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Prevalence of intestinal parasite infection in symptomatic and asymptomatic dogs in southwestern West Virginia: the potential impact on human health

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Prevalence of intestinal parasite infection in symptomatic and asymptomatic dogs in southwestern West Virginia: the potential impact on human health.

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ABSTRACT

Prevalence of intestinal parasite infection in symptomatic and asymptomatic dogs in southwestern West Virginia: the potential impact on human health.

By Tashina Marie Savilla

Most dog owners are unaware of asymptomatic infection and of the possibility of contracting zoonotic parasites from their dogs. We hypothesized that parasite infection is: associated with one or more symptoms; independent of gender and age; and independent of anthelmintic usage. Stool samples were collected from 231 dogs in Kanawha County, West Virginia, and were examined by simple fecal flotation. Parasitic prevalence was found to be 23% for *Ancylostoma caninum*, 8% for *Trichuris vulpis*, 7% for *Toxocara canis*, 4% for *Isospora* species, and 32.5% overall. There was no significant relationship between infection and gender, nor between infection and symptom for *A. caninum*, *To. canis*, and *Isospora* species. There were significantly more infections in puppies for *A. caninum*, *To. canis*, and *Isospora* species. Eighty-six percent of the dogs receiving anthelmintics were free of parasitic infection ($P < 0.005$). These results may lead to improved deworming protocols and awareness of dog zoonoses.

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CHAPTER ONE: BACKGROUND & OBJECTIVES

In recent years, many surveys have been conducted to determine the prevalence of intestinal parasites (helminthes and protozoa) in dogs. Such surveys include Ramírez-Barrios et al. (2004) in Venezuela, Martínez-Moreno et al. (2006) in Spain, Katagiri and Oliveira-Sequeira (2007) in Brazil, Dubná et al. (2007) in The Czech Republic, Sowemimo (2009) in Nigeria. Few published surveys, however, have been conducted in the United States, and none have been conducted in the state of West Virginia.

Parasite prevalence surveys often take into account demographics such as the dog's age, gender, breed, and locality. Furthermore, the zoonotic potential of specific parasite species has been reported by McCarthy and Moore (2000), Robertson et al. (2000), Robertson and Thompson (2002), Traub et al. (2005), and Katagiri and Oliveira-Sequeira (2008). The study conducted by Katagiri and Oliveira-Sequeira (2008) assessed the risk perception of dog owners and revealed that most owners are unaware of the zoonotic potential of the parasites carried by their dogs, and thus concluded that this lack of knowledge was a cause for negligence in deworming. It is our belief, however, that both the lack of knowledge of zoonotic potential and a lack of knowledge of asymptomatic infection is cause for this negligence. According to veterinary practitioners (Dr. R. Eric Lee, Dr. Paula M. Lee, and Dr. Chad Brown; pers. comm.), the most common symptoms reported with intestinal parasitic infection include loose stool or diarrhea, vomiting, weight loss, loss of appetite, and blood or mucous in the stool. It is common for owners to assume that their dogs are not infected if they lack these symptoms, and thus often disregard regular deworming practices. As a result, the potential risk of the spread of zoonotic parasites may be greater than anticipated.

The primary objective of the present work is to determine the overall prevalence of intestinal parasite infection in symptomatic and asymptomatic dogs in southwestern West Virginia. A thorough look into the occurrence of infection without symptoms could expose a potentially large public health risk, and ultimately lead to a change in deworming protocols for both veterinary practitioners and owners. It is hypothesized that intestinal parasite infection is associated with one or more clinical symptoms. Another objective of this study is to determine the general prevalence of intestinal parasites, in association with host gender and age. It is hypothesized that the prevalence of intestinal parasite infection is equal in both genders and among all age groups. A third objective of this study is to determine the effect of anthelminthic medication on intestinal parasite prevalence. It is hypothesized that there is no difference in the prevalence of infection in dogs receiving an anthelminthic prophylactic than in dogs that are not. A final objective of this study is to understand the potential risk that common dog zoonotic parasites pose to human health.

CHAPTER TWO: PARASITES

The most common intestinal parasites in dogs of southwestern West Virginia include the helminth species *Ancylostoma caninum* (hookworm), *Toxocara canis* (roundworm), *Trichuris vulpis* (whipworm), and *Dipylidium caninum* (tapeworm), as well as protozoan species of *Giardia* sp. and *Isospora* sp. Species that were given special consideration in the present study were *A. caninum*, *To. canis*, *Tr. vulpis*, and *Isospora* (coccidia) due to their ubiquitous status worldwide. Moreover, *A. caninum* and *To. canis* are etiologic agents of eosinophilic enteritis, and cutaneous and visceral larval migrans in human subjects, and thus represent important zoonoses.

Ancylostoma caninum

Ancylostoma caninum is a nematode of dogs that is found in temperate and tropical areas of the world. The parasite favors moist, sandy soils (Soulsby, 1969) and is so-called the "hookworm" because when viewed from a lateral aspect, the anterior portion of the worm is projected in a decidedly forward or hooklike manner. These nematodes are further characterized by 3 pairs of distinct teeth anteriorly placed in a large buccal cavity (Figure 1). Adult hookworms range from 9 to 18 mm in length.

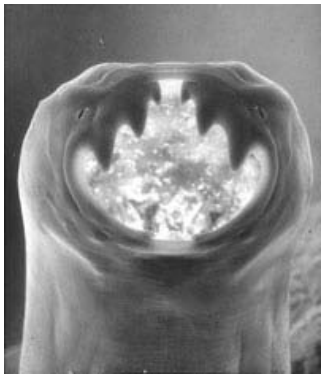

Figure 1. *Ancylostoma caninum*. Note the large buccal cavity with three pairs of teeth (University of Pennsylvania School of Veterinary Medicine, 2008).

The life cycle of *Ancylostoma caninum* is illustrated in Figure 2. *Ancylostoma caninum* infects dogs by oral or percutaneous infection, although it is unknown which mode of infection is most common under natural conditions (Baker and Muller, 1995). Hookworm infection can also be acquired prenatally. Through the percutaneous route of infection, the cycle includes skin penetration, followed by a migratory phase, and finally an intestinal phase. After being deposited into soil through an infected animal's feces, infective larvae migrate to the surface of the soil or up blades of grass or other vegetation and adhere to a host on contact (Prociv and Croese, 1996). Larvae locate the skin by thermotaxis prior to penetration. If adherence occurs on exposed skin or on a host without thick fur, invasion occurs through the hair follicles or the larvae may enter under fragments of epidermal tissue (Soulsby, 1969).

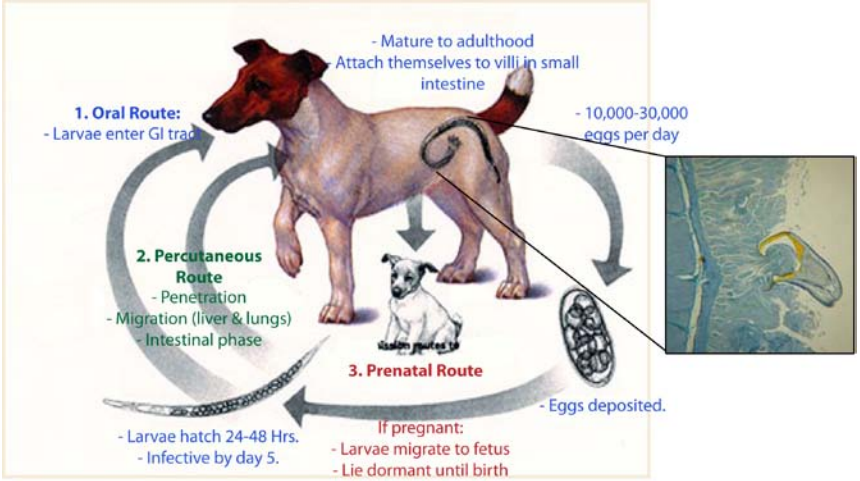

Figure 2. Life cycle of *Ancylostoma caninum* (Adapted from Novartis Animal Health Inc., 2009 and Companion Animal Parasite Council, 2006).

