Hurtado, Tortora, Tsutsumi, & Ortega-Pierres, 2000; Burren, 1972). Possible consequences of this neural affinity include behavioural alterations

resulting in an increased likelihood of falling prey to various canids or felines. This may serve as an evolutionary explanation for the parasite's migratory preference (Chieffi, Aquino, Pasqualotti, Ribeiro, & Nasello, 2010; Cox & Holland, 1998; Hamilton, Stafford, Pinelli, & Holland, 2006).

Numerous species of animals can potentially ingest infective parasite eggs and serve as paratenic hosts of *Toxocara* spp. involving rodents such as mice and rats, avian species including poultry and other mammals such as pigs. In mice, susceptibility varies widely as do larval counts in body tissues and organs with migration behaviour being similar to that seen in the definitive host; a hepato-pulmonary phase and a somatic or neural phase. Evidence has shown that distribution and developmental arrest of the larva results in persistence of the infective stage for at least one year in these animals (Strube et al., 2013). Mice with larval brain invasion exhibit decreased fear and aggressiveness, reduced learning and memory and increased desire to spending more time in open areas; all factors influencing susceptibility to predators, thus assuring the parasite's transmission (Cox & Holland, 1998, 2001; Hamilton et al., 2006; Strube et al., 2013). Rats are also considered to have paratenic potential but are seriously neglected in terms of conducted research compared to mice. High larval counts have also been observed in the lungs, carcass and brain of these animals suggesting a comparable migratory cycle to that seen in mice (Lescano, Queiroz, & Chieffi, 2004). Once again, presence of *T. canis* larva within the brain of rats led to similar behavioural changes as those seen in mice as they lost their natural caution and aggressiveness was decreased, possibly turning them into easier prey for the definitive host in nature (Chieffi et al., 2010).

Another paratenic host of human importance is the pig, an animal that has also been extensively studied in terms of *T. canis* infection. Larval recovery has been more frequent in the liver, but has also been possible in the heart, brain, lungs and muscles not in the eye, however—

indicative of a complex migratory cycle (Helwigh, Lind, & Nansen, 1999; Sommerfelt et al., 2004). Larvae do not seem to persist in the pigs' tissues for long periods as a significant decrease has been observed after only a couple of weeks. After 126 days post infection, no larvae were detectable at all, indicative of self-limiting factors (Helwigh et al., 1999; Sommerfelt et al., 2004). The transmission potential of pigs should not be disregarded, however, as human infection might easily occur after consumption of raw or undercooked pork dishes (Taira, Saeed, Permin, & Kapel, 2004).

Comparable to pigs, chickens can also be paratenic hosts and as such, are of concern due to their possibility of being an infective source for humans as well as other animals. Evidence exists linking consumption of undercooked or raw poultry to *T. canis* infection in humans (Nagakura, Tachibana, Kaneda, & Kato, 1989; Taira et al., 2004; Taira et al., 2011). Additionally, larvae in chicken muscle have been shown to remain extremely infective even after continued exposure to low temperatures, increasing the risk of potential acquisition in humans as chickens are highly likely to ingest *T. canis* embryonated eggs from the soil and in turn, be consumed by various mammals (Strube et al., 2013). Recent literature has also postulated the role of bovine products in the infective pathway of *T. canis,* as calves and cows can become paratenic hosts and harbour arrested larvae in their viscera resulting in documented cases of human infection (Choi et al., 2012). Theoretically, additional animals, ranging from small mammals to invertebrates such as earthworms, have paratenic potential but their contribution to this zoonosis is still under further investigation. Overall, paratenic hosts play an essential role in the zoonotic transmission of *Toxocara* spp. as they present ways for the parasite to infect definitive hosts or other paratenic hosts, including humans. The life cycle of *T. canis*, including definitive and paratenic hosts and their interactions, is represented in Figure 3.
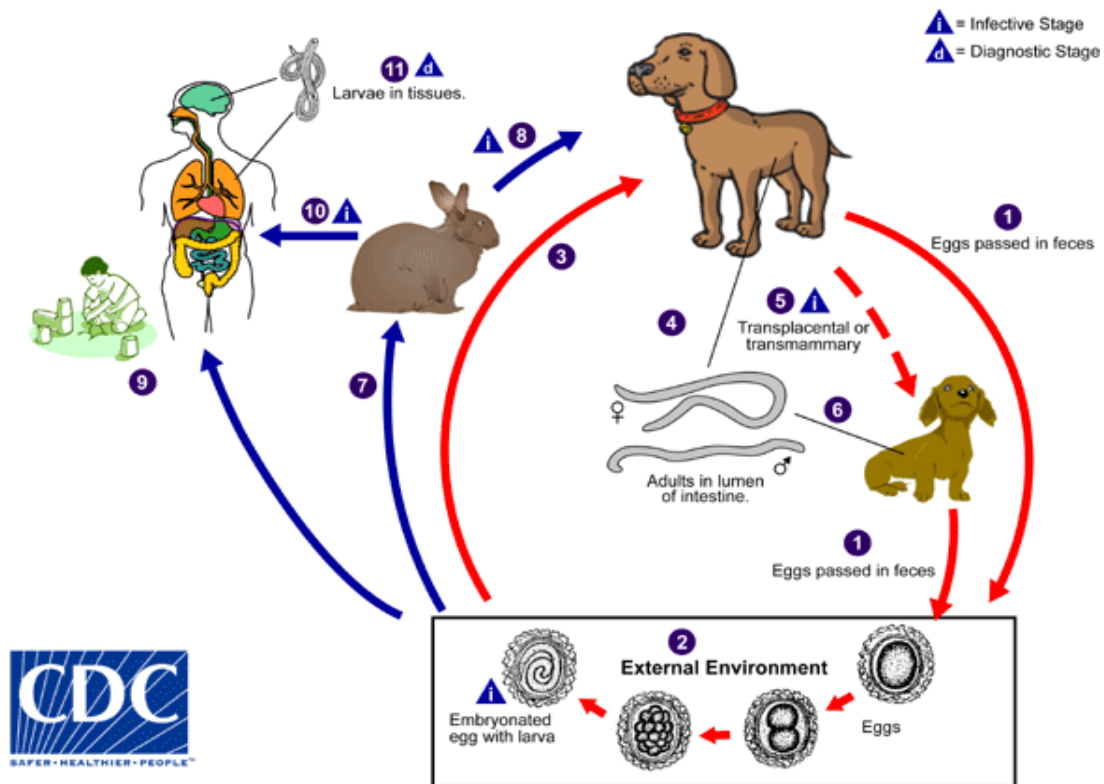
Figure 3: Life cycle of *Toxocara canis* including definitive hosts, paratenic hosts such as humans and the numerous ways of transmission between them (CDC, 2013).

2.3: Humans as Paratenic Hosts

Humans may accidentally acquire *Toxocara* spp. infection by ingesting infective eggs from the soil or through culinary practices leading to consumption of meat from livestock serving as paratenic hosts. More specifically, humans can be infected by: (i) accidental ingestion of eggs from soil containing the L3 infective stage. This can occur through oral contact with contaminated hands or fomites, unwashed or undercooked contaminated vegetables that had been in contact with soil, etc. (ii) Ingestion of encapsulated L3 larvae in undercooked or raw viscera and muscle tissue from paratenic hosts including but not limited to: lamb, cows, chickens, pigs and rabbits (Akao & Ohta, 2007; Nagakura et al., 1989; Salem & Schantz, 1992; Smith et al., 2009; Yoshikawa et al., 2008). Infections have also been recently hypothesized to originate from direct contact with the hair of pet dogs and cats, as experimental data has proven that embryonation of *Toxocara* spp. eggs can occur within the coat of these domestic animals (Amaral et al., 2010; Aydenizoz-Ozkayhan, Yagci, & Erat, 2008; Keegan & Holland, 2010; Roddie, Stafford, Holland, & Wolfe, 2008; Wolfe & Wright, 2003). This occurrence has also been postulated for the raccoon ascarid *Baylisascaris procyonis,* particularly in the few reported cases in Canada where exposure to *B. procyonis* eggs was hard to pinpoint in the patients. The presence of embryonated eggs on the fur coat of pets was deemed as a possible source of exposure in both cases, however it was deemed unlikely to be the cause in each case (Bauer, 2013; Hajek et al., 2009; Hung, Neafie, & Mackenzie, 2012).

The various routes of transmission of *Toxocara* spp. to humans are summarized in Figure 4. Successful infection is predicated by a number of factors ranging from socioeconomic and cultural, to geographic, genetic, nutritive, behavioral, immune and even coinfection with other parasites (Macpherson, 2013; Viney & Graham, 2013b). Untreated or uncontrolled infections in dogs result in substantial environmental contamination with *Toxocara* spp. ova. This along with

poor hygiene, low socioeconomic status and geophagia, all increase human exposure to this specific parasitic infection and its subsequent clinical disease in humans, referred to as toxocariasis (Fan et al., 2015; Hotez & Wilkins, 2009).
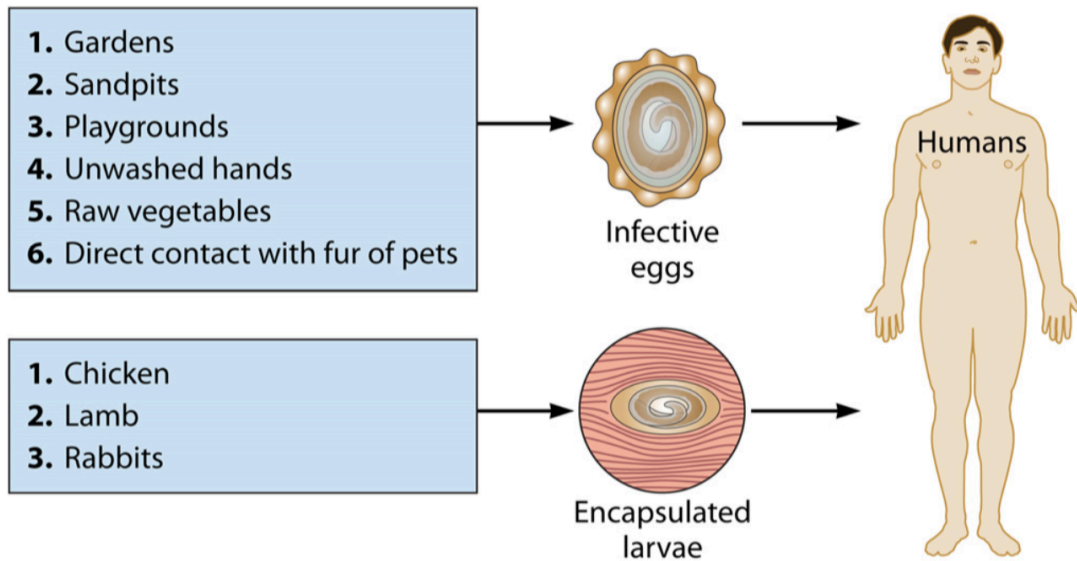
Figure 4: Reported oral transmission routes of Toxocara spp. infection to humans (Fan et al., 2015)

Human toxocariasis and its clinical syndromes are attributed to migrating L3 *Toxocara* spp. larvae as they move through circulation to various organs such as the liver, the heart, kidneys, brain, lungs, eyes and muscle tissues. Infection can remain asymptomatic but can also present with a variety of inflammation-related manifestations such as lymphadenopathy, endomyocarditis, granulomatous hepatitis, endophthalmitis, meningoencephalitis and others (Lopez Mde et al., 2010; Ranasuriya, Mian, Boujaoude, & Tsigrelis, 2014; Salvador, Ribeiro, Winckler, Ohlweiler, & Riesgo, 2010; Vidal, Sztajnbok, & Seguro, 2003). Whether infection exhibits symptoms depends on the organs involved, the intensity of infection and the corresponding host immune response (Fan, Liao, & Cheng, 2013). Due to the wide variation of clinical manifestations

associated with *Toxocara* spp. infection in humans observed during the last three decades, four different forms of human toxocariasis have been established. These include visceral larva migrans and ocular larva migrans in 1988 (Taylor, Keane, O'Connor, Mulvihill, & Holland, 1988), common or covert toxocariasis was described in 1992 and 1993 (Nathwani, Laing, & Currie, 1992; Rasmussen, Dirdal, & Birkebaek, 1993) and finally, neurotoxocariasis which has garnered substantial research interest as of late (Quattrocchi et al., 2012).

2.3a: Visceral Larva Migrans

The first described case of *visceral larva migrans* (VLM) was documented in the U. S. in 1952 by Beaver and colleagues to explain a group of symptoms associated with eosinophilic granulomas that had enclosed *Toxocara* spp. larvae in the livers of children presenting with anemia, hepatomegaly and extreme hypereosinophilia (Beaver, Snyder, Carrera, Dent, & Lafferty, 1952). Later work would identify VLM as the most common manifestation in individuals with *Toxocara* spp. infection, children in particular, with clinical symptoms such as coughing, wheezing, myalgia, rash, eczema and pruritus among others being reported (Ma et al., 2017). Although the majority of VLM cases remain asymptomatic, liver-related pathology including hepatic granulomas, granulomatous hepatitis and fibrous perihepatitis have all been associated with VLM (Hartleb & Januszewski, 2001; Musso, Castelo, Tsanaclis, & Pereira, 2007). Lymphadenopathy, myocarditis, nephritis, arthritis, pulmonary fibrosis and non-specific symptoms like headache, fever, vomiting, diarrhea, weight loss and abdominal pain are also associated with VLM (Despommier, 2003; Fan et al., 2015; Kuenzli, Neumayr, Chaney, & Blum, 2016; Pawlowski, 2001). Although classic VLM occurs typically in children, cases of VLM in adults have increased particularly in Asian countries, (*e.g.,* South Korea and Japan) possibly due to the consumption of raw lamb, beef, chicken or

ostrich liver (Akao & Ohta, 2007; Yoshikawa et al., 2008). While it is not plainly obvious why the development of symptoms in VLM occurs, the main hypothesis points to the human immune response to larval excretory-secretory antigens detected during migration. This response entails mainly elevated levels of eosinophilia and hypergammaglobulinemia as well as increased cytokine production by TH$_2$-cells (Fan et al., 2015).

2.3b: Ocular Larva Migrans

Ocular larva migrans (OLM) also known as ocular toxocariasis was first elucidated by Wilder in 1950 when he used the term nematode endophthalmitis to describe larva-containing lesions in the eyes of children with suspected retinoblastoma (Wilder, 1950). OLM is currently thought to be rare compared to VLM and is usually reported in children between the ages of 3 and 16 years, with highest incidence occurring in those between 5–10 years of age (Arevalo et al., 2013; Despommier, 2003; Pivetti-Pezzi, 2009). OLM typically leads to unilateral visual impairment and can be accompanied by strabismus, endophthalmitis, retinitis or granulomata. It can also cause irreversible blindness by severe vitritis, macular edema, or retinal detachment (Despommier, 2003; Fan et al., 2015). Impairment is associated with migration and/or death of larvae and the resultant immune activity against the worm load in the eye (Fan et al., 2013; Ma et al., 2017). OLM has been hypothesized to stem from ingestion of small numbers of *Toxocara* spp. ova or encapsulated larvae. These low infective doses usually are not identified by the immune system until larval migration has reached the eye, reflecting why anti-*Toxocara* antibody titers are usually decreased in OLM in comparison to VLM and other forms of toxocariasis (Arevalo et al., 2013). Arevalo *et al.* also suggest the further sub classification of ocular toxocariasis into three major clinical types, based on the severity of the observed manifestations: nematode endophthalmitis as was first

proposed by Wilder, peripheral inflammatory mass type and posterior pole granuloma type (Arevalo et al., 2013). Mounting clinical evidence points to the peripheral retinal granulomas as the most common presentation of OLM, seen in 50–64% of cases, followed by posterior pole granuloma in 25–36% of cases and endophthalmitis seen in less than 25% of cases. (do Lago, Andrade, Muccioli, & Belfort, 2006; Fan et al., 2015). Damage to an individual's vision occurs usually over days or weeks with the location of larvae and granulomatous response determining the degree of impairment and it is this formation of granulomas that stimulates sufficient damage leading to blindness, mostly uniocular but if infection persists, bilateral disease has been reported (Cortez, Ramirez, Collet, & Giuliari, 2011). Correct diagnosis of OLM is significantly difficult and is only presumptive at best in most cases since concrete diagnosis would require the observation of larval presence within the eye. Common standard methods for diagnosis are imaging, fundoscopy and serologic testing, the sensitivity of serologic testing for OLM diagnosis is considerably lower compared to that of VLM and is not considered reliable (Arevalo et al., 2013; Fan et al., 2015).

2.3c: Covert or Common Toxocariasis

The proposition that toxocariasis can display clinical scenarios other than VLM and OLM originated from the observation of high seroprevalence in individuals but a relatively small number of VLM and OLM related symptomatology (Taylor et al., 1988). As a result, the disease spectrum for toxocariasis was expanded following the evidence collected in two separate cross-sectional studies done in communities of France and Ireland. The researchers described seropositivity in adults and children but no association with the common manifestations of *Toxocara* spp. infection; instead, they documented mild or non-specific symptoms in these individuals (Glickman et al.,

1987; Taylor, Keane, O'Connor, Girdwood, & Smith, 1987; Taylor et al., 1988). The first study, which was conducted in French adults showed symptoms such as pulmonary dysfunction and insufficiency, abdominal pain, cutaneous rashes and pruritus along with elevated levels of anti-*Toxocara* antibodies, IgE and eosinophilia. The syndrome documented was named *common toxocariasis* (Glickman et al., 1987). In Irish children, the most common clinical signs were fever, appetite loss, vomit, nausea, headache, wheezing, abdominal pain, lethargy, limb pain, sleep and behavioural disorders, hepatomegaly and even cervical lymphadenitis. These were accompanied by higher than normal eosinophil counts in 73% of the children, yet all exhibited high titers of anti-*Toxocara* antibodies. This symptomatology in children was termed *covert toxocariasis* (Taylor et al., 1987). To date, covert and common toxocariasis are believed to go largely undiagnosed due to nonspecific systemic manifestations and generalized lab test abnormalities (Ma et al., 2017; Taylor et al., 1988).

2.3d: Neurotoxocariasis

Neurotoxocariasis (NT) is a manifestation that is related to the migration of *Toxocara* spp. larvae into the CNS. It is dependent on the number of ova or larvae ingested, previous exposure, host immunity and even genetic factors, all of which contribute to the complex pathology of NT (Fan et al., 2015; Fan et al., 2013). It was first recognized by Beautyman and Woolf in the early 1950's following their discovery of a larva in the left thalamus of the brain during the autopsy of a child (Beautyman & Woolf, 1951). Subsequently, over the last 30 years improved diagnostic techniques have led to an increasing number of NT cases, which in turn have been linked with neurological damage, epilepsy, meningitis, encephalitis, cerebral vasculitis, myelitis, eosinophilic meningoencephalitis and even neuropsychological deficits (Caldera, Burlone, Genchi, Pirisi, &

Bartoli, 2013; Deshayes, Bonhomme, & de La Blanchardiere, 2016; Finsterer & Auer, 2007; Holland & Hamilton, 2013). Possible associations between neurotoxocariasis and neurodegenerative disorders such as seizures, idiopathic Parkinson's disease, schizophrenia, dementia and cognitive deficits have also been posited in the literature and still require further elucidation (Celik, Kaplan, Atas, Oztuna, & Berilgen, 2013; Fan et al., 2015; Ma et al., 2017). Another area of particular concern is the possibility of developmental delays and reduced cognitive function among socioeconomically disadvantaged children harboring infection, as this could lead to the perpetuation of poverty, a phenomenon seen in other parasitic infections that are endemic in developing countries (Ma et al., 2017; Walsh & Haseeb, 2012). An interesting publication from Honduras, that will be discussed further in section 2.5a presents the case of a 14-month-old male suffering from unexplained fever and generalized tonic-clonic seizures. The child was diagnosed with neurotoxocariasis based on clinical presentation, lab reports of hypereosinophilia, an anti-*Toxocara* antibodies in the CSF and response to parasitic treatment (Puerto Sanabria & Tovar, 2016).

