Evaluation of *Echinococcus multilocularis* and other intestinal parasites in off-leash dogs in southern Ontario

By

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ABSTRACT

Evaluation of *Echinococcus multilocularis* and other intestinal parasites in off-leash dogs in southern Ontario

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Parasites can affect dog health, and some parasites, including *Echinococcus multilocularis*, are a zoonotic concern. *Echinococcus multilocularis* has recently been detected in wild carnivores in Ontario; however, little is known about the prevalence of *E. multilocularis* and other intestinal parasite in dogs in Southern Ontario. Therefore, this thesis was designed to investigate the prevalence of intestinal parasites in dogs that reside in southern Ontario. Using fecal floatation, 11 different species of intestinal parasite were identified in 483 fecal samples collected from dogs at 12 off-leash dog parks in southern Ontario in 2018. *Ancylostoma caninum, Trichuris vulpis, Cystoisospora* spp. and *Toxocara canis* were the most frequently identified intestinal parasites. Based on PCR testing of 212 fecal samples from the same dogs, the prevalence of *E. multilocularis* was 0% (97.5% confidence interval [CI] 0 – 1.6%). The information about parasite prevalence could assist veterinarians with deworming protocols targeted for off-leash dogs.

STATEMENT OF WORK

The preparation of this thesis was the sole responsibility of Tyler Greer. Assistance with field sampling was provided by the summer student Melanie Moore. Assistance with the analysis of intestinal parasites via the Cornell Wisconsin Double Centrifugation method was completed by Jacob Avula and Melanie Moore. Joyce Rousseau and Jonathon Kotwa provided assistance with laboratory testing for *Echinococcus multilocularis.*

TABLE OF CONTENTS

ABSTRACT ii

STATEMENT OF WORK iii

TABLE OF CONTENTS iv

LIST OF TABLES viii

LIST OF FIGURES ix

LIST OF ABBREVIATIONS x

LIST OF APPENDICES xi

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW 1

INTRODUCTION 1

TOXOCARA CANIS 2

ANCYLOSTOMA CANINUM 4

TRICHURIS VULPIS 5

CYSTOISOSPORA SPP. 6

GIARDIA SPP. 8

ECHINOCOCCUS MULTILOCULARIS 9

DIAGNOSTIC METHODS FOR HELMINTHS 12

DIAGNOSTIC METHOD FOR *ECHINOCOCCUS MULTILOCULARIS* 13

PREVALENCE OF INTESTINAL PARASITES IN DOGS IN NORTH AMERICA 15

PREVALENCE OF INTESTINAL PARASITES IN ONTARIO 17

STUDY RATIONALE AND OBJECTIVES....18

REFERENCES....20

TABLES....34

CHAPTER 2 - PREVALENCE OF INTESTINAL PARASITES IN THE FECES OF DOGS VISITING OFF-LEASH DOG PARKS IN THE NIAGARA AND HAMILTON REGIONS OF ONTARIO, CANADA. 36

ABSTRACT....36

INTRODUCTION....37

MATERIALS AND METHODS....38

Dog and dog park selection....38

Fecal collection....39

Laboratory analysis....39

Statistical analysis....39

RESULTS....40

DISCUSSION....41

LIMITATIONS....44

CONCLUSION....45

CONFLICT OF INTEREST....45

ACKOWLEDGMENTS....45

FUNDING....45

REFERENCES....46

FIGURES AND TABLES....52

CHAPTER 3 – INVESTIGATION OF *ECHINOCOCCUS MULTILOCULARIS* IN THE FECES OF DOGS VISITING OFF-LEASH DOG PARKS IN THE NIAGARA AND HAMILTON REGIONS OF ONTARIO, CANADA. 58

ABSTRACT 58

INTRODUCTION 59

MATERIALS AND METHODS 60

Dog, and dog park selection and fecal collection 60

PCR analysis 61

Data analyses 61

RESULTS 62

DISCUSSION 62

LIMITATIONS 63

CONCLUSION 63

CONFLICT OF INTEREST 64

ACKNOWLEDGMENTS 64

FUNDING 64

REFERENCES 65

CHAPTER 4 – GENERAL DISCUSSION 69

SUMMARY AND SIGNIFICANCE 69

LIMITATIONS 72

FUTURE DIRECTIONS 72

REFERENCES 74

APPENDICES 78

LIST OF TABLES

Table 1.1. Prevalence (%) and 95% confidence interval (CI) of *Ancylostoma caninum* and *Trchuris vulpis, Toxocara canis* and *Cystoisopora* spp. found from studies in Alberta, Ontario and Saskatchewan and the United States...……...….34

Table 2.1. Demographics of dogs stratified by sex that had fecal samples collected from off-leash dog parks between May – November 2018……...……...…52

Table 2.2. Prevalence (%) and 95% confidence interval (CI) of intestinal parasites in 483 dogs that visited off-leash dog parks in southern Ontario between May – November 2018…….53

Table 2.3. Fecal samples from dogs that were identified with more than one intestinal parasite.54

Table 2.4. Prevalence (%) and 95% confidence interval (CI) of *Ancylostoma caninum, Trichuris vulpis and Cystoisospora* spp. in fecal samples from dogs visiting off-leash dog parks in southern Ontario between May – November 2018……...55

Table 2.5. Monthly prevalence (%) and 95% confidence interval (CI) of intestinal parasites in fecal samples from dogs visiting off-leash dog parks in southern Ontario between May - November 2018……..……56

LIST OF FIGURES

FIGURE 2.1 Locations of dog parks selected for fecal collection from May – November 2018. 57

LIST OF ABBREVIATIONS

C – Celsius

CI – Confidence interval

ELISA – Enzyme-linked immunosorbent assay

L1 – First stage larvae

L2 – Second stage larvae

L3 – Third stage larvae

L4 – Fourth stage larvae

MC – PCR – Semi-automated magnetic capture probe DNA extraction and real-time PCR

NADH – Nicotinamide adenine dinucleotide

PCR – Polymerase chain reaction

SCT – Sedimentation counting technique

LIST OF APPENDICES

Appendix 1. Survey that contains questions regarding the dog owner's personal information, their dog's physical and behavioral characteristics, travel and medical history……78

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The relationship between humans and dogs began during the Pleistocene period when early hominids were found in association with wolves (Serpell and Barrett 2016). Today, approximately 35% of households in Canada own a dog (Andreassen et al., 2013). In addition to household companions, dogs assist with a variety of occupations, including national security and assistance for people with physical and mental disabilities (Robertson et al., 2000; Vilà and Leonard 2001). Dogs rely on humans for medical treatment and overall welfare (Serpell and Barrett 2016).

Parasites are an animal health concern because it is common for dogs to become infested with a parasite at some stage of their lives (Roberston et al., 2000). Macroscopic parasites, like most helminths, can be seen with the naked eye. Conversely, microscopic parasites such as protozoa, are single-celled organisms that require a microscope for identification. Both classes of parasites can infect the gastrointestinal system of dogs and may cause a range of diseases and sometimes death, if not treated (Roberston et al., 2000;Oliveira-Sequeira et al., 2002; Raza et al., 2018). In addition, some parasites can be passed from dogs to humans and are known as zoonotic parasites (Little et al., 1983). So, the close association between dogs and humans raises concern about zoonotic parasite transmission (Little et al., 1983; Prociv and Croese 1990; Croese et al., 1994; Prociv 1998; Deplazes et al., 2011; Mateus et al., 2014). Therefore, humans must be aware of the prevalence of zoonotic pathogens in domestic dogs and risk factors associated with acquiring these pathogens to alleviate the risk of transmission to humans (Mateus et al., 2014).

Off-leash dog parks are an enriching area that provides space for dogs to exercise, socialize and improve their well-being (Gómez 2013; Urbanik and Morgan 2013). Exercise can

help dogs stay at their optimal weight which can reduce their risk of weight-related diseases (Lund et al., 2006; Hurley et al., 2011). As urbanization increases, the demand for off-leash dog parks will likely increase (Rahim et al., 2018). However, dog parks also provide an area for parasite transmission (Westgarth et al., 2009; Smith et al., 2014). The overall parasite prevalence in dogs visiting off-leash dog parks was found to range from 4.4% in Saskatoon Saskatchewan, to 50.2% in Calgary Alberta (Gaunt and Carr, 2011; Smith et al., 2014). To the best of the author's knowledge, there is only one study that have evaluated the prevalence of *Giardia* spp. in dogs visiting off-leash dog parks in southern Ontario (Procter et al., 2013). Additional intestinal parasite prevalence data could be used to increase parasite awareness for dog owners and veterinarians for evidence-based preventive measures to reduce the risk of infections in dogs. The remainder of this literature review will focus on common intestinal parasites that can infect dogs, diagnostic methods used and the prevalence within the United States of America and Canada.

TOXOCARA CANIS

Toxocara canis is a roundworm that belongs to the phylum Nematoda. *Toxocara canis* has a complex life cycle with multiple routes of transmission which makes it a common intestinal parasite in dogs (Macpherson 2013; Taylor et al., 2016). The adult stage of *T. canis* resides in the small intestine of dogs and releases its eggs into the environment via the dog's feces (Macpherson 2013). The eggs are dark brown, spherical, and measure 85µm - 90µm by 75µm - 80µm (Deplazes et al., 2016). Once in the environment, eggs become infective in approximately 3-4 weeks (Macpherson 2013). A dog can become infected by the ingestion of infective eggs, or ingestion of a paratenic host (Macpherson 2013). Paratenic hosts are organisms that harbour the sexually immature parasite in an encysted state until the parasite has an

opportunity to move to a definitive host species (Porta and Last 2018). For *T. canis*, some species of rabbits, rodents and birds can be paratenic hosts (Fok and Kassai 1996; Rahbar et al., 2015). Once infected, two possible mechanisms of larval migration can occur: tracheal migration or somatic migration (Macpherson 2013). Tracheal migration results in intestinal infections and occurs when the ingested egg hatches and the larva penetrates the mucosa of the small intestine, enters the blood stream via the portal circulation, and moults in the lungs (Taylor et al., 2016). Subsequently, the larva migrates to the trachea, where it undergoes the final moult (Taylor et al., 2016). The larva is then swallowed by the dog and reaches the small intestine where it matures into the adult stage (Taylor et al., 2016). Somatic migration may result in larval cysts and occurs when the larvae hatch from eggs and migrate to tissues, mainly skeletal muscle, and go dormant (Taylor et al., 2016). Both aforementioned routes are possible in dogs of all ages. However, tracheal migration is most commonly observed in puppies less than three months of age; somatic migration is commonly observed in dogs older than 6 months (Taylor et al., 2016). The prepatent period following egg ingestion is approximately 4 – 5 weeks (Taylor et al., 2016). Additionally, *T. canis* may be transmitted through vertical transmission; an infected pregnant female dog may transmit the infection to her puppies through transmammary or transplacental routes these routes shorten the prepatent period to less than 4 weeks (Taylor et al., 2016).

In moderate *T. canis* infections, there are no clinical signs during the pulmonary phase of larval migration, but adult worms can cause potbelly, vomiting, and diarrhea mostly in puppies (Taylor et al., 2016). In heavy infections, clinical signs such as frothy nasal discharge, coughing, and an increased respiratory rate can occur during the pulmonary phase (Deplazes et al., 2011; Taylor et al., 2016). Deaths from *T. canis* infection occur during the pulmonary phase with

puppies at risk of death within a couple of days after birth because of transplacental transmission (Taylor et al., 2016).