Once larvae have penetrated the skin, they begin their migratory phase that involves the liver and lung. Some larvae may enter superficial venules or lymph vessels, while others may simply die. Those that are able to enter venules and vessels are then carried to the liver and pulmonary circulation by the heart, where they then break out of capillaries and enter alveoli. From here, larvae make their way up the bronchial tree to the esophagus, are swallowed, and then enter the digestive tract where they begin their intestinal phase and grow to adulthood. Adult hookworms attach themselves to villi of the mucosa of the small intestine, particularly in the duodenum. Here, the worm buries its large buccal capsule between villi and sucks blood from the intestinal mucosa. The hookworm's blood appetite is so voracious, in fact, that it has been referred to as, "the luxurious consumer, being excessive of the blood of its host" (Soulsby, 1969). Hookworms reach maturity at approximately 5 weeks after infection. Each female worm may lay between 10,000 to 30,000 eggs per day, which are shed in the dog's feces at the

4-8 blastomere stage (i.e., "segmented egg" stage). The eggs are oval-shaped and approximately 70 to 80 μm in length (Figure 3). Larvae hatch within 24-48 hours after deposition, depending on environmental temperature, and reach an infective form by day 5, at which time they can infect another host by skin penetration and complete the cycle.

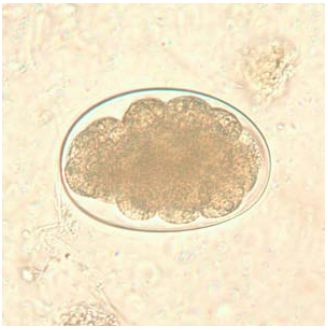

Figure 3. *Ancylostoma caninum* segmented egg (Center for Disease Control: Division of Parasitic Diseases, 2009).

During the hookworm's migration from the skin to the lungs, some larvae enter the dog's systemic circulation, where they may be carried to various other organs. If the dog is a pregnant female, these larvae may even migrate to the fetus through the circulation and cause prenatal infection. Within the fetus, larvae lie dormant until the puppy is born, at which time the larvae continue to develop into adults and complete the infective cycle as described above.

Toxocara canis

Toxocara canis, or roundworm, is a relatively large nematode that can grow up to 10-18 cm in length (Figure 4). Roundworms have 3 well-developed lips and no buccal capsule (Figure 5). They infect dogs through complex somatic and tracheal routes of

infection (Parsons, 1987). *Toxocara canis* is considered a significant parasite of young dogs since it also causes prenatal infection, as well as infection *via* lactation.

Figure 4. *Toxocara canis* (Mark Blaxter et al., 2007).

Figure 5. Well-developed lips of *To. canis* (Novartis Animal Health Inc., 2009).

The infective life cycle of *To. canis* begins when a thick-shelled, unembryonated egg is passed in the environment through the feces of an infected host (Figure 6). An incubation period of several days, depending on ambient temperature and conditions, is required to develop an infective larva within the egg. When a dog ingests an egg, the infective larva hatches from the egg into the duodenum, penetrates the intestinal wall, and migrates *via* the circulation to the liver and then the lungs, taking in nutrients along the way. In mature dogs, most larvae migrate from the lungs to the heart, and then are

widely distributed *via* the bloodstream to other tissues, especially skeletal muscle and the kidneys where development ceases and the larvae become dormant.

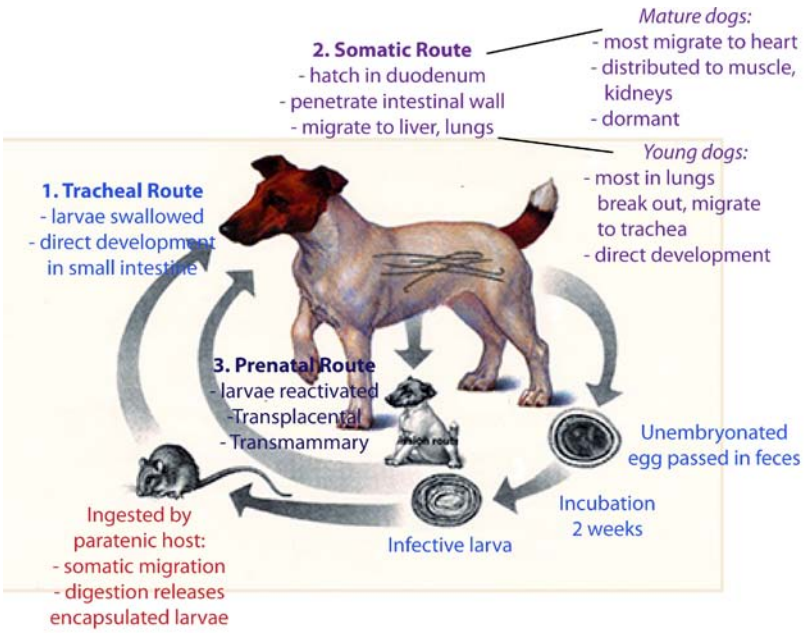

Figure 6. Life cycle of *Toxocara canis* (Adapted from Novartis Animal Health Inc., 2009).

The somatic route of infection results in a reservoir of encapsulated larvae that are very important in the subsequent infection of a dog's puppies. In young dogs (3 months old or younger), most larvae in the lungs break out of the alveoli and migrate to the trachea. Here, they are swallowed, and are able to complete development in the stomach and small intestine. However, if an infected dog is pregnant or becomes pregnant, somatic larvae are reactivated. Factors responsible for this reactivation are unknown at this time. The reactivated somatic larvae then migrate from the bitch's tissues to the umbilical vein, and then to the fetal liver *via* the placenta where they will remain until

birth. In neonates, larvae migrate from the liver to the lungs, and then to the trachea where they are swallowed and migrate to the small intestine. Puppies infected prenatally pass eggs in their feces by 3 weeks of age. This is the major route of infection for neonatal pups.

During late pregnancy and early lactation, somatic larvae can migrate to mammary glands and be passed in the colostrum. Infective larvae can be found in the milk by day 5. Puppies then ingest the larvae and they complete a direct development in the small intestine. This route of infection is considered to be relatively unimportant compared to the transplacental method of infection.

After it has reached maturity within the small intestine, the adult roundworm sheds unembryonated eggs *via* the host's feces. The eggs are approximately 75-80 μm in diameter, are subglobular, and are encased within a thick, pitted shell (Soulsby, 1969) (Figure 7).