Currently, knowledge on the mechanism that underlies NT is incomplete and with seroprevalence surveys indicating high levels of *Toxocara* exposure in a variety of populations across the globe, this disease may be an underestimated public health issue (Fan et al., 2015). In addition, the number of cases still may not be truly reflective of the magnitude of the problem, due to the non-specificity of the clinical signs, the negative antibody titers in blood commonly seen as well as the unavailability of appropriate testing in regions where it could be needed most (Holland & Hamilton, 2013; Hotez & Wilkins, 2009). Nevertheless, testing of the cerebrospinal fluid (CSF) in the presence of abnormal neurological symptomatology is what is currently relied upon, as CSF has shown to be antibody positive in NT cases, sometimes being accompanied by eosinophilia also

in the CSF. Furthermore, it should be noted that serological testing is unreliable as reactivity can persist after clinical recovery of disease activity in the CNS (Caldera et al., 2013; Fan et al., 2015; Vidal et al., 2003).

2.4: Diagnosis of Human *Toxocara* Infection

In OLM, the diagnosis can sometimes be made by direct visualization of the larvae. In VLM, histopathological examination and observation of encapsulated larvae in tissues is sometimes possible but extremely difficult and thus rarely attempted (Macpherson, 2013). Specific detection of larval DNA if present in tissue or body fluid samples can be accomplished through PCR-based tools, using genetic markers found in the first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal RNA or selected mitochondrial genes. These sequences and their detection can enable the identification of *Toxocara* spp. larvae and other ascarid nematodes, even in human CSF, irrespective of life stage (Caldera et al., 2013; Gasser, 2013; Ishiwata et al., 2004; Jacobs, Zhu, Gasser, & Chilton, 1997; Li, Lin, Song, Wu, & Zhu, 2008; Rai, Uga, Wu, Takahashi, & Matsumura, 1997). However, procedures for obtaining tissue biopsies and CSF are invasive and impractical. Therefore, diagnosis of toxocariasis in humans relies on clinical presentations, history of exposure, and the use of serological antibody detection through immunological techniques (de Savigny, Voller, & Woodruff, 1979; Fillaux & Magnaval, 2013; Magnaval, Fabre, Maurieres, Charlet, & de Larrard, 1991).

Immune diagnosis is based on the use of the ELISA technique employing *Toxocara* excretory-secretory (TES) antigens (de Savigny et al., 1979), with ELISA test kits being commercially produced and widely used for diagnosis and seroprevalence studies. Differences in the quality of antigens used and lack of standardization of thresholds for meaningful results

coupled with polyparasitism in certain populations, cross-reactivity and exposure to other pathogens have all increased the difficulty of comparison and affected the reliability and quality of use of ELISA for diagnosis (Holland & Smith, 2006; Macpherson, 2013). Polyparasitism is of major concern, as coinfection with gastrointestinal helminths can reduce specificity below 50% (Ma et al., 2017; Macpherson, 2013). Thus, any serological findings should be confirmed by immunoblotting in order to avoid false positive results and to evaluate cross-reactivity with other pathogens (Moreira et al., 2014). Positive serum samples are most commonly confirmed through Western blotting (WB). WB is a technique based on the use of native TES antigens of *Toxocara* spp. larvae that has been shown to increase specificity due to the observed reactivity to bands of low molecular weight, between 24–35 kDa, that have previously been shown to be *Toxocara* specific (Fillaux & Magnaval, 2013; Mohamad, Azmi, & Noordin, 2009; Smith et al., 2009).

The specificity and sensitivity of serological and immunological assays used for the diagnosis of human toxocariasis depend on the antigens used and the type of antibodies that they measure for (*e.g.,* Total IgG, the different subclasses of IgG, or IgM) (Roldan, Elefant, & Ferreira, 2015). Numerous modified TES-ELISAs have been developed and implemented and of note, an IgG4-ELISA using recombinant TES-120 (rTES-120) and rTES-30 was reported to have increased sensitivity to 93% (Mohamad et al., 2009), while a total IgG-ELISA incorporating deglycosylated TES was reported to increase both sensitivity and specificity to 100% (Ma et al., 2017; Roldan et al., 2015). The performance of both modified assays still needs to be validated. Besides specificity and sensitivity, serological methods currently do not have the capability of distinguishing active *Toxocara* infection from past exposure —a problem that is causing much discussion and research among investigators (Ma et al., 2017; Smith et al., 2009).

Examination of serum antibody presence is not reliable for NT and OLM. Anti-*Toxocara* serum antibody titers may be low or completely absent in these two manifestations. Instead, NT should involve examination of CSF in addition to serum to identify anti-*Toxocara* antibodies and eosinophil levels (Caldera et al., 2013; Fan et al., 2015; Vidal et al., 2003). Imaging through computed tomography and MRI scans can also aid in diagnostic conclusions as diffuse lesions or granulomas can be revealed (Finsterer & Auer, 2007; Macpherson, 2013). The diagnosis of OLM is also challenging through serology, instead specific antibody detection is done in aqueous or vitreous humor (Yokoi, Goto, Sakai, & Usui, 2003). A wide variety of imaging techniques can also be done in conjunction with antibody testing to help diagnose and localize larvae in the eye (Arevalo et al., 2013; Macpherson, 2013).

2.5: Epidemiology and Public Health Relevance

At present, toxocariasis is not a notifiable disease in any country, which hinders a correct estimation of its true prevalence and human health impact. Nevertheless, its importance as a public health burden is confirmed by the increasing number of studies demonstrating *Toxocara* infection as one of the most ubiquitous zoonotic parasitoses occurring throughout the globe (Jenkins et al., 2013; Mattia et al., 2012; Pinelli, Herremans, Harms, Hoek, & Kortbeek, 2011). Highest seroprevalences have been documented in developing and mainly tropical countries, where studies have focused on mainly children, as they are a major risk group. Studies have found 30% seroprevalence in Nigeria, between 3.6 – 50% in Brazil, 37% in Peru, 45% in Swaziland, 58% in Malaysia, 63% in Indonesia, 81% in Nepal and 93% in La Reunion (Africa) (Espinoza et al., 2010; Holland & Smith, 2006; Liao et al., 2010; Roldan, Cavero, Espinoza, Jimenez, & Gutierrez, 2010; Schoenardie et al., 2013). Seroprevalences appear to be greater in socioeconomically

disadvantaged groups (Ma et al., 2017). Unlike many helminthiases, certain populations in industrialized countries have shown high degrees of seropositivity to *Toxocara* spp. Prevalence is reported in wide ranges, with 0.7% in New Zealand, 1.6% in Japan, 2.4% in Denmark, 6.3% in Austria, 7% in Sweden, 7.5% in Australia, 14% in the U.S. and up to 15% in Poland (Fan et al., 2013; Jarosz et al., 2010; Macpherson, 2013; Nicholas, Stewart, & Walker, 1986; Poeppl et al., 2013; Stensvold et al., 2009). These high percentages reported in developed countries and the estimated 2.8 million people infected in the United States (Hotez & Wilkins, 2009) indicate that this helminthiasis poses an important burden even in the richest countries in the world.

The prevalence of *Toxocara* spp. in canids and felids should not be overlooked as they play the biggest role in the spread of toxocariasis. Reported prevalence of *T. canis* and *T. cati* are typically low; 1.2% and 3.2% in Australia respectively, 4.4% and 4.6% in Holland, 6.1% and 4.7% in Germany (Barutzki & Schaper, 2011; Palmer, Thompson, Traub, Rees, & Robertson, 2008; Roddie et al., 2008). However, studies in countries like Nigeria, India, China and Portugal have published prevalence rates of as high as 50 to 100% in puppies and between 45 to 91% in cats (Sowemimo & Asaolu, 2008; Traub, Robertson, Irwin, Mencke, & Thompson, 2005; Waap, Gomes, & Nunes, 2014). In the U. S., relatively small prevalence can translate to major health risks, as the population of dogs was estimated at 77 million and of cats at 93 million (Ma et al., 2017). Their potential spread of eggs, through fecal excretion into gardens, parks, playgrounds, and sand pits is of concern as these spaces are heavily trafficked by the public and it puts children, an already vulnerable population, at an increased risk (Manini, Marchioro, Colli, Nishi, & Falavigna-Guilherme, 2012). Notably, the large-scale analysis by Won *et al*. in the U. S. showed that seroprevalence differed among races with non-Hispanic blacks having 21.2% seropositivity, non-Hispanic whites having 12.0% and Mexican-Americans having 10.7%. These differing results

are most likely due to socio-economic factors rather than ethnicity and genetic differences. The researchers also highlighted the possible usage of health education messages as a way to diminish the disparity and high degree of seroprevalence (Won, Kruszon-Moran, Schantz, & Jones, 2008).

Human toxocariasis is expected to be quite common based on the data that has been published to date, especially in communities where there is close contact between human inhabitants and domestic or wild canids and felids. Global seroprevalences and distribution of *Toxocara* spp. are depicted in Figure 5. However, assessing the global effect of *Toxocara* infection is still an obstacle mainly due to the limitations of the current diagnostic tools (Fillaux & Magnaval, 2013; Moreira et al., 2014). Expansion of epidemiological data is also hindered by the difficulty in distinguishing exposure to *Toxocara* spp. versus infection (Poulsen et al., 2015). While the last 3 decades have yielded an expansion on understanding *Toxocara* infection and assessment of epidemiological burden, there is still an urgent need for further studies addressing both of these issues in order to improve large-scale prevention and detection programs among those populations most affected.

2.5a: Epidemiological and Clinical Data in Honduras

No peer-reviewed literature measuring anti-*Toxocara* antibodies in Honduran populations could be found at this date. Therefore, no reference point is available for the present study. However, two relevant studies were found. Kaminsky (2014) assessed the prevalence of *T. canis* infection in pet canines, dogs from a dog pound and stray dogs in the areas of Tegucigalpa, Tatumbla, Zambrano and Danli. Fecal samples collected from each corresponding group were examined through direct microscopy using a direct wet mount with iodine as well as after a passive flotation method using hypertonic saline solution. 82 fecal samples were collected from pet dogs, 69

samples from a dog pound and 56 from strays of 207 samples. *T. canis* prevalence was 3.8% (8 cases). Five cases were from stray dogs, two cases were from dogs that were kept as pets and only a single case was identified in dogs from the local dog pound. The authors concluded that the findings of *T. canis* infection in Honduran dogs creates the necessity for improvement of diagnostic techniques for human toxocariasis as the risk of zoonotic transmission is present (Kaminsky et al., 2014).

The second study further underscores the spillover potential of *Toxocara* spp. in the country, as it presents a clinical case found in Danlí. A 14-month-old male patient presented to a regional hospital with a four-day old fever of unidentified origin, with no progression or exacerbation, diarrhea for three days containing blood and mucus, postprandial vomiting, a productive cough and rhinorrhea. Two days prior to the hospital visit, the patient suffered three episodes of generalized tonic-clonic seizures. Upon physical examination, hepatomegaly of 7 centimeters was found along with hyperreflexia and a positive Babinski response during neurological examination. A hemogram revealed hypereosinophilia with a count of 44,500/μL. The child was then transferred to a pediatric hospital in the capital city of Tegucigalpa, where electroencephalography, MRI and an analysis of cerebrospinal fluid were conducted. Due to the results of these tests, mainly the presence of a high number of leukocytes in the CSF, an anti-*Toxocara* antibody ELISA was performed, with the patient testing positive. Following treatment and improvement with antiparasitic medication, the infant was concluded to have been suffering from neurotoxocariasis (NT) (Puerto Sanabria & Tovar, 2016).

Unpublished academic papers, mainly theses and dissertations were also searched for through the use of repositories and online databases. Grey Literature Report ([www.greylit.org)](www.greylit.org),

GreyNet (www.greynet.org), Open Grey (www.opengrey.eu), OAIster/Worldcat (www.oasiter.worldcat.org), UNAH Repository (www.bibliovirtual.unah.edu.hn), Unitec (www.crai.unitec.edu), Google Scholar (www.scholar.google.ca), the LILAC, Honduran Medical Journal and Virtual Health Library databases (www.bvsalud.org/en/) were all searched to obtain any pertinent work by utilizing the following terms in both English and Spanish languages; "*Toxocara*" "*Toxocara canis*" "T. *canis*" "*Toxocara cati*" "T. *cati*" "*Toxocara* prevalence" "T. *canis* prevalence" "*Toxocara* antibodies" "*Toxocara canis* antibodies" "T. *canis* antibodies" "*Toxocara* seroprevalence" "*Toxocara canis* seroprevalence" "T. *canis* seroprevalence".

The only relevant document obtained was that of a study conducted by UNAH students during July and August of 2013 in Tegucigalpa, Honduras that aimed to measure the prevalence of intestinal parasites in both stray and domestic canines. Researchers looked at fecal samples of the canine subjects using direct wet mounts, Sheather's flotation technique and employed McMaster counting chambers to quantify parasitic load. Out of 101 total dog samples examined, they observed *T. canis* eggs in a single sample. Other intestinal parasites that were documented included *Ancylostoma* spp., *Trichuris vulpis* and *Dipylidium caninum* (Portillo & Martínez, not published). Upon communication with the first author, it was concluded that this low result was likely because most samples examined were from adult dogs. Conversely, puppies are known to excrete eggs in stools.
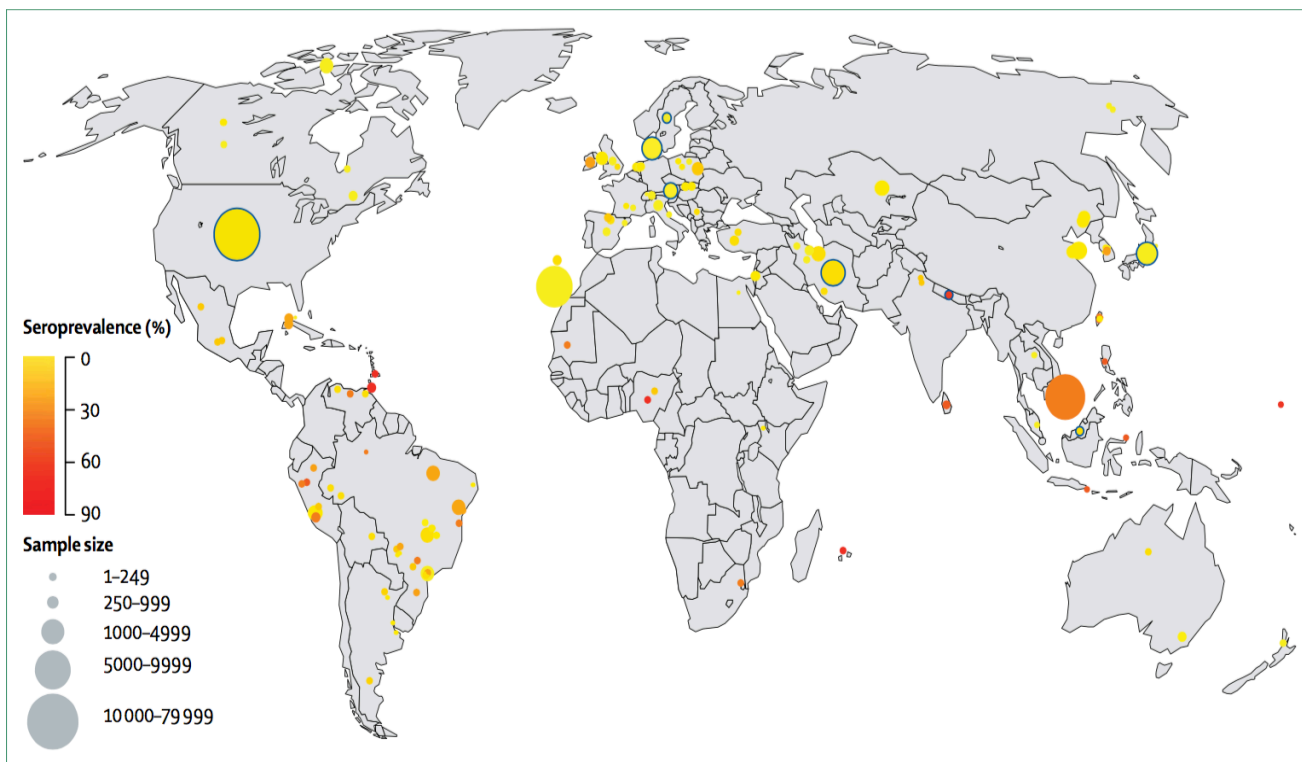
Figure 5: Reported seroprevalences of *Toxocara* spp. around the world. Sample sizes are displayed by circle size. Blue outlines represent a well-defined national survey or meta-analysis

CHAPTER 3: METHODOLOGY

3.1: Study Design

Since there are no previous studies on this topic in Honduras, the present study had an exploratory design with the aim of generating a hypothesis and insights to guide further investigations. The study used a non-probability and purposive sampling method.

3.1a: Study Area

The study was conducted in the village of Santa Cruz del Junco, in the municipality of Tela, department of Atlántida, situated on the northern Caribbean coast of Honduras. The town of Tela, named after the municipality is located between 15°47′ 00″ North latitude and 87° 28′ 00″ West longitude, placing it approximately 67 km north-east of the city of San Pedro Sula, the primary industrial centre in Honduras and the nation's second largest city after the capital (http://tools.wmflabs.org/geohack/, accessed September 14th, 2017). Tela has a total area of 1,163.3 km^2, an elevation of 3 m above sea level. In the most recent census in 2013, a population of 96,758 was documented by the Honduran National Institute of Statistics. In terms of distribution, 32.8% of the overall population was listed as being 14 years old or younger ($\leq$ 14 years), while 48% of inhabitants were reported to be residing in a rural setting (http://www.ine.gob.hn/, accessed September 14th, 2017). According to the Köppen Climate Classification, Tela exhibits a tropical rainforest climate. This type of weather is characterized by a lack of dry or wet seasons, as all months present at least 60 mm of precipitation, a condition that contributes to high levels of humidity. There is also no defined summer or winter and it is typically hot and wet year-round, with the wettest months being November and December and the driest being April and May (https://weather-and-climate.com/average-monthly-precipitation-Rainfall,Tela,Honduras, accessed December 12th 2018). The annual climatic average in 2017 was

26.4°C, >75% relative humidity with 2,801 mm of precipitation. The highest temperatures of the year are reached during the month of May and only begin to drop after the month of September, with the coolest month of the year being February (https://www.weather-atlas.com/en/honduras/tela-climate., accessed December 12th, 2018). The selection of the town of Tela as an ideal site is due to its environmental conditions, especially the hot and humid climate, as well as the disadvantaged socio-economic circumstances that lead to a lack of sanitary infrastructure. These conditions are regarded as ideal for the development and transmission of a variety of parasites, most importantly of *Toxocara* spp. (Hotez & Wilkins, 2009; Mizgajska, 1997). Previous research investigating prevalence and intensities of STH in Honduran schoolchildren has identified Tela as belonging to a sanitary region characterized by poverty and a STH prevalence of almost 50% (Torres et al., 2014). Such high endemicity in a region known to be impoverished further solidifies the plausibility of *Toxocara* spp. spread. Additionally, an earlier study undertaken in Santa Cruz del Junco's primary school also supports this selection as a high level of prevalence for STH has been found (Rodríguez et al., unpublished).

Figure 6: Location of Study Area (Tela) in Relation to Tegucigalpa & San Pedro Sula

3.1b: Study Population

Schoolchildren from the only existing school in the village, a primary school consisting of grades one through six, were the target population for the study. As mentioned, a prior collaboration already existed with the school and Honduran co-investigators from the National Autonomous University of Honduras (UNAH), thus facilitating the communication, socialization and implementation of this project. All children enrolled in the school were invited to participate.

Studying school-age children for exposure to *T. canis* is based on the following factors; First, young children tend to more frequently harbour parasitic infections as compared to adults (Hotez, 2008). More specifically, infection with *Toxocara* spp. is markedly more associated with children, a finding related to certain determinants such as geophagia, children's playing habits and their lack of hygienic practices (Despommier, 2003; Macpherson, 2013; Magnaval, Glickman, et al., 2001). Pre-school children, especially aged one year or older could also be at risk of exposure and worth studying; however, enrolling this population was not possible as there is no day-care or kindergarten in the village. A house-to-house survey was not feasible, as it would exceed our logistic capabilities.