Finally, *T. canis* poses a zoonotic threat (Deplazes et al., 2011). *Toxocara canis* causes visceral larva migrans in humans, which can result in infections in a variety of organs including the skin and the eye (Taylor et al., 2016). In European countries, play areas for children, such as sand-pits, backyards and playgrounds, were found to be contaminated with *Toxocara canis* eggs ranging from 2 – 56% (Mizgajska 2001). In addition, serological tests found that 87% of children, aged between 5 and 7 years old, had been exposed to *Toxocara* spp (Worley et al., 1984; Taylor et al., 2016). However, cases of visceral larva migrans have been documented in adults (De Melker et al., 1995; Lim 2008).

ANCYLOSTOMA CANINUM

Ancylostoma caninum is a hookworm that also belongs to the phylum Nematoda (Bowman 2014). Adult stages of *A. caninum* reside in the small intestine of dogs (Bowman 2014; Deplazes et al., 2016). The eggs shed by the adult stage of *A. caninum* are usually 56µm - 75µm by 34µm - 47µm and are barrel shaped with a thin smooth shell (Deplazes et al., 2016). Eggs enter the environment via the feces (Raza et al., 2018). The eggs then hatch into the first larval stage (L_1) and after approximately 5 days they will moult via the L2 stage into the third larval stage (L_3) stage if optimal conditions are present (Raza et al., 2018). The L_3 stage can infect dogs through ingestion or by skin penetration (Raza et al., 2018). If a dog is infected by ingestion, the L_3 penetrate the epithelium lining the mouth and enter the systemic circulation. Alternatively, the L_3 will pass directly to the intestine where they will moult into the adult stage (Robertson and Thompson 2002). When infected though skin penetration, they enter the blood stream and travel to the lungs (Robertson and Thompson 2002). Once in the lungs, the L_3 moult

into the fourth stage larva (L_4) either in the lungs or trachea (Robertson and Thompson 2002). Then, the L_4 will be swallowed and arrive at the small intestine where it will undergo a final moult to the adult stage (Robertson and Thompson 2002). A phenomenon known as the larval leak occurs when larval hookworms in the somatic tissue of a dog leak out and enter the gastrointestinal system (Raza et al., 2018). It is also important to note that transmammary transmission is possible for puppies who are nursing from an infected dam. The prepatent period is 14-21 days and *A. caninum* worms are highly prolific resulting in a large number of eggs being passed in a short time period (Raza et al., 2018).

Ancylostoma caninum intestinal infections can cause anemia in dogs (Wells 1931; Bowman 2014). However, the worm burden, nutrition status, and host resistance are factors that influence the risk of this occurring (Bowman 2014). Acute hookworm disease can occur if a dog is exposed to large numbers of L_3 larva; this can lead to melena, lethargy, poor coat, pale mucosa, and possibly death (Bowman 2014; Raza et al., 2018).

Ancylostoma caninum has been associated with eosinophilic enteritis in human cases from Australia (Prociv and Croese 1996). Both routes of infection, ingestion or skin penetration are possible however ingestion of infectious larval was the predominate route for the acquisition of eosinophilic enteritis in experimentally infected humans (Landmann and Prociv 2003). So, *A. caninum* could pose a zoonotic threat to dog owners.

TRICHURIS VULPIS

Trichuris vulpis, the dog whipworm, also belongs to the phylum Nematoda (Traversa 2011). The worm resides in the large intestine of an infected dog (Traversa 2011). *Trichuris vulpis*' eggs are usually 70µm - 92µm by 32µm - 40µm and have two distinct polar plugs and a lemon-like shape (Traversa 2011). The life cycle of *T. vulpis* is simple (Traversa 2011). Adult

worms that reside in the large intestine of an infected dog release eggs that are excreted in the feces (Traversa 2011). The L_1 stage develops in the eggs after one or two months in the environment; dogs become infected by ingesting infective eggs (Traversa 2011). Once ingested, the eggs hatch and release the L_1 stage which penetrate the crypt of Lieberkühn, a gland in the small and large intestine (Traversa 2011). Once mature, the worm will penetrate the mucosa layer within the large intestine and embed in the epithelium of the colon (Traversa 2011). Adults start releasing eggs 7-10 weeks after a dog has ingested infective eggs (Traversa 2011). Few papers have been published concerning the pathology of *T. vulpis* infections, but this parasite has been associated with acute or chronic inflammation of the mucosa of the caecum and colon, intermittent diarrhea, and anaemia in dogs (Traversa 2011).

Importantly, egg development of *T. vulpis* is highly dependent on environmental conditions. The eggs thrive in high humidity and are temperature resistant (Raza et al., 2018). Thus, contaminated environments, such as off-leash dog parks, could provide optimal conditions for whipworm development and increase the risk for infection in dogs.

The role of zoonotic properties for *T. vulpis* is controversial. One human case report has been identified but the diagnosis was based on egg morphology without molecular diagnostic tests (Dun et al., 2002). The human case could have been infected with *Trichuris trichiura,* a human whipworm, since the egg morphology and size is similar to *Trichuris vulpis* (Dun et al., 2002).

CYSTOISOSPORA SPP.

Cystoisospora species belong to the phylum Apicomplexa (Dubey et al., 2009). Dogs can become infected with *Cystoisospora canis*, *Cystoisospora ohioensis*, *Cystoisospora neorivolta* and *Cystoisospora burrowsi* (Dubey et al., 2009). *Cystoisospora canis* oocysts are the largest of

the four, measuring 38µm by 30µm (Deplazes et a., 2016). Dogs are directly infected by ingesting sporulated oocysts (Dubey et al., 2009). Once ingested, the oocyst releases sporozoites that penetrate the intestinal epithelium cells of the small intestine (Dubey et al., 2009). Once the sporozoite has entered the cell, it undergoes one round of merogony, a form of asexual replication (Dubey et al., 2009). The final product of merogony is a meront (Dubey et al., 2009). The meront releases merozoites which subsequently rupture the epithelial cells (Dubey et al., 2009). In order to develop into a mature male (microgametocyte) or female (macrogametocyte), the merozoites infect an epithelial cell and undergo another round of merogony (Deplazes et al.,2016). Finally, the microgametocytes are released from the epithelium cell, reinvade an epithelial cell to undergo sexually reproduction with a microgametocyte and form a zygote (Dubey et al., 2009). The zygote is released from the epithelial cell (Dubey et al., 2009). Over time, the zygote will develop a cyst wall and become an unsporulated oocyst (Dubey et al., 2009). However, *Cystoisospora* species are only infective once the oocyst becomes sporulated in the environment (Dubey et al., 2009). Development to sporulated oocysts occurs between 20° Celsius (C) and 40°C with rapid sporulation occurring between 30°C and 37°C. Sporulated oocysts can survive in moist environments up to a year, if protected from extreme temperatures and thrive in crowded environments, making transmission to uninfected dogs possible (Deplazes et al., 2016). Enzootic infections are frequently found in kennels or other areas where dogs are held in close quarters (Oduyne and Bobade 1979; Kirkpatrick and Dubey 1987; Dubey and Greene 2006). Importantly, recurrent infections of *Cystoisopora* spp can occur if treatment protocol is not followed or if there is inadequate cleaning of the dog's environment (Dubey et al., 2009). Clinical signs such as diarrhea, weight loss, and dehydration, are prominent in newborns

or puppies less than a year of age (Dubey et al., 2009; Barutzki and Schaper 2011; Houk et al., 2013).

GIARDIA SPP.

Giardia spp. are a genus of anaerobic flagellated protozoa that belong to the Metamonada phylum (Payne and Artzer 2009). A total of 40 species of *Giardia* have been recognized with *Giardia duodenalis* being the most recognized (Štrkolcová et al., 2015). *Giardia duodenalis* has been organized into 8 assemblages (A-H) with assemblage C and D being found only in dogs (Payne and Artzer 2009). However, assemblage A and B are zoonotic with the capability to infect both dogs and humans (Payne and Artzer 2009). Dogs become infected with *Giardia duodenalis* by direct infection from contaminated soil or water (Taylor et al., 2016). Once a cyst is ingested, it will begin the transformation process in the stomach and become two trophozoites once it reaches the small intestine (Payne and Artzer 2009). Trophozoites are 12µm - 17µm in length by 7µm - 10µm in width and have a distinct morphology (Payne and Artzer 2009); composed of four pairs of flagella with a flattened ventral surface occupied by a ventral adhesive disk tapering posteriorly to a tail (Payne and Artzer 2009). Trophozoites transform back into a cyst before being excreted into the environment via the feces (Midlej and Benchimol 2009). Cysts are immediately infective once they enter the environment and are 8µm – 12µm by 7µm – 10µm and contain four nuclei (Payne and Artzer 2009).

Dogs can be infected with *Giardia* and not have clinical signs. However, once infection with *Giardia* causes disease, typical signs are chronic pasty diarrhea, lethargy, and weight loss (Taylor et al., 2016). Signs and symptoms of human infection include diarrhea, stomach cramps, and vomiting, which can lead to dehydration (Farthing 1996; Gardner and Hill 2001; Roberston et al., 2010). Although some *Giardia* assemblages are zoonotic, the role of dog ownership as a

risk factor for human infection is unclear (Warburton et al., 1994; Hoque et al., 2002; Pereria et al. 2007; Taylor et al., 2016).

ECHINOCOCCUS MULTILOCULARIS

Echinococcus multilocularis is a small tapeworm (1-3mm) that belongs to the Platyhelminth phylum. Its lifecycle typically involves wild canids as the definitive host and rodents as the intermediate host (Deplazes and Eckert 2001). The adult stage resides in the small intestine of the canid and sheds its eggs, which are immediately infective, into the environment via the feces (Deplazes and Eckert 2001). Once in the environment, rodents become infected through ingestion of eggs when they scavenge for food (Deplazes and Eckert 2001). The larvae hatch and migrate from the small intestine via the portal circulation to the liver where the metacestode stage develops (Deplazes and Eckert 2001). The metacestode undergoes exogenous budding, similar in behaviour to a tumour, ultimately weakening the rodent's chance of survival (Deplazes and Eckert 2001). The resultant disease is known as alveolar echinococcosis (Deplazes and Eckert 2001). The lifecycle is completed once the rodent is ingested by a canid (Deplazes and Eckert 2001).

In some areas around the world, dogs can perpetuate the lifecycle, and most importantly dogs may facilitate transmission of *E. multilocularis* to humans (Stehr-Green et al., 1988; Deplazes et al., 2011). A case-control study in Alaska investigated associations between human alveolar echinococcosis and dog related exposures (Stehr-Green et al., 1988). Alveolar echinococcosis diagnosis was based on serological testing and medical imaging or histological confirmation (Stehr-Green et al., 1988). They found that cases were 6-times more likely to own a dog than controls (Stehr-Green et al., 1988). Additionally, cases were 8.5-times more likely to have their dog tethered to their house than control individuals. However, there were only 19

cases included in this study, so the study had low statistical power to identify weaker associations. Ultimately, this study found dog ownership could be significantly associated with alveolar echinococcosis in humans (Stehr-Green et al., 1998; Kern et al., 2004; Massolo et al., 2014).

