A Thesis Presented to

The Faculty of Alfred University

Addressing *Toxocara* in humans and companion animals as a public health issue

Allison Naclerio

In Partial Fulfillment of the Requirements for

The Alfred University Honors Program and

Biology Department Honors

April 16, 2018

Supervised by:

Committee Chair:

Dr. Jean A Cardinale

Committee Members:

Dr. Robert Myers

Dr. Heather Zimbler-DeLorenzo

Department of Biology

Alfred University College of Liberal Arts and Sciences

Alfred, NY

Table of Contents

Abstract

Toxocara canis and *Toxocara cati* are zoonotic parasites that primarily infect dogs and cats. *Toxocara* is especially relevant to human health because humans frequently live in close contact with companion animals. Young animals are at risk for complications while adult dogs and cats rarely experience severe illness due to *Toxocara* infection. Infected humans can develop toxocariasis, which can be very dangerous. Both humans and animals can become infected by accidently ingesting *Toxocara* eggs. Because *Toxocara* eggs can persist in the environment under harsh conditions and for very long periods of time, infection is significant health concern. The issue of *Toxocara* is not currently being addressed properly. In order to comprehensively and effectively address *Toxocara* infection, public health initiatives targeting human health, animal health, and *Toxocara* in the environment must be implemented to decrease incidence and prevalence of *Toxocara* infection.

Introduction

Toxocara canis and *Toxocara cati* are two parasites largely ignored in the public health sphere, despite causing disease in humans, canids, and felids. These parasites are only treated as if they are an animal-specific problem; yet, because *Toxocara* has a complex biology, it must be treated in humans, animals, and the environment. Oversight results in the occurrence of preventable infections, whose implications are not entirely understood. This thesis will examine the relevant biology of *Toxocara* in order to propose practical recommendations that could decrease the incidence and prevalence of *Toxocara* infection. *Toxocara* is an issue that requires further attention. In order to control his parasite, public health initiatives targeting human health, animal health, and Toxocara in the environment must be implemented.

T. canis and *T. cati* are zoonotic parasites, meaning they can be transmitted from animals to humans. Zoonotic diseases are a major concern because at least 60% of all human pathogens have zoonotic origins (Bueno-Marí et al. 2015). Zoonotic diseases may be of bacterial, fungal, viral, or parasitic origins. There are four major ways a person might become infected with a zoonotic agent: direct contact (directly touching or getting bitten or scratched by an infected animal), indirect contact (touching an area where an infected animal was), through a vector (being bitten by an infected arthropod), or via food and water (consuming the infected animal). Some well-known examples of zoonotic agents are the rabies and Ebola viruses, *Bacillus anthracis* (which causes anthrax), and *Borrelia burgdorferi* (which causes Lyme disease). Because animals act as reservoirs, zoonotic disease is particularly difficult to control and requires a multidisciplinary approach. Addressing zoonotic issues requires the cooperation of physicians, veterinarians, and environmental scientists. Another major component of responding to zoonotic disease is public health efforts. Public health is the science of organized efforts to improve the

health of a community through surveillance, prevention, and treatment of disease. Because of their pervasiveness and complexity, zoonotic diseases require further research to diminish their impact on human, animal, community, and global health.

Pets can pose a significant zoonotic threat because humans have frequent interaction with these animals. Companion animals are becoming fully integrated into all aspects of their owner's lives. Therefore, it is important that the zoonotic risks of pets are assessed and reduced. One example is *Toxocara*, an intestinal parasite. Canids, such as foxes and domestic and wild dogs, are the definitive hosts for *T. canis* while felids, cats of all types, are the definitive hosts for *T. cati* (Macpherson 2013). The definitive host is the host in which a parasite can mature and develop to complete its life cycle. The definitive host can therefore shed many *Toxocara* eggs into the environment as long as the internal parasites continue to reproduce. Other mammals, such as humans and rodents, are paratenic hosts, species in which the parasite can survive but will not reach sexual maturity. These hosts serve as reservoirs to harbor the parasites until they can reach a definitive host (Despommier 2003). Although the parasites do not reproduce within the paratenic host, they may still cause harm to the host as well as exit the host and infect other organisms.

Toxocara infection in humans was first described in 1950 (Despommier 2003). In humans, *Toxocara* causes toxocariasis, which is the result of larvae migrating through various tissues throughout the body. There are multiple manifestations of this disease with varying severities dependent upon the specific tissues impacted. Each manifestation of toxocariasis is discussed later, in further detail. Recently, links between toxocariasis and more serious conditions, such as asthma and epilepsy, have been suggested (Ogundipe et al. 2017; Quattrocchi et al. 2012). While the relationship between these conditions has not yet been proven, this

association could mean that toxocariasis is even more dangerous than previously thought. Humans are at risk for developing toxocariasis because they oftentimes spend lots of time in close proximity to dogs and cats.

Dogs and cats are among the most popular pets worldwide, and there are also extensive feral populations of both animals. *Toxocara* infections are common in both cats and dogs; however, the prevalence of infections is unknown because it is not a reportable disease. Additionally, the estimated prevalence values would only be from domestic animals that visit veterinarians. The total prevalence of all owned and unowned animals is likely to be significantly higher than that of just owned animals that are brought to a veterinarian. An estimation of prevalence is known for humans. Based on one national estimate, between 4.6% and 7.3% of children in the United States have *Toxocara* antibodies in their serum, indicating that they have had exposure to the parasite (Herrmann et al. 1985). In adults, this number is estimated to be approximately 13.9% (Woodhall et al. 2014). Clearly, *Toxocara* is a widespread issue with the capacity to impact both human and animal populations.

Toxocara is classified in a much larger context of similar and related organisms, some of which have been studied extensively (Fig. 1). *Toxocara* is a genus within the phylum Nematoda. Nematodes, otherwise known as roundworms, are a highly diverse group of Ecdyzoa that have adapted to live in almost any environment including desert, marine, and tropical habitats (De Ley 2006). Many nematode species have an important ecological role in nutrient cycling in the environment, while other species can be much more detrimental, acting as vectors for plant viruses or other parasites (De Ley 2006). *Toxocara* belongs to the class Chromadoria, which are generally terrestrial nematodes. Another member of Chromadoria is *Caenorhabditis elegans*, which has been studied extensively. *Toxocara* share several traits with *C. elegans* including the

ability to arrest development in unfavorable conditions (Maizels et al. 2000). Studying other similar nematodes may be useful in better characterizing physiological details of *Toxocara*. Within Chromadoria is the subclass Ascaridida, comprised completely of animal parasites. All of these ascarids are very similar, differing primarily in their definitive hosts and morphology (De ley 2006). Because of their similarity, ascarids can potentially all be treated using similar protocols.

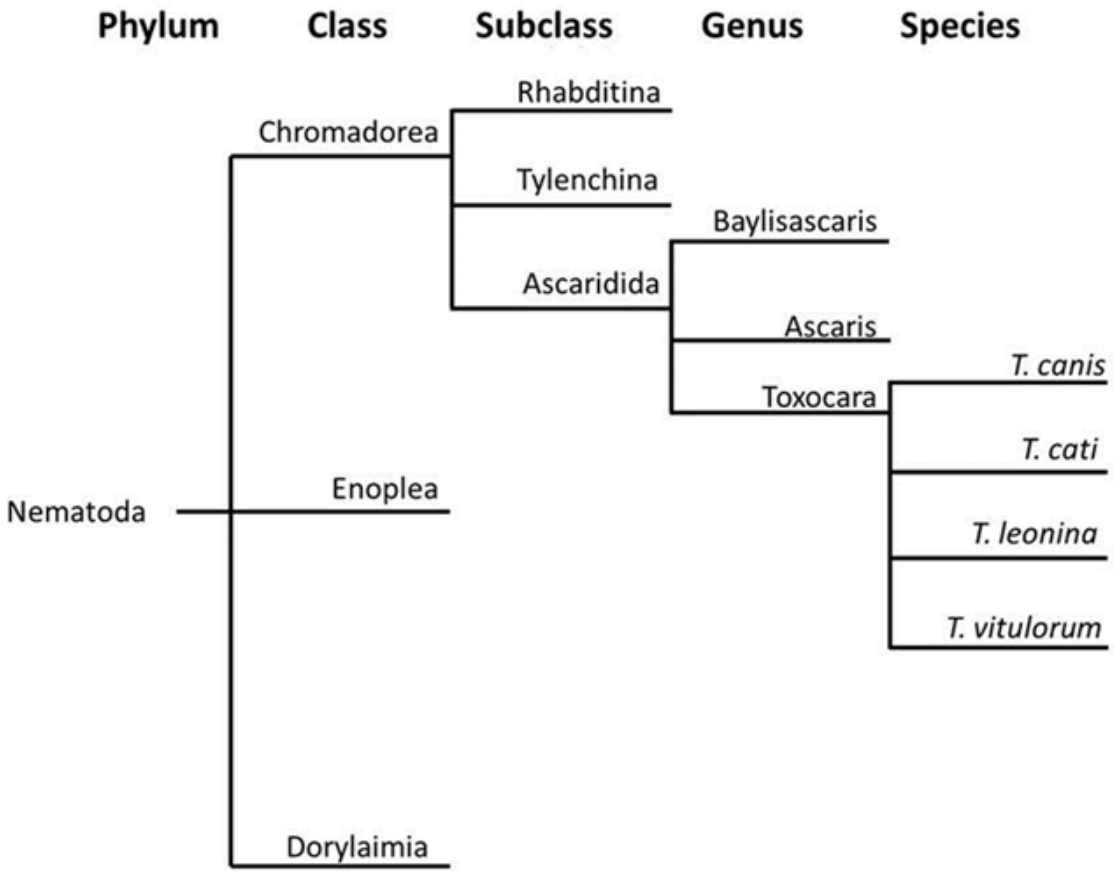


Figure 1. Phylogenetic tree of the phylum Nematoda, including branches of the three major classes. The class Enopla contains groups of marine and freshwater nematodes, some of which are parasitic. The class Dorylaimia contains primarily predatory and omnivorous nematode species. Chromadorea includes primarily terrestrial nematodes. Within Chromadorea, the subclasses Rhabditina and Tylenchina are composed primarily of non-parasitic species. The subclass Ascardida contains parasitic species whose definitive hosts include mammals, fish, amphibians, and birds. Ascaris, Baylisascaris, and Toxocara are all genera which contain parasites whose definitive hosts are mammals. The definitive hosts for *T. leonina* are canids and felids. The definitive hosts for *T. vitulorum* are Bovids (cattle). Neither *T. leonina* nor *T. vitulorum* are considered zoonotic threats to humans. However, both *T. canis* whose definitive hosts are canids and *T. cati* whose definitive hosts are felids can both use humans as a paratenic host (De Lay 2006).

All *Toxocara* species follow similar life cycles in their definitive hosts (Table 1). Understanding this life cycle is crucial in order to formulate methods to interrupt it. *Toxocara* eggs develop from the unembryonated stage (fertilized egg that does not yet contain a developed larva) to the infective stage (egg containing a larva) outside of a host (Schnieder et al. 2011, Sprent 1956). This development takes about 9-15 days under optimal temperature and moisture conditions (25º Celsius, 90% humidity). When embryonated eggs are ingested by a definitive host, the eggs will hatch in the duodenum within 2 to 4 hours. The larvae then penetrate the intestinal wall, likely by secreting proteases, enzymes that aid the larvae in permeating the epithelial and mucosal layers of the intestine. Once released from the intestine, larvae will travel to mesenteric lymph nodes (the lymph nodes located within the mesentery associated with the intestines). Larvae then travel to the liver via venous capillaries associated with the mesenteric lymph nodes. Some larvae may remain trapped in the liver while others will continue to the heart through the vena cava and subsequently to the lungs via the pulmonary artery. During this portion of the life cycle, the larvae will develop by molting periodically, which changes the composition of the proteins secreted by the larvae helping them evade the immune system (Schnieder et al. 2011). This also makes creating a vaccine against the parasites difficult because the parasitic antigens change as the worm develops.