3.1c: Timeframe of the study

The study took place during August 2015 and October 2017. Co-researchers at UNAH conducted the first phase in 2015. The second phase also included the author of the current thesis. Data and samples collected by both studies were integrated and are the basis of the current thesis.

3.2: Ethical Considerations

The study received ethics clearance from both participating institutions, both in Canada and Honduras.

1. Brock University, St. Catharines, Ontario, Canada. Bioscience Research Ethics Board-File number: 17-032-Sanchez. Clearance received: September 12th 2017 (Appendix A) and Bioscience Research Ethics Board-File number: 14-224 Sanchez. Clearance received: May 8th 2015 (Appendix B).

2. National Autonomous University of Honduras, Tegucigalpa M.D.C, Honduras. Committee of Ethics, Master's program in Zoonotic and Infectious Diseases School of Microbiology-File number: 04-2017. Clearance received: September 21st 2017 (Appendix C) and Committee of Ethics, Master's program in Zoonotic and Infectious Diseases School of Microbiology- File number: 01-2015. Clearance received: August 1st 2015 (Appendix D).

In addition to these ethical clearances, approval for the implementation of this study was requested from the participating school's principal and its grade teachers. Both parental consent and children's assent were also mandatory prior to an individual's participation.

3.3: Research Participant Recruitment

Once approval from the school principal was attained, parents and guardians of children enrolled in the school were invited to an informational session where the rationale, protocol, benefits, risks and objectives of the study were fully explained. Invitation to this information session occurred through the circulation of letters of invitation, which were sent home with children. It was made clear to parents/guardians that enrollment in this study was voluntary and that there would be no

detrimental consequences had their child not participated. Furthermore, the informational session briefly provided background information regarding *Toxocara* spp., their potential disease and its effects on children along with the importance of hygienic practices as preventive measures. Attendees were encouraged to ask questions and express any doubts or apprehensions they may have had regarding the study at this time. At this stage, individuals who conveyed interest in participating were apprised of the meaning of free and voluntary consent and of their right to refuse or discontinue participation at any time without explanation and, without penalty or loss of benefits to which they were otherwise entitled. This included reassuring the parents that the school was not involved whatsoever in the study and does not have access to any data, or information concerning participation/withdrawal. Once individuals understood the study and verbally communicated their willingness to enroll, they were presented with two informed consent forms (Appendix E and F) that contained the same information that was explained orally to them (study objectives, procedure, risks, benefits etc.). One form was for consent to parental involvement in the study (answering the questionnaire, collecting their child /children's stool sample) and a second form was prepared for their consent to have their children partake in the study and provide both a stool and blood sample. Parents/guardians were then asked to sign both forms in the presence of a witness.

Once a parent/guardian granted consent for their child's participation, the child was then asked if they wished to participate in the study. All details of the study were disclosed and the assent form (Appendix G) was read aloud and explained to the children, including their individual right to refuse participation regardless of their parents' decision without any negative consequences. Only the children whose parents provided consent and who expressed their own assent in supplying stool and blood samples were enrolled in the study. Their assent was documented in the presence of a witness.

3.3a: Inclusion and Exclusion Criteria

All children attending the school were recruited for participation in the study as long as they could provide informed consent from their parent/guardian, informed assent for themselves, a stool and blood sample (from the child) and completion of a face-to-face interview of the parent/guardian. There were no exclusion criteria: all children that met these conditions were eligible to participate in the study regardless of gender, height, weight, ethnicity, etc.

3.4: Data Collection

Each participating parent/guardian partook in a face-to-face interview that used the questionnaire (Appendix H) as a guideline, through which demographic data and information on child behaviour were documented. A face-to-face interview asking the questions outlined on the questionnaire was preferred over administration of a paper based, self-reported questionnaire due to concerns of illiteracy or possible misinterpretation of any questions. A blood sample was necessary to determine the presence of anti-*Toxocara* antibodies in the child's serum. Moreover, a stool sample was also requested to establish the presence or absence of any intestinal parasite infections, most importantly, *Ascaris lumbricoides.*

3.4a: Interviews and Questionnaire

To be interviewed, participating parents must have given informed consent and their children must have given their assent to participate as well as blood and stool samples. To anonymize data, each child that took part in the study was assigned a unique numerical code through which their samples and the questionnaire were identified. Interviews were conducted individually at the school and in such a manner that participants and interviewers were visible to others, but their responses

remained inaudible and private. The questionnaire was structured into different categories that all aimed to gather relevant information across diverging areas, including:

1. Basic information: this section asks things like the child's name, age, date of birth, gender and grade in school.

2. Household characteristics: explores information pertaining to domestic animals in the child's household such as ownership of the animal, age of the animal, contact with the child.

3. Habits: Child behaviours such as playing in/with dirt, geophagia and eating undercooked meat or vegetables were assessed.

4. Knowledge of parasites: Prior exposure to parasites and knowledge of these organisms was asked.

To ensure that the interviewer missed no information on the questionnaire, a different member of the research team checked the questionnaire for completeness. Detection of missing information was remedied by retrieving the participant. In case that a participant was enrolled in both 2015 and 2017, the most recent epidemiologic and demographic information was used.

3.4b: Stool Sample Collection

The necessary materials along with an instruction sheet were provided to the parents/guardians of the children enrolled in the study. These materials and instruction sheet were packaged together into a stool collecting kit. The kit contained a plastic, disposable "chamber pot" (for defecation), vinyl gloves, a wooden tongue depressor (for scooping of the fecal specimen), a plastic, non-breakable container with a lid, a transparent plastic bag, a brown paper bag and a sheet with detailed and numbered instructions with pictures (Appendix I). Stool samples were to be collected in the morning and children were asked to bring their fecal sample back to the research team on the day of blood drawing, at the school.

3.4c: Blood Sample Collection

A single blood sample, totalling no more than 5 mL, was extracted from each child through venipuncture of the main cubital, basilic or cephalic veins. Each blood sample was collected using a Vacutainer^{TM} tube, without anticoagulant to obtain serum and detect the presence of anti-*Toxocara* antibodies. In 2015 only, a second blood sample was obtained using Vacutainer^{TM} tubes with the anticoagulant K2-EDTA to perform a complete blood count. Phlebotomy took place at the school and only those children who provided assent and whose parents/guardians had given informed consent had their blood drawn.

3.4d: Stool Sample Analysis for Soil-Transmitted Helminth Infections

Once the children's stool samples were delivered to the research team, they were kept refrigerated by being placed in a portable cooler to be transported to a laboratory at a local hospital in Tela for further analysis later the same day. Samples were subject to the Kato-Katz technique; a method for microscopic examination that yields qualitative and quantitative results in terms of intensity of infection, as endorsed by the World Health Organization (WHO) (WHO, 1991). Prepared smears were systematically examined after approximately 30 minutes of clarifying time. Any intestinal parasite egg that was identified was counted to help calculate the number of eggs per gram (epg) of stool for each species of soil-transmitted helminths (STH). The calculation of eggs per gram results in an estimate of infection intensity for any helminths found, in accordance with the classification thresholds established by the WHO (WHO, 2012). The classification is based on the biological characteristics of the female worms, mainly their fecundity (Table 1). These findings were documented and were made known to the participants through a lab report form (Appendix J). Any insufficient or unsatisfactory stool sample provided was instead subjected to a direct wet mount examination.

Table 1 Intensity of infection thresholds in eggs per gram (epg) of stool for STH (WHO, 2002)

Soil-transmitted Helminth	Light infection	Moderate infection	Heavy infection
Ascaris lumbricoides	1 – 4,999 epg	5,000–49,999 epg	>50,000 epg
Trichuris trichiura	1 – 999 epg	1,000 – 9,999 epg	>10,000 epg
Hookworms	1 – 1,999 epg	2,000 – 3,999 epg	>4,000 epg

3.4e: Blood Sample Analysis for Anti-*Toxocara* Antibodies

Following phlebotomy, blood drawn from study participants was stored within Vacutainer^{TM} tubes, which were maintained on ice inside of a portable cooler for refrigeration. Serum was separated by centrifugation at 2,500 rpm for 5 minutes and subsequently stored at –20 ^{o}C until assayed. Using this serum derived from a venous blood sample, anti-*Toxocara* IgG antibodies were detected through both an ELISA and a Western blot.

3.4f: *Toxocara* Excretory/Secretory Enzyme-Linked Immunosorbent Assay (TES-ELISA)

The AccuDiag^{TM} *Toxocara* IgG ELISA kit (TES-ELISA), manufactured by ImmunoDiagnostics was used. The assay involves the covering of wells in a well-plate with *Toxocara* spp. purified excretory-secretory antigens (TES) which are obtained from the worm's second larval stage. In general, TES refers to a set of proteins that are heavily glycosylated, made up of approximately 40% carbohydrates, mainly *N*-acetylgalactosamine. They are composed of similar but not identical antigenic properties to proteins expressed on the larval surface, as determined by detailed molecular comparisons (Maizels, de Savigny, & Ogilvie, 1984). There are five major TES antigens used in this assay, of the following sizes: 32, 55, 70, 120, and 400 kDa. According to size, names for the different TES antigens have been proposed; TES-32, TES-55, TES-70, TES-120 and TES-400 (Holland & Smith, 2006; Maizels et al., 1984). TES-32 and -70 are members of the C-type (calcium-dependent) lectin family and are the most prominent larval proteins. Meanwhile, TES-120 has been shown to be a complex constituted of the mucin proteins MUC-1, -2 and -3 with MUC-1 and MUC-3 being the most eminent (Loukas, Doedens, Hintz, & Maizels, 2000; Loukas, Hintz, et al., 2000; Loukas, Mullin, Tetteh, Moens, & Maizels, 1999). TES-55 and -400 are made

up of proteoglycan-like material and a number of active enzyme molecules (Holland & Smith, 2006).

Briefly, the participants' serum is added to the antigen-covered wells with the premise being that if antibodies are present in the serum, they will react with the TES antigens on the well-plate. This is then followed by the addition of an anti-antibody containing a conjugate enzyme, which when a substrate is introduced, reacts with the substrate. This resulting reaction between substrate and conjugate enzyme induces a colour change in the solution and it is this colour change, that can be measured through spectrophotometry, which dictates the presence or absence of anti-*Toxocara* antibodies in a participant's serum depending on the absorbance value generated, expressed as optical density (OD).

3.4g: Western Blot

In addition to the described TES-ELISA, any positive result was further tested by a Western blot (WB) procedure that detects IgG antibodies specific for TES antigens (LDBIO Diagnostics). Only three negative ELISA results were further subjected to Western blotting, as negative controls. Per manufacturer instructions, the simultaneous presence of 2 low-molecular-weight (LMW) bands (24 – 35 kDa) following the WB procedure indicates the presence of specific anti-*Toxocara* antibodies. Besides TES-32, described in the previous section, these LMW bands are believed to be associated with antigens TES-24, -28, -30 and -35 that are now characterized as phosphatidylethanolamine-binding proteins and other mucins (Magnaval et al., 1991; Roldan & Espinoza, 2009). Any serum that yielded 2 or more of these low-molecular weight bands was considered to be seropositive.
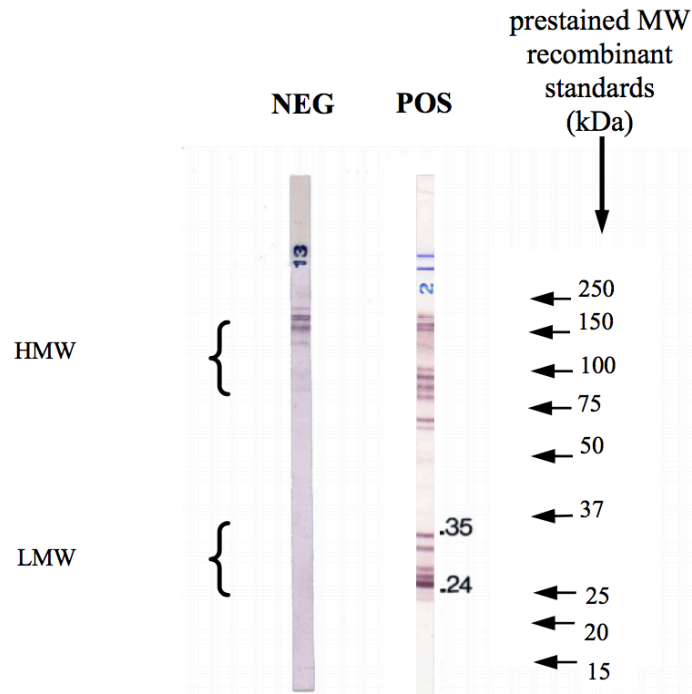
Figure 7: Depiction of positive vs a negative Western blot result showing the relevant bands and their corresponding molecular weights.

The need for confirmation of a positive result from TES-ELISA differs around the world. Concurrent parasitoses are less frequent in developed and temperate regions of the world compared to tropical, underdeveloped regions where polyparasitism can be endemic. While TES-ELISA can be an effective and reliable serodiagnostic tool in temperate and developed countries, its use for serodiagnosis in tropical countries such as Honduras is fraught with concerns about the presence of cross-reacting antibodies from other common infections. Most notably seen in this area are trichinellosis, strongyloidiasis, and particularly, ascariasis (Fillaux & Magnaval, 2013; Jacquier, Gottstein, Stingelin, & Eckert, 1991; Lynch, Wilkes, Hodgen, & Turner, 1988). There has also been discussion elsewhere of reported observations indicating that human antibodies from *T. trichiura* cross-react more frequently with TES-ELISA than those from *A. lumbricoides* (Holland & Smith, 2006). Regardless of species, cross-reactivity can lead to false positive results, which

would reduce the value of TES-ELISA when used in people from tropical areas where polyparasitism is common, hence the reasoning for WB as an additional confirmatory measure.

3.4h: Eosinophil Determination

For children who participated in the 2015 portion of the study, an absolute eosinophil count was realized using the blood sample that was collected with K2-EDTA through the automatic counter ABX Pentra 210, manufactured by ABX Diagnostics. This system utilizes cytochemistry, impedance, absorbance and flow cytometry. Eosinophil counts were expressed as the number of eosinophils/µL, where values $\leq$ 450 eosinophils/µL were considered normal and those $\geq$ 500 eosinophils/µL were classified as eosinophilia (Kovalszki & Weller, 2016).

3.4i: Biosafety Considerations

Due to the biohazardous potential of the samples being collected and worked with, containment level 2 (CL-2) practices were employed throughout the implementation of this project. Beginning with the extraction of blood samples, researchers donned personal protective equipment (PPE), including lab coats, gloves, lab goggles or protective eyewear, long pants and closed-toed shoes. The storage of these samples was discussed in an earlier section. While participants provided stool samples, their handling also required adherence to CL-2 biosafety practices. The non-breakable, leak proof containers that participants were provided were stored in a cooler for transportation to a CL-2 laboratory. Here, investigators were again donning PPE including lab coats, long pants, gloves and close-toed shoes. Following analysis, all the materials and tools utilized in the Kato-Katz technique including microscope slides and cover slips were decontaminated using a 10% bleach solution.

3.5: Data Management and Statistical Analyses

Ensuing its collection and accumulation, all data including from Kato-Katz, ELISA and Western blot results along with epidemiological and demographic data collected during the interview process were inputted into a Microsoft Excel 2016 spreadsheet (Microsoft Corp., Redmond, WA, USA) for the creation of a master database. A second, anonymized database was derived from this master database by replacing participant names with unique participant identification numbers. Datasets from both phases of the study were cleaned and integrated into a single database.

Data cleaning involved inspecting the transcribed dataset for errors or missing values. Wherever possible, participant data were simplified into dichotomous variables. For example, the continuous variable of the age of dog(s) owned was dichotomized into ownership of dog(s) either

younger than 1 year or older, based on biological relevance of *Toxocara* oviposition in canines. Statistical analyses were carried out using the STATA 13 software package (StataCorp LP., College Station, TX, USA).

For characterization of the study population, descriptive statistics were documented for both continuous and categorical variables. Seroprevalence of anti-*Toxocara* antibodies within the study sample was calculated based on TES-ELISA and further confirmation of all positive TES-ELISA results via Western Blot. Discrepant serology results for 2015 and 2017 were in all cases considered positive for the following reasons: seroconversion (from negative in 2015 to positive in 2017) would signify that exposure to the parasite occurred in the interim period. Seroreversion (from positive in 2015 to negative in 2017) would most likely represent previous exposure with successful infection clearance and ensuing decreasing antibody titres, as seropositivity can remain for up to 2 years following exposure, even if infection has been cleared (Boldis, Ondriska, Spitalska, & Reiterova, 2015; Fillaux & Magnaval, 2013; Macpherson, 2013).

Assessment of the significance of univariate associations with positive serology (as confirmed by WB) was done by Fisher's exact test. A logistic regression model was applied to investigate the relationship between the various epidemiological risk factors with anti-*Toxocara* antibody presence. It should be noted that data regarding the majority but not all of these risk factors was collected only in the second segment (October 2017) of the study. Hence, some epidemiological information is available only for the 46 individuals that enrolled in October of 2017. Despite this, it was deemed appropriate to include both years in the statistical analyses because of the lack of sanitary infrastructural changes within the community and the participating school and no implementation of any preventive or awareness-raising programs in the 2 years.

Sensitivity, specificity, positive predictive and negative predictive values were calculated for the TES-ELISA used, compared to the Western blot. The kappa statistic for level of agreement between both of these diagnostic tools was also evaluated. For the eosinophilia observed in the first phase of the study, a Mann-Whitney U test was also employed to determine if there was a significant difference in circulating eosinophil counts in seropositive vs. seronegative individuals. Level of significance was established at $\alpha = 0.05$.

CHAPTER 4: RESULTS

4.1: Study Enrollment

The school enrolment for 2015 and 2017 was 92 and 71 children, respectively. Of those, 73 (79%) and 46 (66%), respectively, completed the study. Initially in 2017, 47 children agreed to participate, but one withdrew from the study due to fear of providing a blood sample. This was the only case of participant withdrawal for both years. In total, 89 children enrolled in the study, again one child did not partake in the study because of needle phobia, leaving a total of 88 schoolchildren: 31 participated in both years (referred to as dual participants) and 57 participated in either 2015 or 2017 but not both (unique participants). Figure 8 outlines what was collected and analyzed for each year.
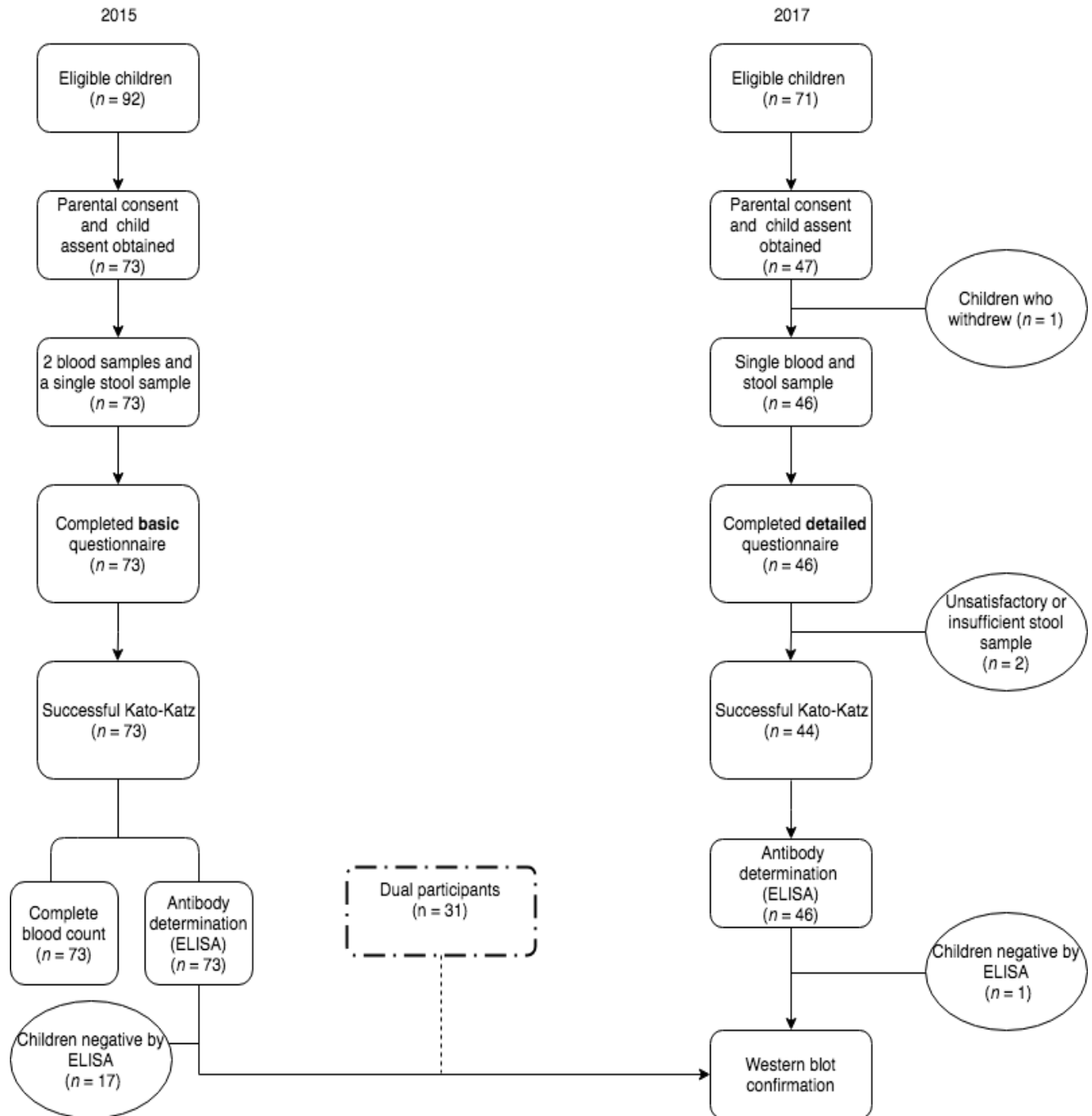
Figure 8: Flowchart outlining the study procedures for schoolchildren enrolled in both years of the study (2015 & 2017). Western blot confirmation was done for both years in 2018.