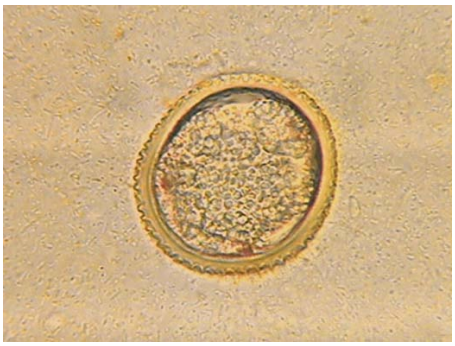

Figure 7. *Toxocara canis* unembryonated egg (Oklahoma State University College of Veterinary Medicine, 2007).

Once the eggs are passed to the environment, they then embryonate over a period of 2 weeks or more before reaching an infective state. Infection in dogs may include a

paratenic host (a host that is not normally found in a parasite's life cycle and in which a parasite will not complete normal development). Mice, a common paratenic host to the roundworm, may ingest infective larvae from the environment, and the larvae then begin somatic migration to the mouse's tissues, including skeletal tissue, and become encapsulated as described above. When a dog eats a paratenic host, the process of digestion releases the encapsulated larvae and they then undergo tracheal migration within the dog. Larvae develop into adulthood within the dog's small intestine by 19 days post-infection.

Trichuris vulpis

Trichuris vulpis is a nematode that infects foxes and dogs. It is widely distributed in all parts of the world and infects the cecum and colon of its hosts. *Trichuris vulpis* is a relatively small worm, ranging from 4-7 cm in length. It is named for its characteristic body shape that resembles a whip: an elongate body with a long, flagellum-like anterior end that makes up approximately ¾ of its total body length, and a short, thick posterior end (Figure 8).

Figure 8. Characteristic "whip" shape of *Tr. vulpis* (Companion Animal Parasite Council, 2009).

The infective cycle of *Tr. vulpis* is simple and direct (Figure 9). Unembryonated eggs are passed into the environment through the feces of an infected animal, where they undergo development for 3-4 weeks under normal conditions, but have been reported to reach their infectious stage within 9-10 days under optimal conditions.

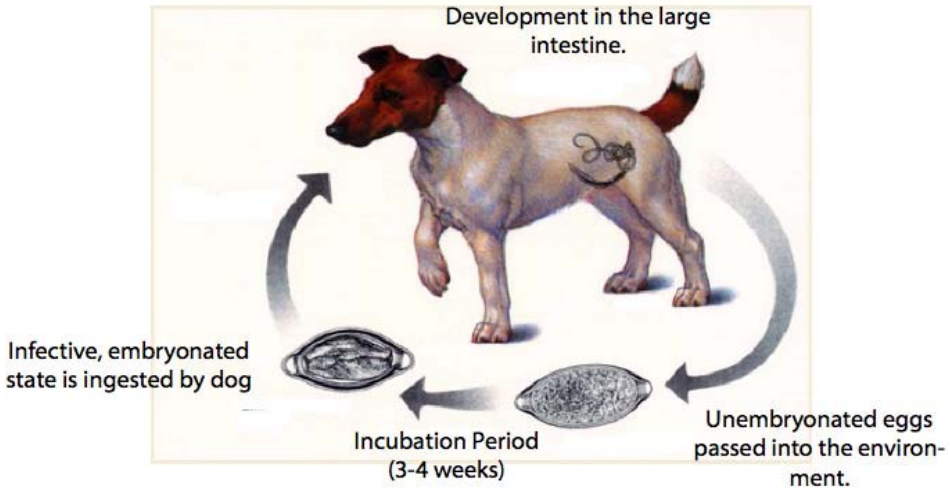

Figure 9. Life cycle of *Tr. vulpis* (Adapted from Novartis Animal Health Inc., 2009).

The eggs are approximately 70-90 μm in length, have a distinct, double-plugged barrel shape, and are encased within a thick shell (Figure 10). This thick outer membrane enables *Tr. vulpis* to remain very resistant to environmental conditions. In fact, Soulsby (1969) reports that whipworm eggs can persist in a suitable environment for up to 5 years. They are very resistant to constant cold and heat (at temperatures of -20^{o}C and 112^{o}C, respectively), as well as freezing, for as long as 12 days. Desiccation, however, is rapidly lethal to them.

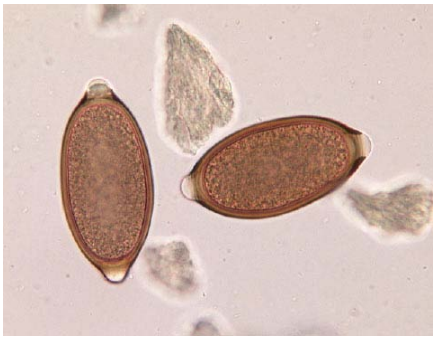

Figure 10. *Tr. vulpis* eggs (Companion Animal Parasite Council, 2009).

Infection is through the fecal-oral route. When a dog ingests eggs from a contaminated area, the eggs enter the digestive tract and develop in the large intestine. Here, they attach to the intestinal mucosa using the narrow, anterior end that can penetrate deeply into the tissue (just before the submucosa). It then obtains blood by absorption through capillary action. Whipworm infections are generally asymptomatic. However, symptoms such as weight loss, diarrhea, and blood in the feces are sometimes associated with whipworm infection. Due to the traumatic impact on the cecum and colon mucosa, irritation can occur, resulting in an inflammatory response that may be hemorrhagic in severe cases.

Isospora species

Isospora species, commonly referred to as coccidia, belong to a group of protozoans referred to as the Apicomplexa. Some authors consider the Apicomplexa a distinct phylum of organisms (Roberts and Janovy, Jr., 2000). Coccidia are small (approximately 20 μm), single-celled organisms that infect a wide variety of vertebrates (Figure 11). Some can infect mice, rats, hamsters, cats, cattle, sheep, and camels as paratenic hosts.

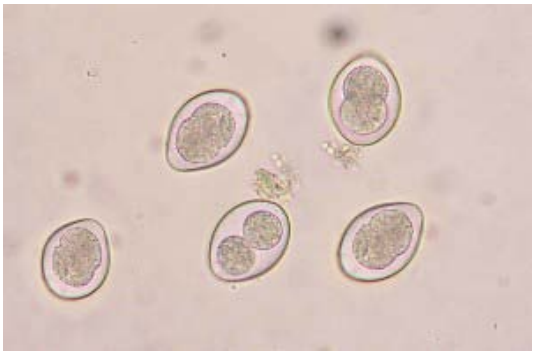

Figure 11. Oocysts of *Isospora* species (Joel Mills, 2006).

As illustrated by Figure 12, *Isospora* species have very complex exogenous and endogenous cycles during their life history (Dedrickson, 2009). In dogs, unsporulated or sporulated oocysts are passed through the feces into the environment. Once in the environment, sporogeny, the production of infective sporozoites within sporocysts in the oocyst, occurs. This is the exogenous phase of the life cycle.