Table 1. Size of various stages in *Toxocara* development and location of molt. (Webster 1958; Schacher 1957).

Stage	Location of molt	Length
Ova	--	0.087 x 0.075 mm
L1	Within ova	0.4 mm
L2	Within ova	0.4 - 0.5 mm
L3	Lungs	0.8 - 0.95 mm
L4	Stomach	1.0 - 1.5 mm
L5	Small intestine	2 cm-4 cm
Adult	Small intestine	10 - 18cm

* Unembryonated ova are fertilized but do not contain larva, when L1 larvae develop the ova is considered embryonated.

When the larvae reach the lungs, they can follow one of two routes: the tracheal route or the somatic route. In younger animals (less than 1 year old) or animals that have not yet been exposed to *Toxocara*, larvae tend to follow the tracheal route. In this route, larvae penetrate the alveolar wall and continue their migration through bronchioles to the trachea and pharynx. The larvae are coughed up and swallowed by the host and will develop into adults in the small intestine (Appendix 1). In older animals or those which have been previously exposed to *Toxocara*, larvae tend to follow the somatic route. In this route, larvae will penetrate the alveolar wall and travel throughout circulatory system to various tissues including skeletal muscles, kidneys, and the liver (Appendix 2) (Schnieder et al. 2011). The larvae that follow the somatic route will remain inactive but can reactivate later in life upon receiving a chemical signal, usually in female hosts during pregnancy or lactation (Traversa 2012; Macpherson 2013).

The route in which larvae proceed is significant to their infective potential because the route determines whether the parasites will reproduce within the host. When larvae migrate through the tracheal route, the animal will begin to shed *Toxocara* eggs in their feces, approximately 3-4 weeks after initial infection (Schnieder et al. 2011). Whereas when the larvae utilize the somatic route, the animal will not shed *Toxocara* eggs in their feces until the larvae are reactivated. Therefore, the somatic route is favorable in the short term to prevent the spread of *Toxocara*; however, eggs will be shed if the worms become reactivated.

Toxocara eggs that are shed into the environment are important because they may lead to infections of humans or other animals. The eggs are extremely resilient to harsh environmental conditions, discussed in a latter section. Small mammals and invertebrates can also spread *Toxocara* eggs to new areas. The resilience of *Toxocara* eggs outside a body and their ability to be spread by animals contribute to the worldwide prevalence of *Toxocara*.

There are three major approaches to reducing the impact of *Toxocara*: control of the parasite in animals, control of the parasite in the environment, and preventing toxocariasis. Currently, measures to control *Toxocara* are mainly focused on treating infected, domestic animals. Dogs and cats are routinely dewormed; however, *Toxocara* is rarely addressed in the environment, in ownerless animals, or as a human health issue. In order to fully and effectively address *Toxocara*, public health initiatives targeting human health, animal health, and *Toxocara* in the environment must be implemented to decrease incidence and prevalence of infection. This thesis will review aspects of *Toxocara* biology that are relevant to transmission and pathophysiology of the parasites as well as how *Toxocara* is currently being handled in human and veterinary medicine. This information will inform recommendations, such as education, hygiene, and environmental control measures, to most effectively address *Toxocara* in public health.

Transmission of *Toxocara* in dogs and cats

Reducing the population of *Toxocara* is dependent upon preventing the transmission of the parasite. *Toxocara* can be transmitted between definitive hosts in two ways: vertical or horizontal transmission. Vertical transmission occurs when a parasite is transferred from parent to offspring at or before birth. This is a very efficient route of transmission for *Toxocara* because an entire litter of offspring become infected at once, increasing the parasite population efficiently. Another advantage is that the parasite is transferred to young animals with less developed immune systems. This results in *Toxocara* larvae in the young following the tracheal route resulting in reproduction of the worms and shedding of eggs.

One common form of vertical transmission in dogs is trans-placental transmission, which occurs when parasites from the mother's tissues cross the placentas to infect the fetuses before birth. It is disputed if trans-placental infection occurs in cats as well, as it has not yet been directly observed (Despommier 2003; Traversa 2012). Trans-placental infection can occur when the bitch is exposed to *T. canis* orally before or during pregnancy. Subcutaneous infection of the bitch, when eggs enter the body through an opening in the skin, may also result in trans-placental infection of puppies. Finally, dormant worms held in tissues from previous infections may become reactivated upon pregnancy and infect puppies through the trans-placental route (Schnieder et al. 2011).

Current research suggests that when levels of estrogen and progesterone increase during pregnancy, these signals trigger *Toxocara* to migrate throughout the body. Dormant parasites may reactivate up to a year after the initial infection of the bitch (Schnieder et al. 2011). Once reactivated, the worms migrate to the circulatory system where they may travel to the placenta. The worms penetrate tissue layers of the placenta which separate the maternal and fetal blood and thus the parasites are able to infect the fetus (Schnieder et al. 2011). Reactivated larvae take at least 42 days, about three-quarters of the total gestation, to reach a fetus (Macpherson 2013). Approximately 16 days after birth, puppies infected through the trans-placental route will begin to shed *T. canis* eggs. Eggs can then proceed to infect other animals or persist in the environment for extended periods of time (Macpherson 2013).

Another route of vertical infection that has been documented in both cats and dogs is trans-mammary or lactogenic infection, when parasites migrate to the mammary glands and are expelled in the milk (Traversa 2012; Macpherson 2013). The hormone prolactin, which enables milk production in mammals, triggers the migration of *Toxocara* larvae to the mammary gland

(Schnieder et al. 2011). Much like trans-placental transmission, lactogenic infection can occur when the female is infected before or during pregnancy. However, lactogenic transmission can also occur in cases where a female becomes infected after giving birth (Schnieder et al. 2011). Milk can remain infected with larvae for at least 38 days after delivery, meaning there is a notable period of time after birth when vertical transmission can still occur (Stull et al. 2007). Kittens begin shedding *T. cati* eggs approximately 47 days after birth after ingesting contaminated milk while puppies will shed *T. canis* eggs roughly 27 days after ingestion of larvae-containing milk (Schnieder et al. 2011). Vertical transmission is extremely efficient because a whole generation of offspring are infected at once. Because these newly infected animals are young, *Toxocara* larvae will follow the tracheal migration route within the host. The tracheal route results in reproduction of the parasite, meaning the animal will shed a large number of *Toxocara* eggs into the environment where it can spread to new hosts through horizontal transmission. Thus, the deworming of young cats and dogs is critical in order to reduce the spread of *Toxocara*.

Horizontal transmission of parasites occurs when an animal becomes infected through contact with another animal or from the environment. This accounts for a significant number of *Toxocara* infections of adult animals. Both dogs and cats can become infected by ingesting free living embryonated eggs from the environment (Macpherson 2013). Another route of infection is consuming soil-dwelling invertebrates, including earthworms and insects, or small mammals (such as rodents) that contain *Toxocara* (Macpherson 2013). Invertebrates and small prey animals are significant to the geographic spread of *Toxocara* as they can act as reservoirs until the parasite can reach a definitive host. Dogs may also become infected or re-infected via coprophagy, the behavior of eating their own or another animal's feces. Meanwhile, cats are

more likely to be infected from ingesting embryonated eggs in their fur, obtained from the environment because of self-grooming and social grooming behaviors (Traversa 2012). Dog coats can also harbor infective *Toxocara* eggs obtained through scent-rolling behaviors, the behavior of rolling in materials with a strong odor, such as feces (Lee et al. 2010).

Horizontal transmission may not be as efficient as vertical transmission; however it does spread *Toxocara* among adult animals and over long distances and results in inactive larvae retained in the tissues of adult animals. In a female animal, these larvae may reactivate during pregnancy resulting in vertical transmission of the parasite. For this reason, deworming should continue periodically throughout the entire life of an animal to prevent the spread of *Toxocara* to other animals and into the environment.

Manifestation of *Toxocara* infection in dogs and cats

Reducing the prevalence of *Toxocara* through public health measures would reduce the negative impacts on the health of dogs and cats. In mild cases of toxocariasis in adult dogs, there are generally no clinical symptoms (Traversa 2012). However, when the parasite load is high (there is an unusually large number of organisms present), larval migrations through the chest and trachea may cause cough, nasal discharge, pneumonia, and pulmonary edema (Traversa 2012). Toxocariasis can be much more severe in puppies. Infected puppies can exhibit extreme discomfort and often appear pot-bellied due to dysbacteriosis (rapid changes in the intestinal microflora) and gas formation. Consequently, they demonstrate a straddle-legged posture in their hind legs. In the most serious cases, the small intestine may become perforated leading to blood loss and can result in death (Neves et al. 2014). Three week old puppies infected with adult *T. canis* experience inflammation of the intestines and increased mucus production. This condition

presents with vomiting, diarrhea, anemia, and emaciation. Other symptoms include weakness, poor coat condition, and nasal discharge. Many of these symptoms are the result of malnutrition as *T. canis* consumes nutrients obtained by the host. When left untreated, puppies may have thickened intestines and obstruction or occlusion of the intestines. Other gastrointestinal consequences include duodenum dilatation, peritonitis, and blockage of the bile and pancreatic ducts (Traversa 2012). Intestinal and duct blockages are caused by masses of adult worms that cannot be passed (Webster 1958). Thickening and inflammation of the tissues may also increase the likelihood of blockages. Although *Toxocara* infections do not usually result in serious illness in adult dogs, there are still rare cases of severe complications, especially when compounded with other health conditions. The risk of severe complications in young dogs justifies both therapeutic and prophylactic deworming treatment against *Toxocara* in dogs.

Similar to dogs, adult cats rarely exhibit any symptoms of *Toxocara* infections, but with a high parasite load, cats may exhibit vomiting, enlargement of the abdomen, and anorexia (Traversa 2012). Kittens, however, present with variable appetite, vomiting after feeding, and alternating diarrhea and constipation. Kittens may also have inflamed mucosal layers of the small intestine and anemia. Those exposed early in life may suffer from developmental delays or disturbances (Traversa 2012). This effect on kittens is likely because they suffer from malnutrition as the parasites feed off the nutrients consumed by the host. Small, developing kittens would be at a higher risk of suffering from reduced nutrient availability. While intestinal blockages caused by worm masses have been described in cats, they occur much less frequently than in dogs (Iqbal et al. 2017). This may be because cats experience much lower levels of intestinal inflammation than dogs. This proposition is consistent with the two species' response to another parasitic intestinal nematode *Ancylostoma*, otherwise known as hookworms. Dogs

infected with *A. canis* experience intestinal inflammation (Sattasathuchana and Steiner 2014), whereas cats infected with *A. tubaeforme* do not experience any intestinal inflammation (Bowman et al. 2003).

From a veterinary perspective, the cited complications of *Toxocara* in dogs and cats signify that measures are needed to prevent infections in these animals. *Toxocara* infection is still extremely common, indicating that current attempts for control are insufficient. While deworming will contribute to the solution, treating all aspects of *Toxocara* is necessary to prevent infection in definitive hosts.

Diagnosis of *Toxocara* in dogs and cats

In many cases, veterinarians require a positive diagnosis of *Toxocara* infection before deworming an animal. A common diagnostic tool for intestinal parasites is the copromicroscopic examination, also known as fecal flotation (Traversa 2012). This is a relatively simple and inexpensive technique based on separating parasitic eggs from fecal matter by utilizing their difference in specific gravity (Dryden et al. 2005). To perform a fecal flotation, a small amount (2-5g) of feces is added to a flotation solution which is a dense liquid made with salts such as magnesium sulfate, zinc sulfate, sodium nitrate, or sodium chloride dissolved in water. The specific gravity of the flotation solution causes the *Toxocara* eggs to float to the top of the solution for collection and observation. The fecal mixture is then strained to remove large particulates, and centrifuged. A coverslip is placed on the top of the centrifuge tube to collect floating eggs, which can be seen under 40x magnification (Dryden et al. 2005). Fecal examinations are recommended periodically throughout the life of any companion animals as

they can become re-infected at any point and many animals appear asymptomatic (Traversa 2012).