4.2: Characterization of the Study Sample

Of 88 participants, 45 (51.1%) were girls and 43 were males (48.9%). The mean age of the study was 9.83 years (± 2.25). A questionnaire to collect epidemiological and behavioral characteristics was only administered in 2017. As a result, variables including dog age, cat ownership, cat straying behaviours, soil contact, eating practices and others such as onychophagia, geophagia and thumb-sucking were only recorded for the 46 participants from 2017 (Figure 8; Table 2). An important proportion (71.6%) of interviewed participants reported dog ownership by their family, and over half of these dogs were under a year of age (Table 2). Most participants with household pets, either dogs or cats, specified that these animals strayed from the household freely (72.6% and 66.7%, respectively). As for habitual practices, 80.4% of children said they had some sort of contact with soil, mostly with soil from the playground adjacent to the school. Most children mentioned consumption of raw or undercooked vegetables and fruits (79.5%) and 45.6% of them reported eating raw or undercooked meat at home.

4.3 STH Infection Results

For both 2015 and 2017, two children provided insufficient or unsatisfactory stool samples for the Kato-Katz technique, so the samples were instead analyzed with the direct wet mount technique. Approximately 40% of the 88 children were found to be infected with at least one species of STH, with *T. trichiura* being the most prevalent helminth. The majority of these children (70.5%), were familiar with what STH were, and an even higher percentage (71.1%) actually recalled having had an STH infection in the past (Table 2).

Table 2: Study sample descriptive characteristics and parasitological examination results (n = 88)

Characteristics	N (%)
Males	43 (48.9%)
Females	45 (51.1%)
Age	Mean: 9.83 years (SD: 2.25)
Soil Transmitted Helminth (STH) Profile	**n = 88**
Overall STH Prevalence	34 (38.6%)[‡‡]
Ascaris lumbricoides Infection	10 (11.4%)[‡‡]
Trichuris trichiura Infection	30 (34.1%)[‡‡]
Hookworm Infection	6 (6.8%)[‡‡]
Polyparasitic Infections	11 (12.5%)
Awareness Of STH	62 (70.5%)
Recalled Having STH Infection	54 (71.1%)[§]
Anti-*Toxocara* Antibody Serology	**n = 88**
Positive by TES-ELISA	80 (90.9%)
Positive by Western Blot (WB)	78 (88.6%)
Domestic Animal Conditions	
Dog Ownership (n = 88)	63 (71.6%)
Puppy Ownership (Dog Age ≤ 1 Year)	13 (56.5%)[†]
Straying of Owned Dog(s)	45 (72.6%)[+]
Cat Ownership (n = 46)	24 (52.1%)[†]
Straying of Owned Cat(s)	16 (66.7%)[†]
Habits/Behaviours	**n = 46**
Contact with Soil In the Village	37 (80.4%)[†]
Contact With Soil In School Playground	41 (91.1%)[†, ““]
Geophagia	2 (4.3%)[†]
Onychophagia	11 (23.9%)[†]
Thumb-Sucking	4 (8.7%)[†]
Consume Undercooked Beef	21 (45.6%)[†]
Consume Raw Fruits/Vegetables	35 (79.5%)[†, ◊◊]

[†] Data collected only for the 46 participants in 2017; [‡‡] Two children did not provide sufficient/satisfactory stool samples for Kato-Katz examination
[§] Ten children did not recall whether they had previously suffered STH infection; [+] One child did not recall whether the owned dog strayed or not
[◊◊] Two children did not recall whether they consume raw fruits/vegetables; [““] One child did not recall coming in contact with playground soil

4.4: General Seroprevalence of anti-*Toxocara* Antibodies

A total of 88 unique blood samples from schoolchildren were collected and examined for the presence of circulating antibodies against *Toxocara* spp. Of the 88 samples, 80 were positive by TES-ELISA, representing a seroprevalence of 90.9%. Under manufacturer's instructions, an absorbance equal to or greater than a 0.3 optical density (OD) value was categorized as positive. The 80 positive sera were then subjected to Western blotting. Western blot results confirmed that 78 out of the 80 sera were indeed positive for anti-*Toxocara* antibodies and hence, an overall seroprevalence of 88.6% was documented. Table 3 presents the seropositivity data observed by year and exclusivity of participation.

Table 3: Seroprevalence results grouped by year of participation and commonality status

	2015				2017			
	ELISA		WB		ELISA		WB	
	+	-	+	-	+	–	+	-
Unique participants (*n* = 57)	35	7	35	0	15	0	14	1
	ELISA +		ELISA -		WB +		WB -	
Dual Participants (*n* = 31)	30		1		29		1	

4.4a: Seroprevalence Analysis of Dual Participants

Dual participants were schoolchildren who were enrolled and contributed samples in both years (2015 and 2017). The number of these overlapping participants along with their serological results from both years are demonstrated in Table 4. The proportion of seropositive children increased from one year of the study to the next.

Figure 9 also deals with the results of the dual participants but exclusively displays the ELISA absorbance values of each participant in both years. Some notable changes include the seroconversion of eight out of the 31 individuals, as well as an increase in ELISA absorbance values of over 30% in 8 individuals when comparing 2015 to 2017. In contrast, only five children showed a decrease of over 30% in ELISA absorbance during the same time span. Lastly, one individual appeared to have become borderline seropositive after initially testing negative in 2015, however this contentious seroconversion was dismissed by a negative Western blot result.

Table 4: Serological changes of schoolchildren who participated in both phases (2015 & 2017)

	ELISA Positives 2015 N (%)	ELISA Positives 2017 N (%)	Western Blot Positives (2017) N (%)	Seroconverted Subjects by 2017
Males ($n = 15$)	12 (80%)	15 (100%)	15 (100%)	3 (20%)
Females ($n = 16$)	9 (56.3%)	15 (93.7%)	14 (87.5%)	5 (31.3%)
Total ($n = 31$)	21 (67.7%)	30 (96.7%)	29 (93.5%)	8 (26%)
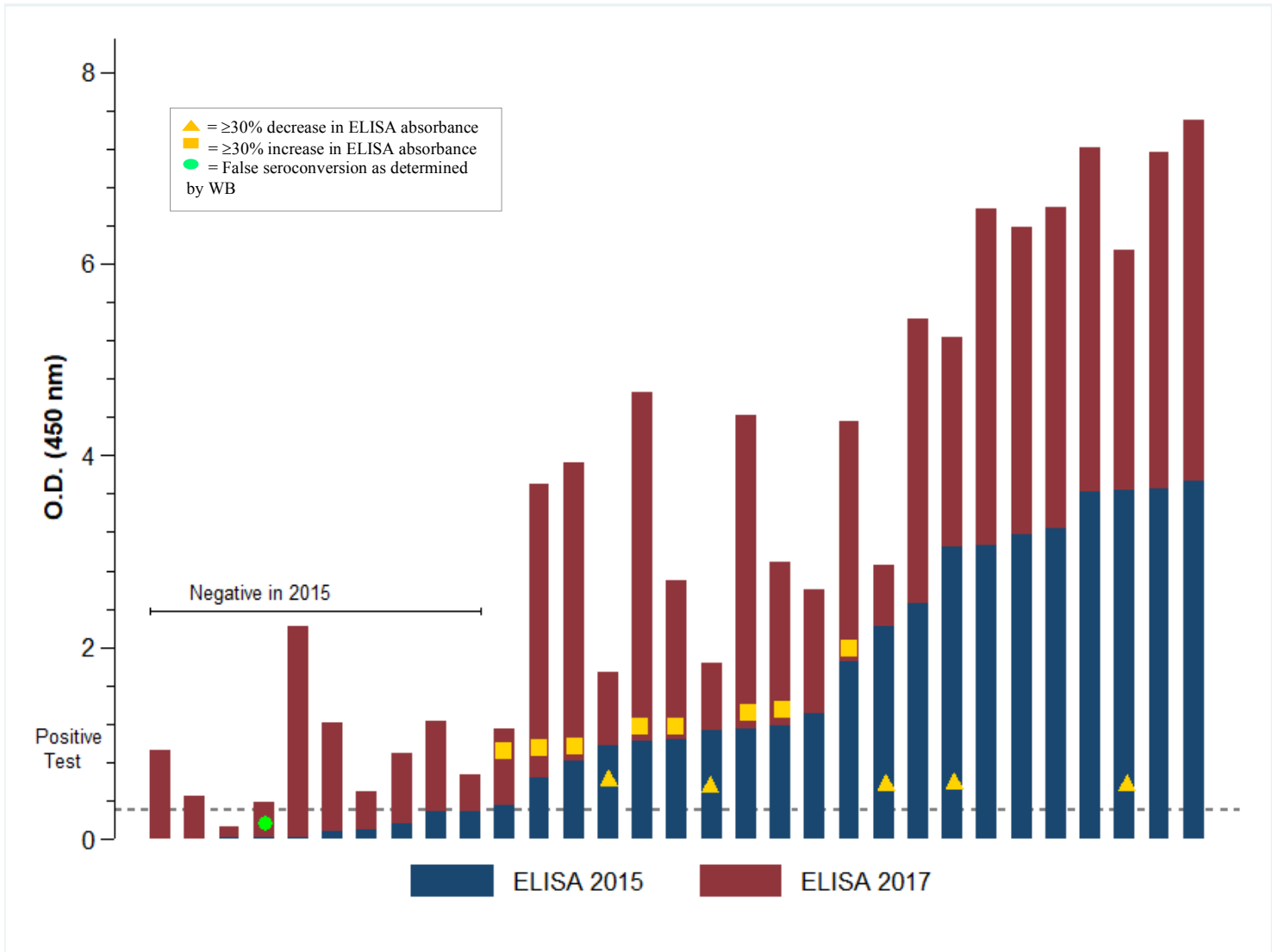
Figure 9: Differences in ELISA absorbance values between 2015-2017 in children who provided samples both years. Dotted line represents the 0.3 absorbance value cut-off for positives

4.4b: Univariate Associations

As previously mentioned, Fisher's exact test was utilized to examine the significance of association between anti-*Toxocara* antibody seropositivity and various reported variables, both demographic and habitual, among the schoolchildren. Table 5 presents the findings of this analysis as well as the percentage of seropositivity among participants who gave an affirmative response to each of the listed variables. No statistically significant associations were found for any of the variables tested.

It should be noted that due to reliability issues, not all of the variables for which information was collected were included in the statistical analysis shown in Table 5. The straying behaviour of companion animals (dogs or cats) was excluded due to the constant wandering and continuous presence outside of households by dogs and cats within the community, as directly observed by researchers. Cat ownership was also excluded due to cultural customs in the community and the country of Honduras as a whole. Owners are typically not in constant contact with felines, as cats spend minimal time in the home and do not defecate indiscriminately. In contrast, dogs are kept more closely and with a higher degree of physical contact. Similarly, geophagia and thumb sucking were not tested for association with seropositivity, yet onychophagia was maintained. This is because of the social stigma attached to the two former habits, especially among older children, and the possible social desirability bias that may have resulted.

Table 5: Univariate analysis of reported characteristics among anti-*Toxocara* spp. seropositive schoolchildren

Variable	Seropositive N (%)	Fisher's Exact p value
Male (*n* = 43)	41 (95.35%)	0.090
Female (*n* = 45)	37 (82.22%)	0.090
Soil Contact (*n* = 37) [†]	35 (94.59%)	0.488
Onychophagia (*n* = 11) [†]	10 (90.91%)	1.000
Undercooked Beef Consumption (*n* = 21) [†]	21 (100%)	0.239
Raw Fruit/Vegetable Consumption (*n* = 35) [†]	34 (97.14%)	0.371
Playground Contact with Soil (*n* = 41) [†]	38 (92.68%)	1.000
Dog Ownership (*n* = 63)	57 (90.48%)	0.461
Dog Age ≤ 1 Year (*n* = 13) [†]	12 (92.31%)	1.000

[†] Data collected only for the 46 participants in 2017

4.4c: Degree of Antigen Recognition

Along with the 80 sera that tested positive via TES-ELISA, 12 positive controls that were provided by the kit manufacturer and three negative control sera were included in the Western blotting procedure. A positive control was ran using the first of every octet of strips (i.e., strips #1, #9 and #17 in every series), as recommended by the kit manufacturer (LDBIO Diagnostics). Following the high positivity observed, three sera that tested negative by ELISA were subjected to immunoblotting as negative controls. A photograph of all individual strips and their resulting bands makes up Figure 11, with positive and negative controls indicated by plus and minus signs, respectively. The majority of positive sera reacted to all five low-molecular-weight glycoprotein bands embedded in the strips. The number of glycoproteins recognized and frequency of recognition by children's sera is displayed in Figure 10, whereas the specific antigenic glycoproteins recognized are shown in Figure 11. Sixty-nine children had antibodies to four or five antigenic glycoproteins of low-molecular-weight. Of the 30 dual participants that were immunoblotted, 24 displayed antibody reactivity to five LMW bands. Similarly, sera from 27 children from the 2015 only group, were also reactive to the maximum number of LMW bands following the immunoblotting procedure.
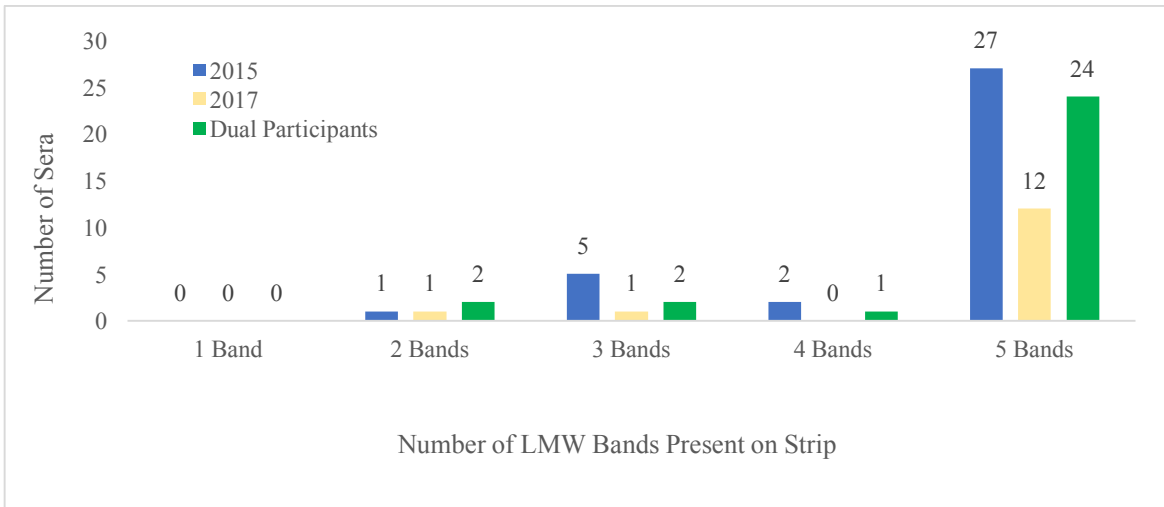
Figure 10: Frequency of different banding patterns expressed following immunoblot assay among schoolchildren who were examined in 2015 only, 2017 only or both years.
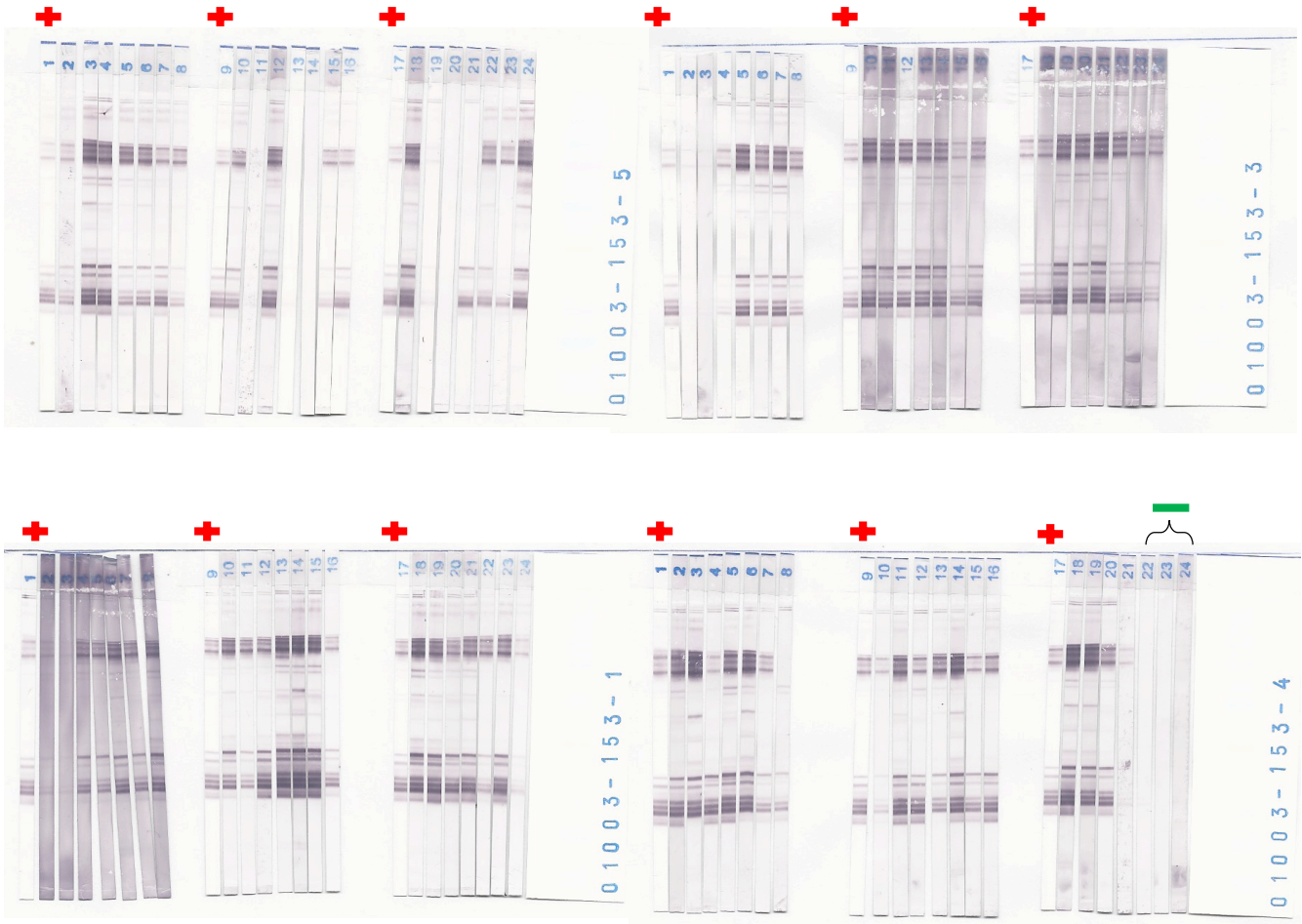
Figure 11: *Toxocara* spp. antigen-embedded strips following immunoblotting with all 80 sera tested for anti-*Toxocara* antibodies along with positive and negative controls, depicted by a red plus and green minus sign, respectively.