To the best of the authors knowledge, there is no information on the distribution and prevalence of *E. multilocularis* intestinal infections in dogs in North America (Massolo et al., 2014; Deplazes et al., 2017). However, Asia has one of the highest prevalence of alveolar echinococcosis in humans and dogs are speculated to be the definitive host and transmit *E. multilocularis* to humans (Wang et al., 2001; Budke et al., 2004). Specifically, two studies from Asia investigated associations between the free movement of dogs and development of *E. multilocularis* intestinal infections in dogs (Budke et al., 2005; Ziadinov et al., 2008). The first study administered 371 surveys to dog owners residing in Shiqu county of China. The survey included questions regarding the dog owner and their dog's behavioural characteristics (Budke et al., 2005). It was found that dogs not tied up all the time were 2.5 (95% confidence interval [CI] 1.3 – 5.0) times more likely to get infected with *E. multilocularis* compared to dogs tied up all the time (Budke et al., 2005). The second study had a similar design and obtained similar results, however it had a larger sample size of 466 surveys and was conducted in Kyrgyzstan (Ziadinov et al., 2008). It is important to note that the traditional cultural beliefs in Kyrgyzstan prevent the use of anthelmintics in dogs, and dogs are not privately owned: the community shares the responsibility of caring for the dogs (Budke et al., 2005; Ziadinov et al., 2008). In areas of Europe, intestinal infection prevalence in dogs ranges from approximately 0-7% (Deplazes et al., 2011). The range in prevalence is impacted by the geographic location of the dogs, the dogs lifestyle, prevalence in wildlife, and diagnostic test conducted (Deplazes et al., 1999; Gottstein et

al., 2001; Dyachenko et al., 2008; Comte et al., 2010). Specifically, in the Slovak Republic, the prevalence of *E. multilocularis* intestinal infections was 2.8% (95% CI 1.2 – 5.4%) in dogs used for guarding, hunting and herding sheep (Antolová et al., 2009; Nagy et al., 2011). Conversely, the prevalence of *E. multilocularis* intestinal infections was 0.13% (95% CI 0.07 – 0.23%) in privately owned dogs from Germany (Dyachenko et al., 2008). So, within endemic areas, dogs that free roam and display high prey drive characteristics are at higher risk of developing *E. multilocularis* intestinal infections (Svobodova and Lenska 2002; Budke et al., 2005; Szabová et al., 2007; Ziadinov et al., 2008).

Alveolar echinococcosis is the disease that occurs in intermediate or accidental hosts that ingest *E. multilocularis* eggs (Deplazes and Eckert 2001). For humans, the metacestode stage begins with asexual reproduction in the liver and develops into nodular cysts no larger than 3 cm in diameter (Deplazes and Eckert 2001). Each cyst is surrounded by granulation or connective tissue and has the capability of developing new cysts that bud off of the developed cysts (Deplazes and Eckert 2001). The exogenous budding property of the parasite gives it the ability to spread to adjacent organs or distant organs via contact or infiltration of the bloodstream, respectively (Deplazes and Eckert 2001; Kern et al., 2003). The clinical incubation period for an individual with alveolar echinococcosis is 5-15 years with symptoms and signs ranging from jaundice, abdominal pain, fatigue, and death if left untreated (Deplazes and Eckert 2001; Kern et al., 2003). Treatment for individuals includes surgical resection of the damaged liver tissue or chemotherapy for potentially the remainder of the individual's life (Deplazes and Eckert 2001; Kern et al., 2003). In Switzerland, the average treatment cost per patient with alveolar echinococcosis was estimated to be €103,312 (~ $150,615 Canadian dollars) (Torgerson et al., 2008). Ultimately, the exogenous budding characteristic and long incubation period of alveolar

echinococcosis make the disease difficult to treat and can cause an economic burden on the health care system (Deplazes and Eckert 2001; Torgerson et al., 2008).

Dogs can also develop alveolar echinococcosis (Deplazes and Eckert 2001). In Switzerland, Corsini et al. (2015) evaluated the treatment of 23 dogs diagnosed with alveolar echinococcosis between 2004 and 2014. It was found that treatment by surgical resection or medical treatment with albendazole increased the lifespan of dogs, compared to those who did not receive any form of treatment (Corsini et al., 2015). Unfortunately, the sample size for this study was low making it difficult to extrapolate to larger populations, but it provides evidence that treating dogs with alveolar echinococcosis can increase survival times (Corsini et al., 2015).

Before 2012, Ontario was considered to be free of *E. multilocularis.* Since then, six dogs have been diagnosed with AE in southern Ontario in the Golden Horseshoe region, the area surrounding the western shores of Lake Ontario (Oscos-Snowball et al., 2014; Skelding et al., 2014; Pinard et al., 2019). Importantly, four of the dogs had no travel history, suggesting they must have become infected locally. The cases of alveolar echinococcosis prompted an investigation into the prevalence of *E. multilcoularis* in wild canids in southern Ontario which, overall, was found to be 23% (95% CI 20 –27%) with a hotspot of infection detected along the northern shore of Lake Erie and the western end of Lake Ontario (Kotwa et al., 2019). Within the southern Ontario hot spot, the overall prevalence of *E. multilocularis* infection in wild canids was 34% (95% CI: 26 – 40%) (Kotwa et al., 2019).

DIAGNOSTIC METHODS FOR HELMINTHS

Fecal flotation and microscopic examination are the most common methods used in veterinary medicine for diagnosing helminth parasites in feces (Zajac and Conboy 2012). Such methods can be used to identify a variety of eggs and parasite stages (Deplazes et al., 2017). The

methods remove debris and concentrate parasite eggs (Zajac and Conboy 2012). Parasite eggs float when suspended in a saturated salt/sugar solution (Zajac and Conboy 2012). In addition, the methods are non-labour intensive and cheap compared to other tests (Zajac and Conboy 2012). However, sensitivity can be altered based on the liquid solution used and the test procedure conducted (Zajac and Conboy 2012). For example, zinc sulfate solution is most effective for recovery of *Giardia* spp. cysts (David and Lindquist 1982); sugar solutions are more effective for *T. canis* and *A. caninum* eggs. However, sugar solutions can destroy *Giardia* cysts (David and Lindquist 1982; Zajac and Conboy 2012). Therefore, solutions and test procedures should be chosen based on the parasite of interest.

DIAGNOSTIC METHOD FOR *ECHINOCOCCUS MULTILOCULARIS*

The identification of *E. multilocularis* intestinal infections in definitive hosts can be based on necropsy, fecal floatation methods, and a variety of immunological or molecular techniques. Each approach has its advantages and disadvantages (Conraths and Deplazes 2015). Necropsy methods, including the sedimentation and counting technique (SCT) and intestinal scraping technique, can be used to identify *E. multilocularis*. Both methods identify the adult stage of *E. multilocularis* and have a specificity close to 100% (Conrath and Deplazes 2015). However, they both depend on examination of the gastrointestinal tract (Umhang et al., 2011; Conraths and Deplazes 2015). Freezing the carcass at -80^{o}C is used as a safety precaution for individuals handling the carcass (Conraths and Deplazes 2015). However, the freezing can reduce the sensitivity of both methods (Conraths and Deplazes 2015). Obviously, necropsy methods are not suitable diagnostic methods for examining live animals (Conraths and Deplazes 2015).

Fecal floatation methods are relatively cheap and less labour intensive than necropsy and molecular techniques (Dryden 2005; Epe 2009). However, they cannot detect prepatent and single-sex infections (Epe 2009). When compared to necropsy, the centrifugal fecal floatation method only identified 57.1% of shelter dogs infected with *Taenia* spp. (Adolph et al., 2017); cestodes release dense eggs that are intermittently shed or remain in proglottids (Burrows and Lillis 1960; Dryden 2005; Adolph et al., 2017).

The coproantigen ELISA is an alternative for diagnosing *E. multilocularis* (Deplazes et al., 2003; Conraths and Deplazes 2015). Coproantigen excretion is correlated to the presence and amount of intestinal immature and mature parasite stages (Deplazes et al., 2003). The sensitivity can range between 84-95% with a specificity greater than 95% (Deplazes et al., 2003). However, cross-reactivity with *Echinococcus granulosus* is possible with this test (Deplazes et al., 2003); this is only relevant if the test is used in areas where *E. multilocularis* and *E. granulosus* are both present (Deplazes et al., 2003; Staebler et al., 2006). The non-invasive characteristic of the coproantigen *ELISA* allows it to be a simple and quick test. However, the test should be paired with molecular techniques such as Copro-DNA detection by PCR to confirm the presence of *E. multilocularis* intestinal infections (Eckert and Deplazes 2001; Deplazes et al., 2003).

Copro-DNA detection by PCR typically targets the NADH dehydrogenase 1 gene located on mitochondrial DNA of *E. multilocularis* (Mathis et al., 1996; Monnier et al., 1996; Dinkel et al., 1998; Van de Giessen et al., 1999; Trachsel et al., 2007; Boufana et al., 2013; Knapp et al., 2014; Isaksson et al., 2014). However, inhibitory materials in fecal samples have been shown to interfere with DNA amplification (Monnier et al., 1996; Dinkel et al., 1998; Knapp et al., 2014). Mathias et al. (1996) modified the sampling preparation stage to remove the inhibitory material. Specifically, *Taenia*-type eggs were isolated in a liquid suspension; PCR was carried out on

samples that contained *Taenia*-type eggs (Mathis et al., 1996). This method is suitable for hosts that have patent infections (Al-Sabi et al., 2007). Lastly, a recent semi-automated method has been developed and is currently used in national surveillance monitoring for *E. multilocularis* in Sweden (Isaksson et al., 2014). The semi-automated magnetic capture probe-based PCR method limits the impact of inhibitory factors found in the feces; when its performance was compared to the SCT technique, it had a sensitivity and specificity of 88% and 99.9%, respectively (Isaksson et al., 2014). The semi-automated magnetic capture probe-based PCR is used for large scale sampling in countries with low estimated prevalence or incidence (Isaksson et al., 2014).

PREVALENCE OF INTESTINAL PARASITES IN DOGS IN NORTH AMERICA

Based on fecal floatation methods in North America, the prevalence of intestinal parasites has been found to range between 0.2 – 14% (Table 1.1) in dogs. The remainder of this section will cover a summary and critical evaluation regarding the parasite prevalences identified. A study conducted in the United States estimated that the overall prevalence of intestinal parasites in dogs in the USA was 12.5% (Little et al., 2009). For this study, researchers used intestinal parasite data obtained through voluntary submissions to Antech Diagnostics, a national service laboratory, from veterinary clinics within 50 states of the USA. Antech Diagnostics completed 1,199,293 fecal diagnostic tests in 2006. The results for each fecal diagnostic test were grouped into four geographical regions, Northeast, Midwest, South and West (Little et al., 2009); the prevalences between each region were compared (Little et al., 2009). Hookworms were detected most commonly out of all the helminths with an overall prevalence of 2.46% (95% CI 2.44 – 2.49%) (Table 1). An explanation for this prevalence could be resistance of *A. caninum* to anthelmintic drugs (Kaplan et al., 2018). Resistance to canine dewormers in the literature dates to 2007, when Kopp et al. confirmed pyrantel resistance in a placebo-controlled trial experiment

in dogs from Australia. Since 2007, research by Kitchen et al. (2019) in Washington DC confirmed multi-drug resistance in *A. caninum* to fenbendazole, thiabendazole, and ivermectin. However, Little et al., (2009) did not specify the types of hookworms found, therefore it cannot be concluded that all the hookworms found in this study were *A. caninum.*

In 2009, researchers from the University of Pennsylvania evaluated the apparent prevalence of intestinal parasites from 6555 dog fecal samples examined between January 1st, 1997 and December 31st, 2007 (Gates and Nolan 2009). Fecal samples were examined for tapeworm proglottids and zinc-sulfate centrifugation was conducted to examine for other intestinal parasites (Gates and Nolan 2009). Each species of parasite found was recorded and classified according to its genus (Gates and Nolan 2009). A total of 932 dogs had intestinal parasite infections (Gates and Nolan 2009), but some dogs had multiple parasites present and some dogs were repeatedly sampled (Gates and Nolan 2009). *Giardia* spp., *Trichuris vulpis,* and *Toxocara canis* had the highest prevalence at 3.3%, 3.0% and 2.0%, respectively (Gates and Nolan 2009). Interestingly, *Trichuris vulpis* was identified in 3.0% of dogs with most infections identified in dogs older than 7 years, which has been reported previously (Visco et al., 1977). Most intestinal parasite infections were observed in dogs less than 6 months of age (Gates and Nolan 2009) which may be due to their naive immune system (Greely et al., 1996; Felsburg 2002; Day 2007; Bowman 2014).

