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The Comparison of Three Different Fecal Egg Counting Techniques and Their Ability to Perform a Fecal Egg Count Reduction Test

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THE COMPARISON OF THREE DIFFERENT FECAL EGG COUNTING TECHNIQUES AND THEIR ABILITY TO PERFORM A FECAL EGG COUNT REDUCTION TEST

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By

Diana Summitt

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ABSTRACT

A comprehensive gastrointestinal parasite control program includes an understanding of common parasites, application of chemotherapeutic agents, as well as frequent and appropriate diagnostic testing. An effective control program is essential for facilities such as animal shelters, that deal with large populations of transient canines with unknown parasite exposure and deworming history. The identification of a sensitive flotation method to evaluate anthelmintic efficacy is critical in monitoring parasite populations for drug resistance. The objective of the current study was to compare three different fecal egg counting technologies and their ability to perform a fecal egg reduction test. The flotation techniques evaluated include a Modified McMasters, a Modified Wisconsin, and the Mini-FLOTAC. Canine fecal samples were obtained from Polk County Humane Society in Bolivar, MO. When possible, three samples were submitted for each canine: the first stool eliminated in the shelter, a sample 7 days after shelter deworming, and a sample 14 days after deworming. Each sample was divided into aliquots of 2 grams for each of the three different diagnostic techniques. Upon examination all ova detected were identified, counted, and the appropriate multiplication factor applied to yield an egg per gram (EPG) result. Where follow up samples were collected, the FEC results were used to preform reduction tests to determine percent ova reduction after anthelmintic treatment. One way ANOVA results determined that the three flotation methods were not different in mean EPG or EPG level for roundworms (p=0.284), whipworms(p=0.130), or coccidia(p=0.315). One way ANOVA found a difference between total Nematode EPG (p=0.002) and hookworm EPG (p=0.033), where the Mini-FLOTAC yielded a higher average EPG than the Modified McMasters or the Modified Wisconsin. No difference was found in FECRT between flotation methods.

KEYWORDS: fecal egg count, fecal egg count reduction test, Modified McMasters, Modified Wisconsin, Mini-FLOTAC,

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INTRODUCTION

A successful animal shelter management plan includes a comprehensive approach to parasite control. Managing parasite prevalence in a shelter is vastly different from individual management of pets. Shelters manage a colony of canines and are responsible for the health of the entire group. It is essential that their goals include managing and reducing the transmission of a variety of parasites among the colony in their care. A comprehensive approach to parasite control includes: a basic understanding of the various parasites commonly found in shelter environments; frequent and appropriate diagnostic testing; appropriate application of chemotherapeutic agents as well as post-treatment examination of drug efficacy; and environmental control measures. Focusing on parasite management will not only ensure the health and welfare of the animals in the shelter but will also contribute to protecting humans from zoonotic parasites.

Endoparasites that commonly infect dogs include helminths and protozoans. The word helminth refers to several taxonomic phyla that include numerous and diverse species of parasitic worms. Helminths belong to the phyla Nematoda and *Platyhelminthes*. Nematodes are often given common names associated with their physical morphology. That common name may encompass numerous species. Nematodes that infect dogs include groups of parasites commonly referred to as “roundworms”, “hookworms”, “whipworms” and “threadworms”. Roundworms belong to the order Ascaridida and are often referred to ascarids. Ascarids include the species *Toxocara canis* and *Toxasaris leonina*. Hookworms known to infect dogs include species such as *Ancylostoma caninum,* and *Uncinaria stenocephala.* The canine whipworm is a species identified as *Trichuris vulpis* and the canine threadworm is *Strongyloides stercoralis*.

Platyhelminthes can further be divided into two classes, Cestodes and Trematodes. Cestodes are more commonly known as Tapeworms. *Dipylidium canium* and *Taennia pisiformis* are the species of cestodes known to infect canines.

Protozoa are single celled eukaryotes. There are protozoan species that are free living in the environment, and there are species of protozoa that are parasitic. Parasitic protozoa that commonly infect dogs include *giardia*, *Cryptosporidium* and *Isopora* species of coccidians, and *Sarcocystis*.

Symptoms of both helminth and protozoa infections are generally associated with the gastro-intestinal tract. However, symptoms range from subclinical to clinical. The severity of symptoms is dependent on the age, nutritional status, and immune competency of the host.

While there are multiple types of parasite diagnostic techniques, this study focuses on the comparison of quantitative fecal floatation methods. Flotation methods detect patent parasite infections by mixing feces with a flotation solution that will concentrate parasite ova to the top of the solution to be evaluated. The test is quantitative as parasitic evidence (ova and cysts) are counted, and results used to make an estimation of the number of ova/cysts per gram. Clinically, fecal floats may be performed qualitatively as the objective is just to determine the presence or absence of a parasite. Having a sensitive and effective FEC method allows for an estimation of parasite load, as well as provide a means for monitoring the reduction of parasite load using follow up samples.

LITERATURE REVIEW

Overview of Intestinal Parasite Species

Ascarids. *Toxocara canis* and *Toxasaris leonina* are the species that have been identified to infect dogs. Adult ascarids can grow to be 7 to 18 centimeters long in the small intestine where the adults will produce ova that will be shed in the feces to perpetuate infections (Foreyt, 2001). The ova shed in the feces do not yet have a viable embryo and are considered unembryonated. The egg must first develop under desirable conditions that allow the larvae inside to molt twice, producing a L3 stage larvae capable of infection (Schnieder et al., 2011). If conditions are favorable, between 25 and 30^{o}C with a relative humidity between 85% and 95%, the ova develop to infective L3 stage larvae in as little as 9 to 15 days (Schnieder et al., 2011). However dynamic environments do not always maintain favorable conditions for larvation, resulting in delayed development of ova. *T. canis* ova may remain viable for up to a year in the environment (Schnieder et al., 2011). After ingestion of material contaminated with ova containing L3 larvae, the ova pass through stomach to the duodenum where it they will hatch in 2-4 hours (Schnieder et al., 2011). Free larvae penetrate intestinal mucosa, where they infiltrate the lymphatic system, migrate to the mesenteric lymph nodes and enter venous capillaries that will guide them to the liver. From portal circulation the migration continues to the lungs via the pulmonary artery where the larvae may cause coughing, nasal discharge, and pneumonia (Raza et al., 2018). Schnieder et al., 2011 reported seeing larvae in the liver in as little as 24 hours after ingestion and larvae in the lungs in 24-36 hours. The numbers in the lungs continued to increase up to 96 hours. This larval migration can cause liver damage, lung damage, and even death. Once in the lungs there are two possible migration routes the parasites utilize. The larvae may

penetrate the alveoli to either migrate up the bronchioles, trachea and pharynx or enter the circulatory system (Schnieder et al., 2011). Those that migrate up the respiratory tract are swallowed and find themselves in the intestines once again. Those that reenter the circulatory system end up in the somatic tissues of the body. Schnieder et al., 2011 discusses that though it is unclear exactly where L3 larvae molt into L4 larvae, the molt takes place somewhere between tracheal migration and passing through to the stomach. The larvae will molt again in the small intestine producing a pre-adult L5 stage worm (Schnieder et al., 2011).

Dogs may also contract parasites such as ascarids by ingesting the flesh of parenthetic hosts infected with L3 larvae. Paratenic hosts are intermediate hosts that may become infected with larvae, however, as paratenic hosts are not the definitive host the larvae do not develop nor behave the same as in the intermediate host. Paratenic hosts of *T. canis* include birds, rodents, rabbits, pigs, foxes, and humans (Schnieder et al., 2011). Upon ingestion of a paratenic host harboring ascarid larvae, the larvae find themselves in the intestines of the definitive host and may either migrate or mature to reproductive status (Schnieder et al., 2011).

Another important mechanism that roundworms utilize for transmission of infection includes trans-placental/trans-mammary transmission. Puppies may contract the infection in utero from arrested L3 stage larvae in the somatic tissue of the bitch that have been activated to transfer to puppies prior to whelping. Schnieder et al., 2011 reports that the mechanisms that activate the arrested larvae remain unclear, but that assumes activation is based on hormonal changes. The reactivated L3 stage larvae migrate through the placenta to the puppies livers where they will remain until after birth. After whelping the larvae will begin tracheal migration. Three days after whelping, the majority of larvae will reach the lung, and in seven days the larvae reach the intestines (Schnieder et al., 2011). After whelping, *Toxocara* larvae may also be

transmitted to the puppies via trans-mammary transmission. Migration pattern is correlated with age. Tracheal migration is more commonly observed in puppies, while in adults it is more common to see arrested larvae in the tissues as a result of somatic migration (Raza et al., 2018). Schnieder et., al 2011 reported that in three-week-old puppies almost all of the larvae migrated using the tracheal route. Symptoms of infection include vomiting/diarrhea, anorexia, anemia, epileptiform seizures, mucoid enteritis, emaciation, or a pot belly appearance (Raza et al., 2018). Additionally, ascarids may respond to stimuli such as an acidic pH and entangle into knots causing obstruction or even ruptures in the gastrointestinal tract (Raza et al., 2018).