One shortcoming of the fecal flotation tests is that false negatives can occur for a variety of reasons. First, detection of eggs in feces occurs only after nematode development and mating, which may take place weeks after initial infection (Traversa 2012). Also, a low egg count could lead to undetected infections. Depending on the health of the *Toxocara* population, eggs may not be constantly shed in the animal's feces (Lucio-Forster et al. 2016). Currently, veterinarians either perform in-house fecal examinations or send fecal samples to a diagnostic laboratory. While information from these laboratories could potentially be used to assess the prevalence of *Toxocara*, the data would likely be inaccurate because it would only represent animals that are brought to a veterinarian. According to the 2012 U.S. Pet Ownership and Demographics Sourcebook, 44.9% of cat owners and 18.7% of dog owners did not take their pets to see a veterinarian in 2011 (American Veterinary Medical Association 2012). In addition, owners may be more likely to provide a fecal sample when their pet displays symptoms of gastrointestinal parasites. Therefore, samples from a laboratory do not accurately represent the domestic animal population. Some veterinarians only deworm animals that have tested positive for intestinal parasites, while others will deworm animals without fecal examinations. Because copromicroscopic examinations are not always accurate, it is practical to deworm animals without a fecal diagnostic in order to ensure that all *Toxocara* infections are appropriately treated. While it is not standard protocol at all veterinary hospitals, I recommend that a fecal examination is performed after an animal has been dewormed to confirm that the treatment has completely cleared the infection. Despite its limitations, fecal flotations are an important tool

used to detect quantify *Toxocara* infections. Fecal flotations should be used to confirm gastrointestinal parasite diagnoses but should not be necessary before each deworming treatment.

Anthelmintic treatments

The predominate technique used to manage Toxocara is deworming domestic animals with anthelmintics. Anthelmintics refer to a group of antiparasitic drugs that kill or disable helminths, which are worms such as Toxocara. Once treated, a host is better able to expel the worms. Because anthelmintics are the only widely practiced method of reducing *Toxocara* populations, they are essential to preventing infection. Four major classes of anthelmintic drugs are routinely used to treat intestinal nematodes in dogs and cats (Traversa 2012). These different drug classes are available in formulations including palatable tablets, liquid, paste, and topical gels ensuring that the veterinarian can administer the treatment regardless of the animal's compliance level (Traversa 2012).

One class of anthelminthic is benzimidazoles that include febantel and fenbendazole (Mottier et al. 2006). Benzimidazoles work by binding to a receptor found on β-tubulin. When bound, the β-tubulin can no longer form microtubules; therefore, cells are unable to secrete proteins, which results in death of the parasites. Benzimidazoles have a much lower binding affinity to β-tubulin of mammals meaning the drug will not bind as strongly to the host's proteins (McKellar and Jackson 2004). For this reason, benzimidazoles are safe for use in mammals within therapeutic dosages. The drug is also excreted from the body relatively quickly; therefore, it will not cause harm to the host by building up in the body over time (McKellar and Jackson 2004).

Another class of anthelmintic is tetrahydropirimidines such as pyrantel. Tetrahydropirimidines act as acetylcholine agonists (Köhler 2001). This drug binds tightly to acetylcholine receptors leading to continual muscle contraction causing paralysis and death in ascarids (Robertson et al. 1994). This particular class of anthelmintic is extremely safe as it works in the mammalian intestine and cannot be not absorbed into the rest of the body. For this reason, it is often the preferred dewormer for very young puppies and kittens (Robertson et al. 1994).

A third type of common anthelmintic is cyclooctadepsipeptides, such as emodepside and praziquantel. Cyclooctadepsipeptides work by binding to latrophilin receptors causing an influx of calcium. The change increases intracellular calcium levels resulting in activation of calcium-activated potassium channels which results in muscle relaxation and death of nematodes (Harder et al. 2003). Cyclooctadepsipeptides do not harm the host because the drug is quickly broken down into inactive metabolites by the liver and kidneys (Lemmens-Gruber 2009).

Finally, a fourth class of commonly used anthelmintic is macrocyclic lactones. Macrocyclic lactones include ivermectin, selamectin, moxidectin, and milbemycin. Macrocyclic lactones work by binding to a receptor on glutamate-gated chloride channels, blocking neural signals (Mottier et al. 2006). Mammals do not have any glutamate-gated chloride channels; therefore, macrocyclic lactones are very safe because they have no effect on the host in therapeutic doses (Campbell and Benz 1894).

Because the four classes of anthelmintics are equally effctive against parasitic nematodes, the choice of drug is inconsequential to the reduction of *Toxocara* (Traversa 2012). This equivalency is valuable because if *Toxocara* developed a resistance to a drug, or even an entire

class of drug, there are alternative options. Deworming drugs should be selected based on the drug's efficacy against other parasites. For example, fenbendazole is effective against some tapeworm species, making it a favorable choice (Roberson and Burke 1982).

The Canadian Communicable Disease Control Division (CDCD) as well as the Centers for Disease Control and Prevention (CDC) in the US recommend worming treatments at 2, 4, 6, and 8 weeks of age for puppies and 3, 5, 7, and 9 weeks for kittens. This schedule ensures that infection cannot become established in the young animals while maintaining that the animal is large and old enough to safely tolerate the treatment (Stull et al. 2007). The CDCD recommends that this procedure is followed up by treatment at least every six months through adulthood (Courbet et al. 2008). The CDC recommendations for adult animals are much less specific; the guidelines simply advise periodic treatment for older pets. The Companion Animal Parasite Council also recommends treating the bitch or queen within the 8-week period after birth to both eradicate any reactivated larvae and prevent transmission from neonates (Stull et al. 2007). These recommendations are devised to mitigate the impact of horizontal and vertical transmission of *Toxocara*. Deworming protects animals from experiencing any of the potentially dangerous symptoms of *Toxocara* infection. In addition, deworming animals will lower the amount of *Toxocara* eggs shed into the environment, decreasing the risk of infection for humans or other animals.

Although anthelmintics are generally very effective, treatment may need to be repeated if the animal becomes re-infected (Traversa 2012). One concern about frequent use of anthelmintic drugs is parasite resistance because some livestock parasites have become resistant to anthelmintic drugs. For example, the nematode *Haemonchus contortus*, which infects ruminants, has developed resistance to phenothiazine, thiabendazole, and benzimidazoles (Kaplan 2004).

Routine, unnecessary use of anthelmintics may promote resistance, especially if used over a long period of time. However, the only known anthelmintic resistance in companion animal parasitic nematodes is the hookworm *Ancyclostoma caninum* to the drug pyrantel (Traversa 2012). No changes in deworming protocols have been made to address the concerns of resistance. Anthelmintic treatment is the most widely used means of controlling *Toxocara*. These drugs have proven to be both safe and effective. Therefore, their use in domestic animals must continue and must be encouraged by veterinarians.

Transmission of *Toxocara* to humans

In order to prevent the transmission of *Toxocara* to humans, it is critical to analyze the routes in which humans can become infected. Humans develop toxocariasis by ingesting embryonated *Toxocara* eggs. Taking certain precautions to limit the contact with *Toxocara* eggs could certainly reduce the risk of toxocariasis. One way humans may ingest *Toxocara* is through eating contaminated food sources. Unwashed vegetables grown in fertilizer made with untreated human or animal feces may harbor infective eggs (Lee et al 2010). In addition, incidental hosts, such as soil-dwelling invertebrates, present in unwashed food may also harbor *Toxocara* eggs (Despommier 2003). Thus, washing vegetables before consumption will lower the risk of infection. Another food source that may contain *Toxocara* eggs is uncooked meat of infected paratenic hosts. In particular, raw livers of chickens, ducks, cows, and pigs have been cited as a source (Lee et al. 2010). The prevalence of *Toxocara* in these animals in not known. In a study investigating the seroprevalence (prevalence detected by antibodies in serum) of *Toxocara* in free–range chickens, 12.7% tested positive for high levels of *Toxocara* antibodies, indicating a current infection (Campos-da-Silva et al. 2015). This estimation indicates that there may be a

large number of food animals exposed to *Toxocara*. To better understand the extent of *Toxocara* infections in food animals, more serological studies need to be performed.

To reduce the chance of *Toxocara* infection, meat should be thoroughly cooked. The U.S. Food and Drug Administration guidelines advise cooking poultry to a minimum internal temperature of 165^{o}F, and beef and pork to a minimum internal temperature of 145 ^{o}F. Adhering to these guidelines will ensure that all *Toxocara* larvae will be killed as the parasites cannot tolerate temperature above 140 ^{o}F (Food and Drug Administration 2013).

Accidental ingestion of non-food materials also contributes to transmission of *Toxocara* to humans. Eating or touching the mouth after contact with animal fur or feces may facilitate the ingestion of *Toxocara* eggs as eggs can be harbored in the coats of animals (Lee et al. 2010). Handwashing after contact animal feces or animal fur is critical to reduce the possibility of accidental ingestion of eggs. Regularly deworming of pets reduces the likelihood that *Toxocara* eggs will be present on their coats or in their feces (Lee et al. 2010). Accidental ingestion may also occur after contact with soil containing eggs. This has been a problem particularly for children who play in sandboxes or in the dirt on playgrounds and have a tendency consume non-food items (Despommier 2003). People who display pica (cravings for non-nutritive substances), as a symptom of iron deficiency anemia or other another disorder, are at an increased risk of *Toxocara* infection as they are more likely to eat soil (Rohilla et al. 2013). To reduce risk, sandboxes or other areas where children play should be covered to prevent animals from defecating in them.

Humans can become infected with *Toxocara* through the environment and contact with animals. Therefore, it is critical to address both of these sources of infection. Each of the routes

of infection observed in humans is a potential area to focus preventative efforts including food safety, hygiene, and environmental treatment. Strategies to decrease *Toxocara* infection in these areas are outlined in further detail in an upcoming section.

Variations of toxocariasis in humans

Toxocara can cause potentially dangerous disease in humans. In addition, the full ramifications of infection are not yet known. Consequently, *Toxocara* infection in humans is a major cause for concern. The only *Toxocara* species known to cause toxocariasis in humans are *canis* and *cati* (Fillaux and Magnaval 2013). When embryonated *Toxocara* eggs are ingested, larvae hatch in the intestines and escape the digestive tract by penetrating through the duodenum to access other parts of the body (Beaver 1959). *Toxocara* then migrate indiscriminately through the body using the circulatory system as a means of transportation (Appendix 3) (Strube et al. 2013). Larvae have been found in nearly every organ (Despommier 2003, Beaver 1959). While the parasites themselves do not cause any damage, they continuously shed execratory/secretory protein antigens that elicit a potentially dangerous immune response. A hallmark of all types of toxocariasis is eosinophilia, an increased number of the white blood cells eosinophils (Despommier 2003). Toxocariasis in humans can vary greatly in its presentation and severity depending on which tissues the parasites are found in. Generally infections are categorized into three groups: Visceral Larval Migrans, Ocular Larval Migrans, and Neurotoxocariasis.