In Calgary, a study was conducted to identify zoonotic parasites in dogs that visit dog parks (Smith et al., 2014). Dog fecal samples were opportunistically collected by researchers from dog owners visiting nine dog parks once a week from June to September 2010. In addition, a survey was administered to each dog owner to obtain information regarding their dog's health status and owner information (Smith et al., 2014). Samples were stored at -80^{o}C for six weeks

before analysis. Analysis was comprised of PCR for *E. multilocularis*, a modified Wisconsin double centrifugation method for helminths, and a direct immunofluorescence assay for protozoa (Smith et al., 2014). The most common parasites identified were protozoa such as *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp. at 24.7%, 14.7% and 16.8%, respectively. Conversely, *Trichuris* spp., *Toxocara canis* and *Taenia*-type eggs were 1.2%, 0.3% and 0.3%, respectively (Smith et al., 2014). However, freezing fecal samples at -80^{o}C prior to analysis may distort, rupture, or affect the colour of intestinal parasite eggs and oocysts (Zajac and Conboy 2012; Schurer et al., 2014), so the true prevalence of the intestinal parasites detected could have been under estimated.

PREVALENCE OF INTESTINAL PARASITES IN ONTARIO

Studies on the prevalence and distribution of intestinal parasites in dogs in Ontario are limited. One study investigated the prevalence of *Cryptosporidium* spp. and other intestinal parasites in dogs and cats in the Niagara region of southern Ontario (Shukla et al., 2006). Twenty-six veterinary clinics were contacted via mail and asked to report intestinal parasite infections in dogs that were examined from 2001 – 2003. Subsequently, 70 canine fecal samples collected from January – April 2004 were analyzed using a sodium nitrate fecal floatation method at a diagnostic laboratory at Brock University. The prevalence of *T. canis* was 14.8% (95% CI 11.3% - 18.9%) in dogs less than 6 months of age (Shukla et al., 2006). However, the study focused on samples from dogs that are considered high risk including puppies at their first veterinary visit, older dogs from shelter organizations, and animals who had gastro-intestinal signs, and animals who were recently treated for intestinal parasites (Shukla et al., 2006).

Another study focused on evaluating the prevalence of intestinal parasites in fecal samples from shelter animals (Villeneuve et al., 2015). For Ontario, 352 fecal samples were

collected and analyzed using a sugar double centrifugation method. Overall, 36.6% of the fecal samples tested positive for an intestinal parasite, with *T. canis*, *Cystoisospora* spp. and *T. vulpis* having a prevalence of 11.9%, 11.6% and 10.2%, respectively. However, the inclusion criteria for shelter animals were not described. Current animal shelters are participating in rescue and relief missions across the continent. So, each dog's place of origin was unknown. It is likely that at least some dogs originated from outside Ontario.

Finally, a study in southern Ontario investigated the prevalence and risk factors for zoonotic pathogens (Procter et al., 2013). Specifically, the prevalence of *Giardia* spp. was determined using the *Giardia* antigen SNAP test (IDEXX Laboratories, Westbrook, Maine) that has a sensitivity and specificity range of 90-92% and 96-99%, respectively (Groat 2003). The prevalence of *Giardia* was found to be 6.4% (95% CI 3.3 – 9.4) from 251 dog fecal samples collected from dog parks located in southwestern Ontario during the summer months (Procter et al., 2013). However, the prevalence estimate was considered low and not analyzed for risk-factors (Procter et al., 2013). Interestingly, the majority of the assemblages were type D which is not considered a zoonotic threat (Payne and Artzer 2009). Nonetheless, the data we collect on *Giardia* spp. can be added to the prevalence data on *Giardia* spp. in southern Ontario that can be used for veterinarian deworming protocols.

STUDY RATIONALE AND OBJECTIVES

Prior to 2012, *E. multilocularis* had not been reported in Ontario. However, since 2012, six cases of alveolar echinococcosis were diagnosed in dogs in southern Ontario. Five of the dogs did not have a travel history outside of Ontario, meaning they must have been infected in Ontario. Due to public health and veterinary concerns, a study was recently carried out to determine the prevalence and geographic distribution of *E. multilocularis* in wild canids across

southern Ontario; overall, approximately 25% of wild canids were fecal positive, with the highest infection prevalence estimates (24-46%) observed in ten southern Ontario public health units around the western region of Lake Ontario and the northern region of Lake Erie (Kotwa et al., 2019). Since *E. multilocularis* can be a large threat to human and canine health, there is a great need to identify the prevalence of *E. multilocularis* in dogs that reside in the ten public health units mentioned above. Dogs that are allowed off-leash and display high prey drive behavior are considered at greatest risk. This project was therefore designed to provide information on the prevalence of intestinal parasites, including *E. multilocularis*, in dogs that visit off-leash dog parks in the aforementioned area; the parasites described in the literature review pose a threat to canine health and data on their prevalence in Ontario is limited. Ultimately, the resultant data could help to inform appropriate preventive deworming program practices for high risk dogs in areas endemic for *E. multilocularis.* Therefore, the primary objectives of this study were:

1. Determine the prevalence of intestinal parasites, including *T. canis, A. caninum*, *T. vulpis* and *Cystoisospora* spp. in off-leash dogs within the Niagara and Hamilton regions of southern Ontario.
2. Determine the prevalence of fecal shedding of *E. multilocularis* in off-leash dogs within the Niagara and Hamilton regions of southern Ontario.

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TABLES

Table 1.1. Prevalence (%) and 95% confidence interval (CI) of *Ancylostoma caninum* and *Trichuris vulpis, Toxocara canis* and *Cystoisopora* spp. found from studies in Alberta, Ontario and Saskatchewan and the United States.

Parasite	Prevalence (%)	95% CI	Sample size	Fecal flotation solution	Location	Authors
Anclostoma caninum	0.4	0.05 – 1.6	457	Sucrose	Saskatchewan	Gaunt and Carr 2011
	0.8	0.2 – 1.7	619	Zinc sulfate	Alberta	Joffe et al., 2011
	1.8	1.5 – 2.2	6555	Zinc sulfate	Pennsylvania	Gates and Nolan 2009
	2.46	2.44 – 2.49	1 199 293	Zinc sulfate	United States	Little et al., 2009
	4.8	2.8 – 7.6	352	Sucrose	Ontario	Villeneuve et al., 2015
Trichuris vulpis	0.7	0.1 – 1.9	457	Sucrose	Saskatchewan	Gaunt and Carr 2011
	0.8	0.79 – 0.81	2 785 248	Zinc sulfate	United States	Mohamed et al., 2009
	1.2	1.18 – 1.22	1 199 293	Zinc sulfate	United States	Little et al., 2009
	2.9	0.4 – 9.9	70	Sodium nitrate	Ontario	Shukla et al., 2006
	3.0	2.6 – 3.4	6555	Zinc sulfate	Pennsylvania	Gates and Nolan 2009
	10.2	7.2 – 13.8	352	Sucrose	Ontario	Villeneuve et al., 2015
Toxocara canis	0.2	0.01 – 1.2	457	Sucrose	Saskatchewan	Gaunt and Carr 2011
	2.0	1.7 – 2.4	6555	Zinc sulfate	Pennsylvania	Gates and Nolan 2009
	2.2	2.17 – 2.23	1 199 293	Zinc sulfate	United States	Little et al., 2009

	5.0	4.97 – 5.03	2 785 248	Zinc sulfate	United States	Mohamed et al., 2009
	11.9	8.7 – 15.8	352	Sucrose	Ontario	Villeneuve et al., 2015
	14.3	7.0 – 24.7	70	Sodium nitrate	Ontario	Shukla et al., 2006
Cystoisopora spp.	3.2	2.8 – 3.6	6555	Zinc sulfate	Pennsylvania	Gates and Nolan 2009
	4.4	4.40 – 4.47	1 199 293	Zinc sulfate	United States	Little et al., 2009
	11.6	8.7 – 15.4	353	Sucrose	Ontario	Villeneuve et al., 2015

CHAPTER 2 - PREVALENCE OF INTESTINAL PARASITES IN THE FECES OF DOGS VISITING OFF-LEASH DOG PARKS IN THE NIAGARA AND HAMILTON REGIONS OF ONTARIO, CANADA.

This chapter is formatted for submission to Veterinary Parasitology: Regional studies and reports as: Tyler Greer, Claire Jardine, David L. Pearl, Scott Weese, Nicola Mercer, Andrew S. Peregrine. Prevalence of intestinal parasites in the feces of dogs visiting off-leash dog parks in the Niagara and Hamilton regions of Ontario, Canada.

ABSTRACT

A significant proportion of the Canadian dog population lives in southern Ontario. However, few data are available on the prevalence of intestinal parasites in dogs in this area. The objective of this study was therefore to determine the prevalence of intestinal parasites in dogs visiting off-leash dog parks within the Niagara and Hamilton region of Ontario. Twelve off-leash dog parks were visited five times between May – November 2018. During each visit, dog owners provided a fecal sample from their dog and completed a survey to obtain demographic and health management information about their dog. Samples were analyzed using the Cornell Wisconsin Double Centrifugation method. A total of 483 fecal samples were collected and a total of 11 different intestinal parasites identified. *Ancylostoma caninum*, *Trichuris vulpis*, *Cystoisospora* spp. and *Toxocara canis* were the most frequently identified intestinal parasites with a prevalence of 5.4% (95% confidence interval [CI] 3.5 – 7.8), 5.2% (95% CI 3.4 – 7.5), 3.5% (95% CI 2.1 – 5.6) and 1% (95% CI 0.3 – 2.4), respectively. *Taenia*-type eggs were detected in one dog (prevalence of 0.2% [95% CI 0.0 – 1.1]). Future work could focus on identifying risk factors associated with *A. caninum*, *T. vulpis* and *Cytoisospora* spp. in off-leash dogs in southern Ontario.

INTRODUCTION

Approximately 35% of households in Canada own a dog (Andreassen et al., 2013). In addition to providing companionship, dogs assist with multiple occupations and improve mental health in people (Robertson et al., 2000). Many pet dogs live with their owners in urban environments, but in these urban areas there can be limited yard space for dogs to exercise (Urbanik, and Morgan 2013). Off-leash dog parks are locations within urban areas that allow for socialization and physical exercise for dogs. They also facilitate social interactions between dog owners (Rock et al., 2016). As urbanization increases, the demand for, and use of, off-leash dog parks are likely to increase (Rahim et al., 2018).

