The epidemiology and control of *Toxocara canis* in puppies

Regan Schwartz [a,b,d], Satesh Bidaisee [a,b,c,d], Paul J. Fields [b,d], Maxine L. A. Macpherson [a,b,d], Calum N.L. Macpherson [a,b,c,d,*]

[a] *School of Veterinary Medicine, St. George's University, Grenada*
[b] *School of Graduate Studies, St. George's University, Grenada*
[c] *School of Medicine, St. George's University, Grenada*
[d] *Windward Islands Research and Education Foundation, Grenada*

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ABSTRACT

Toxocara canis is a helminth zoonosis that is estimated to infect more than 100 million dogs and 1 billion people, mostly in the tropics. Humans can be infected by accidentally ingesting embryonated *T. canis* eggs from the environment or occasionally after ingesting L_3 larvae from paratenic hosts. This study investigated the importance of vertical transmission and the role of puppies in the epidemiology of *T. canis* through the examination of fecal samples from dogs less than one year of age in Grenada, West Indies, a small island tropical developing country. Samples were stored at 4 °C or in 10% formalin until microscopic examination for helminth eggs or using a rapid antigen test for the presence of protozoan species. A knowledge, attitudes and practices study was completed among dog owners, physicians and veterinary students.

Of 306 dogs less than one year of age, 147 (48%) were found to have *T. canis* eggs. Vertical transmission was indicated by the proportion of infected dogs increasing from 50% at two weeks of age (from in utero transmission) to 70% by 12 weeks (in utero and lactogenic transmission). After 12 weeks the positivity rate dropped rapidly with no dogs over 40 weeks of age being infected. As *T. canis* eggs were found in puppy feces at two weeks of age, initial treatment of puppies should begin earlier, at twelve days post-partum, than currently recommended to prevent shedding of eggs. Perhaps even more importantly, treatment of pregnant dogs, preventing vertical transmission, would have a major impact on the control of *T. canis* infection.

Knowledge of *T. canis* and other zoonotic helminths such as *Ancylostoma caninum* was found to be low among dog owners, physicians and veterinary students. None of the dog owners treated their dogs for helminths, all were unaware of the risk of zoonoses, and only 9% picked up dog feces. Efforts to prevent vertical transmission and to increase awareness and knowledge of these zoonoses could result in reducing their public health impact.

1. Introduction

Toxocara canis is a helminth parasite with a worldwide distribution estimated to infect tens of millions of people annually (Fakhri et al., 2018; Ma et al., 2018; Dutra et al., 2014; Morgan et al., 2013; Macpherson, 2013). A recent meta-analysis suggests that over 100 million dogs are infected with *T. canis,* shedding billions of eggs into the environment annually (Rostami et al., 2020). The highest

prevalence of *T. canis* was reported in young (< 1 year of age), stray, rural, male dogs, that live in tropical countries with a low Gross Domestic Product (Rostami et al., 2020; Hotez and Wilkins, 2009). Such conditions are ideal for the propagation of this helminth due to free roaming definitive hosts with unchecked dog reproduction ensuring a large percentage of young dogs that are untreated for the parasite while living in environmental conditions favorable to the parasite.

There are many routes of transmission of *T. canis* in dogs and the prepatent period (PPP) varies with the mode of transmission. Vertical transmission occurs when somatic larvae are reactivated during the third trimester of pregnancy, invading the gestational sac, and infecting the developing embryos (Roberts et al., 2013; Schnieder et al., 2011). Physiological changes during pregnancy alter the signaling pathways of the larvae leading to reactivation and migration of larvae (Schnieder et al., 2011; Ma et al., 2019). Puppies are born with prepatent worms and egg expulsion has been reported to start around day 16 postpartum (Lloyd et al., 1983). Vertical transmission continues postpartum as larvae can be passed lactogenically to the newborn pups (Ma et al., 2019; Burke and Roberson, 1985) with a PPP of 28 days (Stoye, 1976). Horizontal transmission is facilitated through ingestion of embryonated eggs with a PPP of 32–35 days (Dubey, 1978) or through ingestion of paratenic hosts with a PPP of 34–48 days (Manhardt and Stoye, 2010).