The term Visceral Larval Migrans (VLMs) is used to describe any larval parasite, especially nematodes, that migrate through the viscera (soft internal organs) of humans (Beaver 1959). VLMs are the broadest category of toxocariasis and thus make up the majority of cases

(Fillaux and Magnaval 2013). Several organs (especially the liver and lungs) are more commonly impacted by larvae. The increased likelihood that larvae will reach the liver is due to the transportation of the worms through the hepatic portal vein (Rohilla et al. 2013). Enlargement and necrosis of the liver may occur if the larvae migrate through it. Likewise, the same symptoms or larval migrations occur in spleen (Despommier 2003). While it has not yet been directly observed, it is possible that a disproportionally large number of *Toxocara* larvae migrate directly to the lungs via the caudal vena cava. When in the lungs, *Toxocara* causes respiratory symptoms that closely resemble asthma including bronchospasm (contraction of the bronchiole wall) (Despommier 2003). These respiratory symptoms may explain the proposed association between toxocariasis and asthma. Asthma is much more common than toxocariasis; therefore, children with asthma- like respiratory symptoms of toxocariasis may be misdiagnosed with asthma.

Another form of toxocariasis is Ocular Larval Migrans (OLM), where larvae migrate to eye. This is less common than VLM, but it may have several long lasting symptoms. Larvae damage the retina by inducing eosinophilic granulomas on the retina resulting in impaired or loss of vision (Overgaauw and van Knapen 2001). Increased inflammation in other areas of the eye may result in endophthalmitis (inflammation of the interior of the eye) or papillitis (inflammation of the optic disk) (Despommier 2003). These forms of inflammation will increase the pressure in the eye and may lead to glaucoma (Despommier 2003). The eye seems to be particularly sensitive to larval migration.

The final form of toxocariasis is neurotoxocariasis, which results when larvae migrate to the central nervous system. *Toxocara* larvae can cause meningitis (inflammation of brain and spinal cord membranes), meningoencephalitis (inflammation of the brain), or transverse myelitis

(inflammation of the spinal cord) (Fillaux and Magnaval 2013). The relationship between symptoms and *Toxocara* larvae is not yet known. In mice with neurotoxocariasis, levels of pro-inflammatory cytokines, IL-6 and TNF-α, were unusually high (Holland and Hamilton 2013). Further investigation into which immune cells are responsible for the inflammation associated with neurotoxocariasis is needed to elucidate the specific origin of symptoms. In this case, the role of neuro-immune system is also significant as the nervous system has particular regulations of inflammation involving specialized microglial cells. It is still controversial whether certain symptoms including seizures and neuropsychiatric disorders are related to neurotoxocariasis (Despommier 2003). In mouse models, it has been observed that mice with neurotoxocariasis are less active and responsive to novel or predatory stimuli (Holland and Hamilton 2013). Although the mechanism is unknown, these results indicate that it would not be completely implausible that psychological or behavioral changes could occur in humans infected with neurotoxocariasis as well.

The symptoms of each form of toxocariasis have the potential to be both severe and long lasting, warranting major concern. In the United States, seroprevalence is estimated to be 13.9%, meaning that a large portion of humans have come in contact with *Toxocara* (Woodhall et al. 2014). Toxocariasis is often neglected by human physicians. However, the disease is preventable through control of the parasite and proper hygiene. Preventative measures proposed in latter sections can protect humans from developing toxocariasis.

Immune evasion of *Toxocara* in humans

Toxocara larvae have the highly unusual ability to stay alive inside paratenic hosts for many years. This property is largely because of *Toxocara*'s ability to evade the immune system.

It is thought that execratory/secretory proteins help the larvae avoid harm from the immune system (Despommier 2003). By leaving a trail of antigens behind them and periodically switching antigenic identity of the execratory/secretory proteins, larvae can travel through the body unperturbed (Despommier 2003). Larvae also have a 10–20 nm thick extracuticular mucin coat. When the coat is recognized by antibodies, it can be shed and the larvae can escape the immune response (Maizels 2013). Dead and dying larvae tend to induce faster and more intense immune responses (Despommier 2003). This is perhaps because the larvae are no longer mobile leading to a higher concentration of unchanging antigens. Understanding the ways in which *Toxocara* evades the immune system is significant because it may lead to novel treatments of toxocariasis. This information may also be useful in developing toxocariasis treatments that mitigate the symptoms caused by the host's immune response to the parasite.

Diagnosis of toxocariasis in humans

Effective treatment of toxocariasis is fully dependent upon appropriate diagnosis of the disease. Diagnosis based solely on symptoms can be difficult due to variation depending on the tissues impacted. One general diagnostic tool that can be used to is a blood count. Patients with any of the groups of symptoms typical of VLM, OLM, or neurotoxocariasis should first be tested for eosinophilia. Levels of eosinophils in the blood of toxocariasis patients are typically 10,000 cells/mm^3 (Fillaux and Magnaval 2013). However, in some cases of OLMs, blood eosinophil levels can be within normal range (Fillaux and Magnaval 2013). This is because OLMs are often caused by a single larva, resulting in a weaker, more localized immune response. In suspected cases of neurotoxocariasis, in addition to examining blood eosinophil levels, cerebrospinal fluid should be tested for pleocytosis (increased level of white blood cells) (Fillaux and Magnaval 2013).

Another method of diagnosing toxocariasis is through imaging or biopsy. Distinct lesions and granulomas caused by *Toxocara* can be visualized by both computed tomography (CT scan) and magnetic resonance imaging (MRI) (Lim 2008). Ultrasonography can also be used to visualize granulomas and lymph-node enlargement typical of toxocariasis (Baldisserotto et al. 1999). Tissue samples taken from a biopsy of the affected tissue can be examined microscopically to reveal *Toxocara* larvae (Fillaux and Magnaval 2013). While biopsy is an effective means of diagnosis, it may cause damage to sensitive organs, such as the eye (Park et al. 2016). Therefore, less invasive methods, such as serological diagnostics, are often preferred.

Serological diagnostics test for the presence of certain antibodies in serum. In cases of suspected toxocariasis, the first serological test performed is an enzyme-linked immunosorbent assay (ELISA) test. The ELISA test is performed by using an ELISA plate coated with an excretory/secretory antigen (a parasitic molecule that is bound by an antibody). Serum is then added and antibodies that recognize the *Toxocara* antigen will bind and stick to the plate. The addition of a fluorescent reagent allows the amount of antibodies that are bound to antigens to be measured. The ELISA test is positive when antibodies for the *Toxocara* antigen are present in the serum because this means the body's immune system has come in contact with this antigen. This test has a sensitivity of 78%, meaning that of 78% of samples that are positive for *Toxocara* antibodies result in a positive ELISA test. This test also has a specificity of 86%, meaning that 86% of samples that are negative for *Toxocara* antibodies result in a negative ELISA test (Fillaux and Magnaval 2013). Specificity is a measure of the expected number of false positives while sensitivity is the expected number of false negatives. One drawback of the ELISA test is that it has shown cross reactions with antibodies for other parasitic nematodes, *Strongyloides stercoralis* and *Trichinella spiralis* (Maizels et al. 1984). Therefore, someone who has

antibodies for either *S. stercoralis* or *T. spiralis* may have a false positive ELISA test. Cross reaction likely occurs because the excretory/secretory antigens of all of these nematodes are very similar.

Positive ELISA tests for toxocariasis are followed up by a Western blot to confirm the diagnosis. This procedure begins with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) that separates proteins by molecular weight. SDS-PAGE is followed by a Western blot, which separates proteins by antigen reactivity. The diagnostic procedure distinguishes between the low molecular weight *Toxocara* antibodies from the much higher molecular weight antibodies of *S. stercorali*s and *T. spiralis* (Maizels et al. 1984). Consequently, the Western blot for *Toxocara* has a sensitivity of 98%, much higher than that of the ELISA test (Fillaux and Magnaval 2013). Due to the range of diagnostic tests, when used in combination, accurate diagnosis for all forms toxocariasis is possible. Physicians must be educated on the variety of symptoms that may present with the various forms of toxocariasis so that appropriate diagnostic tests are performed. Much fewer cases will go undiagnosed if toxocariasis were more included as part of a patient's differential diagnosis.

Toxocara eggs in the environment

The environment is a major source of infection of *Toxocara* for both humans and animals. Reducing the amount of environmental *Toxocara* would reduce the incidence and prevalence of infection. However, *Toxocara* eggs are extremely resilient to most environmental conditions. The durability of *Toxocara* eggs causes them to remain viable in the environment for many years (Overgaauw and Van Knapen 2012). The eggs can survive at temperatures as low as -29° Celsius and as high as 40° Celsius (Mizgajska 2001; Papini et al.2012). Although,

Toxocara eggs can be killed by rapid changes in temperature (Mizgajska 2001). *Toxocara* eggs can also tolerate a wide range of humidity; as high as 100% humidity to as low as 3% at 19° Celsius. However, survival rate is positively correlated with percent humidity (Gamboa 2005). In addition, *Toxocra* can withstand a number of chemical stressors, including 10% hydrochloric acid, 4% Benzalkonium chloride (a strong surfactant), and toxins such as 40% formalin.

One of the main factors that contribute to *Toxocara*'s resilience is the outer shell layers of the eggs. While there is not much research available on the egg shell of *Toxocara canis* and *Toxocara cati* specifically, the structure and function of the egg shell can be inferred using information about other on nematode eggs in the order Ascaridida. In *Toxocara* eggs, there are four major layers of the egg shell. The outermost layer is the uterine layer, which in the ascarid *Ascaris lumbricoides* is comprised of lipoprotein originating from the uterus that clings to the outside of the egg (Wharton 1980). The uterine layer has adhesive properties allowing the eggs to adhere to surfaces such as animal fur (Overgaauw et al. 2009).

The second layer is the vitelline layer, which, in ascarids, is composed of lipoprotein originating from the vitelline membrane of the egg (Wharton 1980). The major difference to the uterine layer is the electron density and the origin (Wharton 1980). These two layers appear to decrease permeability of the egg, keeping harmful compounds out.

Beneath the vitelline layer is the chitinous layer. In ascarids, this is generally the thickest layer of the egg shell and primarily composed of chitin and associated protein fibers (Wharton 1980). Chitin is a fibrous polysaccharide with a high tensile strength. The purpose of this layer is to provide mechanical strength. The protein elements of this layer add flexibility to the shell, making it resistant to mechanical pressure in any direction (Wharton 1980). The protein-chitin

complex may also be responsible for the irregular ridges and pits visible on the surface of *Toxocara* eggs as they appear simultaneously during development (Ubelaker and Allison 1975). Pitting increases the surface area to volume ratio and may also increase the permeability of the egg, which allows more oxygen to diffuse into the egg. However, these two characteristics also have the potential to increase water loss, leaving the egg vulnerable to desiccation.

The innermost layer is the lipid layer, which is comprised of 25% protein and 75% lipids (primarily ascarosides) in the ascarid, *Ascaris suum.* The purpose of this layer is to provide a certain degree of impermeability to the egg. Due to the largely hydrophobic nature of this layer, only gases and some lipid solvents can pass through.

Toxocara eggs may also endure the wide temperature range as well as low humidity through the use of compatible solute, which accumulates in the cytoplasm to increase the osmolarity. This prevents water loss as well as protects against protein destabilization in hot or cold conditions. One compatible solute present in most nematodes is trehalose, a major metabolite that is found in high quantities both *Toxocara* eggs and mature worms (Behm 1997, Learmonth et al. 1987). Trehalose is a disaccharide of glucose whose production is upregulated in times of stress such as dehydration, heat, or cold. In unfavorable temperatures, trehalose can stabilize membranes to prevent damage that would render the egg more permeable (Behm 1997). In cold, dry conditions, trehalose vitrifies rather than crystalizes, making it an amorphous solid rather than an organized crystalline structure (Behm 1997). Vitrification prevents harmful ice crystals from forming. Trehalose also inhibits the Maillard reaction that occurs in nematodes during desiccation when glucose molecules precipitate out of solution and react with nucleophilic side chains of amino acids, resulting in damaged proteins (Behm 1997; Gaugler 2002). Because trehalose is a non-reducing sugar, it will not participate in this type of reaction.