Although there are many benefits associated with off-leash dog parks, these areas may present health risks to dogs due to a potential elevated risk of parasite transmission from dog to dog (Westgarth et al., 2010). In western Canada and the United States, the overall parasite prevalence in dogs visiting off-leash dog parks has been found to range from 4.4% to 50.2% (Gaunt and Carr 2011; Wang et al., 2012; Smith et al., 2014). Some of these parasites, such as *Ancylostoma caninum*, may cause significant disease when parasite burdens are high (Bowman 2014; Raza et al., 2018), and others, such as *Toxocara canis* and *Echinococcus multilocularis* may pose a zoonotic risk (Deplazes et al., 2011). In North America, *E. multilocularis* intestinal infections prevalence estimates have been investigated in wild canids but only one study in Alaska has investigated it in dogs (Schantz et al., 1995; Massolo et al., 2014). Recently, cases of the metacestode stage of *E. multilocualris* have been diagnosed in dogs in southern Ontario (Skelding et al., 2014; Oscos-Snowball et al., 2015; Pinard et al., 2019). In European countries where *E. multilocularis* is endemic, the prevalence of *E. multilocularis* intestinal infections in dogs has ranged from 0-14% with a median of approximately 0.3% (Deplazes et al 2011); dogs

that ingest rodents are at risk of developing intestinal infections and are a public health concern (Antolova et al., 2009; Deplazes et al., 2011; Gottstein et al., 2015).

Limited information about parasite prevalence in dogs located in Ontario is available. In addition, dogs infected with parasite can become diseased or transmit zoonotic parasites to humans (Kostopoulou et al., 2017; Raza et al., 2018). Therefore, there is a need to investigate the prevalence of intestinal parasites to assist with evidence based deworming protocols in Ontario. The objective of this study was therefore to determine the prevalence of intestinal parasites in dogs visiting off-leash dog parks within Niagara and Hamilton regions in southern Ontario.

MATERIALS AND METHODS

Dog and dog park selection

Off-leash dog parks in southern Ontario, Canada were identified in March 2018, using the Google search engine; a total of 85 off-leash dog parks were identified with 44 located in an area where *E. multilocularis* had been recently detected in the wild canid population (Kotwa et al., 2019). To accurately estimate a prevalence of 1% (95% confidence interval [CI] 0.3 – 2.3%) of intestinal infections in dogs, a total of 500 fecal samples would be required. Initially, a convenience sample of ten off-leash dog parks, located in the aforementioned area, was selected for fecal collection with a goal of 50 fecal samples from each off-leash dog park (Figure 1). Two additional parks were added to the original 10 off-leash dog parks because low numbers of dog owners visited two original off-leash parks (Figure 1). To control for temporal effects over the sampling period, each dog park was visited 5 times between May and November 2018 with a goal of collecting ten samples at each visit.

Ethics approval for this study was obtained from the University of Guelph's Research Ethics Board (Certification Number: 18-03-028).

Fecal collection

At each visit, dog owners were asked to provide a fecal sample from their dog immediately before leaving the park. In addition, dog owners were administered a survey to obtain information regarding their dog's physical and behavioral characteristics (i.e., age, sex, spayed/neutered) (Appendix 1). The inclusion criteria for the study were as follows: dog owners had to be at least 14 years old, the dog owner must have owned the dog for at least 6 months and the dog had to be greater than 6 months of age. Exclusion criteria were: dogs that were previously sampled for this study and owners who did not wish to complete the survey.

Laboratory analysis

After each visit, fecal samples were stored in a refrigerator for up to 1 week prior to processing. Each sample was analyzed using the Cornell Wisconsin Double Centrifugation method (Egwang and Slocombe 1982). The analytical sensitivity of the diagnostic method is approximately 0.3 eggs per gram. Intestinal parasites were identified to species by egg morphology and size (Zajac and Conboy 2012). However, *Taenia*-type eggs were only identified to genus (Zajac and Conboy 2012).

Statistical analysis

A data set was created that included dog and dog owner information as reported by the owner and results of the diagnostic data. This included the dog's sex (male or female; intact or neutered), weight, breed, the number of veterinary visits (zero, one, two or more than three) and use of heartworm preventive/deworming drugs in the 6-month period prior to filling out the survey, and the frequency and duration of visits to off-leash dog parks.

The data were analyzed using STATA/SE 15.1 (Stata Corp, College Station, Texas) statistical software. Proportions were reported for sex, breed and deworming status of dogs.

Weight was tested for a normal distribution using the Shapiro-Wilks test. In addition, median and range was reported for weight. The overall prevalence with 95% confidence intervals was calculated for each of the parasites identified. A one-sided 97.5% confidence interval was reported when the parasite prevalence was 0%.

RESULTS

Fecal samples were collected from 483 dogs with 59.2%, 40.4% and 0.4% classified as male, female, or unidentified (Table 2.1). The age of the dogs ranged from 7 months to 15 years with a median of 2.5 years. The weight of the dogs ranged from 2 to 83 kg with a median of 25.0 kg. Finally, 62.9% (304/483) were pure breed and 36.2% (175/483) were mixed breed dogs. The breed of four dogs was not identified.

Examination of the 483 fecal samples revealed 11 different intestinal parasites (Table 2.2). Single infections were identified in 13.3% (64/483) of samples. Co-infections with two (1.4%, 7/483) or three (0.41%, 2/483) parasites were identified in a small percentage of cases (Table 2.3).

Ancylostoma caninum was the most frequently identified intestinal parasite with an overall prevalence of 5.4% (95% confidence interval [CI] 3.5 – 7.8%; ranging from 0% (97.5% CI 0 – 7.1%) in dogs to 10% (95% CI 3 - 22%) among parks (Table 2.4). *Trichuris vulpis* was the second most common parasite identified with an overall prevalence of 5.2% (95% CI 3.4 – 7.5%); ranging from 0% (97.5% CI 0 – 7.1%) to 14.3% (95% CI 3 - 36.3%) among parks. Finally, *Cystoisospora* spp. was the third most frequently identified intestinal parasite with an overall prevalence of 3.5% (95% CI 2.1 – 5.6%); ranging from 0% (97.5% CI 0 – 70.7%) to 6.3% (95% CI 0.2 - 30.2%) among parks. The median ages of dogs infected with *A. caninum, T. vulpis* and *Cystoisospora* spp. were 1.5 years (age range from 6 months to 13 years), 3.0 years

(age range from 8 months to 14 years) and 1.5 years (age range from 8 months to 10 years), respectively.

Ancylostoma caninum infection ranged from a low prevalence of 0% (97.5% CI 0 – 13.7%) in November to a high of 7.3% (95% CI 2.4 - 16.1%) in May. *Trichuris vulpis* ranged from a low of 2.1% (95% CI 0.3 - 7.3%) in July to a high of 12.0% (95% CI 2.5 – 31.2%) in August. Finally, *Cystoisospora* spp. ranged from a low prevalence of 0% (97.5% CI 0 – 13.7%) in November to a high of 5.0% (95% CI 0.1 - 13.9%) in October (Table 2.5).

Finally, 91.3% (441/483) of dog owners reported visiting a veterinarian at least once in the year prior to providing a fecal sample from their dog, and 65.4% (316/483) had given a heartworm preventive or dewormer (as reported by the owner) to their dog within the previous 6 months.

DISCUSSION

This study provides a baseline prevalence for several intestinal parasites in dogs that visit off-leash dog parks in southern Ontario. A total of 11 intestinal parasites were found with *A. caninum*, *T. vulpis* and *Cystoisospora* spp. being detected most commonly. Additionally, one fecal sample tested positive for *Taenia*-type eggs. Interestingly, the prevalence of *Toxocara canis* was 1% (95% CI 0.3 – 2.4%), which is numerically lower than the prevalence of 11.9% (95% CI 8.7 – 15.8%) found by Villeneuve et al. (2015) who used the same diagnostic method but focused on shelter dogs from Ontario and other Canadian provinces between May 2009 and November 2010. The difference in prevalence could be explained by the difference in inclusion criteria. Villeneuve et al. (2015) excluded dogs if they had a history of being dewormed within 5 months of sample collection, unlike the present study that did not use deworming as an exclusion criterion. Additionally, in the work by Villeneuve et al. (2015), information on each dog's place

of origin was unknown; it is likely that at least some dogs originated from outside Ontario. Animal shelters are a temporary housing environment where dogs are either returned to their owner or adopted. The temporary housing allows for increased traffic of dogs that have an unknown medical history (Raza et al., 2018). In addition, animals housed in a shelter experience a variety of stressful factors, such as a new environment, isolation, change of diet and noise (Raza et al., 2018). In combination, stress and animal movement could provide a reason why *T. canis* prevalence was higher in shelter dogs compared to the dogs in our study.

The prevalence of *Ancylostoma caninum* detected in this study was 5.4% (95% CI 3.5 - 7.8%). This figure is higher compared to 0.8% (95% CI 0.2 - 1.7%), 0.4% (95% CI 0.05 – 1.57%) and 1.8% (95% CI 1.5 - 2.2%) from Alberta, Saskatchewan and Pennsylvania, respectively (Gates and Nolan 2009; Little et al., 2009; Gaunt and Carr 2011; Joffe et al., 2011). The dog population and diagnostic methods used were similar to the present study with samples collected from privately owned dogs and centrifugation methods used for analysis with sugar or zinc-sulfate solutions (Gates and Nolan 2009; Joffe et al., 2011; Gaunt and Carr 2011). Most dewormers (including heartworm preventives) approved in Canada for use in dogs are approved with activity against *A. caninum* and *T. canis*. The present study found that 65.4% of dog owners reported they had given a dewormer within the 6-month period prior to sample collection consistent with the low observed prevalence of *T. canis* (1.0%; 95% CI 0.3 – 2.4%). In work carried out by Mohamed et al. (2009) on privately owned dogs in 44 states in the United States of America, similar prevalence values were observed for *T. canis* and *A. caninum*, 4.5% and 5.0%, respectively. Similarly, prevalence values for *T. canis* and *A. caninum* in privately owned dogs from Pennsylvania with an age range from 1 year to 15 years were 0.68% and 1.38%, respectively (Gates and Nolan 2009; Little et al., 2009). As indicated, the prevalence of *A.*

caninum in this study was 5.4% (95% CI 3.5 – 7.8%), higher than the estimate for *T. canis.* Since most canine dewormers have activity against *T. canis* and *A. caninum,* this observation could be due to the occurrence of drug resistance in *A. caninum* which has recently been described for benzimidazoles and macrocyclic lactones in the southeast USA (Kaplan et al., 2018; Gilleard 2019; Kitchen et al., 2019). However, the present study did not obtain information on the specific drug(s) dog owners had administered to their dog or when it was administered. Alternatively, there may be different risk factors for these two parasites in southern Ontario. Further work on this issue is warranted. However, it should be recognized that obtaining information on specific dewormers and when they were used by owners (either from owners or their veterinarians) is often problematic (Peregrine, personal communication).