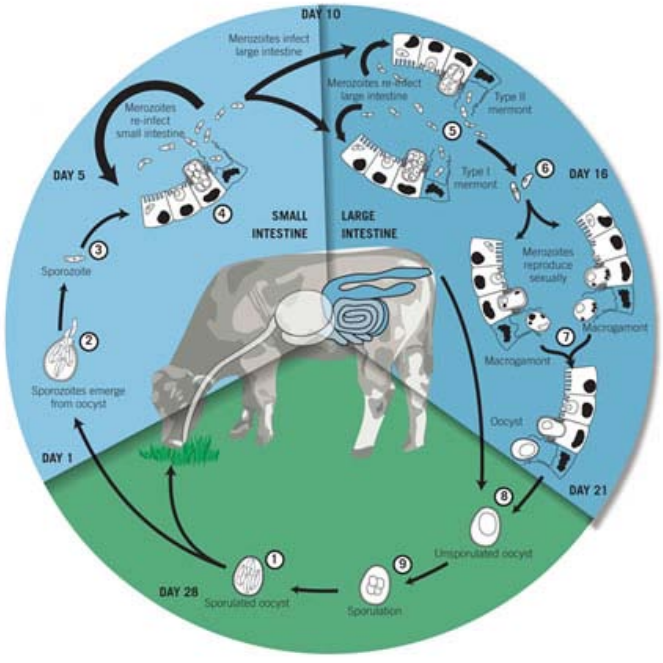

Figure 12. Life cycle of *Isospora* species (Merial, 2003).

Endogenous development begins when a dog ingests the infective oocysts or a paratenic host, allowing excystation, the release of sporozoites from the oocyst, to occur. These sporozoites enter a gut epithelial cell and undergo a multiple-fission process resulting in the production of a schizont that has many asexually produced forms referred to as merozoites. Some merozoites may invade extraintestinal tissues such as mesenteric lymph nodes, the liver, spleen, or other organs, enabling infection through ingestion of a paratenic host. Dedrickson (2009) reports that coccidia are *via*ble for at least 23 months in extraintestinal tissues of mice. This stage of the life cycle is normally subclinical until migration into the large intestine, where the merozoites reproduce. Reproduction begins when the merozoites enter gut epithelial cells, where some may develop into a macrogamete (female), while others develop into microgametes (male). Sexual reproduction occurs when a microgamete fertilizes a macrogamete to form a zygote. The zygote forms a protective wall and becomes an oocyst, causing the host cell to rupture. As oocysts are released from gut epithelial cells, they are then passed, unsporulated, in the feces. As they come into contact with oxygen, they sporulate and become infective, allowing the potential infection of another host, and thus completing the cycle. A single oocyst can produce up to 24 million oocysts in the next life cycle.

CHAPTER THREE: ZOONOSES

Many dog owners are unaware of the potential to contract parasites from their dogs. Katagiri and Oliveira-Sequeira (2007) reported that 70.1% (54/77) of owners questioned were unaware of the possibility of dogs harbouring parasites capable of infecting man. Two common zoonotic parasites relevant to this study are *A. caninum* and *To. canis*.

Ancylostoma caninum: Cutaneous Larval Migrans and Eosinophilic Enteritis

Ancylostoma caninum, or hookworms, are capable of infecting humans by penetrating the skin when the person is in contact with soil or sand contaminated with feces of infected dogs (Robertson and Thompson, 2002). People who sunbathe, walk barefooted, crawl under buildings, and engage in similar activities where there may be indirect contact with contaminated soils are at an increased risk to contract this parasite. In fact, in the 1940's, before sewage sanitation was well-developed, infection with *A. caninum* was often referred to as "Plumber's Disease," since many plumbers became infected after crawling under houses and working with sewage pipes. Robertson and Thompson (2002) suggest daily removal of feces from the environment, as well as regular deworming to eliminate parasite burdens as ways to decrease risk of exposure.

As described previously, when *A. caninum* infects a dog through skin penetration, it often becomes dormant in the tissues and may be encased within a granuloma. In humans, however, infection can cause cutaneous larva migrans (CLM) or eosinophilic enteritis (EE).

CLM is a term used to describe the larval migration under the skin, and is specifically referred to as "creeping eruption" when occurring in humans. This migration causes progressive linear eruption, and sometimes intense pruritis that may subside over a few weeks. In massive infections, however, the larvae may penetrate deeper tissues, causing pulmonary and intestinal symptoms. Enteric infections may also lead to EE, a disease of the small intestine that causes abdominal pain, diarrhea, abdominal distensia, weight loss, rectal bleeding, anorexia, and nausea.

Ancylostoma caninum was originally suspected as the cause of EE in Townsville, Australia (Prociv and Croese, 1996), when several patients presenting these symptoms were found to have been infected with the parasite. According to McCarthy and Moore (2000), a study was performed on 233 people with either abdominal pain or known EE using ELISA and Western Blot analysis through ES antigens from adult *A. caninum*. The results of the study were that all 233 clinical cases tested positive for the parasite. The precise prevalence is not known in other parts of Australia, or in the United States. However, since *A. caninum* has a worldwide distribution, it is expected that *A.caninum*-induced EE is not limited to Townsville, Australia.

Some speculate that a large part of the population is already infected with *A. caninum*, either asymptomatically or symptomatically. Prociv and Croese (1996) report in a study performed in Queensland, Australia, that both clinical and subclinical infection is very common. Similarly, Walker, et al. (1995) proposed that EE was likely to be under-diagnosed and may be more widely distributed than is currently appreciated.

Toxocara canis: Visceral Larva Migrans

Toxocara canis, or roundworm, is capable of infecting humans through the ingestion of embryonated eggs. As previously described, the life cycle of *To. canis* includes complex tracheal and somatic routes of infection. It is this somatic route of infection, during which larvae migrate throughout the body to various tissue and organs, that plays a significant role in human roundworm infection, most notably causing visceral larva migrans (VLM) and ocular larva migrans (OLM).

VLM is a term used to describe *To. canis* larval migration to various organs of the viscera. Once ingested, the eggs hatch, releasing larvae that penetrate the small intestine, enter the circulation, and move throughout the body, potentially invading all organs (Despommier, 2003). The larvae develop into adults in the small intestine approximately 60-90 days after hatching. There, they reproduce and shed nonembryonated eggs that are excreted in the feces. VLM is accompanied by an immediate hypersensitivity response to dying and dead larvae in the viscera, including the lungs, liver, and brain. In addition to this inflammatory response, reported symptoms of VLM include fever, enlargement of the spleen, lower respiratory symptoms that resemble asthma bronchiospasms, myocarditis, nephritis, and sometimes involvement of the central nervous system which can lead to seizures, neuropsychiatric symptoms, and encephalopathy.

A specific type of VLM, ocular larva migrans (OLM), refers to the migration of larvae to the eye. This causes damage to the retina, leading to impaired sight due to granuloma formation. Blindness is common with OLM, and this disease can often be misdiagnosed as retinoblastoma. Another specific form of VLM involves migration of

larvae to the brain. This can cause idiopathic seizure disorders, as well as functional intestinal disorders.

VLM is mainly a disease of young children, less than 5 years of age, due to the likelihood of young children coming into contact with contaminated sand or soils, and then subsequently putting their hands in their mouths (Despommier, 2003). Children often come into contact with embryonated eggs when playing in sandboxes and playgrounds. In fact, according to Muradian et al. (2005), the highest prevalence of *To. canis* eggs was found to be in playgrounds (66.7%) and home yards (66.7%). Infection was more strongly attributed to environmental contamination than to direct contact with dogs, indicating that soil contamination is the main source for human infection.

As with most parasite infections, the risk of *To. canis* infection can be reduced by removing potentially contaminated feces, maintaining a clean environment, and through regular deworming to reduce parasite burdens. It is also important to note that *To. canis* eggs may remain infective for years in a suitable environment, so preventive measures should always be taken, especially in households with small children.