Trehalose will instead coat proteins to prevent amino acid side chains from reacting with glucose (Behm 1997).

The resilience of *Toxocara* eggs accounts for the prevalence of the parasites worldwide. *Toxocara* eggs can be found in equal concentrations in all soil types and textures and generally found in the top 5 cm of soil; although disruptions in the soil or transportation by earthworms may disperse the eggs deeper into the ground, to depths of at least 30 cm (Mizgajska 2001, Despommier 2003). Areas with highest prevalence of *Toxocara* contamination are outdoor parks in both urban and suburban areas (Despommier 2003). There does not seem to be a difference in prevalence among urban, suburban, or rural areas (Mizgajska 2001). Geographical location also does not seem to impact the prevalence of *Toxocara* eggs as concentrations of eggs are similar in soil from all populated continents (Mizgajska 2001). However, one current issue in studying *Toxocara* distribution in the environment is that there is no standard procedure for soil sampling and analysis. This makes it very difficult to compare data on the soil prevalence of separate investigations because different procedures yield different results for the same soil samples. In order to compare the quantity of *Toxocara* in different areas, a sampling and analysis method needs to be created. Data from this type of study could potentially determine if any uninvestigated environmental factors, such as local species, have an impact on the abundance *Toxocara* eggs. Currently, data that has been gathered indicates that there is a consequential amount of *Toxocara* eggs (Mizgajska 2001, Despommier 2003). Because these eggs have infective potential, a method of reducing the amount of *Toxocara* in the environment is needed.

Controlling *Toxocara* in the environment

There are very few known chemicals that can be used to destroy *Toxocara* eggs. There are, however, fungi with ovicidal properties, making them potential candidates to manage environmental *Toxocara*. Fungi are able to kill the eggs through either predation or endoparasitism (Ciarmela et al. 2002). Several species of fungi have been proven to effectively destroy *Toxocara* eggs in a laboratory setting. These species include *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Chrysosporium merdarium*, *Fusarium pallidoroseum* and *Fusarium oxysporum* (Carvalho et al. 2010, Ciarmela et al. 2002). While the detailed mechanism that these fungi utilize to kill *Toxocara* eggs is unknown, the general procedure has been observed and outlined (Carvalho et al. 2010). First, the fungi adhere to the shell of the eggs (Ciarmela et al. 2002). Next, the shape of the eggshell and embryo within is altered (Carvalho et al. 2010). This is thought to be accomplished through the use of exoenzymes including chitinases, glucanases, and proteases secreted by the fungi. These enzymes would be able to effectively breakdown the material that constitutes various layers of the egg shell. Finally, the fungi penetrate the egg with hyphae (filamentous branches) and colonize the internal space (Ciarmela et al. 2002). Regardless of whether the fungi can be used directly, examining the way fungi kill *Toxocara* eggs may lead to the development of a safe and effective means of eliminating the eggs from the environment.

The practical utility of fungi to control *Toxocara* egg levels in soil is dependent upon several factors. First, it is important that the fungi that are introduced to the soil do not have any negative impacts on humans or the existing ecosystem. One of the effective ovicidal fungi species, *C. merdarium* causes skin and nail infections in humans (Ciarmela et al. 2009). Therefore this fungus would not be a good choice to add to soil as the potential risks may

outweigh the potential benefits. Increasing the amount of ovicidal fungi in the ecosystem could cause undesirable density mediated effects, (effect caused by changes in the density of a species). Other species of terrestrial nematodes serve important ecological functions including controlling bacterial populations and nutrient cycling (Freckman 1998; Bardgett et al. 1999). Some of these nematodes are decomposers that breakdown and deposit nutrients from dead organisms into the soil for plants to use. If the populations of these ecologically important nematodes were reduced due to the introduction of a fungal species, the ecosystem could decrease in productivity. A decrease in nematodes that control bacterial species could result in the increase in pathogenic bacteria, which would have a devastating effect on other local species. Another consideration is the efficacy of the fungi destroying *Toxocara* eggs at all stages of egg development. Much of ovicidal fungi research has been conducted using immature (unembryonated) *Toxocara* eggs. However, some parasitic fungi have shown reduced ability to colonize eggs in later stages of development (Bojanicha et al. 2017).

The fungi chosen to eliminate *Toxocara* eggs must also be able to survive in the environment. The most valuable fungal research is conducted on fungi native to the area in which it would potentially be used. Finally, predatory and parasitic fungi each have different nutritional requirements that may impact their utility as an ovicidal agent. Predatory fungi require nematode eggs for survival and therefore do not establish themselves as easily in the environment (Filho 2013). This would be favorable because it may make the fungal population easier to control. However, fungi would need to be periodically reintroduced to the environment to have a lasting effect on *Toxocara* populations. Meanwhile, parasitic fungi can survive in the presence or absence of nematode eggs and can easily establish themselves in an environment (Filho 2013). The disadvantage of this property is that nematode eggs may not be a preferred

nutrient source of these fungi, potentially reducing their efficacy in the environment. Therefore, the use of predatory fungal species is likely more favorable.

Another source of environmental *Toxocara* is waste water. Currently, sewage from waste water treatment plants needs to be tested for viable nematode eggs before it can be used as fertilizer (Dąbrowska et al. 2014). Nematode eggs are detected by performing a flotation examination on a small sample. *Toxocara* eggs are identifiable using a light microscope, due to the distinct pitted surface (Uga et al. 2000). If eggs are detected, their viability must be determined by incubating the eggs. The development of an embryo would indicate viability. Another potentially less accurate method of testing viability is estimating the stage of development by microscopic examination (Dąbrowska et al. 2014). A faster and potentially more accurate method of determining egg viability is a LIVE/DEAD stain kit. This kit stains cells with intact membranes green and dead cells with damaged membranes red (Dąbrowska et al. 2014). I recommend that this easier method of detecting viability should be used for testing both sewage from waste water plants can be and any fertilizers made from human or animal waste, in order to reduce the amount of *Toxocara* eggs in soil where food is grown.

Although *Toxocara* eggs are very resilient to most disinfectants including chlorine, phenol, cresol, and sodium or potassium hydroxide, there are methods to disinfect surfaces (Ayçiçek et al. 2001). Sodium or potassium hypochlorite was previously the recommended disinfectant against *T. canis* eggs (Morrondo et al. 2001). However, research has indicated that iodine solutions at concentrations as low as 2.5% are more effective (Ayçiçek et al. 2001). While this treatment is impractical in an environmental setting, I recommend that a 2.5% iodine solution is used to disinfect areas or cages where infected animals are held to prevent spread.

There are relatively simple measures that should be implemented immediately taken to reduce risk of *Toxocara* infection, particularly in children. One measure is covering sand boxes in parks with a vinyl sheet at night or when not in use. This prevents dogs and cats from defecating in the boxes. Uga and Kataoka (1995) found that a clear vinyl sheet was effective at reducing the number of *Toxocara* eggs over a 42-week period. An additional benefit is that the vinyl sheet can also keep the sand dry and warm in order to kill or inhibit growth of any existing *Toxocara* eggs in the sand (Uga and Kataoka 1995). Another measure to reduce risk of *Toxocara* is to fence in sand boxes to prevent animals from entering. This method is less preferred as it would be more expensive and it may discourage use. However, it might be more reliable as it would not rely on anyone to routinely cover the box. I recommend the use of physical barriers in order to protect areas designated for children's play from the introduction of *Toxocara* eggs. A third simple measure to reduce *Toxocara* in the environment is to encourage pet owners to clean up their pet's feces. To increase compliance, waste bags and receptacles should be made available in all parks. Local pet waste laws should also be posted in parks to encourage waste clean-up. There have been very few efforts focused on reducing the amount of *Toxocara* eggs in the environment. Because *Toxocara* eggs in the environment are a major source of infection for both humans and animals, it is critical to address this issue. Some methods such as testing waste water, covering sandboxes, and disposing domestic animal waste should be implemented immediately. Other methods such as chemical and fungal approaches to controlling *Toxocara* in the environment still require further research.

Controlling *Toxocara* in mammals

Because mammals are a reservoir for *Toxocara*, it is imperative to both prevent and treat infections in mammals. Nijsse et al. (2015) determined that out of definitive *Toxocara* hosts,

stray cats contributed the most *Toxocara* eggs to the environment in urban areas. In rural areas foxes contributed the most *Toxocara* eggs to the environment and domestic dogs were the most impactful contributors to suburban regions (Nijsse et al. 2015). These three animals likely contribute to the spread of *Toxocara* in different proportions worldwide depending on local ecology and culture. While little can be done to change the impact wild animals have on *Toxocara* spread, there are methods to help reduce the impact of stray cat and domestic dog populations.

Stray cats populations are managed most successfully by trap, spay/neuter, release programs (TNR) (Gibson et al. 2002). These efforts not only manage population size, but also promote the health of the population as cats are also vaccinated against feline panleukopenia virus, feline herpes virus, feline calicivirus, feline leukemia virus, and rabies virus (Fischer et al. 2007). Some TNR programs will also deworm cats (Gibson et al. 2002). If successful, deworming treatments could contribute to reducing the amount of *Toxocara* eggs in the environment. However, it often requires multiple doses to fully deworm a cat, especially with a high parasite load. In addition, widespread indiscriminate deworming could lead to anthelmintic resistance. Regardless of whether TNR programs chose to incorporate a deworming component, these efforts are effective at controlling and reducing stray cat populations, which reduces the *Toxocara* input to the environment. I recommend that TNR programs deworm all trapped cats as well as continue to control feral cat populations.

Deworming domestic animals presents different challenges than deworming feral animals. One of the main challenges is client compliance. Of dog owners in the Netherlands that visit veterinary clinics, 40% reported adhering to a twice a year deworming protocol (Nijsse et al. 2015). This is likely lower as pet owners do not always visit veterinarians regularly. One

way to promote client compliance is for veterinarians to discuss the zoonotic risk of *Toxocara*. In a survey of American veterinarians performed in the 1990s, only 29% reported to talking to clients about zoonotic hazards of *Toxocara* (Harvey et al. 1991). Explaining the risk of *Toxocara* to humans could potentially motivate clients to deworm their animals more regularly. In recent years, veterinarians have been collaborating more with human medical professionals and agencies. In 2007, the American Veterinary Medical Association established The One Health Initiative Task Force to address health issues that span across human, animal, and environmental health (King et al. 2008). In 2009, 13.9% of The One Health Initiative Task Force resources were focused on zoonosis; however, no efforts have been specifically focused on *Toxocara* (Bibaisee and Macpherson 2014). The One Health Initiative Task Force should consider *Toxocara* a higher priority. With this effort, veterinarians would be more likely to acknowledge their contribution to public health and educate pet owner about zoonosis.

Deworming compliance is particularly difficult because it needs to be repeated regularly. A possible solution to this problem would be a vaccine against *Toxocara* for animals. One potential antigen for a vaccine is *Toxocara* excretory/secretory proteins (TES). Two lectins (carbohydrate binding proteins) have been identified as possible targets for a vaccine against *T. canis*. The identified lectins, TES-32 and TES-70, are proteins expressed by larvae whose development is arrested inside a definitive host. These proteins are both involved in evading the host immune system. While there are homologues of TES-32 present in mammals, there appear to be significantly different in at least the binding regions that would limit host reactivity. TES-32 contains a cysteine loop while in the same location mammalian homologues contain a histidine residue (Maizels et al. 2000). Vaccines that protect against parasites are not yet common. Parasites have complex antigens and are often able to evade the immune system in

part due to the expression of different proteins at various stages of their life cycle (Mutapi 2013). Additionally, little research has been performed on polymorphisms (different genetically determined variations) of excretory/secretory proteins. While a vaccine would be beneficial in addressing *Toxocara*, development would require a greater understanding of the immune response to *Toxocara* infections in the definitive hosts.