The prevalence of *Trichuris vulpis* observed in this study was 5.2% (95% CI 3.4 -7.5%). This figure is higher compared to 0% (95% CI 0.18 – 1.7%), 0.7% (95% CI 0 – 1.9%) and 3.0% (95% CI 2.6 – 3.5%) found in Alberta, Saskatchewan and Pennsylvania, respectively (Gates and Nolan 2009; Little et al., 2009; Gaunt and Carr 2011; Joffe et al., 2011). Gaunt and Carr (2011) collected fecal samples from owned dogs, and garbage containers located in off-leash dog parks and on walking trails in Saskatoon, Saskatchewan, from May – October 2018. The standard centrifugation fecal examination technique described in Dryden et al. (2005) was used for fecal samples analysis. Parasite egg and oocyst survivability is dependent on the temperature of its environment (Bowman 2014). The colder and drier environment of Alberta and Saskatchewan could affect the prevalence found for *T. vulpis* (Raza et al., 2018).

Finally, the prevalence of *Taenia*-type eggs in dogs from this study was 0.2% (95% CI 0.0 – 1.1%). The prevalence of this parasite is likely underestimated because the fecal floatation has relatively low sensitivity and cannot detect prepatent and single-sex infections (Dryden et al.,

2005; Conboy 2009; Epe 2009). Furthermore, when compared to necropsy, the centrifugal flotation method identified only 57.1% of shelter dogs infected with *Taenia* spp. (Adolph et al., 2017). *Taenia*-type eggs are excreted in proglottids and are not homogenously mixed throughout feces (Adolph et al., 2017). Thus, they can be missed when examined using the double centrifugation technique (Dryden et al., 2005; Liccioli et al., 2012). Since *Taenia*-type eggs are morphologically indistinguishable from *E. multilocularis* eggs, the identity of the parasite is not known.

Lastly, the prevalence of *Giardia* infections was found to be 0.4% (95% CI 0.1 – 1.5%) which is substantially less than estimates of 6 – 7% that have been found in previous studies in the same area (Lefebvre et al., 2006; Procter et al., 2013) using fecal antigen tests that are considered more sensitive than the diagnostic method used in this study (Dryden et al., 2006).

LIMITATIONS

Dog park and fecal sample collection was based on the convenience sampling method. The method is a non-probability sampling method where individuals do not have a specific probability of being selected (Dooho et al., 2003). The method is biased towards dogs who used off-leash dog parks selected in this study and not a representation of all dogs who visit off-leash dog parks in southern Ontario. In addition, our study did not identify the prevalence in dogs who do not visit off-leash dog parks because of their owner's motives to avoid off-leash parks. Specifically, dog owners could avoid off-leash parks based their awareness of their dog's dominant behavior or perception of pathogen transmission at off-leash dog parks. Conversely, dog owners could avoid parks because their dog obtains adequate exercise and socialization from different sources. Ultimately the prevalence and behavioral characteristics of these dogs was not captured.

CONCLUSION

This study provides baseline data for the prevalence of intestinal parasites in off-leash dogs located in southern Ontario. This data could be used by veterinarians to increase parasite awareness and generate evidence-based prophylaxis for dogs residing in the study area. *Anclyostoma caninum*, *T. vulpis* and *Cystotisospora* spp. were the most common intestinal parasites identified. Finally, one fecal sample tested positive for *Taenia*-type eggs. Future studies could identify risk factors associated with *A. caninum*, *T. vulpis*, and *Cystoisospora* spp. infections in off-leash dogs in southern Ontario.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURES AND TABLES

Table 2.1 Demographics of dogs stratified by sex that had fecal samples (N=483) collected from 12 off-leash dog parks located in southern Ontario between May – November 2018.

Dog Classification	Male	Female
Sex (%) [*]	59.2	40.4
Neutered or spayed (%)	43.7	34.0
Intact (%) [+]	15.5	6.4
Weight range (kg)	2.0 – 79	2.0 – 83
Median weight (kg)	25	25
Age range (years)	0.5 – 15.0	0.5 – 14.0
Median age (years)	2.5	2.1

[*]0.4% were unidentified

[+] percentage of all dogs

Table 2.2 Prevalence (%) and 95% confidence interval (CI) of intestinal parasites in 483 dogs that visited off-leash dog parks in southern Ontario between May – November 2018.

Parasite	Frequency	Prevalence (%)	95% CI
Ancylostoma caninum	26	5.4	3.5 – 7.8
Baylisascaris procyonis	1	0.2	0.0 – 1.1
Cystoisospora spp.	17	3.5	2.1 - 5.6
Eucoleus aerophilus	2	0.4	0.1 – 1.5
Giardia spp.	2	0.4	0.1 – 1.5
Sarcocystis spp.	3	0.6	0.1 – 1.8
Strongyloides stercoralis	2	0.4	0.1 – 1.5
Taenia type-egg	1	0.2	0.0 – 1.1
Toxocara canis	5	1.0	0.3 – 2.4
Trichuris vulpis	25	5.2	3.4 – 7.5
Uncinaria stenocephala	1	0.2	0.0 - 1.1

Table 2.3. Fecal samples from dogs that were identified with more than one intestinal parasite.

Parasites found in fecal sample	Dog ID								
	49	153	163	175	197	206	266	368	453
Ancylostoma caninum		X	X		X				X
Baylisascaris procyonis				X					
Cystoisospora spp.	X		X	X	X	X	X		
Eucoleus aerophilus						X			
Giardia spp.								X	
Strongyloides stercoralis	X		X						
Trichuris vulpis	X	X					X	X	X

Table 2.4. Prevalence (%) and 95% confidence interval (CI) of *Ancylostoma caninum, Trichuris vulpis* and *Cystoisospora* spp. in fecal samples from dogs visiting off-leash dog parks in southern Ontario between May – November 2018.

Dog Park #	Number of fecal samples	*Ancylostoma caninum* (%)	95% CI (%)	*Trichuris vulpis* (%)	95% CI (%)	*Cystoisospora* spp. (%)	95% CI (%)
1	50	6.0	1 – 17	4	2.2 – 19.2	6.0	1.3 – 16.5
2	51	3.9	0.5 – 13.5	13.7	5.7 – 26.3	5.9	1.2 – 16.2
3	50	10	4 – 21	0	0 – 7.1[a]	2.0	0.5 – 13.7
4	44	4.5	1 – 15	2.3	0.1 – 12	4.5	0.6 – 15.5
5	50	0	0 – 7.1[a]	2	0.5 – 13.7	6	1.3 – 16.5
6	3	0	0 – 70.7[a]	0	0 – 70.7[a]	0	0 – 70.7[a]
7	50	10	3 – 22	4	2.2 – 19.2	4.0	0.5 – 13.7
8	50	2	0.1 – 10.6	6	1.3 – 16.5	0	0 – 7.1[a]
9	44	6.8	1 – 18	11.4	3.8 – 24.6	2.3	0.1 – 12
10	21	9.5	1 – 30	14.3	3 – 36.3	0	0 – 16.1[a]
11	16	0	0 – 20.6[a]	0	0 – 20.6[a]	6.3	0.2 – 30.2
12	54	5.6	1 – 15	1.9	0 – 9.9	1.9	0 – 9.9
Overall	483	5.4	3.5 – 7.8	5.2	3.4 – 7.5	3.5	2.1 – 5.6

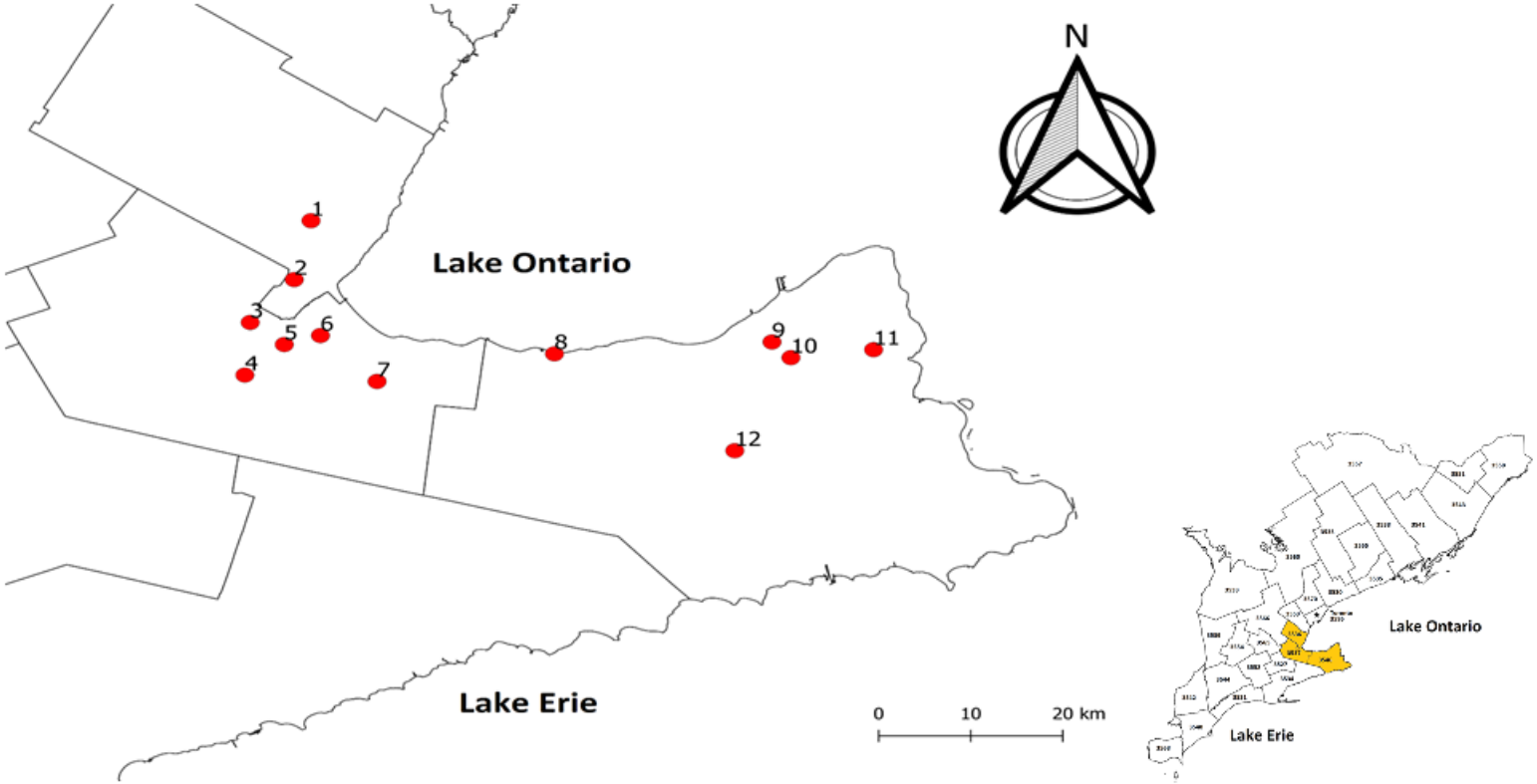
[a] corresponds to a one-sided 97.5% exact binomial confidence interval

Table 2.5. Monthly prevalence (%) and 95% confidence interval (CI) of intestinal parasites in fecal samples from dogs visiting off-leash dog parks in southern Ontario between May – November 2018.