Transmission to humans is via ingestion of larvae from paratenic hosts or eggs from the environment. Once embryonated eggs are ingested, they travel down the digestive tract, hatch in the small intestine, penetrating its wall, enter the circulatory system and migrate to the liver, lungs, eyes or central nervous system, causing both mechanical and immunopathological damage (Hotez and Wilkins, 2009; Lee et al., 2014). Infection can result in any of the four clinical syndromes of toxocariasis: visceral larva migrans (VLM) or visceral toxocariasis (VT), ocular larva migrans (OLM) or ocular toxocariasis, covert toxocariais (CT), and neurotoxocariasis (NT). *Toxocara* larvae will eventually die within the human host, although the internal tissue damage may be irreparable, especially damage to the eye (Azira and Zeehaida, 2011). Many behavioral factors predispose children to infection such as closer contact with contaminated soil in public parks, playgrounds and beaches (Hotez and Wilkins, 2009) and naïve hygiene practices (Sowemimo et al., 2017).

After young dogs ingest embryonated eggs, L_3 larvae are released from the egg, invade the gut wall, and undergo hepatic-tracheal migration ending up in the small intestine where they mature into adult worms (Roberts et al., 2013). With age, larvae tend to become developmentally arrested in tissues. *T. canis* adult worms, like other members of the Family Ascaroidea, have a life span of only a few months (Urquhart et al., 1987). Other zoonotic parasites investigated in this study were *Ancylostoma* spp.*, Strongyloides* spp., and *Trichuris vulpis.*

There are four zoonotic species of *Ancylostoma* spp. (Stracke et al., 2020), with varying zoonotic importance and some are geographically restricted. All can result in cutaneous larva migrans (CLM), a serpiginous, pruritic, creeping dermal eruption, lasting for a few days to weeks but can be prolonged in *A. braziliense* infections. *A. caninum* has recently been shown to reach patency in humans (Ngcamphalala et al., 2020) and is a known cause of eosinophilic enteritis in humans. *A. ceylanicum* and *Uncinaria stenocephala* are geographically restricted to South-East Asia and the Pacific and temperate climates, respectively. *A. ceylanicum* is the only canine hookworm species which is fully adapted to humans, canids and felids and contributes to the burden of disease caused by the soil transmitted helminths. Transmission from dogs to humans is facilitated by percutaneous or ingestion of the L_3 larvae from the environment (Stracke et al., 2020). Of the 6 widely recognized *strongloides* spp., *S. stercoralis* has a global distribution, particularly in rural areas, throughout the tropics and subtropics. In humans, strongyloidiasis can result in larva currens and non-pathognomonic clinical symptoms including abdominal pain, diarrhea and weight loss with autoinfection in some individuals resulting in life-threatening hyperinfection which can be fatal. Studies using nuclear and mitochondrial DNA sequence polymorphisms have demonstrated that humans and dogs can share genetically similar *S. stercoralis* populations providing evidence for zoonotic transmission (Sanpool et al., 2019; Jaleta et al., 2017). *Strongloide*s spp. reproduces in the small intestine, but free-living stages can additionally reproduce in the environment and transmission is facilitated through skin penetration by L_3 larvae. *T. vulpis* has been reported in up to 10% of dogs and has been found very rarely to occur in humans and thought to be of minor zoonotic relevance (Dunn et al., 2002).

Zoonotic protozoans include *Cryptosporidium* spp. and *Giardia* spp. Both protozoans can be transmitted to humans through ingestion and thought to be mostly waterborne and occasionally foodborne. Using a variety of detection methods, *Cryptosporidium* infection was found in 6–8% of approximately 18,000 dogs studied (Taghipour et al., 2020). Most human cases of *Crytposporidium* spp. are due to *C. hominis* and *C. parvum*, while the most common *Cryptosporidium* spp. found in dogs are *C. canis* (3.6%) and *C. parvum* (1.8%) (Taghipour et al., 2020). A meta-analysis provided a pooled prevalence of 15.2% *Giardia* spp. in dogs out of 4.3 million dogs (Bouzid et al., 2015).

This study was conducted to examine the prevalence of *T. canis* and the role of vertical transmission and the epidemiology and potential for control in the small-island developing-state of Grenada, West Indies. Other helminths and protozoan parasites in puppies were also recorded. This study also explored the knowledge, attitudes and practices (KAP) of dog owners, physicians, and Doctor of Veterinary Medicine (DVM) students who had completed courses in veterinary public health and veterinary parasitology. These groups represent stakeholders who would be responsible for implementing a helminth control program using a One Health approach in a sustainable, cost-effective way.