CHAPTER FOUR: TESTING EXPERIMENTAL HYPOTHESES

Materials and Methods

Study Area

This study was carried out in Charleston, West Virginia, USA. All research was conducted at Kanawha Boulevard Animal Hospital under the supervision of Dr. R. Eric Lee, D.V.M. The clientele range of the clinic included much of southern West Virginia, including Kanawha and contiguous counties.

Source of samples

From May to August 2009, fecal samples from 231 dogs (114 males and 117 females) were examined for the presence of parasites. Samples were collected from both asymptomatic and symptomatic dogs presenting at the clinic for various reasons[*], and were obtained using a fecal loop, or were pre-collected by the owner less than 1 hour before examination.

A brief questionnaire was completed by owners to obtain demographic (age, gender) and symptomatic data for each dog, as well as history of anthlemintic usage. Participants were asked if they noticed any symptoms in their dog(s) within the past 30 days, and then were asked about specific symptoms (i.e. diarrhea, vomiting, weight loss, loss of appetite, or mucous or blood in the stool).

[*] Some dogs were presented at the clinic for surgery, including spaying, neutering, routine dental cleaning, etc.; some were presented for routine examinations or vaccinations; some were presented for illness. Samples were taken randomly, disregarding particular reason for presentation.

Parasitological procedures

Unpreserved fecal samples were microscopically examined for parasite eggs immediately after collection by simple fecal flotation method, as described by Dryden, et al. (2005). A small pill *via*l was filled approximately half-full with FecaTect ® zinc sulfate fecal flotation medium. This medium was chosen for its specific gravity of 1.18, based on the information that the specific gravity of most parasite eggs is between 1.05 and 1.23 (Dryden, 2005). A 1.00 – 2.00 specific gravity hydrometer was used to check the specific gravity of the FecaTect ® solution periodically. The pill *via*l was filled approximately half full of FecaTect ®, the fecal sample was added and mixed, and then more FecaTect ® was added to the *via*l until a positive meniscus was formed at the top. A glass coverslip was placed over the meniscus for 20 minutes in order to gain the greatest fecal egg counts without centrifugation. After 20 minutes, the coverslip was removed, placed on a glass slide, and viewed under a compound microscope.

Statistical analysis

After careful examination of each sample, data were recorded regarding the presence or absence of parasite eggs, type of parasite eggs observed, along with the demographic, symptomatic, and anthelminthic information obtained from owners. The observed prevalence and 95% confidence intervals were calculated for each parasite. Associations between parasitism and host factors of age group, gender, and anthelmintic usage, as well as presence or absence of symptoms were calculated for the 231 dogs. The dogs were separated into three age groups: Puppies ($\leq$ 12 months of age), Adults (between 13-83 months of age), and Seniors ($\geq$ 84 months of age). Analyses for

associations between parasitism and age group were performed using chi-squared tests for two independent proportions, with a null hypothesis that the prevalence of parasitic infection is equal in all age groups. All other analyses were completed using general chi-squared contingency tests calculated through GraphPad Software. To determine the association between parasitism and host gender, the null hypothesis was that the prevalence of parasitic infection in males is equal to that in females. To determine the association between parasitism and host anthelmintic usage, the null hypothesis was that the prevalence of parasitic infection in dogs receiving anthelmintic medication is equal to that of those receiving none. To determine the association between parasitism and symptom, the null hypothesis was that intestinal parasite infection is associated with one or more clinical symptoms.

Results

Overall Prevalence

Table 1 summarizes the overall prevalence of each parasite species. A total of 231 dogs were included in the analyses, including 114 males and 117 females.[**] Of the 231 dogs sampled, 39 males (52%) and 36 females (48%) were infected with one or more parasite species, giving an overall prevalence of 32.5%. The remaining 156 dogs (67.5%) were not infected with an intestinal parasite visible by fecal flotation. Of the 231 dogs, the overall prevalence of *A. caninum* was found to be 23.4%; for *Tr. vulpis*, the

[**] A total of 235 dogs were sampled (116 males and 119 females). Four of the dogs sampled were reported by owners to be infected with *D. caninum*, or tapeworm, which is rarely seen under fecal flotation analysis. Therefore, since these infections were not confirmed *via* fecal flotation, these samples were excluded from the data set.

prevalence was 7.8%; approximately 7% for *To. canis*; and for *Isospora* species, the prevalence was 4.3%. No *Giardia* species were found.

Table 1. Overall prevalence of parasite species.

Parasite Species	Female	Male	Overall Prevalence (%)
A. caninum	24	30	23.4
To. canis	9	7	6.9
Tr. vulpis	10	8	7.8
Isospora sp.	8	2	4.3

Asymptomatic versus Symptomatic Infection

Overall, of the 75 dogs infected with one or more parasite species, 36 (48%) showed one or more of the previously mentioned symptoms, while 39 (52%) were asymptomatic. Of the 156 dogs without infection, 67 (43%) had one or more of the mentioned symptoms, while 89 (57%) were asymptomatic. Overall symptomatic and asymptomatic data, as well as for each individual parasite species, is summarized in Table 2.

With *A. caninum*, *To. canis*, and *Isospora* species infections, there was no significant correlation between infection and symptom, with χ^2 and *P*-values of 0.011 and 0.98, 0.28 and 0.55, and 0.72 and 0.37, respectively (d.f. = 2). However, there was a significant relationship between infection and symptom for *Tr. vulpis* ($\chi^2 = 4.129$, d.f. = 1, $P = 0.049$). A total of 6 out of the 8 dogs infected with *Tr. vulpis* were symptomatic, consistently exhibiting diarrhea (50%), vomiting (37.5%), and mucous or blood in the stool (25%).

Table 2. (a.) Relationship between infection and symptom overall (includes all parasite species). **(b.)** Relationship between infection and symptom for *A. caninum*. There was no significant relationship between infection and symptom ($P > 0.05$). **(c.)** Correlation between infection and symptom for *To. canis*. There was no significant relationship between infection and symptom ($P > 0.05$). **(d.)** Relationship between infection and symptom for *Tr. vulpis*. There was a significant relationship between infection and symptom ($P < 0.05$). **(e.)** Relationship between infection and symptom for *Isospora* sp. There was no significant relationship between infection and symptom ($P > 0.05$).

Test of overall independence of infection & symptoms			
	Infected	Not infected	Totals
symptoms	36	67	103
no symp.	39	89	128
Totals	75	156	231
P-value = 0.46954		$x^2 = 0.271$	

a. Test of overall independence of infection and symptom.

Test of independence of A. caninum infection & symptoms			
	Infected	Not infected	Totals
symptoms	24	79	103
no symp.	30	98	128
Totals	54	177	231
P-value = P = 0.9805		$x^2 = 0.011$	

b. Test of independence of *A. caninum* infection and symptom.

Test of independence of T. canis infection & symptoms			
	Infected	Not infected	Totals
symptoms	6	97	103
no symp.	10	118	128
Totals	16	215	231
P-value = P = 0.5543		$x^2 = 0.279$	

c. Test of independence of *T. canis* infection and symptom.

Test of independence of T. vulpis infection & symptoms			
	Infected	Not infected	Totals
symptoms	12	91	103
no symp.	6	122	128
Totals	18	213	231
P-value = 0.04971		$x^2 = 4.129$	

d. Test of independence of *T. vulpis* infection and symptom.