Another way to increase client deworming compliance would be to deworm animals without a prior fecal diagnostic. Of a sample of 450 American, small-animal veterinarians, 54% self-reported that they do not treat animals with anthelmintics without performing a diagnostic fecal examination first (Harvey et al. 1991). Requiring a diagnostic fecal examination gives the client the additional responsibility of collecting, storing, and bringing a fresh fecal sample to veterinary appointments. A fecal diagnostic also adds and additional cost to the client, which may further discourage deworming. Clients may be more likely to accept a deworming treatment if they are not required to provide a fecal sample at appointments. I recommend that veterinarians deworm all animals on a regular basis without requiring a diagnostic fecal sample.

Human medicine also has a role in addressing the problem of *Toxocara*. Toxocariasis is often detected accidentally (Gawora et al. 2008). Indicators of toxocariasis such as eosinophilia, anemia, and elevated Immunoglobulin E levels may appear on a diagnostic test performed for different reasons. The CDC considers toxocariasis one of the five most important neglected parasitic diseases (Moreira et al. 2014). Seroprevalence of *Toxocara* infection is estimated to be 13.9% in the United States (Woodhall et al. 2014). The fraction of these patients who show clinical toxocariasis is unknown. There is a major lack of research on the serological prevalence of toxocara infection and the prevalence of clinical toxocariasis. This warrants the need for a large-scale national serological study to determine the actual prevalence of toxocara infections.

A database is also needed for physicians to report cases of toxocariasis. With this data, the impact of *Toxocara* on humans can be better characterized. Comparing the rate of infection to the rate of disease incidence will help determine the ratio of clinical to subclinical infections.

Family physicians also have a role in educating patients about the risks of zoonosis and methods to reduce these risks. For this reason, I suggest general practitioners and pediatricians should ask patents about their pets or contact with other animals. Health care providers should then provide practical suggestions on how individuals can prevent zoonotic infections, such as deworming pets, properly disposing of feces, and hand washing after contact with animals. Physicians have a unique role in which they have the ability to educate a large portion about health precautions; therefore, physicians must accept their role in preventive medicine and educate patients about zoonosis.

The major risk factors for toxocariasis in humans are pet-ownership and poverty (Won et al. 2008; Schantz et al. 1980; Congdon and Patsy 2011). Measures to reduce the incidence of toxocariasis should therefore be aimed at these at-risk populations. One measure targeting pet owners in poverty is providing reduced-cost or free deworming programs. While deworming medication itself is relatively inexpensive, the cost increases with an office visit fee as well as a fecal examination fee. A number of low cost-veterinary clinics funded by non-profit organizations or veterinary colleges exist through the U.S. These clinics exist to provide affordable, basic veterinary care for animals. Because *Toxocara* can impact human health, it is important that these services are continued and expanded in order to deworm pets that may not be taken to a veterinarian. Another way to protect at-risk human populations is to educate the public about the risks of zoonosis and proper hygiene. Public health departments often offer

hygiene and education programs to the public. These programs could incorporate hygiene habits that prevent the spread of zoonotic diseases such as *Toxocara*.

It is currently thought that *Toxocara* has a relatively even distribution worldwide and affects those in poverty evenly in both developed and developing countries (McGuinness and Leder 2014). However, it is unlikely that the distribution of *Toxocara* and the disease burden of toxocariasis are proportional throughout the world. There are no current disease-burden estimates for toxocariasis (Hotez et al. 2014). Disease burden can be estimated using values (adjusted for each country or region) of years of life lost and years lost due to disability (Steenland and Armstrong 2006). To better characterize *Toxocara*, a large scale investigation needs to be conducted, comparing not only distribution of Toxocara and prevalence of toxocariasis, but also the disease burden of toxocariasis in different areas. A study of this scale would be challenging because it would require the cooperation of physicians worldwide to thoroughly document cases of toxocariasis and its effects on patients' lives. However, this information would be extremely valuable in characterizing the scope an impact of *Toxocara* and aiding in the establishment of more targeted public health efforts.

Conclusion

Toxocara remains a threat to both human and animal health and thus this issue requires a more intensive treatment. Young dogs and cats, who are more likely to become infected with *Toxocara,* can suffer severe complications. Each variation of *Toxocara* infection in humans has significant risks as well. VLMs can result in bronchospasm and contraction of the bronchiole wall. OLMs can result in inflammation of the eye and vision loss. Neurotoxocariasis can result

in meningitis or transverse myelitis. Further investigation is needed into associations of toxocariasis and other conditions such as asthma and epilepsy.

Currently, *Toxocara* is only controlled by periodically deworming domestic dogs and cats with anthelmintics and controlling feral cat populations. While both of these methods are beneficial, they are both underutilized. Many pet owners do not chose to have their animals dewormed regularly. Veterinarians must help improve the number of animals that are dewormed by discussing the zoonotic potential of *Toxocara* with their clients and deworming animals without performing a diagnostic fecal examination. TNR programs also have the potential to expand their impact to underserved communities. TNR programs need to incorporate deworming trapped cats as part of their contribution to the health of the feline community.

There are several simple measures that can be applied immediately to reduce the spread of *Toxocara*. Covering sandboxes while not in use will reduce the chance of an animal defecating and shedding *Toxocara* into the box. This will protect children, who are at particularly high risk to become infected with *Toxocara* because of their poor hygiene and tendency to eat non-food items. Another simple measure to prevent the spread of *Toxocara* is disinfecting areas where animals with *Toxocara* infections are kept with a minimum of 2.5% iodine solution. I suggest using this solution in veterinary hospitals in any areas where an animal with a *Toxocara* infection is held.

Some potential measures to reduce *Toxocara* require further research before they can be implemented. One of these measures is using fungi to control the number of *Toxocara* eggs in the environment. The efficacy of this idea in a natural setting needs to be evaluated. The ecological impact of introducing an ovicidal fungus species into soil also needs to be assessed.

Another potential area of research is finding a safe chemical agent that can be used to kill *Toxocara* eggs in soil. While this has proved to be exceptionally challenging, with more information about the biochemical structure of *Toxocara* eggs, a chemical with high specificity to *Toxocara* eggs would be extremely valuable. A third area that requires further research is the possibility of a vaccine against *Toxocara* for animals. Proteins expressed in different life stages of each *Toxocara* species need to be better characterized to identify potential antigens for a vaccine.

Toxocara presents a complex challenge. The issue itself is multifaceted; therefore, the solution must also be multifaceted. Currently, *Toxocara* is being managed as if it were an animal-specific disease. The impact *Toxocara* has on humans needs to be better characterized through serological studies as well as investigations of the distribution and disease burden of toxocariasis. Reducing the impact of *Toxocara* will require the collaboration between human and animal health professionals to increase awareness of the problem and address *Toxocara* in the environment and in human health.

References

American Veterinary Medical Association. 2012. U.S. pet ownership & demographics sourcebook. Schaumburg (IL): American Veterinary Medical Association. p. 225.

Ayçiçek H, Yarsan E, Sarimehmetou HO, Tanyüksel M, Girginkardesler N, Özyurt M. 2001. Efficacy of Some Disinfectants on Embryonated Eggs of *Toxocara canis*. Turk J Med Sci. 31(1): 35-39.

Baldisserotto M, Conchin CF, Soares G, Araujo MA, Kramer B. 1999. Ultrasound findings in children with toxocariasis: report on 18 cases. Pediatr Radiol. 29(5):316-9.

Bardgett RD, Cook R, Yeates GW, Denton CS. 1999. The influence of nematodes on below-ground processes in grassland ecosystems. Plant Soil. 212(2):23-33.

Beaver PC. 1959. Visceral and cutaneous larva migrans. Public Health Rep. 74(4): 328–332.

Behm CA. 1997. The role of trehalose in the physiology of nematodes. Int J Parasitol. 27(2): 215-229.

Bidaisee S, Macpherson CNL. 2014. Zoonoses and One Health: A Review of the Literature. J Parasitol Res. 76(1):1-8.

Bojanicha MV, Basualdo JA, Giusiano G. 2017. In vitro effect of *Chrysosporium indicum* and *Chrysosporium keratinophylum* on *Toxocara canis* eggs. Rev Argent Microbiol. doi: 10.1016/j.ram.2017.08.001.

Bowman DD, Barr DC, Hendrix CM, Lindsay DS. 2003. Companion and Exotic Animal Parasitology. Ithaca (NY): International Veterinary Information Service.

Bueno-Marí R, Almeida APG, Navarro JC. 2015. Editorial: Emerging Zoonoses: Eco-Epidemiology, Involved Mechanisms, and Public Health Implications. Front Public Health. 3(157). doi: 10.3389/fpubh.2015.00157

Campbell WC, Benz GW. 1984. Ivermectin: a review of efficacy and safety. J Vet Pharmacol Therap. 7(1):1-16.

Campos-da-Silva DR, da Paz JS, Fortunato VR, Beltrame MAV, Valli LCP, Pereira FEL. 2015. Natural infection of free-range chickens with the ascarid nematode *Toxocara* sp. Parasitol Res. 114(11): 4289–4293

Carvalho RO, Arau´ jo JV, Braga FR, Araujo JM, Alves CDF. 2010. Ovicidal activity of *Pochonia chlamydosporia* and *Paecilomyces lilacinus* on *Toxocara cani*s eggs. Vet Parasitol. 169(1-2):123–127.

Ciarmela ML, Arambarri AM, Basualdo JA, Minvielle MC. 2009. Effect of saprotrophic soil fungi on *Toxocara canis* eggs. Malays J Microbiol. 6(1):75-80.

Ciarmela ML, Minviell MC, Lori G, Basualdo JA. 2002. Biological interaction between soil fungi and *Toxocara canis* eggs. Vet Parasitol. 103(3): 251-257.

Congdon P, Patsy L. 2011. Toxocara infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. Int J Public Health. 56(1): 15-24.

Courbet T, Bour S, Rochet J, Thibault-Fayard A, Boda C. 2008. Comparison of the acceptance of 2 anthelmintic formulations, in the dog. Revue Méd Vét. 159 (10) 508-513.

Dąbrowska J, Zdybel J, Karamon J, Kochanowski M¸ Stojecki K, Cencek T, Kłapeć T. 2014. Assessment of viability of the nematode eggs (Ascaris, Toxocara, Trichuris) in sewage sludge with the use of LIVE/DEAD Bacterial Viability Kit. Ann Agric Environ Med. 21(1): 35-41.

De Ley P. 2006. A quick tour of nematode diversity and the backbone of nematode phylogeny. In: WormBook: The Online Review of *C. elegans* Biology. Pasadena (CA): WormBook; 2005-2009.

Despommier, Dickson. 2003. Toxocariasis: Clinical Aspects, Epidemiology, Medical Ecology, and Molecular Aspects. Clin Microbiol Rev. 16(2): 265–272.

Dryden MW, Payne PA, Ridley R, Smith V. 2005. Comparison of Common Fecal Flotation Techniques for the Recovery of Parasite Eggs and Oocysts. Vet Ther. 6 (1): 15-28.

Filho FDM, Vieira JN, Berne MEA, Stoll FE, Pötter PNL, Pereira DIB. 2013. Fungal ovicidal activity on *Toxocara canis* eggs. Rev Iberoam Micol. 30(4):226–230.

Fillaux J, Magnaval JF. 2013. Laboratory diagnosis of human toxocariasis. Vet Parasitol. 193(4): 327-336.

Fischer SM, Quest Cm, Dubovi EJ, Davis RD, Tucker SJ, Friary JA, Crawford PC, Ricke TA, Levy JK. 2007. Response of feral cats to vaccination at the time of neutering. J Am Vet Med Assoc. 230(1):52-58.