2. Material and methods

Fecal samples were collected between January and November 2017 from Pothounds less than one year of age. Pothounds are nondescript, medium sized dogs, weighing up to 50 pounds, usually brown or black in colour, and are the most common dog found throughout the Caribbean and tropics (Catan and Macpherson, 2007). They are often loosely owned within a community, and are allowed mostly to roam free. The sample size was determined based on the intent to use point biserial correlation to test for a

relationship between parasite positivity and age with 95% confidence, 95% power, and an effect size of 20%.

Fecal samples were collected from puppies following consent from the owner and confirmation of the puppies' ages and lack of any prior treatment with anthelmentics from all communities throughout Grenada. Every effort was made to ascertain the exact age of the puppies sampled from the owner and from physical examination of the puppies. Despite this, some minor variation in recollection may have occurred, especially with puppies older than 1 month of age. Samples were located on each owner's property, usually from under the house or porch where the puppies were seen to congregate.

Collection was from the top of a fresh fecal deposit, and immediately split into two aliquots with one being submerged in 10% formalin and the other stored fresh, placed into a cold box for transportation and stored at 4 °C until examined. Two grams (g) (+/− 0.02 g) of each sample was removed from the formalin, weighed in a small weigh boat, transferred to a paper cup and mixed with 13 mL of zinc sulfate (specific gravity of 1.18). The mixture was poured through a fine mesh tea strainer, and then transferred to a pre-numbered conical tube to be centrifuged at 1200 rpm for 5 min. Additional zinc sulfate was pipetted into each tube to create a positive meniscus onto which a coverslip was placed. After 5 min, the coverslip was removed and placed on a pre-numbered slide for examination by light microscopy using 40× magnification. A sensitivity of almost 90% has been described using this method for detecting helminth eggs, when present, including *Toxocara* (Dryden et al., 2005). Eggs are not shed continuously and are not evenly distributed in feces. These factors should also be considered when estimating the true prevalence of a particular parasite species in cross-sectional point prevalence surveys. As a consequence, the *T. canis* prevalence found in this study's puppy population represents the minimum prevalence in this population. *T. canis* eggs were identified from all other helminth eggs as they have a unique appearance, being 80-85um, round, thick shelled and dense. These characteristics separate them from other helminths, in particular, *Toxascaris leonina,* which are smaller (55-75um), oval, translucent and are largely geographically restricted to Africa and the Eastern Mediterranean with rare reports from the Western Hemisphere (Rostami et al., 2020).

Fresh fecal aliquots were tested using the Quick Check assay (Thermo Fisher Scientific, USA) on a proportion of the samples to examine the presence of *Cryptosporidium* and *Giardia* antigens.

Approval for this study was obtained from the St George's University (SGU) Institutional Animal Care and Use Committee (IACUC) to examine the dogs. All puppies found to be positive for *T. canis* or other helminths were subsequently treated. Approval from the SGU Institutional Review Board (IRB) was obtained to complete oral and on-line knowledge, attitudes, and practices (KAP) questionnaires from a convenient sample of dog owners, physicians and veterinary students.

3. Results

A total of 306 fecal samples were collected from dogs ranging in age from 2 weeks to 48 weeks with a median age of 8 weeks. There were 183 puppies (60%) 12 weeks of age or younger and 123 puppies (40%) over 12 weeks (Fig. 1).