Test of independence of Isospora sp. infection & symptoms			
	Infected	Not infected	Totals
symptoms	3	98	101
no symp.	7	123	130
Totals	10	221	231
P-value = 0.371102		$x^2 = 0.718$	

e. Test of independence of *Isospora* sp. infection and symptom.

Parasitic Infection by Gender

A total of 75 dogs (52% male, and 48% female) were infected with one or more parasite species. Of the 75 dogs that were infected, 54 (72%) were infected with *A. caninum*, including 24 (44%) females and 30 (56%) males (Table 3). A total of 18 dogs (24%) were infected with *Tr. vulpis*, including 8 (44%) females and 10 (56%) males. Sixteen dogs (21%) were infected with *To. canis*, including 9 (56%) females and 7 (44%) males. A total of only 10 dogs (13%) were infected with *Isospora* species, including 8

(80%) females and 2 (20%) males ($P = 0.058$) (Table 3). The null hypothesis that the prevalence of parasitic infection in males is equal to that in females was accepted for *A. caninum*, *To. canis*, and *Tr. vulpis*; however, since the *P*-value of *Isospora* species was very near the 95% confidence interval (a difference of 0.008), the data are strongly suggestive of a relationship between *Isospora* sp. infection and gender.

Table 3. Prevalence of parasitic infection by gender. A total of 75 dogs were infected with one or more parasite species. Host sample size (n) for each gender shown in parentheses. The prevalence of parasitic infection in males is not significantly different than in females ($P > 0.05$).

	Prevalence (%)			
Parasite species	Female (n = 36)	Male (n =39)	X^2	P-value
Ancylostoma caninum	44.4	55.6	1.08	0.29
Toxocara canis	56.3	43.8	0.30	0.58
Trichuris vulpis	44.4	55.6	0.22	0.64
Isospora sp.	80.0	20.0	3.6	0.058

Parasitic Infection by Age

Table 4 summarizes the prevalence of parasitic infection by age group. There were a total of 86 dogs within the puppy age group ($\leq$ 12 months), 64 dogs in the adult age group (13-83 months), and 81 dogs in the senior age group ($\geq$ 84 months).

Table 4. Chi-square equality of proportions test for prevalence of parasitic infection by age group. Numbers in the rows for each age group represent: [1]number of dogs negative or positive for individual parasite species; [2]negative or positive cell contribution to the total chi-square value. Note: ***** denotes statistically significant.

Age	*A. caninum*		*T. canis*		*T. vulpis*		*Isospora sp.*	
(Mo.)	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
≤ 12	55 [1]	31	71	15	79	7	76	10
[n= 86]	1.8 [2]	5.90	1.02	13.70	0.00	0.01	0.48	10.60
13-83	52	12	64	0	58	6	64	0
[n= 64]	0.17	0.60	0.33	4.43	0.02	0.20	0.13	2.78
> 84	70	11	80	1	76	5	81	0
[n= 81]	1.00	3.30	0.28	3.79	0.02	0.27	0.16	3.51
Total n = 231	181	54	215	16	213	18	221	10
Overall Prevalence (%)	76.60	23.40	93.10	6.90	92.20	7.80	95.70	4.30
Total X^2	12.8, d.f. = 2		23.6, d.f. = 2		0.53, d.f. = 2		17.7, d.f. = 2	
P-value	0.0016*		< 0.0001*		0.7700		0.0001*	

There were significantly more infections within the puppy age group among *A. caninum* (57.4%), *To. canis* (93.8%), and *Isospora* species (100%), with χ^2 and *P*-values of 12.820 and 0.0016, 23.562 and <0.0001, and 17.654 and 0.0001, respectively (d.f. = 2). There was no significant difference in *Tr. vulpis* infection among the different age groups (χ^2= 0.527, d.f. = 2, *P* = 0.7684). Figure 13 gives a graphical representation of the overall parasite prevalence by host age. Figure 14 gives this information by means of individual parasite.

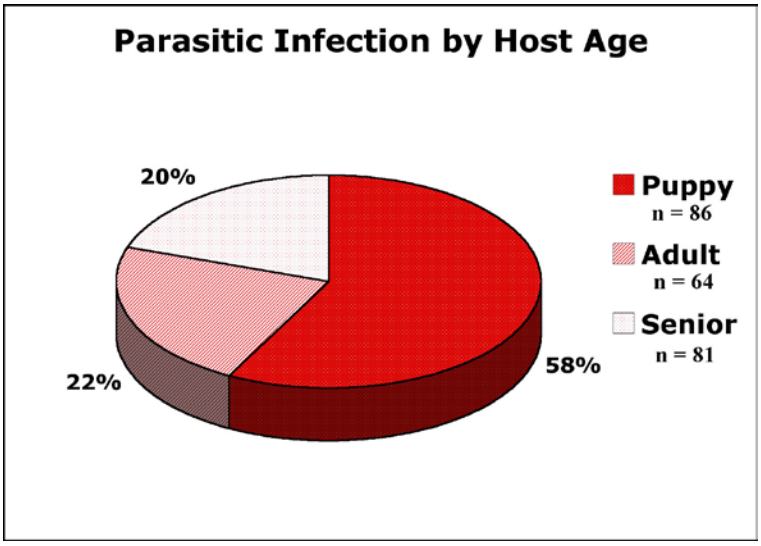

Figure 13. Prevalence of parasitic infection by host age for puppies (≤ 12 months of age), adult (13-84 months of age), and senior dogs (≥ 84 months of age).

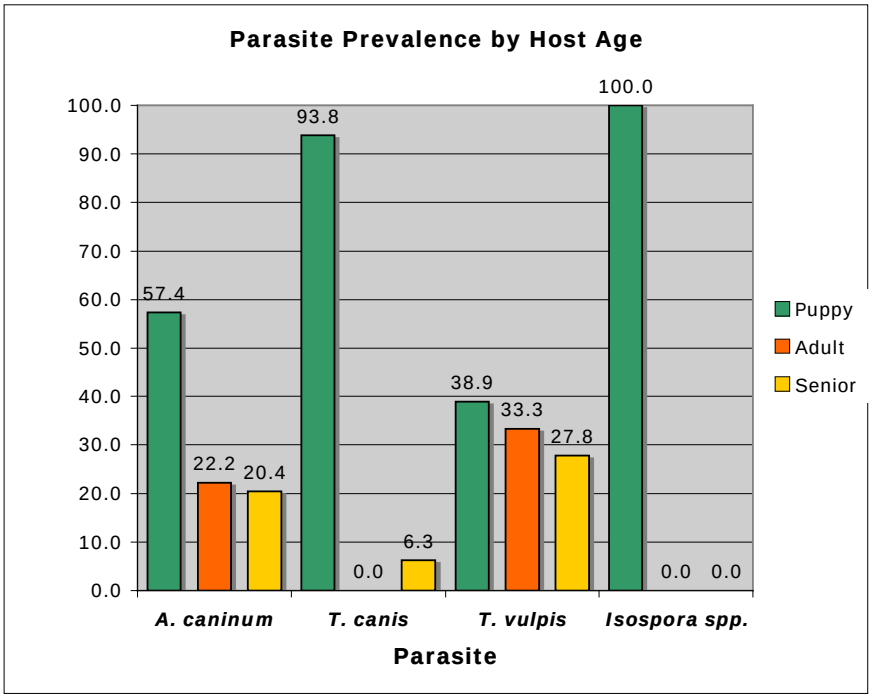

Figure 14. Prevalence of individual parasitic infection by host age for puppies (≤ 12 months of age), adult (13-84 months of age), and senior dogs (≥ 84 months of age).