Hookworms. Another common nematode known to infect dogs are those species referred to as hookworms. *Ancylostoma caninum* (Southern hookworm), *Uncinaria stenocephala* (Northern hookworm), *Ancylostoma duodenale* (Old world hookworm), *Necator americanus* (New world hookworm), *Ancylostoma braziliense*, and *Ancylostoma ceylanicum* are all species of hookworms that have been documented to infect canines. Much like ascarids, a hookworm infection is contracted via the ingestion of infective stage larvae. Additionally, hookworms may also infect their host via transdermal penetration of infective L3 stage larvae. Should the larvae penetrate through the skin, they will then migrate through the lymphatic system to the lungs. Once reaching the lungs the larvae will utilize tracheal migration to reach the intestines. *A. caninum* is capable of infecting nursing puppies via trans-mammary transmission. Trans-placental transmission of *A. caninum* remains unclear (Raza et al., 2018). *A. braziliense* does not utilize either trans-mammary or trans-placental transmission (Dias et al., 2013). Hookworm larvae can be protected in areas with unpaved soil and fecal contamination. Transmission can be exacerbated during wet seasons with increased humidity (Raza et al., 2018). Hookworms are hematophagous, meaning that they feed on the blood of their host. Hookworms use their hook to

pierce the intestinal mucosa in order to feed, resulting in lesions. Their feeding patterns often lead to fluid loss, malabsorption, diarrhea, pale mucous membranes, reduced hemoglobin, reduced packed cell volume (PCV) and reduced red blood cells (RBCs) (Raza et al., 2018). Symptoms of infection is dependent on the pathogenesis of the species and is related to the amount of blood consumed. *Ancylostoma caninum* is generally considered to be the more pathogenic than *Uncinaria stenocephala* (Raza et al., 2018).

Whipworms. The canine whipworm, *Trichuris vulpis,* is contracted via ingestion of embryonated ova. Once ingested the larvae hatch, pierce the intestinal glands, molt, and begin to colonize the large intestine (Raza et al., 2018). They pierce the mucosa with their slender anterior end while their posterior end remains free in the intestinal lumen. *T. vulpis* cannot utilize trans-placental or trans-mammary transmission and therefore most infections are observed in adult dogs who are at risk of reinfection (Raza et al., 2018). *T. vulpis* infection symptoms can range from subclinical to G.I. upset. G.I. upset may present as alternating seemingly normal stool with looser watery stools that may contain mucus or blood. Infection may also cause typhlitis (inflammation of the cecum) and weight loss. *T. vulpis* infections are generally considered less pathogenic than infections with *T. canis* or *A. caninum* (Raza et al., 2018).

Threadworms. The canine threadworm is the nematode *Strongyloides stercoralis*. *S. setercoralis* infective larvae can also utilize transdermal migration to penetrate the skin and migrate to the lungs in order to complete tracheal migration and begin to colonize the small intestine. In the small intestine they will develop and produce ova to be passed in the stool to perpetuate future infections. *S. setercoralis* also utilizes trans-mammary transmission to infect nursing puppies. Infections may range from subclinical to G.I. upset. The parasites damage the

G.I. tract and the lungs during migration. Puppies are more prone to clinical infections than older dogs (Raza et al., 2018).

Tape Worms. Cestodes are commonly referred to as tape worms. *Dipylidium caninum* is the most common cestode observed to infect canines (Raza et al., 2018). *Taennia pisiformis* is another cestode capable of infecting canines. *D. Caninum* is transmitted via ingestion of intermediate hosts such as fleas and biting lice. Infection is often asymptomatic, however a heavy load in a puppy can lead to poor growth and even intestinal obstruction (Raza et al., 2018).

There are pre-patent and patent parasite infections. Pre-patent infections are infections harboring immature parasite stages not yet capable of reproducing. A patent infection is an infection harboring adult stage parasites that are reproducing and releasing ova into the gastrointestinal tract. The prepatent period is the time between the point of infection to maturation of infective larvae into adult stage parasites capable of reproducing and releasing ova. Each species has a different prepatent period, listed in Table 1.

Coccidia. Species belonging to the class conoidasida and phylum apicomplexa are commonly known as coccidia. Species of the *Cryptosporidium*, *Isopora* (also known as *Cystoisospora*), and *Sarcocystis* genus are all coccidians known to infect canines. Coccidia is contracted via the ingestion of infective cysts. *Cryptosporidium* cysts are immediately infective when shed in feces. Most infections are asymptomatic leaving the immunocompromised individuals most at risk (Raza et al., 2018). In *Isopora*, unsporulated oocytes are passed in feces and 9-12 hours later sporulation occurs (Raza et al., 2018). *Sarcocystis* is heteroxenous, requiring two hosts. Herbivores are the intermediate host species while carnivores act as the definitive host. Twenty-one species of *Sarcocystis* have been identified to infect dogs (Raza et al., 2018). Dogs usually contract *Sarcocystis* by ingesting the flesh of an infected herbivore. *Sarcocystis*

infections are often asymptomatic while symptoms may include fever, lymphopenia, thrombocytopenia, myosistis, lethargy, pain, and muscle wasting (Raza et al., 2018).

Table 1. Common intestinal parasites, their prepatent periods, and their route of transmission.

Parasite	Prepatent Period	Route of Transmission
T. canis	6 weeks	Oral fecal, Trans-placental, Trans-mammary
A. caninum	2 weeks	Oral fecal, Trans-mammary, Subcutaneously
T. vulpis	3 months	Oral fecal
Giardia	7 days	Oral fecal
Cryptosporidium	2-10 days (Avg 7)	Oral fecal
Isopora	7 days	Oral fecal

Canine Immune Response to Parasitism

The immune system is a complex web of reactions that follow an encounter with a pathogen. The immune system can be divided into two parts: the innate immune system and the adaptive immune system. Once a pathogen has breached the physical barriers of the body, the innate immune system is the first to act. The innate immune system involves a diverse collection of cell receptors capable of recognizing an array of various pathogens (bacteria, virus, fungi, or parasites). Once a pathogen is recognized by the innate immune system, the system will recruit effector cells using chemical signals called cytokines. The innate immune system is essential for the initiation of a pathogen specific response but is not a pathogen specific response itself. The innate immune system does not produce antigen specific antibodies or keep an immunological memory for future responses. The cascade of reactions initiated by the innate immune system

triggers the adaptive immune system. The goal of the adaptive immune system is to eliminate a specific pathogen and retain an immunological memory to combat the pathogen more efficiently if encountered again. Antigen specific antibodies and immunological memory are achieved by the adaptive immune system using B and T lymphocytes. B and T lymphocytes must communicate and work together to mount an effective adaptive response. Millions of inactivated B lymphocytes, circulate the body via the bloodstream attaching to antigens that matches their unique antigen receptor. Once a B lymphocyte attaches its unique antigen, the cell must present the antigen to T lymphocytes. The process of antigen presentation determines the activation of B cells. If the T lymphocyte recognizes the antigen being presented, the T lymphocyte communicates to B lymphocyte via cytokines that influence the B cell to activate. Once activated, B lymphocytes begin to produce their antigen specific antibodies. Antibodies are essentially the B cells antigen receptor, replicated and released to survey the blood plasma, lymph, and mucosal surfaces. Generally, antibodies are found in a free-floating non-membrane bound form. Antibodies respond to pathogens in a variety of ways to either disable the pathogen or to make the pathogen more susceptible to destruction. T cells continue to provide cytokines that influence the B lymphocyte to increase the antigen binding affinity of their antibodies. A small population of activated B cells will develop into memory B cells. Memory B cells do not require T cell activation on subsequent encounters with the pathogen, rather will immediately be able to start producing antibodies. T cells not only activate B cells but also help other effector cells in their actions by producing cytokines. The unique combinations of cytokines produced influence the immune response.

When parasites are the encountered pathogen, the most effective immune response is an adaptive immune response dominated by Th_2 response is the most effective in eliminating a

parasitic disease (Parham, 2015). Th_2 cells are a specific population of T lymphocytes that secrete cytokines such as IL-4, IL-5, IL-10, IL-11, IL-13 and TGF-beta among others (Parham, 2015). The mixture of cytokines secreted by Th_2 cells recruits B-cells, basophils, eosinophils, and mast cells to respond to the infection. Activated B-cells are responsible for producing antibodies of five possible isotypes (G, M, D, A, and E). Under the influence of cytokines produced in a Th_2 response, B-cells produce immunoglobulin isotype E (IgE). IgE is unique from the other antibody isotypes in the way that it binds to basophils, eosinophils, and mast cells in the absence of antigen, unlike the other isotypes which are free-floating. When the variable region of an IgE antibody contacts the antigen on the surface of the parasite, a chain reaction is triggered allowing the effector cells to release the contents of their granules almost immediately (Parham, 2015). Basophils and mast cells release histidine, resulting in muscle spasms and watery feces in hopes to expel the parasite from the intestinal mucosa (Parham, 2015). Eosinophils release Major Basic Protein (MBP) which stimulates basophils and mast cells to release histidine while is also toxic to many helminths (Parham, 2015). Additionally, other cytokines associated with a Th_2 response increase the production of goblet cells resulting in the increased production of mucus to act as a lubricant to expel the parasite while protecting endothelial cells (Parham, 2015). Figure 1 provides a visual summary of the parasitic immune response.

Though the ideal response to a parasite infection is a Th_2 response, some parasite-host interactions can have immunomodulatory effects that push the immune response in the direction of a Th_1 response. A Th_1 response is characterized by the production of INF- gamma, TNF-alpha, and IL-2 and while these cytokines are essential in suppressing intracellular infections by utilizing a strong inflammatory response, this response creates a favorable environment for the

parasite (Parham, 2015). Each species of intestinal parasite has adapted their own mechanisms to compete in their host environment.

Schnieder et al., 2011 discusses the antigenic coat theory regarding how *T. canis* attempts to evade its host's immune system. It was found that when the metabolism of *T. canis* is inhibited by cold temperature and antimetabolites, fluorescently stained antibodies were detected on the surface of the parasite. However, when the metabolism of *T. canis* was not inhibited with antimetabolites and allowed to rest at a normal temperature, no antibodies were detected on the surface of the parasite. This study concluded that the entire surface of the parasite is in involved in excretory and secretory (E/S) product mechanisms that allow the parasite to shed antibodies that bind it. *T. canis* can produce eight nano grams of E/S products in one day and shed 25% of its surface antigens in as little as one hour. The ability of *T. canis* to shed its surface antigens emphasizes the importance of IgE's ability to bind to effector cells before binding to antigen so that the effector cell may act instantly and before the antibody is shed (Schnieder et al., 2011).