4.4d: Comparison of Serodiagnostic Techniques

As an additional step, the validity of the TES-ELISA used as a screening measure in this study was measured against the Western blot, the current gold standard for anti-*Toxocara* antibody detection (Table 6). The ELISA sensitivity was 100% and the specificity was 80%, lower than the 93.7% reported by the manufacturer of the ELISA kit (ImmunoDiagnostics[TM]). With these two parameters, a kappa (κ) statistic was calculated to gauge the degree of agreement between the two serodiagnostic tools. In general, the higher the kappa value is, the stronger the agreement as outlined by the following parameters: a κ between 0.01 – 0.20 indicates slight agreement, between 0.21 – 0.40 a fair agreement, 0.41 – 0.60 a moderate agreement, 0.61 – 0.80 a substantial agreement and anywhere between 0.81 – 0.99 represents an almost perfect agreement. In this case, the resulting kappa statistic was $\kappa = 0.87$, an indicator of an almost perfect agreement between the two diagnostic tools.

Table 6: Level of agreement of TES-ELISA results with gold standard (Western blot)

TES-ELISA Validity Characteristics (in comparison to WB)		95% Confidence Intervals
Sensitivity	100%	95.4 – 100
Specificity	80%	44.4 – 97.5
Positive Predictive Value (PPV)	97.5%	91.3 – 99.7
Negative Predictive Value (NPV)	100%	63.1 - 100
Kappa (κ) Statistic (Between ELISA & WB)	0.8764	--

4.4e: *Toxocara* spp. Exposure and Eosinophilia

Eosinophilia determination was only done in 2015, and 16 cases of eosinophilia were found. A Mann-Whitney *U* test was conducted to see if there was a significant difference in the count of circulating eosinophils in schoolchildren who tested seropositive compared to those who were seronegative. The results obtained are listed in Table 7.

Eosinophil counts were higher by an average of over 100 eosinophils/µL in children who tested seropositive for circulating anti-*Toxocara* antibodies in comparison to seronegative children. To account for possible confounders, the test was also applied when controlling for individuals with any kind of STH infection and *T. trichiura* specifically. The reasoning behind the exclusion of these subgroups is due to the current knowledge that STH infection in general, and trichuriasis in particular, are potent drivers for eosinophilia. While the mean eosinophil count decreased in seropositive children when removing these potential confounders, a statistically significant difference was still found between both subgroups. For those that were found free of any STH infection, seropositive children averaged 191.3 eosinophils/µL, compared to 101.2 eosinophils/µL in those who were seronegative ($p = 0.058$). A similar level of elevation was documented for those with no trichuriasis, as children who were considered seropositive had a geometric mean of 198.5 eosinophils/µL, while seronegative individuals had a lower average of 101.2 eosinophils/µL ($p = 0.035$).

Table 7: Mann-Whitney U test results comparing circulating eosinophil levels in seropositive vs seronegative schoolchildren from 2015 only.

	Anti-*Toxocara* Antibody Western Blot (WB) Seropositives (95% CI)	Anti-*Toxocara* Antibody Western Blot (WB) Seronegatives (95% CI)
G-Mean Eosinophil Count (/µL)	262.1 (211.7 – 324.4)	101.2 (59.6 – 171.9)
p Value	0.004	0.004
G-Mean Eosinophil Count (/µL) (No STH)	191.3 (141.7 – 258.2)	101.2 (59.6 – 171.9)
p Value	0.058	0.058
G-Mean Eosinophil Count (/µL) (No *T. trichiura*)	198.5 (151.2 – 260.5)	101.2 (59.6 – 171.9)
p Value	0.035	0.035

Eosinophilia data collected in 2015 only.

4.5: Epidemiological Factors for *Toxocara* spp. Seropositivity

A total of 12 epidemiological variables were deemed as potential risk factors for participant data collection. As previously mentioned, only eight variables were tested for their association with positive serology of anti-*Toxocara* antibodies in the research participants. However, out of the eight variables analyzed, an additional three had to be omitted from the final model due to the high number of children who turned out to be seropositive, making it mathematically impossible to apply logistic regression. Continuous variables were dichotomized before their integration into a logistic regression model. Results of this model can be found in Table 8.

Gender was the only variable found to be marginally associated with *Toxocara* spp. exposure, as males had over four times the odds of being seropositive (OR = 4.43, p = 0.07). Of the remaining variables, some appeared to increase the odds of seropositivity; however, statistical significance was not reached. These include soil contact (OR = 2.19, p = 0.5), raw vegetable consumption (OR = 4.25, p = 0.3), and dog ownership (OR = 1.80, p = 0.3). Lastly, it was found that onychophagia appeared to have a protective effect against being seropositive for anti-*Toxocara* antibodies (OR = 0.61, p = 0.6).

Table 8: Logistic regression analysis of *Toxocara* spp. seropositivity among schoolchildren in Santa Cruz del Junco, Tela

Variable	Odds Ratio (OR)	95% Confidence Interval (CI)	*p* Value
Gender (Male)	4.43	0.87 – 22.42	0.072
Soil Contact	2.19	0.17 – 27.96	0.547
Onychophagia	0.61	0.04 – 7.61	0.698
Raw Beef Consumption	--		
Raw Fruit/Vegetable Consumption	4.25	0.23 – 78.01	0.330
Dog Ownership	1.80	0.46 – 7.10	0.396
Dog Age ≤ 1 Year	--		
Playground Contact With Soil	--		

(--): Variable omitted from final model

CHAPTER 5: DISCUSSION

Honduras is considered a middle-income country with a national human development index (HDI) of 0.617 for 2017. Globally, the country ranks 133 out of 189 countries and territories (UNDP, 2018). Further, when the HDI indicator is discounted for inequality, Honduras' HDI falls to 0.459, "a loss of 25.6 percent due to inequality in the distribution of the HDI dimension indices" (UNDP) (UNDP, 2018). The country's estimated population in 2016 was 8,189,501 (PAHO, 2018). Access to sanitation, safe water and hygiene (WASH) by the population is a challenge for Hondurans. Despite reports stating that drinking water was accessible for 83.8% and 77.7% of urban and rural residents, respectively, real access to drinking water is much lower: 90% of the supply is intermittent and only 44% of the water systems purify it effectively. Moreover, only 52% of the sewers have a purification system (PAPSAC, 2014).

These conditions are conducive to the transmission of various pathogens, including soil-transmitted helminths (STH), and in fact, a high level of STH endemicity has been documented in the country (Bearman et al., 2016; Sanchez et al., 2014; Sanchez et al., 2013; Torres et al., 2014). As well, there are a number of zoonotic diseases identified with the national health system (PAHO, 2018). One such zoonosis is toxocariasis, whose definitive host and source of infection for multiple hosts is the domestic dog. Not much is known about the size of dog population, dog registration, breeding policies, vaccination, and deworming requirements in Honduras. Important for this thesis, studies in dog ownership patterns in the country are not available. By direct observation, one can conclude that dog ownership can be in the form of pet dogs and guard dogs, and that regardless of ownership, most dogs may be allowed to wander free on the streets, especially during the day. This is particularly true in the rural areas, where free-range dogs (*i.e.,* village dogs) are the norm. Village dogs can be expected to defecate indiscriminately around the village while at the same time are

exposed to all kinds of pathogens including *T. canis*, either by ingestion of contaminated soil or paratenic hosts. The combination of all these conditions make Honduras a likely candidate for endemic animal and human toxocariasis.

To explore this hypothesis a widespread search of both peer-reviewed published and grey literature was conducted. The search yield two publications: a clinical case of neurotoxocariasis in a 14-month old child (Puerto Sanabria & Tovar, 2016) and a parasitological survey of 207 dogs from the capital city and 2 nearby communities which only found a 3.8% prevalence (Kaminsky et al., 2014). The present study is the first of its kind aiming to elucidate the prevalence of human toxocariasis and its potential risk factors in Honduran schoolchildren.

5.1: Seroprevalence of anti-*Toxocara* Antibodies

Even though there have been no previous studies of a similar nature in the country the finding of an overall seroprevalence of 88.6% exceeded any *a priori* expectations. It is not altogether surprising however, to find relatively elevated prevalence in a developing country like Honduras where other parasitoses are endemic. The international literature examined for this thesis revealed a wide range of prevalence values across the globe, with the vast majority of studies reporting lower figures than the present study. One study with comparable prevalence (84.6%) is one carried out with 117 Indonesian schoolchildren aged 10 – 15 years old (Hayashi, Tuda, Imada, Akao, & Fujita, 2005). Conversely, only one published study, conducted in La Réunion had a higher seroprevalence (92.8%) (Magnaval, Michault, Calon, & Charlet, 1994).

High prevalence data have been documented in other countries across the globe, mainly Brazil, Cuba, India, Indonesia, Iran, Marshall Islands, Nigeria, Peru, Philippines, Saint Lucia, Sri Lanka, Swaziland, Taiwan and Trinidad & Tobago (Alderete et al., 2003; Baboolal & Rawlins, 2002; Dar et al., 2008; Espinoza et al., 2008; Fajutag & Paller, 2013; Fan, Hung, Du, Liao, & Su,

2004; Fan et al., 2005; Fu et al., 2014; Gyang et al., 2015; Hayashi et al., 2005; Iddawela, Kumarasiri, & de Wijesundera, 2003; Liao et al., 2010; Mendonça et al., 2013; Roldán et al., 2008; Sariego et al., 2012; Schoenardie et al., 2013; Sharif, Daryani, Barzegar, Nasrolahei, & Khalilian, 2010; Thompson, Bundy, Cooper, & Schantz, 1986). In the Butanta region of Sao Paulo, Brazil a study investigating prevalence of *Toxocara* spp. infection in schoolchildren (those aged between 7 – 16 years, in this study) revealed that out of the 399 tested participants, 38.8% of them were seropositive (via ELISA) for anti-*Toxocara* antibodies (Alderete et al., 2003). More recently, in the south of Brazil a seroprevalence of 50.6% among 427 samples originating from children of 1 to 12 years of age was found (Schoenardie et al., 2013). An approximately similar result was also documented in the Northeastern zone of the country, with 48.4% seropositivity being demonstrated in over 1,300 children of ages 4 – 11 (Mendonça et al., 2013). While these figures do not reach or surpass the rate described in the current study, it is important to note that any seropositivity should be a sign of concern and that clinical evaluation of the study population is warranted.

Sariego and colleagues measured anti-*Toxocara* antibody frequency in schoolchildren from two different municipalities in Cuba and found a seroprevalence of 38.8% in 1,011 studied children (Sariego et al., 2012). Literature from Trinidad & Tobago listed an ELISA based seropositivity of 62.3% in schoolchildren based on a sample size of over 1,000 children (Baboolal & Rawlins, 2002). An even higher figure of 86.0% was documented in 203 preschool-aged children from a coastal community in Saint Lucia (Thompson et al., 1986). More recently, investigations involving 646 children 5-12 years of age residing in two separate areas of Peru reported seroprevalences of around 30% for each population (Roldán et al., 2008). Likewise, children of a similar age range (5 – 10 years old) from the Morrope district in Peru yielded a very

comparable seroprevalence result of 32.4%, albeit with a much smaller sample size of 182 schoolchildren (Espinoza et al., 2008).

Meanwhile in Africa, a study of over 1,000 Sri Lankan preschool and school-aged children (1 – 12 years old) found a seroprevalence of 43.0% (Iddawela et al., 2003). Findings from Swaziland using a more modest sample size (92 children) within a very comparable age range but using Western blot instead of the more popular ELISA method, resulted in 44.6% seropositivity (Liao et al., 2010).

Furthermore, Taiwanese schoolchildren from rural, mountainous communities between the ages of 7-12 years were examined by Fan *et al.* One of their studies, which only included 72 participants, had a 57.5% seroprevalence, while the second study incorporating 329 participants found a more elevated proportion of 76.6% (Fan et al., 2004; Fan et al., 2005). In India, a small epidemiological study of 110 children between 5 and 16 years of age resulted in a 32.3% seroprevalence (Dar et al., 2008), while a larger study encompassing sixteen different schools throughout northern Iran and over 1,200 children found a seropositivity rate of 25.0% (Sharif et al., 2010). As mentioned above, a drastically superior prevalence of 84.6% was found in Indonesian schoolchildren aged 10 – 15 years old in a study that included 117 participants, a finding that approximates the extent of seropositivity found in Honduran schoolchildren within our study (Hayashi et al., 2005). In the urban community of Los Baños, Philippines a seroprevalence among schoolchildren of 49.0% was published by Fajutag and Paller when examining 75 children from public schools among the ages of 8 – 13 years (Fajutag & Paller, 2013). Finally, in the Marshall Islands, a series of isles located in the Pacific Ocean between Hawaii and the Philippines, Fu *et al.* assessed 166 primary schoolchildren sera. These researchers employed an ELISA and Western blot combination for their seroepidemiological survey and a

staggering seroprevalence of 86.8% was detailed, a finding that also resembles that of this study (Fu et al., 2014). While most of this literature demonstrates considerably lower seropositivity compared to the one reported here, all the results underscore the worrying tendency of developing countries, especially those with tropical climate and inadequate sanitation, to be under a considerable threat for human toxocariasis.

In contrast, more developed nations such as England, Denmark, Holland, Italy, Japan, Poland, Spain, Sweden, Switzerland and the United States all paint a very different picture in terms of human toxocariasis frequency (Akao & Ohta, 2007; Buijs et al., 1997; Cilla, Perez-Trallero, Gutierrez, Part, & Gomariz, 1996; Genchi, Di Sacco, Gatti, Sangalli, & Scaglia, 1990; Jimenez, Valladares, Fernandez-Palacios, de Armas, & del Castillo, 1997; Josephs, Bhinder, & Thompson, 1981; Kondo, 1998; Kroten, Toczylowski, Kiziewicz, Oldak, & Sulik, 2016; Ljungstrom & van Knapen, 1989; Łużna-Lyskov, 2000; Stensvold et al., 2009; Sturchler & Peter, 1981; Won et al., 2008). Amid these countries, the highest rate of seroprevalence originates from young children in Bedfordshire, England and the general American population; both with rates of approximately 14% (14.3% and 13.9%, respectively) (Josephs et al., 1981; Won et al., 2008). Elsewhere, seropositivity is even more scarce, with children of ages 2 – 16 years in the Basque Country of Spain registering a rate of 12.1% as found by Cilla *et al.* in an investigation of over 500 children (Cilla et al., 1996). A statistically powerful Spanish study conducted by Jimenez *et al.* in the Canarian Archipelago found an even more diminished seropositivity among the general population. Of the 14,000 human blood samples analyzed by ELISA, only 3.4% of the Archipelago was found to be positive (Jimenez et al., 1997).

Poland, a hotbed for toxocariasis research over the past two decades, has produced varying results in terms of seroprevalence. A study, taking place in the northeast of the country, evaluated

1,025 individuals of no specified age and published a resulting prevalence of 20.7% (Hermanowska-Szpakowicz, Kondrusik, Swierzbinska, Zajkowska, & Pancewicz, 2001). The publication does not specify the exact methods of antibody detection nor the demographics of its participants and instead focused on clinical symptoms and their possible association. However, a more recent Polish study from the same northeastern region of the country incorporating individuals between 2 -17 years of age and using an ELISA plus Western blot combination for antibody detection established a modest seropositivity of 4.2% (Kroten et al., 2016). Previous research concentrating on anti-*Toxocara* antibodies seroprevalence in children of ages 2 – 12 from Poznan found a slightly higher proportion of 7.9% in a fairly small sample of 63 children (Łużna-Lyskov, 2000). All findings that have surfaced from seroepidemiology in Poland display a substantially reduced burden of toxocariasis in humans.

Likewise, additional European countries have followed the same theme over the past several decades. A seroepidemiological survey among primary school children in two districts of the Netherlands collected over 1,300 individual blood samples that when evaluated for antibody presence yielded a positive proportion of 8.0% (Buijs et al., 1997). Schoolchildren from the Swiss village of Jura, more specifically 134 of them, aged 7 to 16 years old were tested using an ELISA for anti-*Toxocara* antibodies by Stürchler *et al.* Of these 134 children, 5 of them presented with detectable antibodies for a seroprevalence of 3.7% (Sturchler & Peter, 1981). Seropositivity data from Denmark, was observed as follows; 2.4% of 3,247 Danish adults of 20 – 39 years of age that were tested by an ELISA in conjunction with Western blotting (Stensvold et al., 2009). In Italy via ELISA, 4.0% of 2,112 northern Italians of 18 to ≥51 years of age were positive (Genchi et al., 1990). Also using solely an ELISA, 1.6% of 3,277 Japanese residents of all ages were deemed seropositive (Akao & Ohta, 2007; Kondo, 1998); Finally, 7.0% of 323 twenty-year old adults from

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varying geographical areas of Sweden (ELISA) tested positive (Ljungstrom & van Knapen, 1989). Much of the seroprevalence of anti-*Toxocara* antibodies in published literature regarding the developed world pales in comparison to the 88.6% detected in the present study.

This existing prevalence disparity among the numerous studies worldwide is influenced by a broad and complex number of variables. At the individual level, these include immunity, concurrent infection with other pathogens, genetics, gender, age, nutritional status and behaviour of both definitive and paratenic hosts (more importantly humans) (Macpherson, 2005, 2012; Viney & Graham, 2013a). On a population scale, human toxocariasis has been linked to environmental, cultural, educational, socioeconomic and geographic factors (Macpherson, 2013). These, along with poverty and uncontrolled canine and feline populations can help explain in part, the variability in global seroprevalence rates. For example, in Mexico, two different studies, one testing 183 participants while the other tested 108, both involving children between 2 – 16 years of age and both using an ELISA as the serodiagnostic method found prevalence rates of 12.0% and 22.2%, respectively (Cortés, Núñez, Guiliana, García, & Cárdenas, 2015; Romero Núñez et al., 2013). Meanwhile, the general population of the French island of La Réunion when assessed by Magnaval *et al.* was found to have a positivity rate of 92.8%, a result which was obtained through Western blotting of 387 individuals (Magnaval et al., 1994). The degree of immune reactivity to *Toxocara* spp. antigens observed among residents of this island, which is the highest that has been documented in humans, eclipses that seen in Honduran schoolchildren. Yet the comparability of these findings in two seemingly unrelated communities conceivably point to the unpredictability and variability of toxocariasis prevalence around the globe due to the multitude of determinants at play. Diagnostic techniques, study sample size, climatic conditions, sanitation and lifestyle of each population could also have contributed to the discrepancies observed in seroprevalence rates.