Anthelmintic Effect on Parasitism

Only 51 dogs (22%) out of 231 were reported to be actively taking a prophylactic anthelmintic drug, and 180 (78%) were not (Table 5). Of the dogs taking anthelmintics, 44 (86%) were not infected, while 7 (14%) were infected with one or more intestinal parasites.

Table 5. Prevalence of parasite infection in association with anthelmintic usage. Note: ***** denotes statistical significance.

	Anthelmintic	**No Anthelmintic**	**Total**
Infection	7	68	75
No Infection	44	112	156
Total	51	180	231
X^2	10.5, d.f. = 1		
P-value	0.001*		

Of the 7 infected dogs, 3 (43%) were infected with *Tr. vulpis*, 2 (29%) were infected with *A. caninum*. One dog was infected with *T.canis*, and one was infected with both *To. canis* and *A. caninum* (Figure 15). Of the 180 dogs not receiving anthelmintic drugs, 68 (38%) were infected with one or more parasites, while 112 (62%) dogs were not infected. These results were statistically significant with a high degree of confidence ($\chi^2 = 10.5$, d.f. = 1, $P = 0.001$).

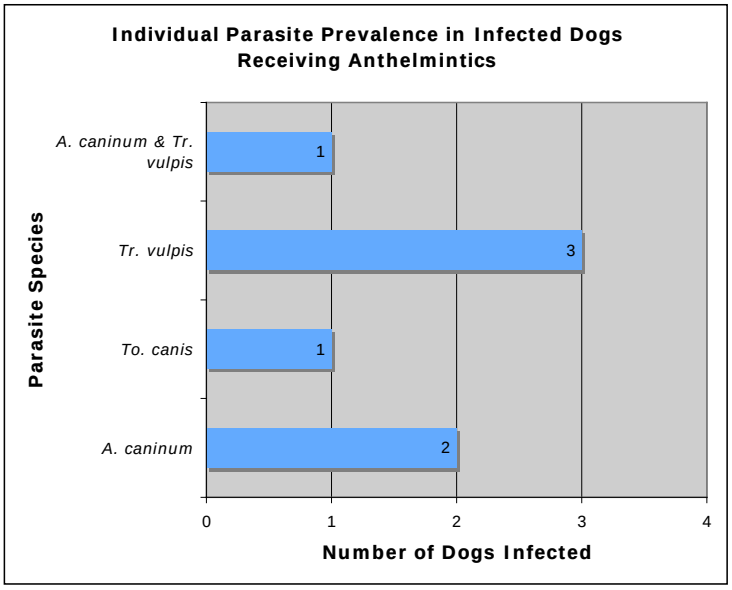

Figure 15. Prevalence of individual parasite infection in infected dogs receiving an anthelmintic medication.

CHAPTER FIVE: DISCUSSION AND FUTURE STUDIES

The objectives of this study were to determine the overall prevalence of intestinal parasite infection in symptomatic and asymptomatic dogs in Southern West Virginia; to determine the general prevalence of intestinal parasites in association with host gender and age; to determine the effect of anthelminthic medication on intestinal parasite prevalence; and finally, to use this information to recognize the potential risk that common dog zoonotic parasites pose to human health.

This study found an overall prevalence of parasitic infection of 32.5%. This was lower than the 52.4% prevalence found in the study conducted by Fontanarrosa et al. (2005) in Buenos Aires, Argentina, but much higher than the overall prevalence (12.5%) reported by Little (2009) in a study conducted in the United States. Dubna et al. (2007) reported a similarly low prevalence of 17.6% in Czech Republic. Katagiri and Oliveira-Sequeira (2008) found an overall prevalence of 54.3% in their study in Brazil, while Martínez-Moreno (2007), in Spain, found a very high prevalence of 71.3%. Similar to the findings in Brazil and Argentina, Sowemimo (2009) in Nigeria found an overall prevalence of 55.0%. Ramirez-Barria (2004) in Venezuela reported a prevalence (35.5%) most similar to that found in this study. The vast differences in overall prevalence was expected and is most likely due to the geographic region of sampling, as well as the type of sampling. The scope of this particular study covers only those dogs whose owners voluntarily submitted a stool sample to this veterinary hospital for analysis, and due to the inability to determine whether or not a dog has been symptomatic or asymptomatic, did not include any stray or shelter dogs. Several of the abovementioned studies did, in

fact, include stray dogs and shelter dogs within their sampling, and some collected stool directly from the environment, rather than from specific dogs. The overall prevalence of the present study would also be greater if noted tapeworm infection had been included in the analyses.

In this study, there appeared to be no significant difference in parasite prevalence between males and females at a confidence of 95%, in agreement with results reported by other prevalence studies conducted in different countries worldwide. However, since $P = 0.058$ (n = 10) in *Isospora* species infections, it is worth mentioning that these values are strongly suggestive of a relationship between infection and gender for this species. Future studies should be conducted to investigate this relationship to determine if infection is more prevalent in females than in males, as the data suggest here.

Also consistent with other studies, the prevalence of infection here was found to be significantly greater in young dogs ($P < 0.05$) than in older dogs. Robertson and Thompson (2002) confirm this finding as well, stating that in their studies of enteric parasitic zoonoses, the prevalence of parasites is higher in younger animals and animals that originate from refuges, shelters, or pounds. This was expected since two of the parasites found in this study, *To. canis* and *Isospora* species, are almost strictly found in puppies due to the parasites' transmission patterns and the dog's immature immunity. The null hypothesis that there is no difference in the prevalence of parasitic infection between males and females was accepted, and the null hypothesis that there is no difference in the prevalence of parasitic infection between age groups was rejected.

The most commonly found parasite in this study was *A. caninum* (23%), followed by *Tr. vulpis* (8%), *To. canis* (7%), and finally *Isospora* species (4%). Similar

prevalences for the three helminth species were also found in Brazil (Katagiri and Oliveira-sequeira, 2008): 38%, 7%, and 9%, respectively. According to the *A. caninum*, *Tr. vulpis*, and *To. canis* species prevalence reported by Little et al. (2009), these percentages are above the United States national average of 3%, 1%, and 2%, respectively. The reported prevalence of *Isospora* species, however, was very similar to the United States national average (4%). This is also comparable to the 4% prevalence reported by Katagiri and Oliveira-Sequeira (2008) in Brazil and the 3% prevalence reported in Argentina (Fontanarrosa et al., 2005). *Giardia* species has been reported as the most frequent protozoan found in dogs (17%) by these authors, as well as in other developed countries. However, as the authors report, a higher frequency of infection was found in stray dogs, which offers a plausible explanation as to why no *Giardia* species were found in this study. Since all samples were taken from dogs under the care of a veterinary practitioner, with no samples taken from dogs that were strays, or that came from a puppy mill or shelter, the presence of *Giardia* sp. was expected to be low (Veterinary Practitioners: Dr. R. E. Lee, Dr. Paula M. Lee, and Dr. Chad Brown, pers. comm.).