Of the 306 samples, 147 (48%: 95% CI, 42.3% to 53.8%) were positive for *T. canis*. The youngest puppies found to be shedding *T. canis* eggs were 2 weeks old and the oldest 40 weeks. Further, 162 samples (53%: 95% CI, 47.5% to 59.0%) tested positive for *Ancylostoma* spp. with 76 samples (25%: 95% CI, 20.1% to 30.7%) testing positive for both parasites. The rates of positivity and
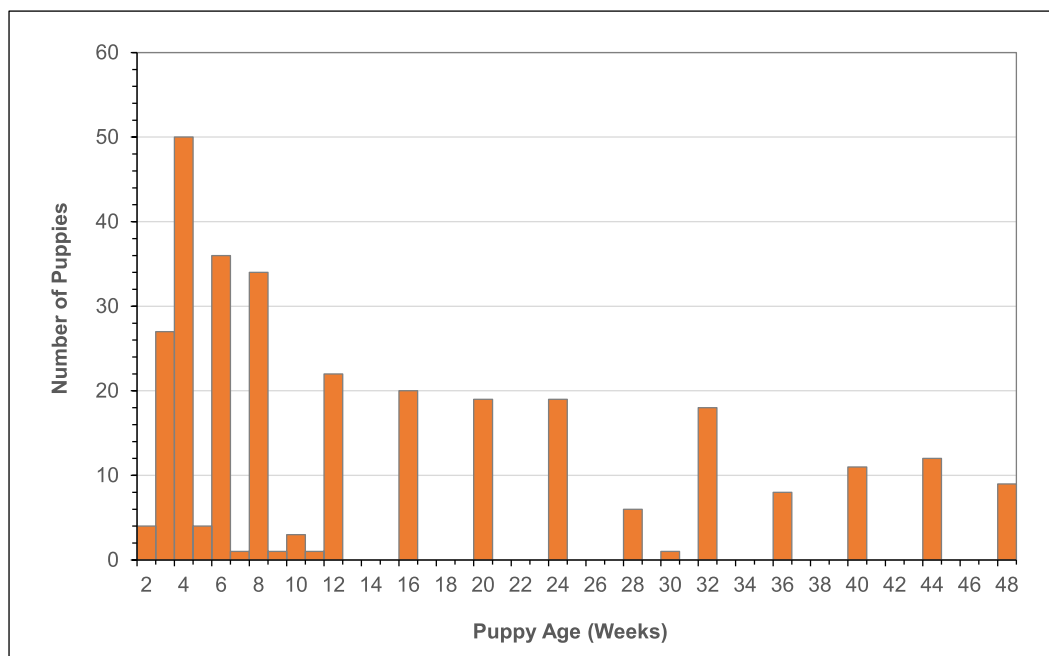

Fig. 1. Age profile of the sample of 306 puppies providing fecal samples examined for *Toxocara canis*.

polyparasitism are shown in Fig. 2. Other parasite species were found in 32 samples including *Strongyloides* spp., *Trichuris vulpis,* and *Isospora* spp. (13%: 95% CI, 9.2% to 17.0%) (Fig. 2).

Point biserial correlation of positivity and age showed evidence of a moderately strong negative relationship between puppy age and testing positive for *T. canis* ($r_{pb} = -0.396$, $p < 0.001$). In comparison, for *Ancylostoma* spp. there was evidence of a weak positive relationship ($r_{pb} = 0.156$, $p = 0.006$). There was no evidence of a relationship between sex and positivity, since 47% of female and 48% of male puppies tested positive ($r_{pb} = 0.058$, $p = 0.310$).

A plot of the proportion of samples positive for *T. canis* by age shown in Fig. 3 displays a clear trend from two weeks of age (50%) increasing to a peak at 12 weeks (70%), and then a logarithmic decrease to 0% by 48 weeks. In comparison, a similar plot for *Ancylostoma* spp. in Fig. 4 displays a possible upward trend in positivity from 2 to 12 weeks, but no strong evidence of a change in positivity on average after 12 weeks.

Puppies 12 weeks of age and younger were 4.8 times more likely to be positive for *T. canis* than puppies over 12 weeks (Odds Ratio = 4.8: 95% CI, 2.9 to 8.0, $p < 0.001$). In comparison, there was no evidence the proportion of positive cases was different below or above 12 weeks of age for puppies with *Ancylostoma* spp. (Odds Ratio = 0.6: 95% CI, 0.4 to 1.0, $p = 0.977$).

A KAP survey was conducted on 35 local puppy owners, 35 community physicians and 60 SGU veterinary students. Based on the small finite size of each target population, the sample sizes were determined with the intent of producing a margin of error of approximately $\pm 10\%$ for the survey results. The KAP survey showed that none of the 35 puppy owners interviewed had any knowledge of zoonoses, 34 (97%) had not spayed or neutered their dogs, 24 (68%) allowed their puppies to roam freely and 3 (9%) cleaned up after their puppies. None of the 35 physicians interviewed had previously diagnosed toxocariasis in children or adults, and consequently, 21 (60%) cited 'no concern' regarding the zoonotic potential of *T. canis* and 6 had ever discussed the potential of any zoonotic diseases with their patients. The majority 39 (65%) of 60 veterinary students surveyed were able to correctly identify the main routes of transmission of toxocariasis to humans, and 4 (7%) ranked *T. canis* as having significant zoonotic potential whilst 2 (3%) were aware of the clinical syndromes produced by *T. canis* in humans.