Apart from numerous individual and population factors, the heterogeneity of serodiagnostic technique(s) and the consequent variance in what is used in each study can also account for disparities. Seroprevalence relies on circulating antibody detection and in immunodiagnosis, the inherent differences in the quality of antigens (*i.e.,* crude *T. canis* larval products, native or recombinant TES antigens, deglycosylated TES antigens) used both in the large assortment of commercial kits and in in-house ELISAs play a role (Fillaux & Magnaval, 2013). This, coupled with a lack of standardization, variation of serum dilutions, different antibody cut-off titres, potential cross-reactants and modification of ELISAs for the detection of specific antibodies, makes comparison and uniformity of seroepidemiological surveys challenging (Fillaux & Magnaval, 2013). Additionally, the need for validation of positive serological findings through ELISA by Western blotting has been well recognized (Fillaux & Magnaval, 2013; Ma et al., 2017; Moreira et al., 2014). Yet, many studies do not implement this confirmatory step, inevitably making their diagnostic specificity worth questioning, especially in those demonstrating extreme seropositivity. Determining prevalence with precision and a true epidemiological understanding of human toxocariasis are still urgent needs for the implementation of large-scale intervention and prevention strategies. While the combined use of ELISA and Western blotting is currently the most widely accepted diagnostic method, future surveys would benefit from further validation of these assays under different conditions globally (Boldis et al., 2015; Moreira et al., 2014). Accordingly, the most pressing issues are the inability to serologically distinguish between active *T. canis* infection versus previous exposure and the futility of serology for the diagnosis of cerebral and ocular manifestations of toxocariasis (Chen et al., 2018; Chen, Gu, Jiang, Zhou, & Chang, 2018; Hotez & Wilkins, 2009; Ma et al., 2017). Serological diagnosis of both neurological and ocular forms of this disease is deemed difficult by the low parasite burden associated with these

infirmities. Thus, an immunodiagnostic test using serum is usually negative (Fillaux & Magnaval, 2013; Smith et al., 2009; Vidal et al., 2003).

With this in mind, along with the absence of pertinent clinical data, the high seroprevalence results reported here should be interpreted cautiously, as immunoreactivity does not signify either active infection or clinical disease. Conversely, seroreversion (the conversion of a seropositive individual to seronegative status) has also been observed in children with persistent symptoms (Rudzińska, Kowalewska, & Sikorska, 2017; Wiśniewska-Ligier, Woźniakowska-Gęsicka, Sobolewska-Dryjańska, Markiewicz-Jóźwiak, & Wieczorek, 2012). Nevertheless, research into the impact of this elevated seropositivity on children's health is necessary in this Honduran community, with potential cognitive and developmental delays among these impoverished children being of particular concern.

5.2 Seroprevalence of Dual Participants

A notable aspect of the present study is the repeated testing of the study population in two different years, which allowed for the collection of data within the same community at two separate time points (2015 and 2017). Moreover, since the second phase of this study (2017) was carried out within the very same school as two years prior, overlap of participants was inevitable. As defined in preceding sections, schoolchildren that were enrolled and contributed to the investigation at both time points were deemed as dual participants. In total, 31 schoolchildren fit the criteria of dual participation, of which 15 were boys and 16 were girls.

Initially, 21 of 31 (67.7%) dual participants were diagnosed as seropositive via TES-ELISA in 2015. The slight majority of seropositive children in 2015 were males, as 12 out of the possible 15 (80%) demonstrated immunoreactivity. In contrast, nine out of the 16 (56.3%) dual participant females were seropositive in 2015 as well. However, upon the second blood sample collection the proportion of ELISA seropositivity saw an increase in both genders. In 2017, all 15 of the boys that had been tested previously now showed anti-*Toxocara* antibody titers. Females saw a sharper surge, as the number of immunoreactive girls rose to 15 of 16 (93.7%) resulting in 96.7% of the 31 dual participants being ELISA seropositive by 2017. The Western blotting procedure then affirmed that indeed 100% of male dual participants were seropositive and that 14 of 16 females possessed anti-*Toxocara* antibodies as of 2017, proving that one of the seroconverted girls as determined by ELISA was a false positive.

The seroconversion of 8 out of 31 schoolchildren in the two-year interim between study phases represents a higher rate of seroconversion than those detailed in Brazil; it also points to the notion of an existing exposure (Correa & Bismarck, 2010; Oliart-Guzman et al., 2014). Conceivably, this phenomenon of seroconversion may be attributed to the normal changes in social

and recreational behaviours of children as they age. In the two-year study gap, this could have resulted in dietary changes or in new or more frequent contact with definitive or paratenic hosts, contaminated environments or other unsanitary objects, or continuance of unhygienic tendencies (*i.e.*, geophagic practices, lack of handwashing) (Chen et al., 2018; Despommier, 2003; Hotez & Wilkins, 2009). Other research has suggested seroconversion during childhood can be explained by a cumulative effect of persistent exposure and is the result of several infections, not of a variation in behaviour (Agudelo et al., 1990; Oliart-Guzman et al., 2014). This alternative explication becomes even more relevant in populations, such as this one, that are exposed continuously due to optimal larvation conditions being present year-round. However, since comparable epidemiological data was not collected at both time points, we were unable to relate seroconversion to any potential differences in habits or behaviours. Nonetheless, the observed seroconversion rate as well as the initial discrepancy between genders, which then turned into a more congruent seropositivity, are curious findings worth investigating further.

Apart from seroconversion, ELISA results from both years also provided the opportunity for direct comparison of the optical density values. Predominantly, OD values either maintained or slightly increased in the two-year span. Since the absorbance of a sample is related to the concentration of antigen-antibody complexes formed within, even a minor increment in absorbance signifies strengthened immunoreactivity. It has been reported elsewhere that the rise in antibody titers is slow and dependent on the larval dose, making it therefore plausible that seemingly insignificant increases in absorbance may translate to a recent exposure (Baaten, Sonder, van Gool, Kint, & Van den Hoek, 2011; Thiha & Ibrahim, 2015). A small reduction meanwhile, can be indicative of a distant former exposure, as an ELISA result will remain unchanged for 2 years and a half, on average, before beginning to fade (Fillaux & Magnaval, 2013;

Magnaval, Glickman, et al., 2001). More importantly, an increasingly discernible reduction in ELISA absorbance values was noted for five of the 31 individuals. These five children showed a decrease of over 30% in their OD values between 2015 and 2017. While their results were still considered positive, this marked decline in immunoreactivity is encouraging, as it possibly demonstrates a discontinued exposure to *Toxocara* spp. (Rudzińska et al., 2017; Smith et al., 2009). Rubinsky-Elefant *et al.* also noted that anti-*Toxocara* IgG levels begin to decline when larvae sequestered in tissues are no longer viable and no re-infection occurs (Rubinsky-Elefant et al., 2010). An alternative explanation for this lowered absorbance is that of transient antibody reactions. Schoolchildren may have been exposed to a minimal number of parasite eggs, with a viable larval infection never being established or immunologically arrested in granulomas at an early stage. Although plausible because larval involvement is dependent on quantity of eggs ingested, evidence of this phenomenon for toxocariasis in humans is lacking. It has been however, previously described for *Taenia solium* cysticercosis and in canine and murine toxocariasis, where the onset, level and duration of responding antibodies has been strictly dose related (Garcia et al., 2001; Glickman & Schantz, 1981; Kanobana, Devleesschauwer, Polman, & Speybroeck, 2013).

In contrast, of the thirty-one dual participants, eight demonstrated an increase in ELISA absorbance values of over 30% from 2015 to 2017. Continuing with the previously mentioned notion published by Baaten *et al.*, this intensification of antibody production could be the result of an exposure or infection which was recent in 2015 but immunologically progressed by 2017 (Baaten et al., 2011). Numerous studies have also discussed the persistence of antibodies for long periods of time as well as potential aggregations as consequences of repeated stimulation either by antigens originating from live larvae in tissues or from periodic reinfection with *Toxocara* spp. (Alderete et al., 2003; Glickman & Schantz, 1981; Roldan & Espinoza, 2009; Roldán, Espinoza,

Huapaya, & Jiménez, 2010; Rubinsky-Elefant et al., 2010). While either of these scenarios are possible in the studied population, the findings of a higher seroprevalence in 2017 and increasingly intensive immune reactions coupled with seroconversion among dual participants all seemingly imply a continuously present source of exposure among the population. However, the unavailability of clinical data makes it unwise to disregard the likelihood of encapsulated larvae in these eight children as the basis for increment in antibody titers.

The importance of the serological results of dual participants should not be underestimated. The elevation in levels of both seropositivity and OD values from 2015 to 2017 emphasize the current exposure that this population of schoolchildren find themselves subjected to. Revealing the source(s) of exposure in this locality would be of great benefit for the prevention and education of human toxocariasis. The decrease in OD values should not be overlooked either, as this connotes that despite the surge in seropositivity, measures can still be taken to avoid infection.

5.3: *Toxocara* spp. Exposure and Eosinophilia

Eosinophilia, which is defined as a count of more than 500 eosinophils/µL in peripheral blood, can be attributed to various causes such as allergic reactions, atopy, hypersensitivity to certain drugs, connective tissue malignancies but more importantly in this case, parasitic infection (Brito-Babapulle, 2003; Kim, Seo, Lee, Choi, & Park, 2017). Due to logistical restrictions, eosinophil count was only possible for the children enrolled in 2015. Of these 73, a total of 16 cases of eosinophilia (as defined previously) were found and upon closer examination, all 16 eosinophilic children were seropositive in 2015.

Naturally, we decided to investigate further and determine if there was a significant relationship between their elevated eosinophil counts and serological status. To do this, a Mann-

Whitney *U* test looking at the differences in geometric means of eosinophil numbers in seropositive children compared to seronegatives was utilized. Unsurprisingly, we observed a higher number of eosinophils in immunoreactive children and a statistically significant difference between the geometric means of seropositive versus seronegative children was calculated ($p = 0.004$). Aware that infection with other helminths, mainly *A. lumbricoides*, *T. trichiura* and hookworms, can also be causes for eosinophilia, a similar model was also run controlling for these confounders. Excluding those who were parasitized with any STH in general and *T. trichiura* in particular, a significant difference was still found in the average number of circulating eosinophils ($p = 0.05$ and $p = 0.035$, respectively).

The presence of eosinophilia in relation to *T. canis* infection has been well documented. Antigens released by migrating larvae drive a vigorous production of type-2 cytokines, mainly interleukins 4, 5, 10 and 13, resulting in increased circulating eosinophils along with IgE antibodies (Maizels, 2013; Mazur-Melewska et al., 2016). More specifically, our finding of a very high prevalence of anti-*Toxocara* antibodies among eosinophilic children reflects those published elsewhere (Kim et al., 2017; Łużna-Lyskov et al., 2000; Obwaller et al., 1998; Park, Lee, Huh, Kong, & Magnaval, 2002; Yoon et al., 2018). Interestingly, although our geometric means for circulating eosinophils were higher for seropositive children compared to seronegatives, they still did not approach the average values reported by other authors, nor did they fall in the range of 400 – 1,000/μL corresponding to asymptomatic infection, according to Pawlowski (Łużna-Lyskov et al., 2000; Pawlowski, 2001; Yoon et al., 2018). In fact, in cases of *visceral larva migrans*, presence of eosinophilia can be massive reaching approximately 10,000 eosinophils/μL (Ehrhard & Kernbaum, 1979; Lassmann, Tsigrelis, & Virk, 2007). Yoon *et al.* speculate that eosinophilia of a Toxocaral origin exhibits a significantly higher rate of normalization and can resolve faster

without treatment when compared to eosinophilia of other etiologies (Yoon et al., 2018). Also proposed is that slight or absent eosinophilia can occur in old infections, as larval destruction takes place in the tissues or alternatively, a light intensity of infection (Łużna-Lyskov et al., 2000; Pawlowski, 2001). The low averages found here could be a consequence of either reasoning. In ocular toxocariasis, blood eosinophilia may also fall within normal or slightly elevated ranges since this disease is usually caused by a small infectious dose of larvae (Glickman & Schantz, 1981; Magnaval, Glickman, et al., 2001). The same is proposed to be true for neurotoxocariasis, however no concrete conclusion has been reached given the studied incidence of this manifestation. Instead, eosinophilia has been confirmed in the cerebrospinal fluid (CSF) in the presence of *Toxocara* spp. larvae in the central nervous system (Fillaux & Magnaval, 2013; Vidal et al., 2003). Ergo, neither ocular nor neurotoxocariasis should be ignored as probable causes of low eosinophilia, especially with a clinical case of neurotoxocariasis already being reported in Honduras.

The conflicting nature of the existing literature around eosinophilia in toxocariasis should not deter clinicians or researchers from utilizing it as an element for diagnosis. Case reports from around the world as well as conclusions from investigations have shown that eosinophilia and hypereosinophilia are mostly indicative of an active *visceral larva migrans* (Akao & Ohta, 2007; Beaver et al., 1952; Hartleb & Januszewski, 2001; Kim et al., 2017; Lassmann et al., 2007; Łużna-Lyskov et al., 2000; Magnaval, Glickman, et al., 2001; Nagakura et al., 1989; Pawlowski, 2001; Ranasuriya et al., 2014; Yoshikawa et al., 2008). Additionally, the hypothesis that toxocaral disease could still occur without peripheral eosinophilia has been put forth and considered possible, as eosinophils can accumulate in tissues, especially if *Toxocara* spp. larvae are localized in tissue (Magnaval, Berry, Fabre, & Morassin, 2001). As this eosinophilia would be difficult to

detect, Magnaval *et al.* carried out an assessment of the worth of eosinophil cationic protein (ECP) levels for diagnosis. Patients with schistosomiasis and filariasis have been shown to exhibit high levels of ECP (Tischendorf et al., 1999). ECP is one of the many granular cationic proteins released upon the stimulation of eosinophils and its concentration in blood can be quantified through specific assays (Fillaux & Magnaval, 2013). Briefly, the researchers identified a significant difference of ECP blood dosage between individuals exhibiting clinical features of toxocariasis and those who presented positive serodiagnosis but were asymptomatic (Magnaval, Berry, et al., 2001). They coupled these findings with serological results of both IgG and IgE antibodies, with ECP proving to be a valuable indicator of current active toxocariasis in patients who lack peripheral eosinophilia (Magnaval, Faufingue, Morassin, & Fabre, 2006). Nevertheless, additional investigations among individuals presenting varying Toxocaral diseases and laboratory features are still required.

5.4: Risk Factors for *Toxocara* spp. Seropositivity

An extensive variety of risk factors for anti-*Toxocara* antibodies have been investigated and postulated in the literature. Some of these include dog ownership, geographic location, socioeconomic status and lack of education (Holland & Smith, 2006; Joy, Chris, & Godwin, 2017; Overgaauw & van Knapen, 2013; Woodhall, Eberhard, & Parise, 2014). Yet there are recognizable conflicts when it comes to the influence of other socio-epidemiological factors. Contributing additional data to the pool that already exists to elucidate the impact of these factors worldwide would help in filling in knowledge gaps regarding human toxocariasis. The present study tried to identify the major risk factors among children of the community of Santa Cruz del Junco, Honduras.

5.4a: Gender

Gender of the participating children was not identified as a statistically significant risk factor in our study. Despite this, it is interesting to note the proportion of boys who tested seropositive (95.35% or 41/45), which was higher than the proportion of seropositive girls (82.22% or 37/45). Although also statistically insignificant, our logistic regression analysis found that being of the male gender increased the odds of possessing anti-*Toxocara* spp. antibodies by over 400%. Several studies from around the world have also found a higher prevalence of seropositivity among boys, likewise not significantly (Fu et al., 2014; Gyang et al., 2015; Martínez et al., 2018; Mendonça et al., 2013; Schoenardie et al., 2013; Sowemimo et al., 2017; Sviben, Čavlek, Missoni, & Galinović, 2009). These results contrast with those published by other authors which have identified a significant association between seropositivity and the male gender (Acero, Muñoz, Flórez, & Nicholls, 2001; Espinoza et al., 2008; Martinez et al., 2015; Romero Núñez et al., 2013; Sharif et al., 2010). It is generally speculated that this potential association is related to the increased exposure of males to sources of infection resulting from their activities and behaviours (Martinez et al., 2015).

Behavioural differences between genders was not an aim of our investigation but some of the data collected in 2017 lends itself to help explain, at least partially, the gender-related differential exposure. Of the 38 children who reported coming in contact with soil within the school playground, 22 were boys. Presumably, this higher contact with soil among boys arises from their preference for games of this nature. Another probable explanation, one that has surfaced in other studied communities, is that boys tend to have more contact with dogs, contributing to their increased exposure (Holland, O'lorcain, Taylor, & Kelly, 1995; Roldán et al., 2009).

5.4b: Soil Contact

Parallel to the high prevalence of seropositivity, there was also a large number of children who confirmed being in constant contact with soil. There was no association found between having soil contact and a positive serological result, despite the crucial role contaminated soil plays in the transmission of this disease. This conclusion has been seen in other research (Gyang et al., 2015), but most studies worldwide have actually found soil contact to be a significant risk factor (Cong et al., 2015; Marchioro et al., 2015; Martínez et al., 2018). The peril of contaminated soil within this community should not be ignored however, as 95% of children who communicated exposure to soil were seropositive. Logistic regression analysis designated those with soil contact as being twice as likely to produce anti-*Toxocara* spp. antibodies, albeit with no statistical significance. Perhaps, this current observation can be explained by the small sample size of the study. Subsequent investigations are required to determine the degree of environmental contamination with *Toxocara* spp. ova as this community may be heavily contaminated, as reflected by the high overall seroprevalence recorded. It is well known that uncontrolled definitive host populations and warm climatic conditions can lead to facilitated transmission, particularly as soil-laden spaces are extremely difficult to decontaminate (Congdon & Lloyd, 2011).

5.4c: Onychophagia

Contradictory to the concept of being at risk of contracting toxocariasis by putting hands or foreign objects in the mouth, the present study found onychophagia or nail biting to have a non-significant protective effect. Elsewhere, other research emanating from Brazil, Serbia and Poland has found onychophagia to have a significant association with positive serology, findings which completely conflict with our current observations in Honduras (Alderete et al., 2003; Cassenote, de Abreu

Lima, Neto, & Rubinsky-Elefant, 2014; Fragoso, Monteiro, Lemos, & Pereira, 2011; Gabrielli et al., 2017; Kroten, Toczylowski, Oldak, & Sulik, 2018). A reason for this discrepancy could be an inaccuracy issue at the time of interviewing. Considering the stigmatization linked with this habit, participating children especially those in the presence of their parent/guardian, may have been inclined to provide more socially acceptable answers. If this was the case, this phenomenon has been constantly seen in research across all disciplines and could have led to the underreporting of onychophagia among children. Besides social desirability bias, an indiscriminate exposure to a substantial source of infection can potentially override or mask the effect of lesser, more subtle modes of transmission. A small sample size, such as the one integrated here, would only exacerbate this occurrence. The possibility of this being a spurious finding also exists.

5.4d: Raw Fruit/Vegetable Consumption

The present study failed to identify the consumption of raw fruits or vegetables as a significant risk factor for anti-*Toxocara* spp. antibody presence, corroborating the findings of a seroepidemiological study in Nigeria among preschool children (Sowemimo et al., 2017). Yet, in our analysis, children who affirmed these dietary penchants were found to be at four times greater odds of being immunologically reactive. Indeed, a great proportion of children who admitted consuming raw fruits/vegetables were found to be seropositive (34 of 35 or 97.14%). Previous research investigating this same risk factor however, did find it to be significantly associated with *Toxocara* spp. exposure (Gyang et al., 2015) with Avcioglu *et al*. actually being able to detect *Toxocara* spp. ova in unwashed and uncooked vegetables (Avcioglu, Soykan, & Tarakci, 2011). In this locality, gathering knowledge of where and how the vegetables consumed are acquired would broaden our understanding of whether or not it is a threatening risk factor.

Similarly, the association between seroprevalence and consumption of raw or undercooked meat has also been looked at and evaluated previously. Studies that differ in terms of the ages of the investigated population have discovered opposing results. In those focusing on preschool or school-aged children, there has been no significant association found between the consumption of raw/undercooked meat and anti-*Toxocara* spp. seroprevalence (Gyang et al., 2015; Sowemimo et al., 2017). Meanwhile, significant associations have been observed in study populations involving adults (Choi et al., 2012; Taira et al., 2004). This distinction may be due simply to the dietary preferences of both the populations due to age and/or customs. Unfortunately, due to the substantial levels of seropositivity within such a small sample size employed in our study, it was mathematically impossible to apply a logistic regression model and determine whether consumption of raw/undercooked meat is a risk factor for toxocariasis in this community.