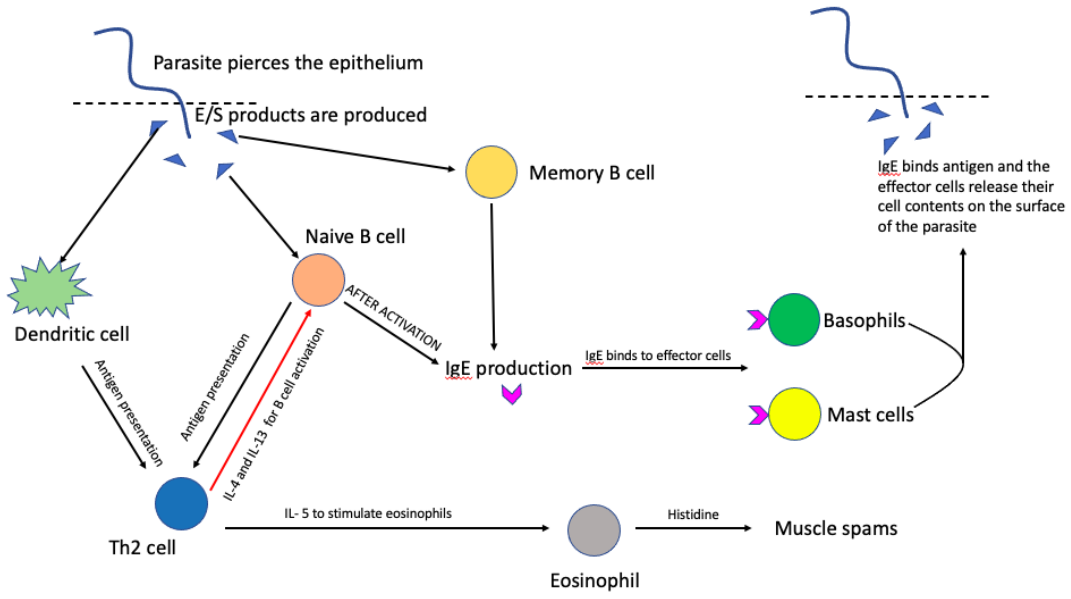

Figure 1. Summary of Immune Response to Parasites, adapted from Parham 2015.

Foth et al., 2014 used RNA sequencing to investigate proteins on the surface of whipworms and gene expression changes in infected host tissue. The whipworm genomes evaluated in this study were of two species: *Trichuris trichura* and *Trichuris muris,* the human and mouse whip worm respectively. It was reported that the anterior end of the parasite is dominated by Chymotrypsin A-like serine proteases and protease inhibitors that are similar to secretory leukocyte peptidase inhibitors. Both are unique to *Trichuris* genus; however their exact functions are unknown (Foth et al., 2014). Foth et al., 2014 hypothesize that Chymotrypsin A-like serine proteases may play a role in digestion, may assist in immunomodulation, or may degrade the hosts intestinal mucins that serve as host protection. Protease inhibitors that are similar to the secretory leukocyte peptidase inhibitors that have a role in the innate immune system may inhibit host inflammation and protect the worm from expulsion. Foth et al., 2014 found that in the tissue of the infected host, genes that are associated with a Th1 response were upregulated. In the mesenteric lymph nodes genes associated with prolonged production of B-cells and IgG were also upregulated. It has been observed that a low dose of whipworms is often the type of infection that leads to a Th1 response resulting in a chronic infection whereas a high dose infection results in a Th2 response and immunological memory (Foth et al., 2014).

Dias et al., 2013 evaluated aspects of acute infections of both *A. canium* and *A. braziliense* in mixed breed dogs. The study used 16 puppies between the ages of 8 and 15 weeks. It was observed that *A. canium* infections resulted in decreasing red blood cells, worsening anemia, increased white blood cells, a decrease in lymphocytes, eosinophils, and monocytes. For *A. braziliense* infections it was noted that there was no difference in red blood cell count, and though there was a decrease in white blood cells the quantity was still within normal physiological limits. *A. canium* infections were observed to shed ova more constantly than *A.*

braziliense while *A. braziliense* had a higher output of ova. However, both infections displayed high variation in shedding rates. Blood sera samples were also evaluated for an antibody response to infection. Both adult crude antigen and E/S antigen were used to determine antibody responses. For *A. canium* the blood sera samples from the time of infection and 7 days after infection were not reactive to the adult excretory and secretory antigens The sera 63 days after infection detected an IgG antibody titer. For *A. braziliense,* IgG for adult crude antigen was high from the moment of infection through the experimental period while antibodies for E/S products were not detected until 21 days post infection. The antibody titers for both infections reached similar levels by 63 days post infection. The authors discuss that in the case of *A. Braziliense*, the IgG antibodies to adult crude antigen detected at the start of infection may have been antibodies acquired from the bitch or a cross reaction as the result of a potential previous infection. The dogs in the study were not confirmed to have had a previous infection but rather that at the start of the experiment all puppies were examined, vaccinated, dewormed, and follow up fecal samples confirmed that the dogs were not harboring parasites before the experimental infection.

Treatment

Anthelmintics. In both the livestock and companion animal markets, antiparasitic products represent the third largest division of the animal health care industry (Colgrave et al., 2008). The Companion Animal Parasite Council recommends the following treatment protocols for canines: puppies should be given anthelmintic treatment effective against heartworms/intestinal parasites/fleas/ticks starting at two weeks of age and treated every two weeks until the canine can be placed on a regular broad spectrum monthly parasite preventative. Broad spectrum parasite control treatment is recommended year-round, regardless of the season,

as the control of zoonotic parasites is critical. A diverse collection of deworming and prophylactic drugs are used to treat and prevent parasitic infections in canines.

Benzimidazoles are a broad spectrum nematicide with narrow spectrum activity on cestodes. Fenbendazole is a commonly used benzimidazole. The drug works by binding the nematode beta-tubulin subunit which prevents the assembly of microtubules. Preventing the assembly of the microtubules effectively disrupts the nematode's cellular division and depletes any energy stores. In cestodes the inhibitory effects disrupt the integument and renders the worm more susceptible to the host's immunity (Elsheikha and Castro, 2015).

Imidazothiazoles are another broad spectrum nematocide. By binding the acetylcholine (Ach) receptors, imidazothiazoles like Levamisole, elicit the same response as acetyl choline and are therefore said to be cholinergic. Cholinergics change membrane permeability allowing for the influx of Ca2+ (Martin and Robertson, 2007). As the permeability of post synaptic membranes to Ca2+ is changed, sustained involuntary muscle contraction, known as spastic paralysis, is induced (Elsheikha and Castro, 2015). The therapeutic margin of this class is low and therefore toxicity can occur; toxicity symptoms include salivation, respiratory distress, seizures, and anaphylaxis (Elsheikha and Castro, 2015).

Tetrahydropyridines such as pyrantel and oxantel, represent another cholinergic drug class that function similarly to imidazothiazoles by targeting Ach receptors. Tetrahydropyridines work on larval and adult nematodes but are not effective against migrating and arrested larvae or cestodes (Elsheikha and Castro, 2015). Pyrantel is effective against both *T. canis* and *A. caninum* however has no action on *T. vulpis*. Oxantel on the other hand is highly specific for *T. vulpis* and has no action on the other nematodes.

Macrocytic lactones represent another antiparasitic drug class comprised of avermectins and milbemycins. Macrocytic lactones work via a dual method of action: first GABA pathways are manipulated to inhibit neurotransmission, then ligand gated chloride channels are stimulated to remain open. These mechanisms result in the inability to control and move muscles, or flaccid paralysis (Elsheikha and Castro, 2015).

Drug Resistance. Anthelmintics are valuable but cannot serve as the only control mechanism for parasites as resistance the drugs in use has been documented. Resistance to drugs can be produced in multiple ways. According to Kopp et al., 2008, the resistance to benzimidazoles is the result of a point mutation at specific locations in the different beta-tubulin gene isotypes. Response to cholinergics depends on the sensitivity of the Ach receptors, specifically how much depolarization (and influx of Ca2+) is produced by the drug, and the sensitivity of muscular contractile proteins to Ca2+ (Martin and Robertson, 2007). The efficacy of cholinergics relies on a neuromuscular signal-transduction pathway. If at any point during that pathway the muscle contraction response is reduced, this is indicative of a parasite that is less sensitive (more resistant) to the drug (Martin and Robertson, 2007). Resistance to cholingerics may develop by four mechanisms: changes in drug translocation, changes in receptor numbers, receptor modifications, and post receptor modifications (Martin and Robertson, 2007). Extensive gene families code for Ach receptor, a pentametric ligand-gated ion channel made up of five glycoprotein unit. The combination of the glycoproteins determines the properties of the receptor (Kopp et al., 2008). Kopp et al., 2008 investigated both a drug sensitive and resistant *A. caninum* isolate for transcription patterns associated with pyrantel resistance. Kopp et al., 2008 reported that while no polymorphism of significant value was identified between the two isolates, there was a significant reduction in the transcription of 3 Ach receptor genes in the resistant isolate.

The three genes reported to be reduced in the resistant isolate compose the genes thought to be responsible for the L-nAch receptor (named L due to sensitivity to Levamisole). However, the two residues that have been reported as essential to sensitivity for pyrantel were 100% present at both larval and adult stages of both *A. caninum* isolates (Kopp et al., 2008).

In 2020 multiple cases of multidrug resistant (MDR) *A. caninum* were identified in greyhounds (Castro and Kaplan, 2020). Multidrug resistant *A. caninum* species are resistant to two or more of the major antiparasitic drug classes (benzimidazoles, tetrahydropyridines, and macrocyclic lactones). According to Castro and Kaplan, 2020, there are three methods to diagnose anthelmintic resistance: a fecal egg count reduction test (FECRT), in vitro bioassays, and molecular testing. The authors discuss that the use of the FECRT may be more practical as the test can readily be performed in a clinic for a lower cost compared to cost of submitting the sample to a specialized laboratory. While the use of a FECRT may be more practical, Kopp et al., 2008 discuss that the use of a molecular based assay has the advantage of being able to detect alleles associated with resistance long before the resistance presents phenotypically. Disadvantages to employing a molecular based assay include the time, resources, and training required to perform the test.