Food and Drug Administration (2013). *Food Code*. College Park, p.80

Freckman, DW. 1998. Bacterivorous nematodes and organic-matter decomposition. Agric Ecosyst Environ. 24(1-3): 195-217.

Gamboa MI. 2005. Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. J Helminthol. 79(4): 327-33.

Gaugler R. Entomopathogenic Nematology. Second edition. Oxfordshire (England): CABI Pub. 2002.174 p.

Gawora J, Borecka A, Żarnowsk H, Marczyńsk M, Dobosz S. 2008. Environmental and personal risk factors for toxocariasis in children with diagnosed disease in urban and rural areas of central Poland. Vet Parasitol. 155(3-4): 217-222.

Gibson KL, Keizer K, Golding C. 2002. A trap, neuter, and release program for feral cats on Prince Edward Island. Can Vet J. 43(9): 695–698.

Harder A, Schmitt-Wrede H, Krücken J, Marinovski P, Wunderlich F, Willson J, Amliwala K, Holden-Dye L, Walker R. 2003. Cyclooctadepsipeptides—an anthelmintically active class of compounds exhibiting a novel mode of action. Int J Antimicrob Agents. 22(3) 318-331.

Harvey JB, Roberts JM, Schantz PM. Harvey JB, Roberts JM, Schantz PM. 1991. Survey of veterinarians' recommendations for treatment and control of intestinal parasites in dogs: public health implications. J Am Vet Med Assoc. 199(6): 702-707

Herrmann N, Glickman LT, Schantz PM, Weston MG, Domanski LM. 1985. Seroprevalence of zoonotic toxocariasis in the United States: 1971-1973. Am J Epidemiol. 122(5):890-6.

Holland CV, Hamilton CM. 2013.The significance of cerebral toxocariasis: a model system for exploring the link between brain involvement, behaviour and the immune response. J Exp Biol. 216, 78-83.

Hotez PJ, Alvarado M, Basáñez MG, Bolliger I, Bourne R, Boussinesq M, Brooker SJ, Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fèvre EM, Naghav M. 2014. The Global Burden of Disease Study 2010: Interpretation and Implications for the Neglected Tropical Diseases. PLoS Negl Trop Dis. 8(7):286-295.

Iqbal N, Mahfooz SA, Khan MK, Rizwan HM, Abbas A, Abbas RX, Ashar A. 2107. Prevalence of gastrointestinal parasites in Faisalabad and comparative efficacy of Ivermectin and Albendazole. Pure Appl Biol. 6(2): 499-504.

Kaplan RM. 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20(1): 477-481.

King LJ, Anderson LR, Blackmore CG, Blackwell MJ, Lautner EA, Marcus LC, Meyer TE, Monath TP, Nave JE, Ohle J, Pappaioano M, Sobota J, Stokes WS, Davis RM, Glasser JH, Mahr RK. 2008. Executive summary of the AVMA One Health Initiative Task Force report. J Am Vet Med Assoc. 233(2): 259-261.

Köhler P. 2001 The biochemical basis of anthelmintic action and resistance. Int J Parasitol. 31(4): 336-345.

Learmonth MP, Euerby MR, Jacobs DE, Gibbons WA. 1987. Metabolite mapping of *Toxocara canis* using one- and two-dimensional proton magnetic resonance spectroscopy. Mol Biochem Parasitol. 25(3):293-298.

Lee ACY, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD. 2010. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol. 26(4): 155-161.

Lemmens-Gruber R, Kamyar M R, Dornetshuber R. 2009. Cyclodepsipeptides - potential drugs and lead compounds in the drug development process. Curr Med Chem.16(9): 122-1137.

Lim JH. 2008. Toxocariasis of the liver: visceral larva migrans. Abdom Imaging. 33(2):151-156.

Lucio-Forster A, Barbecho JS, Mohammed HO, Kornreich BG, Bowman DD. 2016. Comparison of the prevalence of *Toxocara* egg shedding by pet cats and dogs in the U.S.A., 2011–2014. Vet parasitol. 5(1):1-13.

Macpherson CNL. 2013. The epidemiology and public health importance of toxocariasis: A zoonosis of global importance. Int J Parasitol. 43(12-13): 999-1008.

Maizels RM, de Savigny D, Oilvie BM. 1984. Characterization of surface and excretory-secretory antigens of *Toxocara canis* infective larvae. Parasite Immunol. 6(1): 23-37.

Maizels RM, Tetteh KKA, Louka A. 2000. *Toxocara canis*: genes expressed by the arrested infective larval stage of a parasitic nematode. Int J Parasitol. 30(4): 495-508.

Maizels RM. 2013. *Toxocara canis*: Molecular basis of immune recognition and evasion. Vet Parasitol. 193(4): 365–374.

Maya C, Torner-Morales FJ, Lucario ES, Hernández E, Jiménez B. 2012. Viability of six species of larval and non-larval helminth eggs for different conditions of temperature, pH and dryness. Water Res. 46(15): 4770-4782.

McGuinness SL, Leder K. 2014. Global Burden of Toxocariasis: A Common Neglected Infection of Poverty. Curr Trop Med Rep. 1(1):52–61.

McKellar QA Jackson F. 2004. Veterinary anthelmintics: old and new. Trends Parasitol. 20(10): 456-461.

Mizgajska H. 2001. Eggs of *Toxocara* spp. in the environment and their public health implications. J Helminthol. 17: 147-151.

Moreira GMSG, Telmo P, Mendonc M¸ Moreira A, McBride AJA, Scaini CJ, Conceic FR. 2014 Human toxocariasis: current advances in diagnostics, treatment, and interventions. Trends Parasitol. 30(9): 456-464.

Morrondo P, Díez-Morrondo C, Pedreira J, Díez-Baños N, Sánchez-Andrade R, Paz-Silva A, Díez-Baños P. 2006. *Toxocara canis* larvae viability after disinfectant—exposition. Parasitol Res. 99(5):558-6.

Mottier L, Alvarez L, Ceballos L, Lanusse C. 2006. Drug transport mechanisms in helminth parasites: Passive diffusion of benzimidazole anthelmintics. Exp Parasitol. 113(1): 49-57.

Mutapi F, Billingsley PF, Secor WE. 2013. Infection and treatment immunizations for successful parasite vaccines. Trends Parasitol. 29(3): 135–141.

Neves D, Lobo L, Simões PB, Cardoso L. 2014. Frequency of intestinal parasites in pet dogs from an urban area (Greater Oporto, northern Portugal) Vet Parasitol. 200(3-4): 295-298

Nijsse R, Mughini-Gras L, WagenaarJ A, Franssen F, Ploeger HW. 2015. Environmental contamination with *Toxocara* eggs: a quantitative approach to estimate the relative contributions of dogs, cats and foxes, and to assess the efficacy of advised interventions in dogs. Parasit Vectors. 8(1):397.

Obwaller A, Duchêne M, Bruhn H, Steipe B, Tripp C, Kraft D, Wiedermann G, Auer H, Aspöck H. 2001. Recombinant dissection of myosin heavy chain of *Toxocara canis* shows strong clustering of antigenic regions. J Parasitol Res. 87(5): 383–389.

Ogundipe F, Hennis ECA, Mehari A, Gillum RF. 2017. *Toxocara* species exposure, symptoms of asthma, and fractional exhaled nitric oxide in the US population. Ann Allergy Asthma Immunol. 119(6): 569-570.

Overgaauw PAM, van Knapen F. 2001. Veterinary and public health aspects of Toxocara spp. Vet Parasitol. 193 (1):398–403.

Overgaauw PAM, van Zutphen L, Hoek D, .Yaya FO, Roelfsema J, Pinelli E, van Knapen F, Kortbeek LM. 2009. Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. Vet. Parisitol. 163(1-2):115-122.

Pak KY, Park SW, Byon IS, Lee JE. 2016. Ocular toxocariasis presenting as bilateral scleritis with suspect retinal granuloma in the nerve fiber layer: a case report. BMC Infect. Dis. 16(426): 2-6.

Papini R, Campisi E, Faggi E, Pini G, Mancianti F. 2012. Prevalence of *Toxocara canis* eggs in dog faeces from public places of Florence, Italy. Helminthologia (Bratisl). 49(3):154-158.

Quattrocchi G, Nicoletti A, Marin B, Bruno E, Druet-Cabanac M, Preux P. 2012. Toxocariasis and Epilepsy: Systematic Review and Meta-Analysis. PLoS Negl Trop Dis. 6(8):1175-1179.

Roberson EL, Burke TM. 1982. Evaluation of granulated fenbendazole as a treatment for helminth infections in dogs. J Am Vet Med Assoc. 180(1):53-55.

Robertson SJ, Pennington AJ, Evans MA, Martina RJ.1994. The action of pyrantel as an agonist and an open channel blocker at acetylcholine receptors in isolated *Ascaris suum* muscle vesicles. Eur J Pharmacol.271(2-3): 273-282.

Rohilla S, Jain N,Yadav R, Ballabh D, Dhaulakhandi DB. 2013. Hepatic visceral larva migrans BMJ Case Rep. 6(1):56–68.

Safe Food Handling: What You Need to Know (US). Resources for consumers [Internet]. Silver Spring (MD). U.S. Food and Drug Administration. 2017 Nov 30. [cited 2017 Dec 28]. Available from: https://www.fda.gov/food/resourcesforyou/consumers/ucm255180.htm

Sattasathuchana P, Steiner JM. 2014. Canine eosinophilic gastrointestinal disorders. Anim Health Res Rev. (1):76-86.

Schacher JF. 1957. A Contribution to the Life History and Larval Morphology of *Toxocara canis.* J Parasitol. 43(6): 599-612.

Schantz PM, Weis PE, Pollard ZF, White MC. 1980. Risk factors for toxocaral ocular larva migrans: a case-control study. Am J Public Health. 70(12): 1269-1271.

Schnieder T, Laabs E, Welz C. 2011. Larval development of *Toxocara canis* in dogs. Vet Parasitol. 175(3-4):193–206.

Sprent JF. 1956. The life history and development of *Toxocara cati* (Schrank 1788) in the domestic cat. Parasitol. 46(1-2): 54-78.

Steenland K, Armstrong B. (2006). An overview of methods for calculating the burden of disease due to specific risk. Epidemiology. 17(5):512-519.

Strube C, Heuer L, Janecek E.2013. *Toxocara* spp. infections in paratenic hosts. Vet Parasitol. 194(4): 375-389.

Studzińska MB, Demkowska-Kutrzepa M, Borecka A, Meisner M, Tomczuk K, Roczeń-Karczmarz M, Kłapeć T, Abbass Z, Cholewa A. 2017. Variations in the rate of infestations of dogs with zoonotic nematodes and the contamination of soil in different environments. Int. J. Environ. Res Public Health. 14(4): 1003.

Stull JW, Carr AP, Chomel BB, Berghaus RD, HirdSmall DW. 2007. Animal deworming protocols, client education, and veterinarian perception of zoonotic parasites in western Canada Can Vet J. 48 (3): 269–276.

Traversa D. 2012. Pet roundworms and hookworms: A continuing need for global worming. Parasit Vectors. 5(19):1-19.

Ubelaker JE, V. F. Allison VF. 1975. scanning electron microscopy of the eggs of *Ascaris lumbricoides*, *A. suum*, *Toxocara canis*, and *T. mystax*. J Parasitol. 61(5): 802-807.

Uga S, Kataoka. 1995. Measures to Control *Toxocara* Egg Contamination in Sandpits of Public Parks. Am J Trop Med Hyg. 52(1):21-4

Uga S, Matsuo J, Kimura D, Rai SK, Koshino Y, Igarashi K.2000. Differentiation of *Toxocara canis* and *T. cati* eggs by light and scanning electron microscopy. Vet Parasitol. 92(4): 287-294.