Month	Number of fecal samples	*Ancylostoma caninum* (%)	95% CI (%)	*Cystoisospora* spp. (%)	95% CI (%)	*Toxocara Canis* (%)	95% CI (%)	*Trichuris vulpis* (%)	95% CI (%)
May	69	7.3	2.4 – 16.1	2.9	0.4 – 10.1	0	0 – 5.2[a]	4.4	0.9 – 12.2
Jun	92	6.5	2.4 – 13.7	4.4	1.2 – 10.8	3.3	0.7 – 9.2	7.6	3.1 – 15.1
Jul	97	6.2	2.3 – 13.0	3.1	0.6 – 8.8	0	0 – 3.7[a]	2.1	0.3 – 7.3
Aug	62	4.8	1.0 – 13.5	4.8	1.0 – 13.5	1.6	0 – 8.7	8.1	2.3 – 17.8
Sep	78	6.4	2.1 – 14.3	2.6	0.3 – 9.0	0	0 – 4.6[a]	3.9	0.8 – 10.8
Oct	60	1.7	0 – 8.9	5.0	1.0 – 13.9	1.7	0 – 8.9	3.3	0.4 – 11.5
Nov	25	0	0 – 13.7[a]	0	0 – 13.7[a]	0	0 – 13.7[a]	12	2.5 – 31.2

[a] corresponds to a one-sided 97.5% exact binomial confidence interval

Figure 2.1. Locations of dog parks selected for fecal collection from May – November 2018. Map was generated using QGIS.



CHAPTER 3 – INVESTIGATION OF *ECHINOCOCCUS MULTILOCULARIS* IN THE FECES OF DOGS VISITING OFF-LEASH DOG PARKS IN THE NIAGARA AND HAMILTON REGIONS OF ONTARIO, CANADA.

This chapter is formatted for submission to Veterinary Parasitology short communication as: Tyler Greer, Claire Jardine, David L. Pearl, Scott Weese, Nicola Mercer, Andrew S. Peregrine. Investigation of *Echinococcus multilocularis* in the feces of dogs visiting off-leash dog parks in the Niagara and Hamilton regions of Ontario, Canada.

ABSTRACT

Prior to 2012, *Echinococcus multilocularis* had not been reported in Ontario. However, a recent study detected *E. multilocularis* in wild canids across southern Ontario with the highest prevalence in wild canids from 10 public health units located along the northern shore of Lake Erie and the western shore of Lake Ontario. Since dogs can harbour *E. multilocularis* intestinal infections, the risk of human exposure to parasite eggs in this area is a public health concern. The objective of this study was therefore to investigate the prevalence of *E. multilocularis* intestinal infections in dogs in the aforementioned public health units. Fecal samples were collected from 212 dogs that visited twelve off-leash dog parks between May – November 2018. Samples were tested for *E. multilocularis* using a magnetic capture probe-based DNA extraction and real time PCR method. All 212 fecal samples tested negative (97.5% confidence interval [CI] 0 – 1.6%). This suggests dogs who visit off-leash dog parks in the Niagara and Hamilton region of southern Ontario are unlikely to be transmitting *E. multilocularis* to their owners.

Keywords: *Echinococcus multilocularis,* dog, feces, off-leash park, Ontario

INTRODUCTION

Echinococcus multilocularis was not considered an endemic parasite in Ontario prior to 2012. However, between 2012 and 2018, six cases of alveolar echinococcosis were diagnosed in dogs in southern Ontario (Skelding et al., 2014; Oscos-Snowball et al., 2015; Pinard et al., 2019); five of the dogs had no history of travel outside Ontario, indicating they must have been infected locally. In addition to these cases, a wild-caught eastern chipmunk, and three lemurs that resided at a private wildlife sanctuary, were diagnosed with alveolar echinococcosis in southern Ontario (Turner et al., 2016; French et al., 2018). The chipmunk, all three lemurs, and five of the dogs resided at the western end of Lake Ontario. As a result of these diagnoses, a study evaluated the prevalence and geographic distribution of *E. multilocularis* in wild canids across southern Ontario. Infections were detected in 23% (95% confidence interval [CI] 20-27%) of the wild canids, with the highest infection prevalence estimates (24-46%) observed in 10 public health units located in the southern portion of southern Ontario, specifically around the western region of Lake Ontario and the northern shore of Lake Erie (Kotwa et al., 2019). Collectively, these findings indicated that *E. multilocularis* has become established in southern Ontario (Kotwa et al. 2019) and raise concerns for public and animal health.

Since dogs live in close proximity to humans and can be definitive hosts for *E. multilocularis*, they may be involved in transmission of this parasite to humans and other animals (Deplazes et al., 2011). Conraths et al. (2017) concluded that dog ownership is a potential risk factor for human alveolar echinococcosis. So, understanding the prevalence of *E. multilocualris* can be used to assess the risk to humans. In work carried out to evaluate the prevalence of *E. multilocularis* intestinal infections in dogs in areas of Europe considered endemic for this parasite, a higher prevalence of *E. multilocularis* intestinal infections was observed in dogs that are used for hunting, and guarding or herding sheep, compared to privately owned dogs

(Antolová et al., 2009; Nagy et al., 2011); dogs who roam are likely at greater risk of developing *E. multilocularis* intestinal infections from ingestion of rodents (Svobodová and Lenská 2002; Budke et al., 2005; Ziadinov et al., 2008).

In areas of Europe where the prevalence of *E. multilocularis* in wild canids is similar to Ontario, the prevalence of *E. multilocularis* intestinal infection in dogs ranges from 0-14%, with a median of approximately 0.3% (Deplazes et al., 2011). However, such information is lacking for Ontario. Since PCR analysis is considered the most sensitive method for antemortem detection of fecal shedding of *E. multilocularis* in dogs, the objective of this study was to use PCR to determine the prevalence of *E. multilocularis* in dogs visiting off-leash dog parks in the aforementioned area of southern Ontario.

MATERIALS AND METHODS

Dog, and dog park selection and fecal collection

The study area and fecal collection method have been described previously (Chapter 2). Briefly, 12 off-leash dog parks located in 3 of the 10 public health units mentioned above were selected for fecal collection with a goal of 50 fecal samples from each off-leash dog park. Each dog park was visited 5 times throughout the months May to November 2018 with an objective of colleting 10 samples per visit. During each visit, dog owners were asked to provide a fecal sample from their dog immediately before exiting the park. Dog owners had to be at least 14 years old and must have owned the dog for at least 6 months. Dogs had to be greater than 6 months of age. Fecal samples were not accepted if the dog had been previously sampled or if the owner did not wish to complete a survey. Information gathered on the survey included characteristics about the dog and dog owner (see Chapter 2). Fecal samples were stored at -80°C prior to analysis.

PCR analysis

The extraction and analysis of *E. multilocularis* DNA was conducted with a magnetic capture probe-based extraction, real-time hydrolysis PCR method (MC-PCR) that has been previously described (Isaksson et al., 2014). However, the automation washing step with magnetic Dynabeads (Life technologies, Carlsbad, California) was replaced with manual washing because an automated robot was not available (Isaksson et al., 2014). When compared to the sedimentation and counting technique (SCT), considered the gold standard test for detection of *E. multilocularis* infections in wild foxes, the MC-PCR method was found to have a sensitivity and specificity of 88% and 99%, respectively (Isaksson et al., 2014; Wahlström et al., 2016). As such, the MC-PCR is used for large-scale screening of fecal samples from suspected infected canids (Isaksson et al., 2014).

Each MC-PCR analysis comprised 45 dog fecal samples, 2 negative controls and 1 positive control. The negative controls contained lysis buffer (8.32 M Tris HCl pH 8.0, 0.42 M EDTA pH 8.0, 0.2% SDS, 16.6 M NaCl) and 208M NaCl (Isaksson et al., 2014). The positive control was a coyote fecal sample that had previously tested positive with the MC-PCR. All samples were run in duplicate and were considered positive if the PCR Ct value was less than 40 for at least one sample (Isaksson et al., 2014).

Data analyses

One-sided 97.5% confidence intervals were calculated when the parasite prevalence was 0%, using STATA/SE 15.1 (Stata Corp, College Station, Texas). The freedom of infection calculation was based on the infinite population formula and used to estimate 95% confidence of the lowest level of infection in this population (Dohoo et al., 2003).

RESULTS

A total of 212 fecal samples from 12 off-leash dog parks located in 3 of the 10 public health units mentioned above were analyzed with the MC-PCR. All 212 fecal samples tested negative for *E. multilocularis*, with a prevalence of 0% (97.5% CI 0 – 1.6%). All positive controls were positive, with the median and range for the positive controls' Ct values being 22.4 and 20.7 – 25.2 (5 replicates), respectively. Based on a freedom of infection calculation, with 212 negative samples, there is a 95% confidence the prevalence in this population is 1.4% or lower.

DISCUSSION

The prevalence of *E. multilocularis* intestinal infections in our dog population was 0% (97.5% CI 0 – 1.6%). Based on a freedom of infection formula, we are 95% confident that the prevalence of *E. multilocularis* in off-leash dogs in southern Ontario is less than 1.4% (Dohoo et al., 2003). This is similar to the prevalence's of 0.3% (95% CI 0.05 – 1.2), 0.35% (95% CI 0.24 – 0.49) and 0.2% (95% CI 0.02 – 0.64) observed in privately owned dogs in European countries where the prevalence of *E. multilocularis* in wild foxes ranged from 2.1% - 67.0% (Deplazes et al., 1999; Dyachenko et al., 2008; Comte et al., 2010), suggesting that similarly low levels of infection could be present in dogs in southern Ontario. Deplazes et al. (1999) tested 660 fecal samples from dogs in Switzerland via an antigen ELISA and PCR for *E. multilocularis.* Interestingly, only two dogs tested positive and both were Dachshunds that were known to hunt small rodents (Crellin et al., 1981; Thompson and Eckert 1983). Additional studies in the Czech Republic and Slovakia found prevalences of 8.1% (95% CI 4.7 – 13.2%) and 2.8% (95% CI 1.3 – 5.6%) in dogs that had a history of hunting rodents or irregular deworming schedules, respectively (Svobodová and Lenská 2002; Antolová et al., 2009). Few dog owners in our study, 8.9% (N=483), reported their dogs to have eaten a small mammal and most dog owners, 65.4%

(N=483), reported they had given their dog a heartworm preventive or dewormer 6 months prior to fecal collection. The selection process for dogs in the Czech Republic and Slovakia studies could explain the higher prevalence in comparison to our study.

In chapter 2, one fecal sample (n=483) tested positive for *Taenia*-type eggs via the Cornell Wisconsin Double Centrifugation method (Egwang and Slocombe 1982) but the sample tested negative on PCR analysis. Since *Taenia*-type eggs are morphologically indistinguishable the sample is most likely *Taenia pisiformis* eggs (Bowman et al., 2014).

LIMITATIONS

The estimated prevalence of *E. multilocularis* intestinal infections in dogs is likely underestimated because of the sensitivity of the MC-PCR and biological factors of *E. multilocularis.* To begin, the MC-PCR has a sensitivity of 88%, so there is a possibility of false negative test results. In addition, the prepatent period for *E. multilocularis* is approximately 30 days with egg excretion lasting up to 90 days (Deplazes et al., 2011). Kapel et al., 2006 found dogs to have a significantly higher worm burden than foxes, racoon dogs and cats after 90 days, post infection with most egg excretion in dogs occurring by 70 days post infection (Kapel et al., 2006). So, fecal samples that were collected during the prepatent period or after 70 days post infection could test negative on the MC-PCR.