To perform a fecal egg reduction test, a quantitative FEC must be performed pre- and post-treatment. The FEC reduction is then calculated by comparing the egg per gram (EPG) of feces for the pre- and post-treatment samples. Collection time of a post-treatment sample for evaluation is important. At least three days must be allotted after treatment to allow for ova that have already been shed to clear the digestive tract. Additionally, suppression of female fecundity following fenbendazole treatment has been observed. Castro and Kaplan, 2020 reported a 99% ova reduction observed 3 days post-treatment while by 10 days post-treatment ova counts rapidly

rose. By collecting a sample too soon, the observer runs the risk of yielding a false negative result for resistance (Castro and Kaplan, 2020). On the opposite end of the spectrum, if a sample is collected too late post-treatment, the observer runs the risk of obtaining a false positive result for resistance as arrested somatic larvae potentially leak back into the intestines to begin a new cycle of egg shedding (Castro and Kaplan, 2020). Therefore, Castro and Kaplan, 2020 recommend utilizing the following post treatment follow up dates: 14 days after treatment with fenbendazole or moxidectin and between 10- and 14-days post-treatment when using pyrantel. As discussed FECs can be extremely variable, and the same goes for FECRTs, and as such, the tests should be interpreted conservatively. Castro and Kaplan, 2020 recommend the following result interpretations: <75% reduction as indicative of resistance, 75-89% reduction as suggestive of resistance, 90-95% reduction as suggestive of reduced efficacy and should raise suspicion while results should be considered inconclusive, and >95% reduction as suggestive of effective treatment.

While FECs and FECRTs provide a quantitative way to evaluate intestinal parasite infections, Kopp et al., 2007 emphasizes the need to further evaluate the techniques as population density related modulation of egg production of *A. caninum* females has been observed. In 1961, Krupp reported that ova production per female *A. caninum* is correlated with the number of adult parasites present and the amount of space available to them. In the experiment, ova production per female did not differ significantly when less than 200 adults were present. However, when the number of adult worms present increased beyond 200 and the space available to the worms decreased to less than 1cm, ova production was depressed (Krupp, 1961). The concern presented is that should a canine be harboring a large parasite burden with depressed ova production, if the treatment is not 100% effective, any adult parasite remaining

will begin producing ova without the burden constraints and influence the FERT towards a result suggesting resistance.

Kotze and Kopp, 2008 investigated whether a decrease in worm burden will result in increased egg production, influencing the results of the FECRT and suggesting resistant isolates when they are not actually present. Having an accurate and precise FEC method is essential in monitoring parasite populations for resistance to treatment.

Environmental Control. In addition to appropriate anthelmintic use and follow up testing to ensure efficacy, Elsheikha and Castro, 2015 also discuss the importance of environmental control protocols. The Shelter environment brings animals from diverse backgrounds with a varying history of exposure and treatment into very close proximity with one another. Canines may come into the shelter harboring a patent infection and immediately begin to contaminate the environment, or a canine may enter the shelter with a prepatent infection and begin to contaminate the environment days to weeks later. As infection for many of the discussed G.I. parasites follow an oral-fecal route of transmission, fecal contamination in the environment poses a threat to any canines sharing that environment. It is essential to regularly remove feces, and proper cleaning and sanitation practices are critical. Ortuño and Castellà, (2011) surveyed twelve different shelters in Spain to identify parasite prevalence and risk factors associated with the facility and its management. A total of 544 fecal samples were evaluated and information about each facility was collected. Information collected about the facility included box material, floor material, housing (individual vs group), quarantine (yes or no), disinfection protocols (product and frequency) and food (commercial vs fresh cooked). The factors identified to play a role in reducing parasite prevalence included: use of nonporous material, use of an appropriate disinfectant, and the use of periodical fecal evaluations to determine which parasites are present

to develop an effective treatment plan (Ortuño and Castellà, 2011). Higher frequency of infection was found in shelters that used wooden housing boxes rather than metal or concrete (Ortuño and Castellà, 2011). Higher prevalence was also found in shelters that used concrete as a floor material compared to floors with a waterproof sealant on the surface (Ortuño and Castellà, 2011). Interestingly there was no significant difference found between shelters that practice individual housing vs those that group house, or the use of a quarantine period. The authors attribute this to the fact that regardless of housing or quarantine time, the shelters reported using the same area for enrichment/elimination. The study also found no significant difference between infection prevalence and drug protocol. Regarding disinfectants, a lower infection rate was found among the shelters that used an ammonia compound as the main disinfectant (Ortuño and Castellà, 2011).

Ash et al., (2019) evaluated fecal samples from greyhound kennels in five different states of Australia and reported identifying 11 species of intestinal parasites. Species of *Sacrocystis, Anclyostoma, Toxocara*, and *giardia* being the species identified most frequently. Six of the identified parasites in the study are considered to be zoonotic. In addition to age and geographic region, Ash et al., (2019) also identified housing substrate type as a risk factor for parasitism in greyhound kennels. Across all parasite groups, a greater proportion of canines housed in grass and or sand tested positive for infections relative to the canines that were housed on concrete (Ash et al., 2019).

Romero et al., (2020) compared the efficacy of various disinfectants on the hatching of larval *T. canis* ova in vitro using distilled water as the control. The disinfectants evaluated included disinfectants such as sodium hypochlorite, benzalkonium chloride, super oxidation solution with a neutral pH, iodopovidone, quaternary ammonium, and an enzymatic solution

with aniosyme DLT. Of the disinfectants that were compared only the quaternary ammonium and enzymatic solution with aniosyme DLT showed any real advantages. Quaternary ammonium was found to be the second most effective, however proved to be the disinfectant with the highest efficacy after only a ten-minute contact time. The enzymatic solution with aniosyme DLT was found to be the most effective of all the disinfectants after a contact time of 20 minutes (Romero et al., 2020).

Diagnostic Methods

With few exceptions, evidence of parasitism is microscopic and cannot be seen with the naked eye. Therefore, many techniques have been developed to diagnose endoparasite infections. Accurate diagnosis is dependent on the sample and method of evaluation. It is critical for the sample to be as fresh as possible. There are two things that can interfere with the diagnostic quality of the sample as the sample ages. First, as the sample ages the ova develop further, making them more difficult to identify. Secondly, as the sample sits out for longer, the possibility of contaminants also increases. Samples that are more than two hours old should be kept at 4 degrees Celsius (Foreyt, 2001). A stool sample can be evaluated by a variety of tests such as direct smears and fecal floats. Each diagnostic test also has a diverse array of modifications that various labs use.

Direct Smears. A direct smear is a quick and easy way to evaluate a stool sample. However, the test is considered inaccurate with a low sensitivity according to Maurelli et al., 2014. The test allows for examination of a very small amount of feces, and there is no step to remove the debris. Therefore, the debris can make the identification of ova more difficult or obscure them from the visual field entirely.

The Kato-Katz smear method of fecal examination is a direct examination of stool using a slide that has a square template with a hole having a diameter of 6cm and a depth of 1.5mm (Cools et al., 2011). The dimensions of the slide assume evaluation of 41.7mg of sample; to calculate EPG from the sample, a multiplication factor of 24 is applied to the number of ova counted as 24 x 47.1mg = 1,000.8mg or 1 gram. This method is recommended by the World Health Organization for use in humans because it is simple and easy to use in the field.

While the Kato-Katz smear technique serves as quantitative analysis, many direct smear methods provide only a qualitative analysis. Particularly in companion animal veterinary practices, a direct smear will be evaluated from a small sample collected using a fecal loop. This method of evaluation is used to determine the presence of a parasitic evaluation while not trying to quantify the intensity of the infection. While the test is viewed as practical for quick field use requiring little equipment and time, the results are variable. As each diagnostic is preformed using a variable and small fecal sample size, the accuracy and precision of the method is also extremely variable.

Fecal Flotation. Fecal floats concentrate ova and oocysts into a drop of solution to be examined under a microscope. Important factors to consider upon choosing a flotation solution include the specific gravity of the solution, the viscosity of the solution, and the rate of plasmolysis caused by the solution. A solution with a specific gravity between 1.2 and 1.3 will generally float most parasite stages (Foreyt, 2001). If the specific gravity is too low the ova will not float, while if the specific gravity is too high it can cause plasmolysis. According to Foreyt (2001), sugar solutions, such as Sheathers, have a distinct advantage over salt solutions. Using sugar solutions are advantageous as they cause less plasmolysis and distortion of the sample. Salt

solutions quickly crystalize, and distortion of the ova occurs in a little as a few hours. A disadvantage of sugar solutions includes not effectively floating *giardia*.

Common methods of fecal flotation include the McMasters method and the Wisconsin method. Both of these methods are used with various modifications. The McMaster method involves mixing a known volume of fecal material with a flotation solution and straining the sample to remove large debris before evaluation. The traditional McMaster test uses a ratio of 4g of feces to 26mL of flotation solution, creating a solution that contains 0.133g of feces per mL (Slusarewicz). The sample is then moved to a McMasters slide with two 1 cm grids that each have six lanes. Each grid contains a known volume of 0.15mL, allowing for an examination of approximately 0.02g of feces (0.133g x 0.15mL= 0.1995g) in the traditional preparation method (Slusarewicz). Once the sample has been transferred to the slide, it then sits for a predetermined amount of time to allow the eggs to float to the top of the slide. After the flotation period is over, each grid is examined under the microscope and each ova lying with-in the grid lines is counted. After counting each grid, the total count for each grid is added together and a multiplication factor is applied to the total to determine the estimated EPG. The multiplication factor can be determined using the known ratios and volumes used to prepare the test. When counting both grids with the traditional McMaster preparation, a total of 0.04g of feces is examined. As 0.04g x 25 = 1g, the multiplication factor for the traditional McMaster preparation is 25 yielding an estimation of the number of eggs per gram of feces. Many modifications of the McMasters test exist that utilize different sample dilution ratios making it important to understand how EPGS are extrapolated based on the sample size examined. As modifications are applied to the test, it is essential to reevaluate the multiplication factor to ensure the appropriate multiplication factor is applied to the egg count for the most accurate results.