4. Discussion

Vertical transmission was indicated by the proportion of infected dogs increasing from 50% at two weeks of age (from in utero transmission) to 70% by 12 weeks (in utero and lactogenic transmission).

In this study, vertical transmission of *T. canis* accounted for up to ¾ of all the infections found in the puppies confirming the paramount importance of vertical transmission in the maintenance of this parasite. It is likely that many of the remaining infections were also as a result of vertical transmission but the role of horizontal transmission through the ingestion of embryonated eggs cannot be ruled out. Puppies, through the excretion of millions of eggs, with each female worm capable of contributing 200,000 eggs daily (Harvey et al., 1991) are important hosts for potential human exposure. Eggs expelled by puppies, although highly resistant, are susceptible to environmental conditions, and depend on the accidental ingestion by other definitive and accidental hosts for their successful transmission. Vertical transmission removes environmental constraints and its efficiency results in bitches being the most important reservoirs of *T. canis* infection and maintenance of the parasite for subsequent generations of the definitive host population (Morgan et al., 2013; Rostami et al., 2020; Kazacos, 1978).

Finding *T. canis* eggs in puppies two weeks of age strongly suggests that initial treatment of puppies, which for decades has been set at two weeks postpartum (Macpherson, 2013; Nijsse et al., 2015; Harvey et al., 1991; Kazacos, 1978) needs to be re-evaluated. The focus on the treatment of puppies does not prevent the development of *T. canis* adults in puppies nor their associated clinical manifestations and does not prevent environmental contamination with eggs. The prevention of vertical transmission should be prioritized in any control initiative. Such an approach would need to involve the treatment of pregnant bitches with anthelmintics administered under veterinary supervision as some anthelmintics are not recommended for treatment of a bitch during pregnancy due to negative side effects to unborn pups such as palatoschisis and low birth rates (Ziegler and Macpherson, 2019; Bosse et al., 1980; Overgaauw and
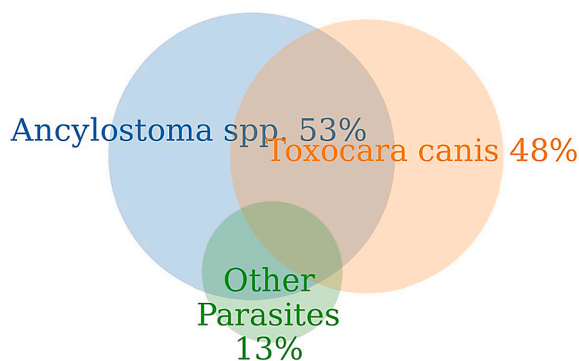