Table 5 summarizes the prevalence of parasitic infection in dogs actively receiving a prophylactic anthelmintic medication such as Interceptor® or Heartgard® compared to those not receiving anthelmintics. A total of 86% of dogs receiving an anthelmintic were free of intestinal parasite infection. The relationship between anthelmintic usage and prevalence of infection was statistically significant (χ^2= 10.5, d.f. = 1, P = 0.001). The null hypothesis that there is no difference in the prevalence of infection between dogs receiving anthelmintics compared to dogs not receiving

anthelmintics was therefore not accepted. Of the three dogs infected with *Tr. vulpis* while receiving an anthelmintic, two (67%) were reported to have been receiving Heartgard® (ivermectin). All other dogs receiving an anthelmintic received Interceptor® (milbemycin oxime). Although ivermectin is designed to prevent heartworm infection, in addition to preventing and treating intestinal parasites such as *A. caninum* and *To. canis*, it does not treat or prevent *Tr. vulpis*. This could explain infections by *Tr. vulpis* in dogs that were receiving Heartgard® as their anthelmintic. Milbemycin oxime, on the other hand, prevents heartworm infection in addition to preventing and treating *A. caninum*, *To. canis*, and *Tr. vulpis*. Still, 5 of the 7 dogs that were infected while receiving an anthelmintic received Interceptor®. This could be a result of owner compliance to the recommended monthly dose. Since the questionnaire was conducted orally, within the veterinary hospital, owners may have been apprehensive in admitting any neglect in administration of the medication. Further studies could reveal a more accurate account of the efficiency of the different anthelmintic drugs on the market today.

As seen in Table 2, there was no significant relationship between parasitic infection and presentation of symptom ($P > 0.05$) overall. The null hypothesis that there is no relationship between infection and symptom was accepted. This was also true in the case of *A. caninum*, *To. canis*, and *Isospora* species when individual parasites were compared with the presence or absence of symptoms (Table 2). This means that the presence or absence of symptoms cannot be considered an indicator of presence or absence of a parasitic infection. Since a majority (52%) of infected dogs lacked any clinical symptom, and many uninfected dogs (57%) displayed symptoms for what must have been some other medical reason, it is clear that the presence of symptoms is not a

reliable cue to begin a deworming program with these particular parasites. In the case of *Tr. vulpis*, however, there was a significant relationship ($\chi^2 = 4.1$, d.f. = 1, $P = 0.049$) between symptom and infection. The most common symptoms associated with whipworm infection, according to this study, are diarrhea, vomiting, and mucous or blood in the stool. These clinical symptoms are probably a result of the traumatic impact on the cecum and colon caused by the whipworm's penetration into the intestinal mucosa.

In lieu of the discovery that most parasite species considered in this study (particularly those that pose a zoonotic risk, *A. caninum* and *To. canis*) cannot be accurately associated with clinical symptoms, we believe that the prevalence of infection, as well as the risk to human health, may be greater than previously anticipated. According to Karagiri and Oliveira-Sequeira (2008), a shocking 48% of owners reported that they sought veterinary assistance only in case of disease or for vaccines. Another 21% of owners reported that they have never sought veterinary assistance, and only 31% sought veterinary assistance regularly. Frequency data similar to this has not been reported elsewhere, but if this data is representative of dog owners worldwide, then this could mean that 1 in 5 dog owners will never keep a regular deworming schedule, and nearly half of all dog owners will present their dogs to a veterinarian for deworming only if symptoms manifest. The potential for zoonotic infections could be huge!

Many authors agree that education is the best way to prevent zoonotic infection. As mentioned previously, many dog owners are unaware that they can contract these parasites from their dogs. Veterinary practitioners are in an ideal position to inform owners of this potential. At each visit, veterinary practitioners should make a point to provide information on zoonotic parasites to their clients, especially if they suspect a

parasitic infection. A standardized deworming protocol should also be put into place for veterinary practitioners worldwide in order to ensure that the same, proper deworming procedures are recommended to owners by all veterinarians. Another way to educate the public on zoonotic potential is to provide pamphlets of information on common zoonotic parasites to clients that order prescription medications, such as anthelmintics. Many owners are aware that heartworms are very bad for their dog, but are unaware that their dog's heartworm medication can also prevent intestinal parasites that are very harmful to humans. If anthelmintics such as Interceptor® and Heartgard® were promoted more as a preventive for zoonotic intestinal parasites rather than pushing its heartworm preventive capabilities, I believe more owners would follow a regular deworming protocol or anthelmintic regimen more closely, thereby decreasing parasite burdens in the environment. The Center for Disease Control (CDC) may also need to be involved in educating people of these risks. For example, in developing countries where veterinary care is less common and uncontrolled populations of dogs exist in close proximity to increasing densities of human populations, education by veterinary practitioners or through printed information with anthelmintic medications would not be as effective. Furthermore, as reported by Traub et al. (2005), a majority (67%) of individuals in some parts of India walk barefoot outdoors. Education regarding hookworm infection, in particular, is vital in cases such as this.

Further studies are needed to verify the overall prevalence of intestinal parasites in the South Central West Virginia area. The present study included samples from dogs that are under veterinary care, often spend time indoors, and whose owners voluntarily submitted stool samples for analysis. Further studies might include stray dogs, or dogs

from shelters or refuges in order to compare the prevalence of parasite infection in those dogs to dogs typically presenting at a veterinary hospital.

In the future, a longer period of data collection could determine any difference in parasitic prevalence from month to month or season to season. A period of approximately one to two years could show significant differences seasonally, or even annually, based on annual temperature and precipitation data. Since the present study was conducted over one summer (a period of approximately four months), significant differences in prevalence from month to month are unlikely.

More sensitive methods of parasite detection could also be used in future studies. Centrifugation with fecal flotation is generally the accepted method of parasite egg detection. However, due to financial constraints, centrifugation was not possible in the present study. In order to compensate for this lack of sensitivity, samples were allowed to float for an additional 10 minutes (a total of 20 minutes) in order to allow as many eggs as possible to float to the top of the meniscus. Although not as efficient as the centrifugation method, this method has been reported to produce greater fecal egg counts than the conventional simple fecal flotation method (Dryden, 2005).

Prevalence studies such as this are important to conduct in various parts of the world so that the public has an accurate awareness of the risks of infection. Furthermore, since so few have been conducted in the United States, it is very important that prevalence studies continue to be conducted here in addition to the developing countries that are typically the areas of interest for this type of study.

Pet animals play a significant role in societies throughout the world. They are important companions, contributing to the physical, social, and emotional development of

their owners. Pet owners are reported to visit their doctor less often, use fewer medications, and have lower blood pressure and cholesterol levels than non-pet owners (Robertson et al., 2000). However, the potential role of companion animals as reservoirs for zoonotic diseases has been recognized as a significant public health problem worldwide (Traub et al., 2005). An awareness of the risks of parasitic zoonoses could lead to improved deworming protocols and potentially decrease this public health concern. Since many owners may consider their dogs to be free of infection when lacking symptoms, knowledge that there is no significant correlation between infection and symptom could force owners and veterinary practitioners to reevaluate their deworming practices. Since dogs, as companion animals, have consistently improved the lives of humans, it is our responsibility to help diminish this public health risk in order to keep "man's best friend" exactly that.

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