A Cornell-Wisconsin/Wisconsin test utilizes a centrifugation step to accelerate the flotations of eggs in a sample while removing heavy debris that may obscure the sample. As previously stated, many modifications exist; some labs may place a coverslip atop of the centrifuge tube prior to centrifugation, while in other labs the sample may be spun in a swinging centrifuge without a cover slip and once removed from the centrifuge additional flotation solution is added to create a positive meniscus before the coverslip is applied. After the determined flotation time, the coverslip is examined and each ova is counted. Typically, one entire gram of feces is used for the test, therefore no multiplication factor is needed to estimate EPG. In some modifications more than one gram may be used and in such situations the multiplication factor will be less than one. For example, if two grams of feces were used to prepare to test, then the total number of ova detected will be multiplied by 0.5 (equivalent to dividing by 2) to yield EPG.

A more novel flotation technique is the FLOTAC method. The FLOTAC technique was inspired by features of the McMaster and the Wisconsin techniques for veterinary parasitology but was quickly adapted for human parasitology as well (Cringoli et al., 2010). In order to perform the FLOTAC method, the entire FLOTAC apparatus and accessories are required. The apparatus consists of a base, a translation disk, and a reading disk. There are two different apparatuses; one that can be read at a maximum of 100x and one that can be read at a maximum of 400x. The apparatus has two 5ml flotation chambers allowing for the examination of a larger sample and the application of a smaller multiplication number. In the basic version of the protocols, the multiplication factor is one as the test is sensitive enough to detect 1 EPG. To prepare the sample for evaluation, the following protocols are suggested; dilute the fecal sample (protocols suggests between 1 and 5 grams) with tap water using a dilution ratio of 1:10,

homogenize the sample and filter using a filter with a 250-nanometer aperture. 11ml of the filtered suspension is transferred to a conic tube and the tube is centrifuged at 170g for three minutes. After removing the tube the supernatant is discarded and 11ml of flotation solution is added to the tube with the sediment (Cringoli et al., 2010). The sample needs to be homogenized once more and then the two 5ml flotation chambers are filled with the suspension. After the FLOTAC apparatus is filled, the entire apparatus goes into the centrifuge using a centrifuge adaptor. The sample is then centrifuged for a second time, this time under 120g for five minutes (Cringoli et al., 2010). After the second centrifugation step, the reading disk of the FLOTAC apparatus is then translated 90 degrees and examined under a microscope under the maximum magnification determined by the apparatus. After counting the ova under each grid, add the grid totals together to determine EPG (determine EPG utilizing the appropriate multiplication factor for the sample size evaluated).

A mini-FLOTAC method has been adapted. The mini-FLOTAC method is simpler and does not require the centrifugation steps while remaining sensitive to ova detection. The mini-FLOTAC has a sensitivity of 5epg when the sample is prepared with 2g of stool and 18mL of solution. The mini-FLOTAC technique utilizes a reading disk that is translated 90 degrees after an appropriate flotation time. The mini-FLOTAC test can be prepared with the fill-FLOTAC apparatus to minimize handler contact with feces, homogenize the sample, filter the sample, and pipette the sample into the mini-FLOTAC disk.

Sources of Variation. It is important to consider that there are many sources of variation between diagnostic tests that may affect a test's accuracy and precision. The accuracy of a test refers to the test's ability to estimate a parameter value from a sample that is as close to the true population value as possible. In FECs the parameter being evaluated is the EPG in feces. The

precision of a test refers to the tests ability to consistently yield results that are close to the true population value as the test is repeated with multiple samples. The more precise a test, the more confident one can be that the value obtained is close to the true value. There are intrinsic factors in sampling a population that affect the accuracy and precision of an estimated value. All parameter estimates, such as FECs, are influenced by sampling error; the fact that all samples will differ from the true population by chance (Schluter and Whitlock, 2020). This is due to the nature of sampling itself. As it is not practical to evaluate the entire fecal mass produced by a canine, a smaller more manageable sample is taken to provide an estimate of the true EPG. As the true distribution of ova in the feces is not known, there is a chance that the sample of feces examined contained more or less ova than another sample of the same original mass of feces. Additional sources of diagnostic variation include the intrinsic variation of testing methodologies, modifications within testing methodologies, and the techniques/training of the lab technicians (Slusarewicz).

Intrinsic variation of testing methodologies refers to the differences in the protocols of the test used. Various tests have been developed to examine the same parameter, and each of these tests will produce results variable in respect to another test due to differences in separate testing protocols. As discussed, the McMasters, Wisconsin, and Mini-FLOTAC fecal egg count methods vary in the preparation and evaluation of samples. Traditionally the McMasters and Mini-FLOTAC diagnostic tests do not require the use of centrifugation while the Wisconsin does. The various slides that each test uses for microscopic examination yield different sensitivities. Even if each test is performed on the same exact sample, the resulted yielded are expected to be reasonably different due to the inherently different way the test was prepared. The same phenomenon exists within the same testing methodologies. While two labs may use the

same general testing method, each lab may apply modifications to the test protocol. Modifications include dilution ratio, flotation solution used, flotation time before reading, etc. Furthermore, the techniques and training of the lab technicians who will be evaluating the sample can provide yet another source of variation even when the test is prepared in a consistent manner. Technicians may evaluate slides at different magnifications, may inherently spend more/less time on a slide, and while one technician might recognize coccidia another might mistakenly dismiss the coccidian as an air bubble. Thus, consistent and explicit lab training is essential in eliminating as much variation as possible.

According to Adolph et al., (2017) fecal floats are likely to underestimate the true prevalence of infection as they are unable to detect prepatent and single sex infections. The study performed by Adolph et al., chose 97 dogs for humane euthanasia to observe differences in the rate of detection of infection via fecal floats, ELISA, and necropsy. Fecal floats failed to detect 100% of the infections that were detected by necropsy. Fecal floats successfully detected 84% of *A. caninum*, 54.5% of *T. canis*, 68.4% of *T. vulpis*, 0.042% of *D. canium*, and 0.14% of *Taenia* infections that were identified by necropsy (Adolph et al., 2017). The study concluded that the flotation method is particularly poor for cestodes as they pass dense egg packets, only intermittently, and in discrete proglottids (Adolph et al., 2017). An ELISA test was also preformed to detect *A. canium*, *T. canis*, and *T. vulpis*. The results for the ELISA were more accurate but still failed to detect 100% of the infections. The ELISA missed those infections that were of a low intensity parasite load (Adolph et al., 2017).

Comparison of Diagnostic Methods

Cools et al., (2011) studied compared the sensitivity of a modified McMaster method and the Kato-Katz thick smear method in regard to soil transmitted helminths in humans. Their study resulted in a significant quantitative correlation between the two FEC methods, however the Kato-Katz smear methods consistently yielded a higher FEC for *A. lumbricoides* (human roundworm) than the McMasters method. Cools et al., (2011) stated that a distinct advantage of the McMasters method is that the sample is homogenized and allows for simultaneous detection for multiple species of ova. The Kato-Katz smear is poor for evaluating a multiple species infection because each species of parasite has a different optimal clearing time (time that the ova become apparent in the sample, and then undetectable again) according to Cools et al., (2011). Cools et al., (2011) also questioned the quantitative performance of the smear test as its calculations are based on the volume of the sample and not the mass of the sample. Cools et al., (2011) reported that the employment of another adjusted fixed multiplication factor still yielded variation. However, trials that utilized a fixed multiplication factor rather than consistently adjusting the multiplication factor for the mass of the sample evaluated consistently gave a higher count of ova (Cools et al., 2011). Ultimately, Cools et al., (2011) concluded that both methods, although variable, are acceptable in monitoring treatment programs.

Katagiri and Oliveria-Sequeira, (2010) compared three concentration methods in their ability to recover evidence of parasitism in canine stool samples. Fecal samples from 254 dogs were evaluated using three techniques which included centrifugation-sedimentation, centrifugation-flotation, and a commercial assay of TF-Test. Katagiri and Oliveria-Sequeira, (2010) reported that in all cases of parasites diagnosed the centrifugation-flotation method was capable of detecting the largest number of ova, however this correlation was only statistically

significant in the case of *A. canium*. The flotation method was reported as significantly more effective at diagnosing cases of polyparasitism and reported as the most sensitive in the detection of all of the parasites. The sedimentation technique was said to be the least sensitive in detection of parasites, while the results for the assay were indeterminate.

As previously mentioned, there are many modifications various labs apply to the preparation of the McMaster test. Vadlejch et al., (2011) examined three different McMaster FEC techniques, specifically the Wetzel (W), Zajicek (Z), and the Roepstorff and Nansen modifications (R&N). Each of these tests differ in the mass of feces used to prepare the test, use of flotation solutions, use of centrifugation, number of chambers read, and multiplication factors. Each test was performed on fecal samples with a known concentration of ova to test the sensitivity and reliability of the methods. In order to achieve this, ova from the nematode *Teladorsagia circumcincta* were collected from experimentally infected naïve sheep feces. Once eggs were collected, a known number of ova were added to parasite negative feces collected from a different population of sheep that remained parasite naïve. Vadlejch et al., (2011) prepared samples with various different ova concentrations to simulate evaluations of samples with low, intermediate, and high ova concentrations. Samples with 20 EPG were considered low concentration, 500 EPG samples were considered a high concentration, and samples prepared with either 50, 100, or 200 EPG were considered intermediate concentrations. Vadlejch et al., 2011 reported that modifications described by Respstorff and Nansen yielded the highest sensitivity and reliability as this technique was able to detect 20 EPG in the low concentration samples 70% of the time while at concentrations of 200 and 500 EPG the accuracy was almost 100%. The lowest reliability was reported with the use of the Wetzel method. The authors of this

study attribute the differences between the testing methods to the mass of feces examined, the dilution ratios, use of centrifugation, and flotation solution used.