Fig. 2. Venn diagram of the proportion of positive cases for *Ancylostoma* spp. (53%) *T. canis* (48%) and other parasites commonly found in puppy feces (13%) – *Stronglyoides* spp., *Trichuris vulpis,* and *Isospora* spp. The intersections indicate the occurrence of polyparasitism. Twenty five percent of the cases were positive for both *Ancylostoma* spp. and *T. canis*.
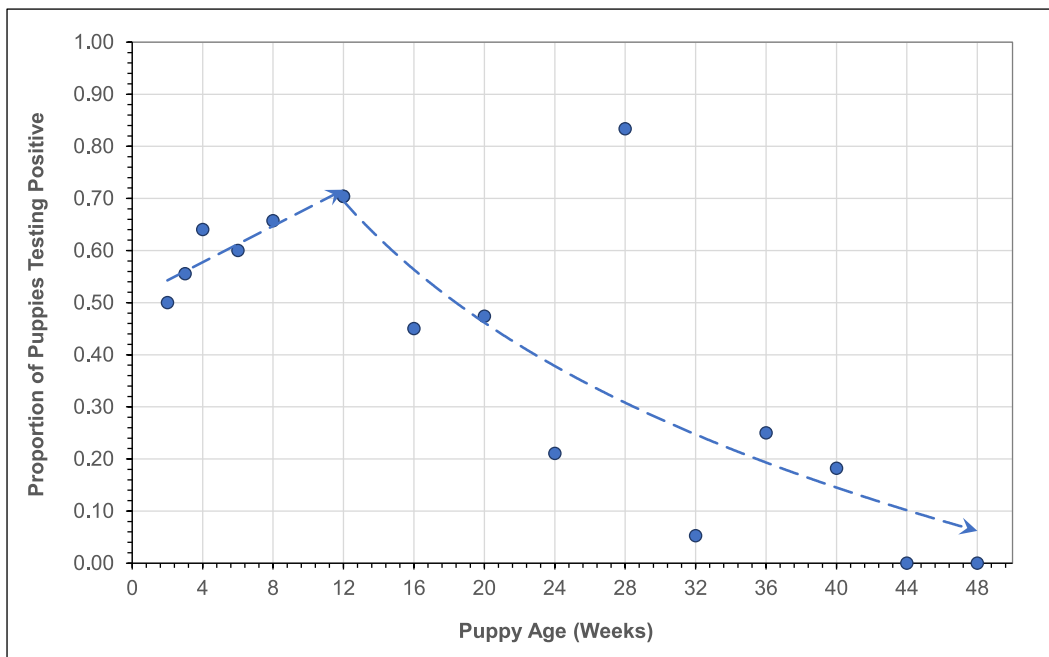
Fig. 3. Proportion of cases positive for *T. canis* by puppy age showing a high rate of positivity at 2 weeks (50%) and increasing to 12 weeks of age (70%) followed by a logarithmic decline through 48 weeks to near zero.
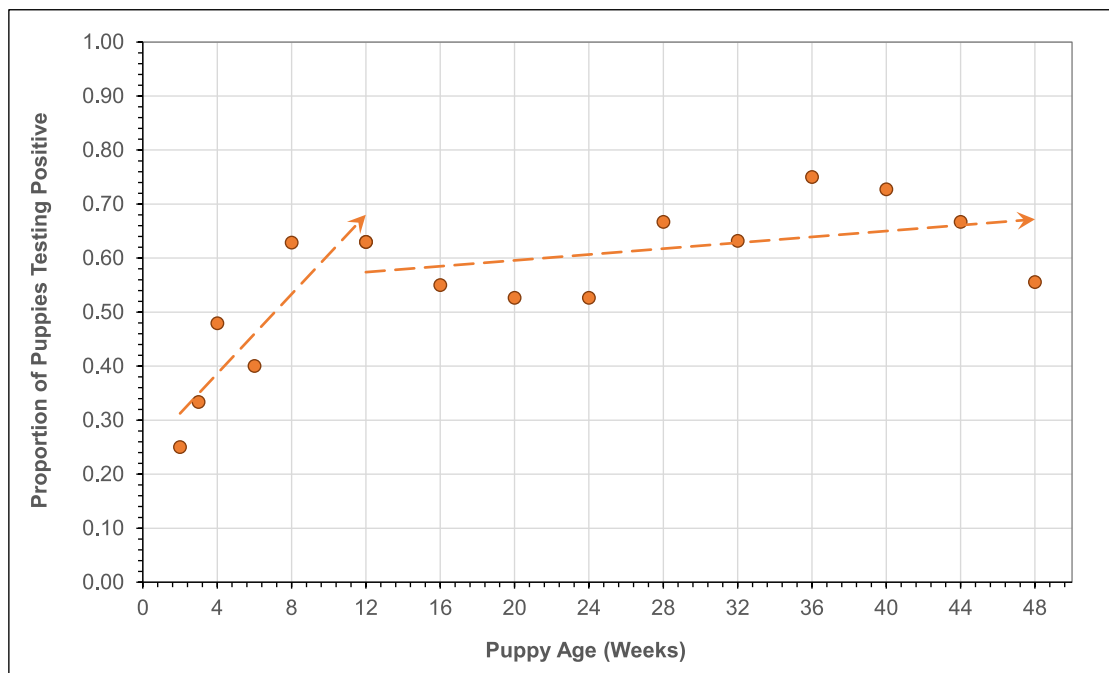

Fig. 4. Proportion of cases positive for *Ancylostoma* spp. by puppy age showing a low rate of positivity at 2 weeks (25%) and increasing to 12 weeks of age followed by a plateau at about 60% positivity through 48 weeks.