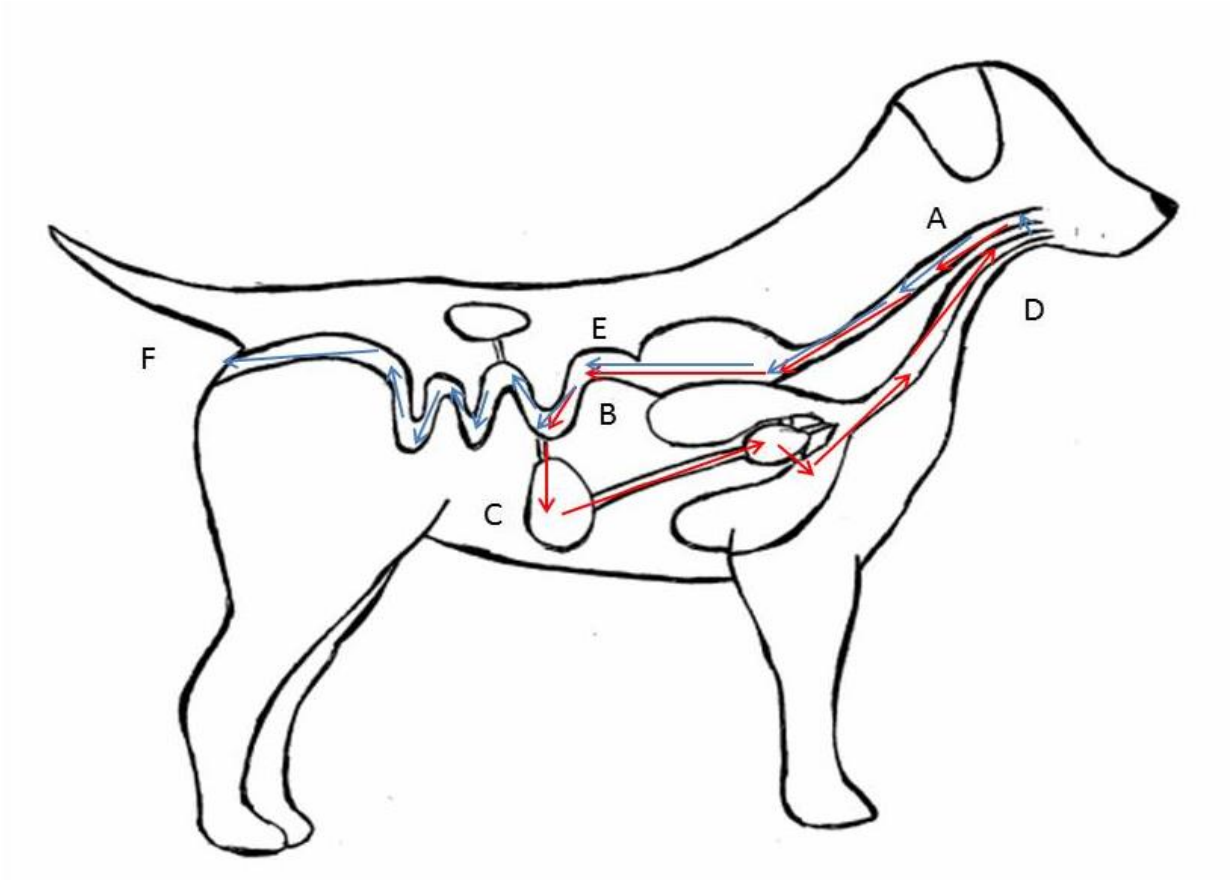
Webster GA. 1958. A report on *Toxocara canis* Werner, 1782. Can J Comp Med Vet Sci. 22(8): 272-274.

Wharton D. 1980. Nematode egg-shells. Parasitol. 81(2): 447-463.

Won KY, Kruszon-Moran D, Schantz PM, Jones JL. 2008. National Seroprevalence and Risk Factors for Zoonotic Toxocara spp. Infection. Am J Trop Med Hyg. 79(4): 552-557.

Woodhall D, Jones J, Cantey PT, Wilkins PP, Montgomery SP. 2014. Neglected parasitic infections: what every family physician needs to know. Am Fam Physician. 89(10):803-81.

Appendix



Appendix 3. The movement of *Toxocara* through the body of a dog or cat following the tracheal route.

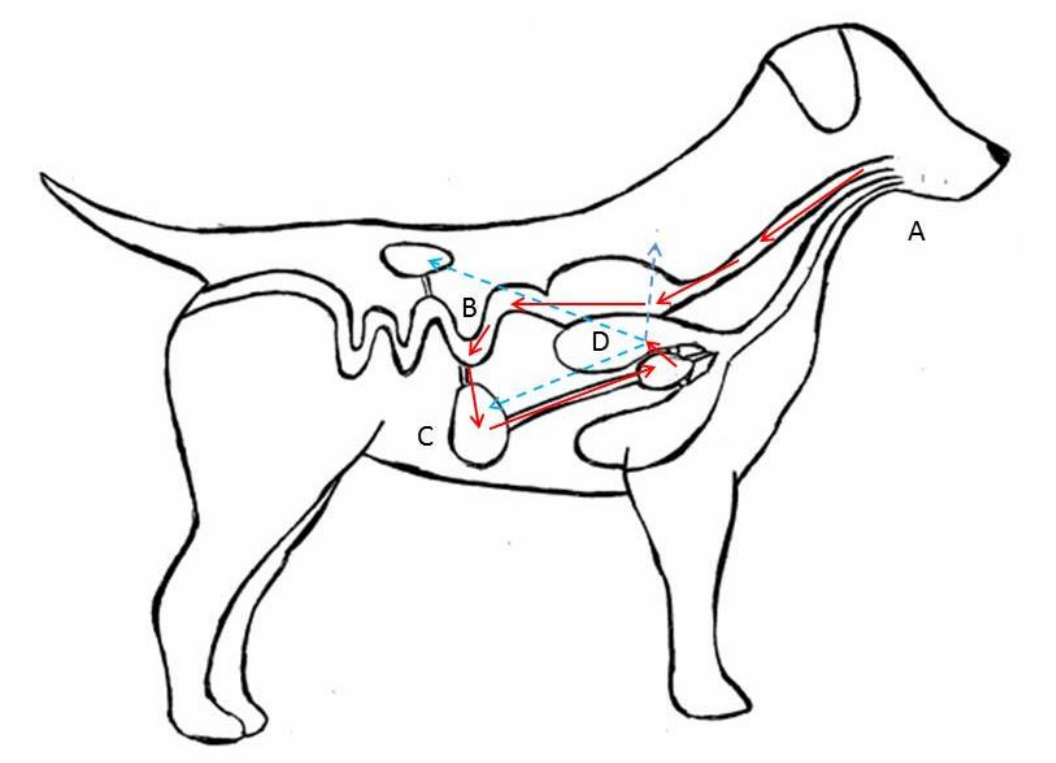
A. Ingested *Toxocara* eggs travel down the esophagus and through the stomach (red arrows).

B. Eggs hatch in the duodenum, penetrate the intestinal wall to reach mesenteric lymph nodes (red arrows).

C. Larvae travel to the liver via venous capillaries. Larvae continue to the heart through the vena cava and subsequently to the lungs via the pulmonary artery. Larvae penetrate the alveolar wall migrate through bronchioles to the trachea and pharynx (red arrows).

D. The larvae are coughed up and swallowed by the host (blue arrows).

E. Larvae mature into adults, reproduce, and release eggs are released into the small intestine (blue arrows).



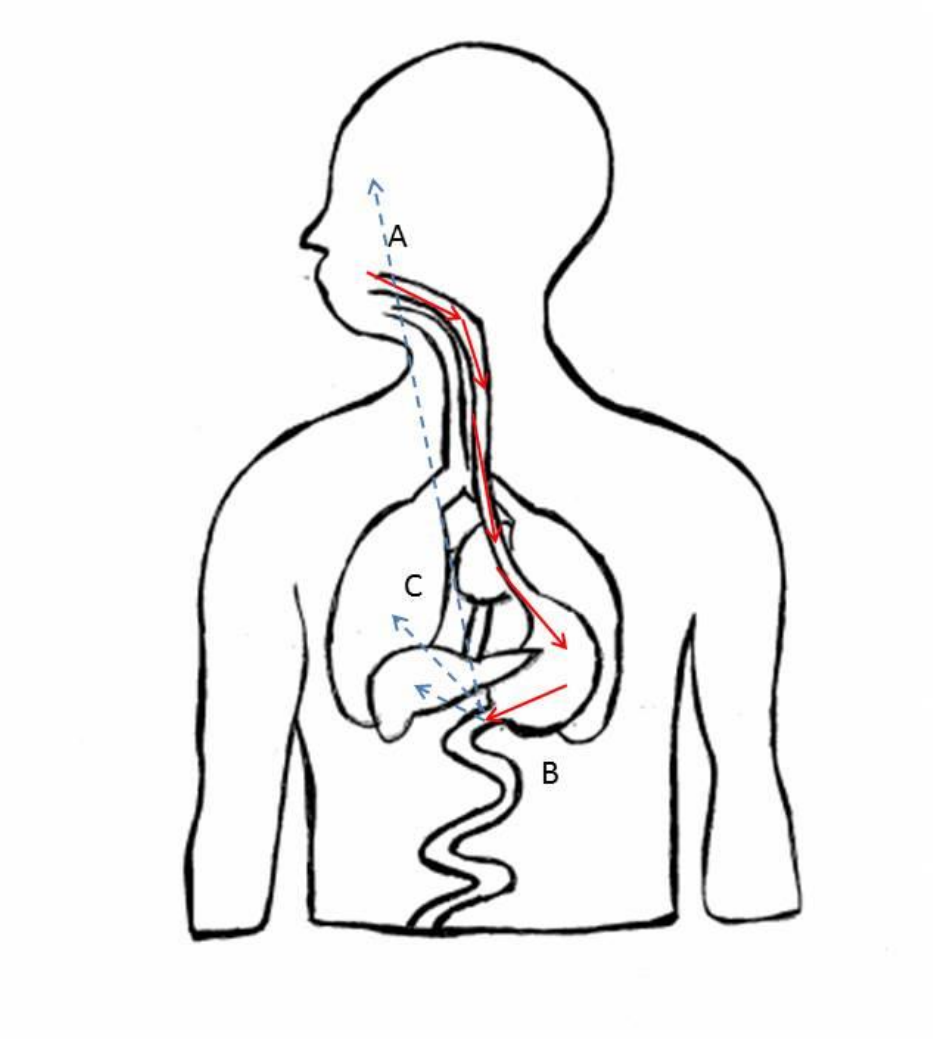
Appendix 2. The movement of *Toxocara* through the body of a dog or cat following the somatic route.

A. Ingested *Toxocara* eggs travel down the esophagus and through the stomach (red arrows).

B. Eggs hatch in the duodenum, penetrate the intestinal wall to reach mesenteric lymph nodes (red arrows).

C. Larvae travel to the liver via venous capillaries. Larvae continue to the heart through the vena cava and subsequently to the lungs via the pulmonary artery (red arrows).

D. Larvae will penetrate the alveolar wall and travel throughout circulatory system to various tissues including skeletal muscles, kidneys, and the liver (blue dashed arrows).



Appendix 3. The movement of *Toxocara* through the human body.

A. Ingested *Toxocara* eggs travel down the esophagus and through the stomach (red arrows).

B. Eggs hatch in the duodenum. Larvae penetrate the intestinal wall (red arrows).

C. *Toxocara* larvae migrate indiscriminately through the body using the circulatory system as a means of transportation. Some organs *Toxocara* may reach include the lungs, liver, or eyes (blue dashed arrows).