5.4e: Dog Ownership/Contact

Even though the association was non-significant, it should still be noted that in this study children reporting dog ownership had almost twice the odds of being seropositive compared to their counterparts that did not report owning a dog. Through univariate analysis, it was found that 57 of the 63 children whose family owned a dog had a positive serological test result. This high reporting of dog ownership seems to be consistent with the observed prevalence of anti-*Toxocara* spp. antibodies and may reflect the considerable impact of these canine populations as a source of contamination and subsequent exposure. Whilst a few older studies also found an insignificant association relating dog ownership and seroprevalence (Ajayi, Duhlinska, Agwale, & Njoku, 2000; Woodruff, De Savigny, & Hendy-Ibbs, 1982), most published work evaluating dog ownership/contact has confirmed its role as a risk factor for humans (Gyang et al., 2015; Mendonça

et al., 2013; Sharif et al., 2010; Sowemimo et al., 2017). Moreover, detailed research from varying locations has also described the presence of embryonated and infective *Toxocara* spp. eggs on the coat of dogs (Amaral et al., 2010; Holland, 2017; Keegan & Holland, 2010; Roddie et al., 2008). Although this may not be a major route of human infection, it still warrants monitoring, especially in a study area such as this one, where stray dogs are rampant, not well cared for and have unrestricted access to public spaces.

Children have the tendency to be in closer contact and be more attracted to dogs, making dog ownership a more relevant circumstance among the pediatric population. Defecation of dogs within close proximity of the household will also subject children to more direct contamination and possible dog pregnancy/parturition would almost ensure exposure to *Toxocara* spp. via infected young dogs (Esch & Petersen, 2013; Fan et al., 2013; Macpherson, 2005, 2013; Overgaauw & van Knapen, 2013). In addition to simply owning dogs, contact with puppies or those dogs younger than 1 year has been documented as posing a comparatively higher risk of acquiring antibodies against *Toxocara* spp. in comparison to older or adult dogs (Fan et al., 2013; Gyang et al., 2015; Soriano et al., 2010). This is of particular pertinence to children as it has been noted that they are generally more attracted to puppies, and surveys have shown that 86 – 100% of puppies are infected with *T. canis* with adult dogs in the same surveys having a lower prevalence of infection between 1 – 45% (Fan et al., 2013; Soriano et al., 2010). The attraction to puppies therefore further exposes children to *T. canis* and highlights the importance of young dogs in the transmission of this parasitosis (Overgaauw & van Knapen, 2013).

A comprehensive One Health approach that takes into account the interaction between humans, animals and the environment should be used for effective surveillance of human toxocariasis in Santa Cruz del Junco, Honduras. Control and monitoring of *Toxocara* infection in

definitive hosts could be of importance in the prospective implementation of public health measures in endemic settings such as this one.

5.5: Comparison of Serodiagnostic Techniques

In many viral and parasitic infections, specific IgA and IgM antibodies are useful characteristic indicators of the stage of infection. In human toxocariasis, both antibodies can be detected in chronic and acute periods (Maizels, 2013; Rubinsky-Elefant et al., 2010). Instead, the serodiagnosis of toxocariasis is dependent on detection of IgG antibodies via the ELISA method. Unfortunately, presence of anti-*Toxocara* IgG maintains at detectable levels for two and a half years, complicating differentiation between an active or past infection (Jacquier et al., 1991; Roldan & Espinoza, 2009; Rubinsky-Elefant et al., 2010). An added complication is that of false positive results that may occur in infections with other helminths due to cross-reactions, since TES antigens that are used in ELISA tests are not 100% specific for the genus *Toxocara* (Jacquier et al., 1991; Magnaval et al., 1991; Roldán et al., 2009; Rudzińska et al., 2017). This indisputable problem with specificity, magnified during the employment of ELISA tests in polyparasitised individuals, gave rise to the development of a Western blotting procedure by Magnaval *et al.* (Magnaval et al., 1991). Usually, two clusters of bands may be detected by Western blotting, but the confirmatory serodiagnosis of human toxocariasis is related to the group of low-molecular weight (24 – 35 kDa) bands corresponding to recombinant *Toxocara* spp. antigens. The high molecular bands of 132 – 200 kDa are unspecific for toxocarosis (Fillaux & Magnaval, 2013; Roldan & Espinoza, 2009; Roldán et al., 2010).

While proving to be more specific without sacrificing sensitivity, immunoblotting processes are also more time consuming, laborious and expensive. For these reasons, comparison

of the simpler, cheaper and quicker ELISA to the Western blot was done as an additional step. Superficial appraisal shows an ELISA sensitivity of 100% while confirmation of all ELISA positive results revealed a specificity of 80%. Only two of the identified 80 ELISA positives were disproved as false by immunoblotting. This improvement in specificity through WB observed here has also been confirmed in other studies evaluating both serological assays (Courtade, Recco, Magnaval, Charlet, & Seguela, 1995; Gueglio et al., 1994; Logar, Šoba, Kraut, & Stirn-Kranjc, 2004; Rudzińska et al., 2017; Smith et al., 2009). Furthermore, calculation of the kappa statistic in our study yielded an excellent level of agreement between both the ELISA used and the confirmatory Western blotting ($\kappa = 0.87$). This finding attests for the usefulness and validity of an ELISA but should still be interpreted carefully. Examination of stool samples within the participating schoolchildren displayed a low prevalence of soil-transmitted helminthiases, a consequence that may have improved the diagnostic reliability of the ELISA test as ascariasis and trichuriasis are recognized drivers of false positive results (Holland & Smith, 2006). Other research has instead confirmed the need for verification of not only all positive ELISA serologies but also of results hovering around the so-called "gray zone", which refers to OD values bordering on the cut-off threshold both negative and positive (Fillaux & Magnaval, 2013; Roldan & Espinoza, 2009; Roldán et al., 2010; Rudzińska et al., 2017; Rychlicki, 2004; Zarnowska-Prymek, 2001). Confirmation of borderline negative and positive absorbance values was not done in this study.

Despite the improvements in specificity seen with immunoblotting and the excellent agreement between the ELISA kit and WB used in our study, the distinction of active versus past infection is still not possible with these two techniques. Avidity of IgG antibodies as a way to determine stage of infection has previously been used for other communicable diseases such as fascioliasis, cysticercosis, strongyloidiasis and schistosomiasis (de Gouvêa Viana, Rabello, &

Katz, 2001; Gonzaga et al., 2011; Manhani, da Silva Ribeiro, Silva, & Costa-Cruz, 2009; Shehab, Allam, & El-Sayad, 2002). Although little is known about the dynamics of antibody avidity in toxocariasis, Rudžinska *et al*. set out to gage the efficacy of IgG avidity as an indicator of clinical diagnosis. In their study, a high avidity was found in the majority of patients upon first serological examination, which was interpreted by the authors as a primary infection older than 7 months. Subsequent low or weak avidities suggested an active stage of infection (Rudzińska et al., 2017). Similar conclusions were derived by Logar and Rychlicki, as they reported that low avidity values helped to confirm early stages of active infection and confirmed that IgG avidity was a valuable diagnostic tool in human toxocariasis (Logar et al., 2004; Rychlicki, 2004). Scientists have also looked at other alternatives for the differentiation of active and old infections such as the measurement of specific IgE antibodies and levels of eosinophil cationic protein. The performance of these assays still needs further validation (Ma et al., 2017; Magnaval, Berry, et al., 2001).

5.6: Degree of Immunoblot Antigen Recognition

Sixty-nine children demonstrated antibody reactivity to at least four of the five recombinant antigens known to be specific to *Toxocara* spp. Sixty-six of these children showed immunoreactivity to all five of the possible glycoproteins. Under manufacturer instructions, an immune reaction to only two of these recombinant antigens is needed for a positive result. Considering this, the excessive recognition of *Toxocara* spp. antigens of low-molecular weight by the children's immune systems may speak to consistent exposure or recent infection. Evidently, toxocariasis is abundant in this studied community but a complete clinical evaluation of health problems potentially caused by this exposure has yet to be done.

This recognition of antigenic glycoproteins should not overshadow the rest of the positive immunoblot results with a lesser number of bands. These lower reactivity seropositives should still be assumed as potentially morbid, especially since studies have drawn a link between low infective doses and the more dangerous clinical manifestations associated with toxocariasis, namely ocular and neurological involvement (Fan et al., 2015; Ma et al., 2017). In turn, this further emphasizes the gap in translation between immunologically positive serology and active clinical disease and symptoms. A more in-depth physical examination of these schoolchildren is urgently needed.

5.7: Study Strengths and Limitations

The present study possesses certain advantages and strengths. First, the inherent cross-sectional design fits the proposed research questions and aims adequately. Since the same design was applied twice but with a two-year gap in between, this allowed for the comparison of results within the same community at two different time points. Furthermore, this repeated implementation also allowed for the monitoring of any changes in children who were enrolled in both years. Our sample from the only primary school in Santa Cruz del Junco, Honduras is considered a strength as it is likely representative of the pediatric population within the locality. The determination of anti-*Toxocara* antibody seroprevalence contributes to the collection of studies investigating this around the world. Lastly, epidemiological data considering plausibility of *Toxocara* spp. transmission was collected from participating children.

The biggest limitation of this current study is that of the small sample size, even after compilation of participants from 2015 and 2017. The sample size in 2017 was even smaller due to a conjunctivitis epidemic in the community that led to large school absenteeism. As a result, this severely hindered the power of our study. In the same token, the small sample size in conjunction with the tremendous levels of seropositivity observed rendered all statistical analysis models insignificant. Also considering the high prevalence of anti-*Toxocara* antibodies, the absence of corresponding clinical data limited conclusions and an appreciation for the true effect of this zoonotic helminthiasis on children's health in a forgotten and medically abandoned area, as is Santa Cruz del Junco.

CHAPTER 6: CONCLUSIONS, RECOMMENDATIONS, FUTURE RESEARCH SUGGESTIONS AND FINAL REMARKS

6.1: Conclusions

The current study is the first of its kind looking at the seroprevalence of anti-*Toxocara* antibodies in schoolchildren conducted in the country of Honduras. The subsequent findings point to the dire reality that exposure with *Toxocara* spp. is rampant in rural Honduras and more attention should be paid to this relatively overlooked helminthiasis.

This study reveals that in rural Honduras, exposure to *Toxocara* spp. is highly prevalent and presents a potential public health problem. Comparison of dual participants also demonstrated that exposure to the source of infection is constant. Risk of transmission is therefore considered high. Although no statistically significant risk factors could be identified, the levels of seropositivity seen in children who reported dog ownership, raw vegetable and meat consumption as well as soil contact suggest that these are still relevant in the transmission of this infection.

Apart from risk factors, this study validated the use of an ELISA as an adequate serodiagnostic tool for detecting anti-*Toxocara* antibodies. Eosinophilia was also found to be strongly associated with seropositivity in this population of children.

6.2: Recommendations

The multiple infection routes (i.e., human-dog/cat contact, food-borne transmission, geophagia) along with the potential associations with neurological disorders require an increased communal awareness of toxocariasis. Enhancing education, as is targeted with STH, is essential in improving the understanding of this disease and its prevention and control. Hygiene, the prevention of children from ingesting soil, material contaminated with feces from definitive hosts and avoiding or limiting the consumption of raw/undercooked meat and vegetables are all central to preventing transmission in endemic communities such as this one appears to be. Moreover, seemingly asymptomatic children should still be monitored for larval invasion of the CNS and eyes.

Surveying of *Toxocara* infections in definitive hosts could reduce the degree of environmental contamination with infective eggs. Ideally, contact with cats and dogs particularly those of young ages should be minimized since straying is a common behaviour of these animals in rural Honduras. The population of stray dogs should be controlled as well, following what rabies initiatives have started. An alignment with rabies control programs could be possible to help reduce these zoonotic diseases. A more profound change would be of cultural attitudes towards dog ownership in terms of contact, as previously mentioned, but also of a higher level of care for owned dogs. In Honduras, like many countries around the world, dogs are not dewormed regularly if ever, and are allowed to scavenge for food while they roam freely. Similarly, it would also be beneficial to analyze common public areas for the presence of *Toxocara* spp. ova in the soil. These recommendations may not be easily achievable or sustainable in underprivileged rural communities.

6.3 Future Research

Given the scarcity of toxocariasis research in the country, it is of utmost important that follow up investigations be conducted to gain a true appreciation of the burden of this zoonosis. It is necessary that more scientific evidence, especially that of prevalence data from other parts of the country, be gathered. The discovery of similarly high seroprevalence to that of the current study will stimulate dedication of efforts to improving policy and control. Even in this community alone, the need for symptomatology data as well as for a more powerful study to elucidate risk factors is obvious. Knowledge gaps in terms of the impact of active *Toxocara* infection and its effect on the cognitive status of children still exist.

Apart from prevalence studies, a diagnostic technique or a way of being able to distinguish between active versus past or cleared infection is urgently required for toxocariasis studies to take the next step. Similarly, translation of seroepidemiological data to clinical relevance poses the biggest challenge for future research. The collection of clinical data and a physical examination following positive serology should be conducted in Santa Cruz del Junco. Because of the high occurrence of seropositivity, future surveys should expand the study population to include not only schoolchildren but also preschool aged children and potentially the families of the schoolchildren studied here.

Expansion of risk factors studied could be an additional avenue of research in the future. While extremely challenging, the feline version of *Toxocara* spp., *T. cati* still needs to be elucidated as an etiological agent of human toxocariasis and evaluated in terms of burden and impact on human populations.

6.4: Final Remarks

The presented thesis research uncovered a surprisingly high seroprevalence of anti-*Toxocara* spp. antibodies in the rural community of Santa Cruz del Junco, Tela, Honduras. It also provides an idea of how serious the issue of human toxocariasis may be in other rural localities where transmission of other parasitic infections is widespread. Our study also identifies the persistence of source(s) of infection with *Toxocara* spp. and opens the door for concrete clarification of risk factors. While these findings indicate that Toxocaral infection is extensive, clinical relevance still needs to be ascertained to understand fully the toxocariasis enigma.

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Appendix A: Ethics Clearance Bioscience Research Ethics Board—Brock University (2017)

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 9/12/2017

PRINCIPAL INVESTIGATOR: SANCHEZ, Ana - Health Sciences

CO-INVESTIGATOR(S): Maria Mercedes Rueda (ma.mercedesrueda@yahoo.com); Maritza Canales (marygchn@yahoo.com); Carol A. Rodriguez (anahelka@yahoo.com)

FILE: 17-032 - SANCHEZ

TYPE: Masters Thesis/Project STUDENT: Sergio Hernandez
 SUPERVISOR: Ana Sanchez

TITLE: Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela

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ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 9/1/2018

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **9/12/2017** to **9/1/2018**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 9/1/2018. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:
  a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
  b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
  c) New information that may adversely affect the safety of the participants or the conduct of the study;
  d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: _____
Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Appendix B: **Ethics Clearance Bioscience Research Ethics Board—Brock University (2015)**

Certificate of Ethics Clearance for Human Participant Research

DATE: 5/8/2015

PRINCIPAL INVESTIGATOR: SANCHEZ, Ana - Health Sciences

CO-INVESTIGATOR(S): Maritza Canales, Maria Mercedes Rueda, and Carol Anahelka Rodriguez

FILE: 14-224 - SANCHEZ

TYPE: Faculty Research STUDENT: Jose Antonio Gabrie (Brock) and Joel Garcia (MB-UNAH)
SUPERVISOR: Ana Sanchez

TITLE: Immunological profile of children with and without soil-transmitted helminth infections: a pilot study in Honduras

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ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 5/31/2016

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from 5/8/2015 to 5/31/2016.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 5/31/2016. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:
  a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
  b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
  c) New information that may adversely affect the safety of the participants or the conduct of the study;
  d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Brian Roy, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

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Appendix C: **Ethics Clearance from the Research Ethics Committee—National Autonomous University of Honduras (2017)**

UNAH
FACULTAD DE CIENCIAS
COMITÉ DE ÉTICA DE INVESTIGACIÓN
MAESTRÍA EN ENFERMEDADES INFECCIOSAS Y ZOONÓTICAS

CONSTANCIA DE RESOLUCIÓN
PROTOCOLO DE INVESTIGACIÓN No. 04-2017

Por este medio el Comité de Ética de Investigación de la Maestría de Enfermedades Infecciosas y Zoonóticas (CEI-MEIZ) hace CONSTAR que el proyecto de investigación:

Título del proyecto: "Toxocariasis en Honduras: Seroprevalencia y Riesgo de Infección en escolares de la ciudad de Tela"

Equipo de Investigación: Ana Sánchez (Investigadora principal), Sergio Hernández (Co-Investigador) María Mercedes Rueda (Co-Investigadora), Maritza Canales (Co-Investigadora) y Carol Anahelka Rodríguez (Co-Investigadora).
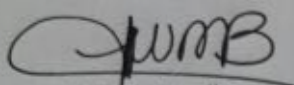
Institución (es): Universidad de Brock y Escuela de Microbiología-UNAH.

Fecha de presentación al comité: 17 / 08 / 2017

Fue sometido a un proceso de revisión y análisis, y cumpliendo con las Normas Éticas Nacionales e Internacionales vigentes, en consecuencia fue **APROBADO**

Duración de la aprobación: 21 / 09 / 2017 al 21 / 09 / 2018

Para los fines de los interesados se les extiende la presente a los veintiún días del mes de septiembre de 2017.

Dra. Wendy Murillo
Presidente CEI-MEIZ

Lic. Judith Bulnes Borjas
Secretaria CEI-MEIZ

UNAH

FACULTAD DE CIENCIAS
COMITÉ DE ÉTICA DE INVESTIGACIÓN
MAESTRÍA EN ENFERMEDADES INFECCIOSAS Y ZOONÓTICAS

CONSTANCIA DE RESOLUCIÓN
PROTOCOLO DE INVESTIGACIÓN No. 01-2015

Por este medio el Comité de Ética de Investigación de la Maestría de Enfermedades Infecciosas y Zoonóticas (CEI-MEIZ) hace CONSTAR que el proyecto de investigación:

Titulo del proyecto: "Perfil inmunológico y epidemiológico de las parasitosis intestinales en escolares hondureños: estudio comparativo entre una población escolar rural y otra urbana durante el periodo comprendido entre agosto del 2015 a junio del 2016"

Equipo de Investigación: Maritza Canales (Investigadora principal), Ana Lourdes Sánchez (Co-investigadora principal), María Mercedes Rueda (Co-investigadora), Carol Anahelka Rodríguez (Co-investigadora), José Antonio Gabrie (Co-investigador) y Joel Saamir García (Co-investigador).

Institución (es): Maestría en Enfermedades Infecciosas y Zoonóticas, Escuela de Microbiología – UNAH y Department of Health Science, Brock University.

Fecha de presentación al comité: 27 / 03 / 2015

Fue sometido a un proceso de revisión y análisis y en consecuencia fue **APROBADO**

Duración de la aprobación: **01 / 08 / 2015** al **31 / 07 / 2016**

Para los fines de los interesados se les extiende la presenta a los treinta días del mes de julio de 2015.

Dra. Wendy Murillo
Presidente CEI-MEIZ

Lic. Judith Bulnes Borjas
Secretaria CEI-MEIZ

Appendix E: Parental/Third Party Consent Form For Participation in the Study

PARENT/GUARDIAN DECLARATION OF CONSENT TO PARTICIPATE IN AN INTERVIEW ON BEHALF OF THEIR

CHILDREN

I understand the contents of this **Consent Form**, and I agree to allow my child to voluntarily participate in this research study, if she/he also assents. Hereby, I am providing my consent to participate in a face-to-face interview responding to questions pertinent to the study. I am also consenting to collect a stool sample from my dog and/or my cat so researchers can determine if they have parasites.