Paras et al., (2018) evaluated the sensitivity of three fecal flotation methods; a 3-chamber modified McMaster technique, a modified-Wisconsin, and the mini-FLOTAC technique. The three methods were tested with clinical samples from four species; cattle, sheep, horses, and llamas. Paras et al., (2018) reported that when using ova spiked samples with a known FEC, the mini-FLOTAC was the most accurate test and recovered 70.9% of the ova, while the modified McMaster method recovered 55.0% of the ova, and the modified- Wisconsin technique recovered 30.9% of the ova. The results from the clinical samples in the four livestock species were reported as in agreement with the ova spiked samples; the Mini-FLOTAC was consistently found to be the most accurate method used. However, it is suspected that there are various sources of potential ova loss during the preparation of the samples, specifically for the methods that consistently yield lower FECs. The study did not evaluate all of the possible sources of ova loss, but Paras et al., (2018) did investigate ova loss via the use of a cheese cloth to strain large debris from the samples. Cheese cloth is commonly used for modified McMasters methods and the modified Wisconsin methods while the mini-FLOTAC method utilizes the fill-FLOTAC that has a built in sieve. In order to examine potential ova loss due to the cheese cloth step, Paras et al., (2018) prepared modified Wisconsin tests using the fill-FLOTAC apparatus in order to use the built-in filter rather than the cheese cloth. When the modified Wisconsin was prepared with the fill-FLOTAC, the test yielded similar fecal egg counts to those obtained using the mini-FLOTAC method (Paras et al., 2018). Paras et al., (2018) reports that this suggests that the small pore size of the cheese cloth is a greater barrier to the passage of ova than of the fill-FLOTAC.

Cringoli et al., (2004) studied the influence of fourteen fecal solutions, six different sample dilutions, and four different volumes of sample evaluated. The volume of sample examined is dependent on the number of McMaster chambers counted. One McMasters grid contains 0.15 ml, two grids contain 0.3 ml, one whole chamber contains 0.5 ml, while both chambers contain a total of 1ml. It is important to note that in this study, Cringoli et al., (2004) appropriately adjusted the multiplication factor as needed for the different volumes of sample examined. The variables were evaluated using a composite fecal sample from naturally infected sheep to detect ova of strongyles and *Dicrocoelium dendriticum*. The fourteen fecal solutions were all reported to be capable of floating the ova for strongyles, however there were statistically significant differences in the number of ova that floated. The following solutions were reported to successfully float the highest number of strongyle ova; sucrose-formaldehyde solution, sucrose-potassium-iodomercurate solution, and sucrose-sodium-nitrate solution (Cringoli et al., 2004). The following solutions were reported to float the least amount of strongyle ova; sodium-chloride, zinc-chloride solution, zinc-sulphate and potassium iodomercurate solution. Cringoli et al., (2004) reported that not all of the solutions were capable of floating ova for *D. dendriticum,* while potassium-iodomercurate was the only solution capable of floating a significantly large number of ova for *D. dendriticum.* In regard to dilution ratios of the samples, the average EPG was positively correlated with increased sample dilutions (Cringoli et al., 2004). For both strongyles and *D. dendriticum*, Cringoli et al., (2004) reported that the highest reliability was observed with a dilution ratio of 1:10. Finally for the volumes of sample evaluated, Cringoli et al., 2004 reported that evaluating 1.0 ml, or the entirety of both McMasters chambers, yielded the highest reliability. Cringoli et al., (2004) noted that 1.0 ml yielded significantly lower EPGs and

had the lowest standard deviation, suggesting that the lower volumes evaluated overestimated the average EPG.

Intestinal Parasite Prevalence

The prevalence of canine parasite infection is varied based on geographic location, season, and whether the pet is owned or living in a shelter. Regarding geographic distribution, Little et al., (2009) explored the national distribution of canine parasite infections in the United States. Distribution patterns were evaluated using the results of 1,199,293 fecal samples that were submitted to veterinary clinics and analyzed by Antech in the year 2006. The study reported that Antech used a modified Wisconsin technique utilizing a 33% zinc sulfate solution. Little et al., (2009) reported that the majority of infections were found in individuals less than six months old. The exception was *T. vulpis* which was found to be more prevalent among canines greater than one year of age. In this population of canines, those less than 6 months old, 29.6% tested positive for intestinal parasites, while 6.1% of dogs greater than one year of age tested positive (Little et al., 2009). The most common parasite infections found in the study were ascardis, hookworms, whipworms, giardia, and cystoisopora. Using contingency and chi squared statistical analysis, the results obtained from Antech were divided into one of the following geographic regions; northeast, midwest, south, and west. Ascarids were found to be more prevalent in the west, while hookworms were identified to be prevalent in the south and midwest. Giardia was most commonly diagnosed in the west, while cystoisopora was more common in the midwest and the west. Whipworms were found with equal frequency in the south, northeast, and the midwest with a three-fold decrease in frequency found in the west.

Little et al., (2009) also discusses other factors that are favorable to parasite transmission. Factors such as temperature, humidity, environmental contamination, and even socioeconomic status can influence parasite transmission. Drake and Carey, (2019) analyzed data collected by the Companion Animal Parasite Council using millions of fecal samples submitted between 2012 and 2018 in the United States. The data sets were investigated to determine changes in parasite prevalence or possible seasonal fluctuations with an emphasis on *A. canium*, *T. vulpis*, and *T. canis*. Drake and Carey, (2019) reported a subtle, yet significant, increasing prevalence of *T. canis* and *A. canium* and a slightly decreasing prevalence of *T. vulpis*. In addition to changing prevalence patterns, the study also reported a seasonality could be determined for different parasites. Drake and Carey, (2019) reported that their study confirmed a previously suggested seasonal prevalence for both *T. canis* and *A. canium* while reporting that to their knowledge, they had for the first time observed a seasonal prevalence in *T. vulpis*. Infections of *T. canis* remained consistent each year with the highest seasonal prevalence occurring during December through January with the lowest prevalence during May through June. The highest monthly *A. canium* prevalence observed in the mid-summer months (July-August) and lowest during the winter. Drake and Carey, (2019) reported that the *T. vulpis* has a seasonal pattern similar to that of *T. canis* with the highest prevalence observed during the winter months of January-February with the lowest prevalence observed in May-June.

Companion Animal Population. According to Little et al., (2009) if this set of data is a good representation of the true population then as many as 9 million pet dogs are infected and shedding at least one of the five most identified parasites. It is important to note that these fecal samples were submitted by clients taking their dog to the vet, so it is assumed that there would be a lower rate of parasitism in this sample (Little et al., 2009).

Shelter Population. Little et al., (2009) report that of 6,000 fecal samples evaluated for shelter dogs in the United States, 36% of the canines were shedding at least one parasite, with rates as high as 50% in the south. Prevalence in shelters has been documented at higher rates than among the pet populations. Out of the 544 samples submitted by the shelters evaluated by Ortuño and Castellà, (2011), 61.8% of the samples tested positive for a parasite. In the Australian greyhound kenneled population evaluated by Ash et al., (2018), parasite prevalence rates ranged between 50-71%. The lower rate of parasite infections in pet dogs vs shelter dogs is attributed to the routine use of anthelmintics and regular veterinary visits.

According to Raza et al., (2018) 4.1 million dogs were estimated to be relinquished to a shelter in 2016. Animal shelters, by their very nature, provide favorable conditions for gastrointestinal parasite infections as dogs with unknown histories enter the shelter and become part of the general population. In addition to inherent environmental contamination in a shelter setting, it is also natural for dogs to feel stressed, and in turn that stress can weaken the immune system.

In 2015 Rindaldi et al., theorized that the "constant presence of parasitic infections in kennel dogs and the reoccurrence of them despite anthelmintic use is probably due to constant reinfection rather than drug efficacy". There are many factors that contribut to the high infection rates among shelter animals. Even if high rates of parasitism are not suspected to be the result of reduced drug efficacy, it is still essential to monitor the efficacy of the parasite control program. Monitoring shelter animals for persisting and potentially resistant infections is essential as emphasized by the discovery of MDR *A. caninum* discussed by Castro and Kaplan, (2020). Castro and Kaplan, (2020) discuss that the resistant parasite likely evolved on greyhound breeding farms and in racing kennels. The authors go as far to say that "most, if not all, actively

racing and/or recently adopted greyhounds appear to be infected with MDR hookworms." Retired racing greyhounds are adopted out to the general public where they may contaminate the environment with MDR ova. As many cases of the MDR *A. caninum* have been reported in non-greyhound breeds, Castro and Kaplan, (2020) suggest there is a spread of the resistant parasite to the general population of canines. Spread of MDR *A. caninum* into the general population of companion animal canines raises public health concerns as *A. caninum* poses a zoonotic threat.

Zoonotic potential

Several of the discussed parasites have the potential to infect a human host, posing a public health threat. Public awareness of zoonoses and associated risk factors is an essential component in management to avoid zoonotic infection. Pereira et al., (2016) published survey results evaluating Portuguese pet owners' awareness and deworming practices. Of the 536 returned surveys only 56.5% of owners reported that they had heard about zoonosis while only 35.2% reported that they knew the meaning of the word. The study concluded that the majority of owners who took their pets to a veterinary clinic were in fact using a prophylactic treatment schedule, but not always on the correct schedule while not truly understanding the purpose of the treatment. Of those that recognized zoonoses, the respondents more frequently reported having an academic degree (Pereira et al., 2016).