CONCLUSION

Our prevalence estimation for *E. multilocularis* intestinal infections was found to be 0% (97.5% CI 0 – 1.6%) in off-leash dogs. So, the likelihood that off-leash dogs are transmitting the parasite to their owners is low. Alternatively, dogs that hunt rodents are more likely to be infected with *E. multilocularis* intestinal infections (Deplazes et al., 2011). Additional studies should investigate the prevalence *E. multilocularis* in other dog populations in southern Ontario.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CHAPTER 4 – GENERAL DISCUSSION

SUMMARY AND SIGNIFICANCE

Because of the recent finding of *Echinococcus multilocularis* in wildlife in southern Ontario and limited information on the prevalence of intestinal infections in dogs in this region this study had the following objectives: (a) Determine the prevalence of intestinal parasites in off-leash dogs within the Niagara and Hamilton regions of southern Ontario using a double centrifugation method (Chapter 2); and (b) Determine the prevalence of fecal shedding of *E. multilocularis* in off-leash dogs within the Niagara and Hamilton regions of southern Ontario using a PCR method (Chapter 3).

From May – November 2018, 12 off-leash dog parks located in the Niagara and Hamilton regions were visited and fecal samples were collected from 483 dogs. We identified a total of 11 species of intestinal parasites in these dogs. The most common intestinal parasites identified were *Ancylostoma caninum, Trichuris vulpis* and *Cystoisospora* spp. with a prevalence of 5.4% (95% Confidence interval [CI] 3.5 – 7.8%), 5.2% (95% CI 3.4 – 7.5%) and 3.5% (95% CI 2.1 – 5.6%), respectively. One sample tested positive for *Taenia*-type eggs. The species identified and prevalence of intestinal parasites found in our study is similar to what has been reported in dogs that visited private vet clinics located in the USA (Gates and Nolan 2009; Little et al., 2009), but the prevalence of *Toxocara canis* (1%; 95% CI 0.3 – 2.4%) was lower than what has been observed in shelter dogs (11.9%; 95% CI 8.7 – 15.8%) and other dogs considered to be at high risk of intestinal parasite infections (i.e., puppies first visit to a veterinary clinic, dogs with gastro-intestinal signs or who have previously been treated for intestinal parasites; 2.9%; 95% CI 0.4 – 9.9%) in Ontario (Shukla et al., 2006; Villeneueve et al., 2015). The lower prevalence of *T. canis* observed in our study may be due to our exclusion of dogs less than 6 months of age,

which is an age group that we know is likely to have a higher prevalence of *T. canis* (Corda et al., 2019). Interestingly studies in Canada and the USA, that used the same diagnostic method found the prevalence of *T. canis* and *A. caninum* was similar. However, based on the 95% confidence intervals, we found a significant difference in the values for *A. caninum* and *T. canis.* In Canada, heartworm preventives have activity against both *T. canis* and *A. caninum* so it is expected that the prevalence values obtained in this study should be similar. One possible explanation for this finding could be that *A. caninum* is developing resistance to anthelmintic drugs (Kaplan et al., 2018). *Ancylostoma caninum* resistance to pyrantel dates to 2007 (Kopp et al., 2007) and since then, *A. caninum* resistance has been found for fenbendazole and ivermectin (Kitchen et al., 2019). Additional investigation on *A. caninum* resistance in our study population is required to investigate this hypothesis.

In addition to providing baseline data on the prevalence of intestinal parasites in well-managed dogs from off-leash dog parks, the results of this study can be used to inform veterinarians and dog owners about the potential risk related to intestinal parasites in dogs that visit off-leash parks in southern Ontario and aid in development of appropriate deworming protocols. Typically, dogs that reside in southern Ontario receive a heartworm prevention that also has activity against *T. canis* and *A. caninum* 6 times per year (Clark et al., 1991). Our study found that 65.4% of dog owners reported they had given a heartworm preventive or dewormer to their dog. However, our survey did not specify the type of heartworm preventive or dewormer that the dog owner administered to their dog or investigate owner compliance. Also, parasites of concern in Ontario has mainly been on roundworms and hookworms (CPEP 2009). However, the findings for our study found hookworms (*A. caninum*) and whipworms (*T. vulpis*) to have a higher prevalence compared to the roundworm (*T. canis).* This suggests that veterinarians may

want to consider using a dewormer that is effective against whipworms, in addition to roundworms and hookworms, in their preventive program.

In the work described here, the prevalence of *E. multilocularis* was found to be 0% (97.5% CI 0 – 1.6%) in 212 dogs located in an area of southern Ontario where the prevalence in foxes and coyotes has recently been shown to range from 24 – 46% (Kotwa et al., 2019). The upper end of our 95% CI is 1.6%. Thus, based on a freedom of infection calculation, we can be 95% certain the prevalence is less than 1.4%. Similar prevalence estimates, ranging from 0.2% to 0.35%, have been found in privately owned dogs that reside in endemic areas of Switzerland, Germany and France, (Deplazes et al., 1999; Dyachenko et al., 2008; Comte et al., 2010). However, higher prevalence estimates ranging from 2.8% to 19.7% have been identified in dogs that hunt rodents in the Czech Republic, Slovakia, Kyrgyzstan and China where *E. multilocularis* is present (Svobodová and Lenská 2002; Budke et al., 2005; Ziadinov et al., 2008; Antolová et al., 2009) Even though *E. multilocularis* is present in the wildlife in southern Ontario, only 8.9% of dogs were reported to have eaten a small mammal (Chapter 3). In addition, the survey did not specify the type of small mammal that was eaten. Nevertheless, dogs that have a history of eating rodents and reside in endemic areas of *E. multilocularis* could become infected with the adult stage of *E. multilocularis* and shed eggs in their feces. Since dogs live in close proximity to humans, this is a zoonotic concern due to the possibility of egg transmission to humans (Deplazes et al., 2011). As such, dog ownership has been associated with alveolar echinococcosis in humans (Conraths et al., 2017). Thus, dog owners in southern Ontario, should be informed and understand the risk of *E. multilocularis* intestinal infections in dogs and decide if deworming with praziquantel is warranted for their dog (Conboy 2009).

LIMITATIONS

Since this study was designed to investigate the prevalence of *E. multilocularis* intestinal infections in dogs, a convenience sampling method was used to select dog parks found in the area of southern Ontario with the highest prevalence of *E. multilocularis* in wild foxes and coyotes (Kotwa et al., 2019). Convenience sampling is a non-probability sampling method that does not give equal chance of selection between units (Dohoo et al., 2003). Despite the properties of this sampling method, it is still appropriate for introductory observational studies like this study (Dohoo et al., 2003). In addition, the internal and external validity of this study is affected by this sampling method (Carlson and Morrison 2009). Dog park selection focused on an area where *E. multilocularis* was established in wild canids (Kotwa et al., 2019). So, our findings cannot be generalized to dogs that visit off-leash dog parks outside of the study area. Finally, dogs and dog owners across Ontario have motives and behaviors that could be different from the results we gathered for off-leash dog park users. So, we cannot extrapolate our findings to all dogs and dog owners in southern Ontario.

An additional limitation is that our survey relied on owner responses to questions, so there may be issues with the reliability of our data, particularly for data regarding weight and administering heartworm preventive or dewormer to their dogs. In addition, owners were not asked to specify what type of heartworm preventive or dewormer they were administering to their dog or how often, so our ability to investigate the relationships between deworming and parasite occurrence is limited.

FUTURE DIRECTIONS

Other dog populations that may be at higher risk of *E. multilocularis* infection should be investigated for intestinal infections with *E. multilocularis.* Studies from China, Slovakia and the Czech Republic have identified higher prevalence of *E. multilocularis* intestinal infections in

dogs who hunt rodents (Budke et al., 2005; Antolová et al., 2009; Nagy et al., 2011) and we could target those dogs for fecal collection and analysis here in Ontario. In addition, dogs who have visited vet clinics in the past and tested positive for *Taenia*-type eggs via fecal flotation, could be followed up and tested for *E. multilocularis* intestinal infections using the magnetic capture probe-based PCR method (Isaksson et al., 2014).

In order to specify what dewormers dog owners are using, a questionnaire could be submitted to dog owners and ask questions regarding the type of heartworm preventives and dewormers they give their dog or alternatively, researchers could work with veterinary clinics to obtain this information. Working directly with veterinary clinics may allow us to get more specific and accurate information about the products that are being used by dog owners.

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APPENDICES

Appendix 1. Survey that contains questions regarding the dog owner's personal information, their dog's physical and behavioral characteristics, travel and medical history.

Participants signature:_____

Date & Location			
Day:	Month:	Year:	Time:
Dog park:			

Dog Information		
Dog's name:	How long have you owned the dog for?	
Dog's weight: Lb □ Kg □	Dog's breed:	
Dog's age:	Sex: Male □ Female □	
Is your dog spayed/neutered: yes □ no □	# dogs in the home:	# cats in the home:

Dog Activity History
In general, when your dog goes for a walk is he/she on a leash? Never (0%) □ Rarely (<20%) □ Sometimes (20% - 80%) □ Always (>80%) □
How long is a typical visit to an off-leash dog park? <30 minutes □ 30 to 60 minutes □ > 60 minutes □
How many times a week do you typically visit an off-leash dog park? <1 per week □ 1 per week □ 2-3 times per week □ 4-5 times per week □ ≥6 times per week □
In general, how often is your dog out of your line of sight when off leash? (e.g. roaming in the woods) Never (0%) □ Rarely (<20%) □ Sometimes (20% - 80%) □ Always (>80%) □

In the last 6 months, where has your dog visited or spent time? *Please check all that apply*

Cottage □	Field □	Inside my house □	Public Park □	Other □ (*please*
Country road□	Forest □	Lake □	Provincial park □	*specify):*
Farm □	In my yard□	Local neighbourhood □		

In the 6 last months, has your dog visited other areas in southwestern Ontario? yes □ no □

If yes, what other areas? *Please check all that apply*

Brant □	Guelph □	Mississauga □	Oxford □	Toronto □	Other □ *(please*
Burlington □	Hamilton □	Niagara □	Perth □	Waterloo □	*specify):*
Caledonia □	London □	Oakville □	St. Thomas □		_____

In the last 6 months, has your dog travelled outside of southwestern Ontario yes □ no □

If yes, where *(please specify)*:_____

Does your dog chase small mammals (i.e.: rodents, squirrels, rabbits)?

No □

Yes □ – How often:

Daily □ Weekly □ Monthly □ Yearly □ Other □ *(please specify):*

Does your dog eat small mammals (i.e.: rodents, squirrels, rabbits)?

No □

Yes □ – How often:

Daily □ Weekly □ Monthly □ Yearly □ Other □ *(please specify):*

Does your dog consume a raw diet?

No □

Yes □ – What type of meat:

Beef □ Fish□ Lamb □ Pork □ Poultry □ Other □ *(please specify):*

How frequently is your dog fed a raw diet? Daily □ Weekly □ Monthly □ Yearly □ Other □ *(please specify):*
Is your dog used for hunting or tracking wildlife (e.g. deer, ducks, bears, moose, turkeys)? yes □ no □
In the last year, how many times have you taken your dog to a veterinarian? 0 visits □ 1 visit □ 2 visits □ ≥3 visits □
In the last 6 months, to your knowledge, has your dog been dewormed or received heartworm preventives? Yes □ No □ I don't know □
Has your dog ever been diagnosed with a tapeworm infection? Yes □ No □ I don't know □

iPad Mini Draw: If you are interested, your name will be entered into a draw for a chance to win a new iPad mini. The prize will be drawn in January 2019.

Please enter my name into the draw for a chance to win an iPad mini: yes □ no □