I declare that I have had the opportunity to ask questions in an information session and all of my questions have been answered to my satisfaction. By signing this consent form, I am not giving up any of my legal rights or those of my child. I am just agreeing to participate in the research study "Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela". In summary, I consent to the following:

A. Interview: I agree to participate in

Face-to-face 10-15 min interview ☐ Yes ☐ No

B. Stool samples from domestic animals: I agree to provide

Stool samples from dogs/cats ☐ Yes ☐ No

C. Data if withdrawal from the study: I authorize

Researchers to keep my data even if I withdraw from the study ☐ Yes ☐ No

I am aware that I can change my mind about any of these agreements ☐ Yes ☐ No
and inform the researchers at any time without any penalty or loss of
benefit to which I might be entitled to by my participation in the study

_____ _____ _____
Participant's name Participant's signature Date (day/month/year)

_____ _____ _____
Witness's name Witness's signature Date (day/month/year)

PARTICIPANT CODE: _____

Study: Tela, 2017. INVITATION AND PARTICIPANT INFORMED CONSENT TO PARTICIPATE IN AN INTERVIEW. PARENT/GUARDIAN. Revised Sept. 2017.
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Appendix F: Parental/Third Party Consent Form for Their Children as Participants

Research Study: Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela
TELA, ATLANTIDA, HONDURAS, 2017

INVITATION AND CONSENT BY AN AUTHORIZED THIRD PARTY: PARENT/GUARDIAN

Date:

Introductions: we are conducting a research study
Hello, we would like to invite your children to participate in a research study about a parasite in school children of your community. We would like to tell you more about us and what is involved in the study, and then you can decide whether or not you want to participate in it. If you want your children to be enrolled in the study, we must obtain your permission first and then we will talk to your child to see if he or she wants to participate.

We have received approval to do this research study:
First of all, we would like to tell you that the study has been reviewed and received ethics clearance both in Canada at Brock University and in Honduras at UNAH. REBs file certificates are dated by Brock on date: September 12th 2017 File #: 17-032 SANCHEZ and by UNAH-CEI-MEIZ, date September 21st 2017 File #: 04-17

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 investigators are Dr. Maritza Canales (professor), Dr. Maria Mercedes Rueda (professor) and Carol Anahelka Rodríguez (professor). They are from the School of Microbiology of the National Autonomous University of Honduras (UNAH).

In **Canada** the investigators are Dr. Ana Sánchez (Professor at Brock University) and Sergio Hernández (Graduate student at Brock University) of the Department of Health Science. Dr. Sanchez is the Project leader. Brock University is located in St. Catharines, Canada and UNAH is located in Tegucigalpa.

Invitation:
We thank you for your time in reading this invitation letter. As mentioned before, the reason why we are here is because we would like to invite your children who are in second, third, fourth or fifth grade to participate in a study about a dog parasite that can also affect humans.

Before you decide you want your children to enroll in the study, it is important that you understand this study well, including its risk and benefits to make an informed decision. We encourage you to ask questions if there is anything that you do not understand.

Purpose of the study
We would like to know if there is risk for the children in this community to be infected with a dog parasite named "Toxocara" (dog roundworm). We plan to achieve this by (i) by examining children's blood and stool samples for the presence of the parasite (ii) by collecting animal and soil samples to detect contamination that could spread to people; (iii) to assess children's practices and behaviours that could increase their risk of infection.

What is involved in the study?

If you are interested in this study, you can expect the following to happen:

1. You will be invited to an information session at your child's school that will cover the basics of the parasite we are studying and its effects on your child.
2. If you are still interested in having your child participate, you will be asked to return the following day to provide your informed consent. Next, you will be asked to meet with one of our interviewers who will ask you very basic information such as your child's name, age, sex, school grade, certain behaviours and some basic information about your household. It will take 10-15 minutes to answer these questions.
3. We will ask your child to give us a small stool specimen. We will provide you with all the instructions needed and with a special container for collecting the sample. The stool sample will be analyzed in the laboratory looking for intestinal parasites, so you can know if your child is infected with them or not.
4. We will also ask your child to give us a blood sample which will be collected in 2 different tubes but through only one needle prick. The amount in each tube will be no more than 5 ml. In total, we will need about 20 minutes of your child's time. While the blood sample will be taken at the school, we will make sure that this time does not interfere with any of your child's school activities.
5. The blood will be analyzed in the laboratory to test for some reactions to parasites.
6. We also want to know if the animals belonging to your family have parasites or not. This is because animals can transmit parasites to you too. By examining their stools we will be able to know this, so we will ask you, not your child to gives us a small stool specimen of any dogs or cats you may have as pets. We will provide you materials and instructions for you to collect these samples in a hygienic way. These samples may contain infectious microbes and they can infect you; that is why we will provide you with materials to protect yourself and instructions to do a safe collection.

Ethical aspects

We would like to assure you that this study has been reviewed and has received ethics clearance from impartial professionals in Honduras and in Canada. More specifically, both Brock University's Research Ethics Board and UNAH's Ethics Review Committee have given the study clearance. Additionally, it has been approved by the principal and grade teachers of your child's school. These approvals mean that the risk of harm to you or your children is very unlikely. 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 your participation in study. We also want to emphasize that your child's school is not part of our study, will not know who has participated or not and will not have access to ANY data.

Risks and discomfort

This study does not pose serious risks to you or your child. There are some minor risks, however, but we will make every possible effort to mitigate those risks so your experience will be overall satisfactory. For example, your child may feel a little embarrassed of providing a stool sample 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. If animal stool sample collection is agreed upon, you may be exposed to any microbes found in the canine/feline stool. That is why we will provide materials and instructions that you must follow very carefully. Also, you may have concerns about you and/or 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 experienced in taking blood samples so we will not cause any more pain than a little prick. Also, some people may feel dizzy or lightheaded when they have their blood drawn, if this happens your child, this will be very transient and we will make sure they recover completely.

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

Potential benefits

Participating in the study has several direct benefits for the child. Firstly, we would let you know whether or not the child has been in contact with the dog roundworm. If this is the case, we will provide you with a report and specific information to seek medical advice. Secondly, since we will also test children for intestinal parasites, you will be informed and referred to the health centre for treatment with albendazole (deworming medicine). Thirdly, if your animals have parasites we will facilitate their treatment so they will have better health too. Finally, we will offer recommendations to reduce children's risk of infection with Toxocara and other parasites.

Confidentiality

We will protect your right to the privacy and confidentially of your information. All information obtained during this study will be kept strictly confidential. Samples collected will be transported to and stored in labs with restricted access that remain locked. Your name and the name of your child will not be given to anyone outside of the research team and electronic information will be in secured computers and paper information locked in a filing cabinet in the investigator's office for a maximum of 5 years after the completion of this study. Only the research team will have access to the information. The results of this study may be published in journals or conferences but your name or the name any participant will never be revealed.

Voluntary participation and withdrawal from this study

Your child's participation in this study is free and voluntary. You may refuse to have them participate. Additionally, 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/when you decide to discontinue your child's participation, please let us know directly or have your child tell us they do not want to participate any longer. If you or your child discontinues participation, no one will suffer any 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 if/when you decide you are unwilling to continue their participation. If you or your child's participation is discontinued at any point in the study, we would like to keep the information you already gave us, if that's ok with you. In that case, we will still provide laboratory results, and if your child is infected, we will make treatment available at the health centre.

Cost and compensation

There are no costs associated with your participation in this study. You will be advised to seek treatment if your child is found to be infected by any parasite. Neither you nor your child will be given any money to participate in this study.

Contact persons

If later on you and/or your child have any questions about the study you can 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 number is: 2252-8089. If you or anybody you know has access to email, you could e-mail her at < macg@unah.edu.hn >. Alternatively, you can also contact the Brock University Research Ethics Office at +1 (905)-688-5550 ext. 3035 or through email at reb@brocku.ca. They will be able to answer any questions on participants' rights as well as provide contact information for the principal investigator

PARENT/GUARDIAN DECLARATION OF CONSENT

I understand the contents of this **Consent Form**, and I agree to allow my child to participate in this research study freely and voluntarily in this research study, if he/she also assents. I have had the opportunity to ask questions in an information session and all of 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 giving up any of my legal rights or those of my child, just agreeing to participate in the research study "Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela". In summary I am consenting to the following:

A. Blood and stool samples: I agree that my child provides

Blood sample (2 tubes)	☐ Yes ☐ No
Stool sample	☐ Yes ☐ No

B. Data if withdrawal from the study: I authorize

Researchers to keep my data even if I withdraw from the study	☐ Yes ☐ No
I am aware that I can change my mind about any of these agreements and inform the researchers at any time without any penalty or loss of benefit to which I might be entitled to by my participation in the study	☐ Yes ☐ No

_____   _____   _____

Participant's name   Participant's signature   Date (day/month/year)

_____   _____   _____

Witness's name   Witness's signature   Date (day/month/year)

PARTICIPANT CODE: _____

Appendix G: Child Assent Form and Letter of Invitation

INVITATION AND INFORMED ASSENT FOR CHILDREN

Date:

Introductions:
Hello, we thank you for giving us the opportunity to speak with you today. Your parents gave us permission to talk to you and invite you to participate in a research study about parasites also called intestinal worms that we are carrying out in your community. Even though 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. We would like to tell you more about us and what is involved in the study, and then you can decide whether or not you want to participate in it

We have received approval to do this research study:
First of all, we would like to tell you that the study has been reviewed by our universities' authorities in charge making sure we conduct our research responsibly and ethically. We have obtained permission (ethics clearance) both in Canada at Brock University and in Honduras at UNAH. The letters of permission are official and they are given official numbers that could be verified if someone wanted to call them. In Honduras, the number is UNAH-CEI-MEIZ file #: 04-2017, date: September 21st 2017, and at Brock, the number is REB file # 17-032 SANCHEZ date: September 12th 2017

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 investigators are Dr. Maritza Canales (professor), Dr. Maria Mercedes Rueda (professor) and Carol Anahelka Rodríguez (professor). They are from the School of Microbiology of the National Autonomous University of Honduras (UNAH).

In **Canada** the investigators are Dr. Ana Sánchez (Professor at Brock University) and Sergio Hernández (Graduate student at Brock University) of the Department of Health Science. Dr. Sanchez is the Project leader.

Why we are we speaking with you if your parents gave you permission to participate in the study?
Even though your parents told us that they would like for you to participate in our study, we still need to ask you directly if you agree. Therefore, we are actually inviting you directly to be part of the study about parasites. Before you decide, you will get a lot of information that we will present. After we give you all this information, we will ask you again if you would like to take part in the study. We will explain why we want to do this study, but basically, we want to find out explanations about how parasites are transmitted to people by animals and what we can do to prevent this risk.

What will happen to you if you agree to participate in the study?

If you decide to take part of this study we will ask you to give us a small stool specimen and a blood sample. It will take a total of about 5 minutes to give us your blood sample. We will ask that you bring us your stool sample that day in a special container that we will provide you. If there are dogs or cats in your house, we will ask your parents to bring us a stool sample from them as well. If we find that you have parasites, we will give your parents a lab report for them to get you treatment. If your animals had parasites, we would provide treatment too.

Are there good things and bad things about the study?

One of the good things is that we will let you know if you have been in contact with the dog roundworm (we will know this by testing your blood). If we find that you have been in contact, we will tell your parents so they can seek medical advice. Also, if you had intestinal parasites, we will provide deworming treatment to help you get rid of them. This means that you will probably feel better and be able to pay more attention in school. Another good thing is that you and your family will have more information about parasites in your animals and this may help you treat them.

By taking part in the study there are some minor risks for you but we will make every possible effort to mitigate those risks so your experience will be overall satisfactory. For example, you could feel anxious about providing the stool sample 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. We also want to know if the animals belonging to your family have parasites or not. This is because animals can transmit parasites to you too. By examining their stools we will be able to know this.

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 the only way to know if you have some parasites 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 only 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 do everything you are asked to do?

If you don't want to do something 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.

What will happen to the information you give us?

The things you tell us and any information about you (for example if you have parasites) will be kept secret in a locked place. Only the researchers will be allowed to see it. Because you are a minor we will give your parents your laboratory results so they can decide what to do with them. When we talk about this study, we will never use your name.

RESEARCH STUDY: Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela
TELA, ATLANTIDA, HONDURAS, 2017

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 want 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 have parasites. If you do, there will be treatment available for you.

Do you have any questions?

You can ask questions at any time. You can ask now or you can ask later; please feel free! You can talk to anyone on the team whenever you want.

If later you have questions about the study you can tell your parents and they can 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 number is: office Tel. 2252-8089. If you or anybody you know has access to email, you could e-mail her at < marygchn@yahoo.com >.

RESEARCH STUDY: Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela
TELA, ATLANTIDA, HONDURAS, 2017

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 in the research study: **Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela**

The child provides verbal assent to:

Providing a blood sample (2 tubes) ☐ Yes ☐ No

Providing stool sample ☐ Yes ☐ No

Authorizing researchers to keep their data even if withdraw from the study ☐ Yes ☐ No

I am aware that I can change my mind about any of these agreements ☐ Yes ☐ No
and inform the researchers at any time without any penalty or loss of
benefit to which I might t be entitled by my participation in the study

_____
Child's name

_____   _____   _____
Researcher's name   Researcher's signature   Date (day/month/year)

_____   _____   _____
Witness's name   Witness's signature   Date (day/month/year)

PARTICIPANT CODE: _____

Appendix H: Parent/Guardian Questionnaire

CUESTIONARIO

Código de identificación del participante:	

BEFORE INTERVIEWING			
ANTES DE LA ENTREVISTA			
Obtained parent/ guardian consent?		Yes	No
1. **¿Se obtuvo consentimiento de sus padres o encargados?**		SÍ	NO
INTERVIEW DETAILS			
IDENTIFICACION DE LA ENTREVISTA			
Date of interview (dd/mm/yyyy)			
2. **Fecha de la entrevista (día-mes-año):**			
Interviewer			
3. **Entrevistador:**			
BASIC INFORMATION			
INFORMACIÓN BASICA			
Name of participant			
4. **Nombre del participante:**			
Name of parent/legal guardian			
5. **Nombre del padre / guardián legal:**			
Community where the child lives			
6. **Barrio o colonia en que vive:**			
Child's date of birth		Child's age	
7. **Fecha de nacimiento (día-mes-año):**		8. **Edad:**	
Grade in school		Sex	
9. **Grado escolar:**		10. **Sexo:** F M	

HOUSEHOLD CHARACTERISTICS			
CARACTERÍSTICAS DE LA VIVIENDA			
Does the family own dogs?			
11. **¿Tienen perros en la casa?**			
Yes/Si	No/No	Don't know/No sabe	How many/Cuantos?:
How old is the dog?			
12. **¿Qué edad tiene el perro?**			
		Don't know/No sabe	
Have you had the dog since its birth?			
13. **¿Ha tenido al perro desde que nació (el perro)?**			
Yes/Si	No/No	Don't know/No sabe	
If not, how long have you had the dog?			
14. **Si no, desde cuando tiene el perro?**			
Do they stray (even partially or all the time)?			
15. **¿Deambulan libres por la calle? (Todo el tiempo o parcialmente)**			
Yes/Si	Enclosed all the time / Encerrados todo el tiempo		Don't know/No sabe
Does the family own cats?			
16. **¿Tienen gatos en la casa?**			
Yes/Si	No/No	Don't know/No sabe	How many/Cuantos?:

125

17. ¿Qué edad tiene el gato?		
	Don't know/No sabe ☐	

Have you had the cat since its birth?

18. ¿Ha tenido al gato desde que nació (el gato)?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

If not, how long have you had the cat?

19. Si no, desde cuando tiene el gato?

Do they stray (even partially or all the time)?

20. ¿Deambulan libres por la calle? (Todo el tiempo o parcialmente)

Yes/Si ☐	Enclosed all the time / Encerrados todo el tiempo ☐	Don't know/No sabe ☐

Does the child come in constant contact with the domestic animals in the house?

21. ¿El niño/a permanece en constante contacto con sus animales domésticos?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Have you ever given deworming medication to your domestic animals/pets?

22. ¿Alguna vez se le ha dado medicamento desparasitante a sus animales domesticos?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

HABITS
HABITOS DEL NIÑO/A

Does the child play with/come in constant contact with soil?

23. ¿El niño/a juega o se mantiene en contacto con el suelo?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child have the habit of eating soil or dirt (pica)?

24. ¿El niño/a tiene la costumbre de comer tierra (pica)?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child have the habit of sucking their thumb?

25. ¿El niño/a tiene la costumbre de chuparse el dedo pulgar?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child have the habit of biting their nails?

26. ¿El niño/a tiene la costumbre de morderse las uñas?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child consume undercooked meat?

27. ¿El niño/a consume carne no bien cocinada?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child consume raw/undercooked vegetables?

28. ¿El niño/a consume vegetales/verduras no cocinados/crudos?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child play in a park/playground?

29. ¿Juega el niño en el parque?

Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	

Does the child have any other contact with soil/dirt?

30. Tiene el niño/a otra clase de contacto con tierra?

KNOWLEDGE OF PARASITES			
CONOCIMIENTO SOBRE LOS PARÁSITOS			
Do you know what parasites are?			
31. ¿Sabe que son los parásitos?			
Yes/Si ☐	No/No ☐		
Has the child ever had any parasites or intestinal worms?			
32. ¿El niño/a ha tenido alguna vez parásitos/lombrices intestinales?			
Yes/Si ☐	No/No ☐	Don't know/No sabe ☐	
HISTORY OF DEWORMING			
HISTORIA DE DESPARASITACIÓN			
Has the child ever received deworming treatment?			
33. ¿Alguna vez ha el niño/a recibido tratamiento desparasitante? Yes/SÍ ☐ No/NO ☐			
Type of treatment		Other/Otros:	
a. Tipo de tratamiento: Pills/Pastillas ☐	Syrup/Jarabe ☐		
Date of last treatment (mm/yyyy)			
b. Fecha del último tratamiento (mes-año):			
Who administered the treatment?			
c. Quien se lo administró			

Appendix I: Instruction Sheet for Human Stool Sample Collection

Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela

Instructions for **human** fecal sample collection

Instructions	Image
1. Inside the package for fecal sample collection, you will find a chamber pot. Make sure the chamber pot is clean and dry. Have your child deposit their feces inside. **2.** Collection of the sample should take place in the early morning. Do not deposit the feces on the ground. **3.** Avoid mixing feces with urine. Have your child urinate before depositing their feces in the chamber pot.	
4. Place the gloves provided on your hands, being careful so that they do not rip or break.	
5. With the wooden spatula, scoop a portion of feces. **6.** Place the feces inside the transparent container that has your child's participant code on it. **7.** Fill half the container with feces and put the lid on it tightly. **8.** Dispose of the wooden spatulas in the garbage.	
9. Place the container with your child's sample inside the transparent plastic bag provided and tie a knot on top.	
10. Place the tied plastic bag with the container inside the brown paper bag.	
11. Dispose of the gloves in the garbage	
12. Wash your hands thoroughly with soap and water.	
13. Take the sample to the designated place as agreed upon with the research team.	

Thank you for your cooperation

Appendix J: Kato-Katz Findings/Results Form

<table>
<tr><td colspan="2">**Toxocariasis in Honduras: Seroprevalence and Risk of Human Infection in the coastal community of Tela**
TELA, ATLANTIDA, HONDURAS, 2017</td></tr>
</table>

INDIVIDUAL LABORATORY REPORT - PARASITOLOGICAL EXAM (FECAL SAMPLE)

<table>
<tr><td>Participant code ID:</td><td>Age:</td><td colspan="2">Community:</td></tr>
<tr><td>Date of sample collection:</td><td colspan="2">Date of analysis:</td><td>Analyzed by:</td></tr>
<tr><td>Lab techniques utilized:</td><td colspan="3"></td></tr>
<tr><td>Fecal sample description:</td><td colspan="3"></td></tr>
<tr><td colspan="4" align="center">**Microscopic Findings**</td></tr>
<tr><td>Potentially pathogenic (harmful) protozoa:</td><td colspan="3"></td></tr>
<tr><td>Helminths (worms) and intensity of infection:</td><td colspan="3"></td></tr>
<tr><td colspan="4">Recommendations / comments</td></tr>
<tr><td colspan="4">Name and signature of researcher:

Date:</td></tr>
</table>