Toxocariasis. Toxocariasis is the infection of *T. canis* in humans. Humans contract *T. canis* infections when they have unintentionally ingested ova. As *T. canis* ova are shed in feces and must become embryonated in the environment before they are infective, one of the most common sources of exposure is contaminated soil; for example, a child who plays in the dirt and promptly puts their hands in their mouth, or ingesting produce that has not been washed. Another

potential source for exposure includes the ingestion of a parenthetic host harboring migrating or arrested larvae. The parasite *T. canis* behaves differently in a human host than in its definitive canine host, so this parasitic infection can take on five different forms categorized by the larval migration patterns: 1) visceral larval migrand (VLM), 2) ocular larval migrand (OLM), 3) neurological larval migrand (NLM), 4) covert, or 5) asymptomatic. How the infection presents is based on the intensity of the exposure, the localization of larvae, the patients history of infection, the patients age, and the efficacy of the hosts immune system. VLM infections are categorized by visceral migration via hepatic and portal circulation and can result in the enlargement of the liver and spleen and may include lung involvement (Mazur-Melewska et al., 2014). According to Raza et al., (2018) VLM is common in children ages 1 to 3 years old. OLM infections can result in sever inflammation and damage to the eyes as larvae migrate via retinal blood vessels (Mazur-Melewska et al., 2014). OLM infections present in children ages 5 to 10 years old, and often the larvae migrate without clear signs (Raza et al., 2018). As the larvae migrate into the orbit the host may experience gradual and permanent visual impairment (Torgerson and Macpherson, 2011). A NLM infection can result in neuro deficits or eosinophilic meningoencephalitis (Mazur-Melewska et al., 2014). An infection with *T. canis* may also present as covert, meaning without specific symptoms due to the immune system being stimulated (Mazur-Melewska et al., 2014). Finally, a *T. canis* infection may also remain completely asymptomatic in nature (Mazur-Melewska et al., 2014). According to Mazur-Melewska et al., (2014), seroprevalence surveys conducted in western countries suggest a positive seroprevalence between 2-5% for populations in urban areas. Rural areas have a positive seroprevalence between 14-20%, while the prevalence in the topics is much greater. Torgerson and Macpherson, (2011) report that as many as 1.3-2.8 million people are infected with *T. canis* in the United States.

Ground Itch/Creeping Eruption. Another canine nematode that is capable of infecting humans is *A. caninum*. This parasite may be contracted via an oral fecal route or may also utilize subcutaneous migration just as the species does in canines. Therefore, not only may someone become infected by accidentally ingesting ova contaminated soil but may also contract the infection by skin to soil contact such as walking barefoot.

Strongyloidiasis. Strongyloidiasis is an infection of *Strongyloides stercoralis* in humans. Torgerson and Macpherson, (2011) reported that the number of estimated *Strongyloides stercoralis* infections range from 3 to 10 million people globally. For many years there has been debate as to whether *Strongyloides stercoralis* could be contracted from dogs, or if there were two septate host specific species. However, Jaleta et al., (2017) utilized PCR for genome mapping to investigate *Strongyloides stercoralis* samples collected from both humans and dogs. Jaleta et al., (2017) reported that there are two different but over lapping populations of *Strongyloides stercoralis.* Two haplotypes, A and B, were discovered, with haplotype A was found in both canines and humans, and haplotype B found only in canine samples. As haplotype A was found to infect both humans and canines, this evidence supports the hypothesis that dogs can serve as a reservoir for the zoonotic parasite. The study also concluded that the free-living worms do in fact reproduce sexually as opposed to asexually as some of the older literature suggests (Jaleta et al., 2017).

METHODS AND MATERIALS

Experimental Design

The present experiment has been designed to observe and compare the performance of three different fecal egg counting technologies and analyze their ability to perform a fecal egg reduction test. Secondarily, the present study also aims to evaluate associations between infection and the canines age, sex, alteration status, as well as the deworming treatments given at the shelter. All methods have been approved by the Institutional Animal Care and Use Committee of Missouri State University (approved protocol #2021-05 in Appendix A).

The fecal samples utilized in this study were provided by Polk County Humane Society in Bolivar, Missouri. Sample collection began in May of 2021 and continued through November of 2021. Shelter staff were asked to collect a total of four samples from each new canine intake over the duration of the study: the first feces passed in the shelter, a sample 7 days after deworming, a sample 14 days after deworming, and finally a sample the day of the canine's adoption. In addition to the fecal samples, shelter staff were asked to submit an intake sheet with the canines age, sex, alteration status, and the deworming treatments given at intake for each canine (appendix B). Shelter staff collected the samples in 2 oz plastic cups, sealed with a lid, and stored at 4^{o} C in a commercial refrigerator, until researchers came to pick the samples up. Every Monday, Wednesday, and Friday during the study period samples were picked up from the shelter and transported to the lab at Missouri State University's Bond Agricultural Learning Center in Springfield, MO in a precooled foam cooler with an ice pack. In the lab each fecal sample was divided into aliquots of 2 g to perform three different parasite examination techniques. The three techniques evaluated include a modified McMasters, a modified

Wisconsin, and a Mini-FLOTAC. Special materials required for the different evaluation methods include McMasters slides (FEC Source, Grand Ronde, OR), and the Mini-FLOTAC reading disk along with Fill-FLOTAC apparatus (University of Naples Federico II, Italy). A total of 6 grams of stool per canine was examined. For consistency, the same dilution ratio was used for each test: 2 g of stool to 18 mL of Sheathers Sugar Solution Jorgensen Labs, Loveland, CO). The solution consists of 454 g of sucrose and 355 mL of water and has a specific gravity of 1.27. Two of the tests, the Modified McMasters and Modified Wisconsin, required the use of a centrifugation step. Samples that required centrifugation were centrifugated at 1500 RPM/88 RCF for five minutes in swinging centrifuge.

Modified McMasters.

To prepare the modified McMasters test, a 2 oz plastic cup is placed on a gram scale sensitive to +/- 0.002 g (METTLER TOLEDO, USA) and the scale is tared or zeroed. Using a tongue depressor, small pieces of feces are gently added to the plastic cup on the scale until a total of two grams (two-thousand milligrams) is obtained. The cup containing the sample is set to the side while 18 mL of Sheathers sugar solution is measured out using a graduated cylinder. The 18 mL of flotation solution is then added to the 2 oz plastic cup that contained 2 g of feces. The flotation solution and the sample are then homogenized using a 3D printed tool with a hollow, lattice lined design to homogenize the solution. After the sample is mixed, the sample is poured through a contrate fecal filter (Jorgensen Labs, Loveland, CO) with a pore size of 0.9 mm that is sitting atop a 15mL glass centrifuge tube. Once the sample has been filtered and transferred into the glass centrifuge tube, the sample is centrifuged at 1500 RPM/8 RCF, for 5 minutes. Following centrifugation, the sample tube is gently removed and placed in a test tube rack and

allowed to sit for 10 minutes undisturbed. Prior to the top of the solution being pipetted off and transferred to both of the McMaster slide chambers for a total of 1.5mLs. After transfer to the slide, the solution then sits for another 5 minutes, before the slide is examined under a microscope using 10x objective lens for a total of 100x magnification. As each lane of the McMasters slide is being scanned, any ova detected with in the grid are identified and counted using a multiple tally counter. The number of ova for each species identified (photo references in appendix C) is then multiplied by 30 to yield an estimate of EPG for each species.

Modified Wisconsin.

Initial preparation steps through centrifugation are shared by both the Modified McMasters and Modified Wisconsin techniques. After centrifugation the sample tube is transferred to a test tube rack, and a few additional drops of Sheathers solution is added to the tube to create a positive meniscus. A coverslip is then placed atop of the meniscus creating a seal with the tube, that then sits for ten minutes undisturbed. After ten minutes, the cover slip is removed from the tube and placed on a microscope slide. The slide is then examined under the microscope using the 10x objective lens for a total of 100x magnification. All ova detected under the coverslip are identified and counted using the multiple tally counter. The total number of ova for each species identified is then multiplied by 0.5.

Mini-FLOTAC.

The mini-FLOTAC examination is performed according to the protocols described by the Parasitology and Parasitic Diseases Department of Veterinary Medicine at the University of Naples Federico II. The test is prepared by placing the fill-FLOTAC container on the milligram

sensitive scale, and the scale is tared to zero. Using a tongue depressor, small pieces of stool are gently added to the container until totaling two grams (two-thousand milligrams). Then 18 mL of Sheathers sugar solution measured in a graduated cylinder is added to the sample. The lid of the fill-FLOTAC apparatus, affixed with the lid to the pipet attachment and the conical pumping mechanism, is then secured atop of the base container. The shaft of the pumping mechanism is then pulled and pushed repeatedly, homogenizing the sample in the container. After mixing thoroughly, a lid is secured to the top of the pumping mechanism, while the lid covering the pipet attachment is removed and the pipette is attached. The sample is then pipetted into the two chambers of the mini-FLOTAC slide for a total of 1 mL/chamber. The reading disk then sits for ten minutes undisturbed prior to translation of the slide 90 degrees and the removal of the small handle on the reading disk. With the aid of a stage adapter, the mini-FLOTAC slide can be evaluated under the 10x objective for a total of 100x magnification. Each grid of the mini-FLOTAC is examined and all ova detected is identified and counted using a multiple tally counter. After counting, the number of eggs for each species is then multiplied by 5 to yield an estimate of EPG for each species identified in the sample.