Boersema, 1998). Anthelmintics that may be considered include ivermectin at 300 μg/kg, which when administered experimentally to greyhounds during gestation on days 0, 30 and 60, was shown to reduce vertical transmission by 90%. In another litter, where ivermectin was administered at days 0, 30, and 60 plus 10 days post-whelping, the worm burden was reduced by 100% (Payne and Ridley, 1999). These dosages are well above the level of ivermectin used in heartworm prophylaxis. Additionally, four experimentally infected beagles treated subcutaneously with moxidectin at 1 mg/kg on days 40 and 55 of pregnancy, completely prevented pre-natal

and lactogenic infections in puppies (Krämer et al., 2006). In the same Kramer et al. study, one untreated bitch who served as a control, showed to have been infected with one adult and 26 somatic larvae of *T. canis* at necropsy.

Anthelmentics administered after 43 days of pregnancy are useful as this timing coincides with the activation of somatic larvae and their migration to the fetuses, which are under the influence of maternal hormonal levels (Ivanova and Georgiev, 2018). Programs implementing the use of ivermectin or moxidectin to prevent vertical transmission of *T. canis* will provide the additional benefit of preventing heartworm disease, treating ear mites, sarcoptic and demodectic mange as well as other intestinal, including zoonotic, nematodes (Sharun et al., 2019).

Implementation of a vertical transmission prevention program would require funding to support the veterinary services which would be required as treatment of pets is not usually performed in most low and middle income countries (LMICs). The high prevalence and favorable environmental conditions that occur in the tropics create a public health threat (Fakhri et al., 2018; Ma et al., 2019; Azam et al., 2012; Rocha et al., 2011). Such regions are thought to have the highest incidence of human infection which results in over ten million people, especially children, being infected with this parasite annually (Fakhri et al., 2018; Dutra et al., 2014; Morgan et al., 2013; Rostami et al., 2020; Cong et al., 2014).

In countries that largely lack both treatment and dog population control options, other appropriate affordable control measures should be implemented, such as education on the need to pick up and remove dog feces. This would require behavioral changes which have proven to be difficult for most parasitic disease control programs (Macpherson, 2005). In this study, only 9% of dog owners regularly picked up dog feces, and none were aware of the zoonotic threat of *T. canis*. Such lack of knowledge and proper behavior presents an intervention opportunity.

A lack of discussion about zoonoses by veterinarians with animal owners is a global problem (Harvey et al., 1991; Robertson et al., 2000; Alho et al., 2018; Kantarakia et al., 2020). This deficiency needs to be addressed not only to practitioners but also in the professional curriculum if any change is to occur. This study also revealed that veterinary students were largely unconcerned about the zoonotic nature of *T. canis* and only a small percentage of students could identify the main route of transmission to humans. This deficiency should be addressed in veterinary education, as veterinarians are the best target group for imparting accurate knowledge to pet owners about this zoonosis (Glickman and Schantz, 1981) and for implementing prevention protocols. Veterinarians would also be responsible for implementing dog population control programs that, if successful, could help reduce the number of young definitive hosts of *T. canis* in the communities.

The lack of recognition by physicians of the public health importance of *T. canis* may be explained by the fact that none of the physicians surveyed had ever diagnosed a case of toxocariasis in humans. The four clinical syndromes of toxocariasis do not exhibit pathognomonic signs and diagnostic tests are locally unavailable, expensive to import and require a cold chain for their importation. There have been no seroprevalence studies conducted in Grenada and the public health and economic importance, as in most other LMICs, of toxocariasis in humans, remains unknown. Enlisting physicians' assistance in an educational control initiative would require evidence of local clinical importance and an ability to make a diagnosis so that treatment can be provided.

Stressing the advantage of reducing the risk of contracting other, more well-known zoonoses such as *Ancylostoma* spp., *Cryptosporidium* spp. and *Giardia* spp., which were found in puppy fecal samples in this study, would be useful to include in any educational message. Molecular differentiation of the *Ancylostoma* spp. in the region to more fully appreciate their zoonotic importance could also be beneficial (Ngcamphalala et al., 2020). Improved hygiene practices, especially in children (Kroten et al., 2018; Moreira et al., 2014; Ahn et al., 2014), picking up pet feces (Vanhee et al., 2015) and increasing dog population control measures, all could lower *T. canis* and other parasitic zoonotic transmission, providing health benefits for both pets and people.

Declaration of Competing Interest

Acknowledgements

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