Procedures

All three methods are prepared simultaneously for a sample. Once sample aliquots have been measured, Sheathers solution has been added, and homogenized as described, the Mini-FLOTAC reading disk is then filled and a time set for 10 minutes. During the ten minutes of flotation time for the Mini-FLOTAC, the samples for the Modified McMasters and Modified Wisconsin are then filtered into their respective test tubes for centrifugation. When the timer signals that the Mini-FLOTAC is ready to be translated and evaluated, the centrifuge will be

started for the other two tests. While the other methods are in the centrifuge the Mini-FLOTAC reading disk is evaluated. Once centrifugation in complete, the test tubes are removed, additional Sheathers solution is added to the tube for the Modified Wisconsin and the cover slip affixed. Both tubes then sit undisturbed for ten minutes. After the ten minutes are up, the sample for the Modified McMasters is gently transferred to the McMaster slide. During the five-minute flotation time for the McMasters test, the Modified Wisconsin is evaluated. The Modified McMasters is evaluated last.

Fecal Egg Count Reduction Test. Each sample submitted by a canine is evaluated by all three methods. Where follow up samples have been provided, fecal egg reduction tests (FERT) have been performed. The equation for the fecal egg reduction test is as follows; (Initial sample EPG – Follow up Sample EPG/ Initial sample EPG) X 100 = % EPG reduced. FECRTs were performed with each FEC method.

Statistics

Samples were blocked by the donor canines age, sex, intake anthelmintic (Table 2). Categories for age include canines less than 6 months old, 6 months to 1 year, and greater than one year of age. These age groups were determined considering parasite transmission patterns (particularly trans-placental and trans-mammary transmission) and the development of adaptive immunity with age. Deworming agents were categorized into groups based on the active ingredient in the product given by the shelter. Samples were additionally categorized into EPG levels based off the results of their FEC. EPG level groups included canines with an EPG of 0, EPG 1-25, EPG 26-50, EPG 51-500, and a group for EPGs greater than 500. When comparing Fecal egg reduction tests, results were categorized into groups based of percent reduction.

Groups for percent reduction include those reduced less than 75%, 75-89%, 90-95% reduced, and those reduced greater the 95% to correspond to Castro and Kaplans, (2020) suggested FECRT interpretations for resistance. In this study, the reduction levels are not indicative of resistance but rather used to interpret the efficacy of the shelter's parasite control protocol.

Table 2. Number of canine intake fecal samples categorized by canine age, sex, and anthelmintic given at shelter intake.

	< 6 months	6 mon- 1 year	> 1 year	Total
Male	11	8	18	37
Female	19	8	21	48
Unknown sex	5	1	0	6
Pyrantel/Praziquantel/Oxantel	8	6	23	37
Levamisole	6	1	0	7
Moxidectin	6	3	5	14
Ivermectin	3	2	2	7
Milbemycin	3	5	7	15
Pyrantel	7	0	1	8
Selemectin and sarolaner	1	0	0	1
No anthelmintic given	1	0	1	2

Data has been analyzed using Minitab 19 (Penn State, State College, PA). A one-way ANOVA was performed to compare the effects of fecal egg counting techniques on FEC per gram of stool and on fecal egg reduction tests. Additionally, Tukey Pairwise Comparisons and Dunnett Multiple Comparisons were made between the FEC methods. Where Dunnett tests required a control treatment group for comparisons, the control was set as the Modified Wisconsin technique as it is widely used in clinical practices and an accepted standard in diagnostic laboratories. A significance level of $\alpha = 0.05$ was used for all statistical analysis.

RESULTS

A total of 91 intake samples, 55 seven-day, and 39 fourteen-day samples were received. All three samples were received for 35 canines, or 38.46% of the canines that submitted an intake sample. Of the 91 initial intake samples, 60 (65.93%) of the canines tested positive for either a nematode, cestode, or protozoa parasite (Table 3). Fifty-seven canines (62.5%) tested positive for either a nematode or cestode parasitic worm, while 18 canines (19.78%) tested positive for Coccidia. A total of 15 dogs (16.48%), tested positive for both a nematode/cestode and Coccidia on their initial intake sample. Many of the infections detected at intake were multi-parasitic infections, or infections harboring more than one parasite at a time. Nineteen canines tested positive for one parasite only, while 34 canines tested positive for two parasites, 5 canines tested positive for three parasites, and 2 canines tested positive for four parasites.

Of the 55 seven day follow up samples submitted; 18 (33%) canines tested positive for parasites, with 13 (72.2%) of the positive follow ups producing ova for the same parasite detected at intake. Six of the 18 individuals (33.3%) that tested positive seven days after deworming were individuals shedding ova of a parasite not detected at intake. All 6 (100%) of the individuals that tested positive for a new parasite seven days after deworming tested positive for Coccidia (Table 4).

A total of 39 fourteen-day follow-up samples were submitted. Fourteen canines (36%) tested positive for parasites fourteen days after deworming. Of the 14 positive samples, 43% of those (6 canines) tested positive for a new infection. All six of the canines that tested positive for a new infection tested positive for coccidia, while one of the canines also tested positive for a *T. vulpis* infection that had not been detected on either of the first two samples.

Table 3. Number of canine fecal samples positive or negative for intestinal parasites at intake, 7 days, and 14 days after shelter administration of anthelmintic.

	Intake Sample	7-day follow up	14-day follow up
Negative	31	37	25
Positive	60	18	14
Total	91	55	39

Table 4. Number of canine fecal samples positive for the same parasite, a new parasite, or both the same and a new parasite 7 and 14 days after shelter administration of anthelmintic.

	Same Parasite	New Parasite	Same and New Parasite
7-day	12	5	1
14-day	8	3	3

When multiple species infections were detected, ova were identified and counted independently from other nematodes/cestodes. In those cases, a canine may have had multiple FEC results; for example, a canine may have an EPG result for both roundworms and hookworms, and a total EPG regardless of the two separate species. Utilizing initial FECs and the 7-day and 14-day post anthelmintic treatment FECs, FECRTs were preformed using both the total EPG for each canine (Table 5) and FECRTs were done using the EPGs for each specific nematode/cestode detected.

Table 5. Fecal egg count reduction test results by percent reduction for 7 and 14 day follow up samples by each FEC method.

Fecal Egg Count Reduction Test	0-75%	75-89%	90-95%	>95%	<0%*	False Negative**	Total***
7-day							
Mini-FLOTAC	0	1	1	33	2	1	38
Modified Wisconsin	1	0	1	34	1	1	38
Modified McMasters	0	0	0	29	0	8	37
14-day							
Mini-FLOTAC	0	0	0	11	2	0	13
Modified Wisconsin	0	0	0	11	1	0	12
Modified McMasters	0	0	1	9	1	1	12

<0%* Indicates an increase in FEC

** FEC method that yielded a false negative on intake.

*** Difference in totals is due to the detection of a new infection on a follow up sample not detected by all methods

A one-way ANOVA was preformed to determine if the mean EPG and mean EPG level yielded by the three methods were statistically different from each other (Table 6). A one-way ANOVA did not detect a difference in mean EPG Level versus flotation method for hookworms, however a difference was detected in the mean EPG versus flotation method for hookworms (p=0.033). A Dunnett Multiple Comparison test determined a difference between the Modified Wisconsin (control) and the Mini-FLOTAC (p=0.030), where the Mini-FLOTAC yielded a higher average EPG for hookworms. The three flotation methods did not yield significantly different mean EPGs for other nematodes: roundworms or whipworms.

When total EPG and EPG level for Nematodes and Cestodes were analyzed, a one-way ANOVA detected differences among the different flotation methods in the total EPG (p=0.002) and total EPG levels (p=0.017). A Tukey Pairwise test determined that the Mini-FLOTAC yielded statistically different mean total EPGs than both the Modified McMasters (p=0.026) and

Modified Wisconsin *(*0.002). The Tukey Pairwise test determined that the Modified McMaster and the Modified Wisconsin Technique were not different in mean total EPGs. The Mini-FLOTAC yielded a higher average total EPG. A Dunnett Comparison determined that the Mini-FLOTAC yielded significantly different mean total EPGs than the Modified Wisconsin control (p=0.001).

Table 6. Comparison of mean eggs per gram detected in canine feces yielded by fecal egg count methods for each group of nematodes using one-way ANOVA with *post hoc* Tukey's.

Nematode	N	Mean EPG	SD	P-value	*Post hoc* Tukey's
Total					
Modified McMasters	75	534.0	1344.0	0.002	B
Modified Wisconsin	75	201.3	382.6		B
Mini-FLOTAC	75	1513.0	3730.0		A
Hookworm					
Modified McMasters	75	173.9	672.3	0.033	C D
Modified Wisconsin	75	77.8	221.7		C
Mini-FLOTAC	75	761.0	2900.0		D
Roundworm					
Modified McMasters	75	132.8	650.9	0.284	E
Modified Wisconsin	75	60.2	194.6		E
Mini-FLOTAC	75	224.1	858.3		E
Whipworm					
Modified McMasters	75	196.0	920.0	0.130	F
Modified Wisconsin	75	62.0	249.9		F
Mini-FLOTAC	75	476.0	1994.0		F

Groups that share a letter in the Tukey's *pos hoc* question are not significantly different from each other

DISCUSSION

As discussed in the literature review, there are a wide array of fecal diagnostic techniques with various specificities, and each lab may utilize their own modifications. The present study aimed to compare three different fecal flotation methods: a Modified McMasters, a Modified Wisconsin, and the Mini-FLOTAC. Statistical analysis of results highlighted a difference in mean EPG for hookworms versus flotation method, as well as a difference in total EPG and EPG level versus flotation method. However, many of the comparisons were not statistically significant. While the higher mean EPG yielded by the Mini-FLOTAC may not always be statistically significant, it may have clinical significance. In a clinical setting or an environment such as an animal shelter, a few false negative tests can inadvertently perpetuate environmental contamination.

Data limitations

One major limitation of this study is the number of data points for the FECRTs, as less than half of the initial intake population submitted all three samples requested. As the nature of shelter canine populations are transient, many follow-up fecal samples were not collected and submitted, limiting the number of samples available for the fecal egg reduction tests. It was difficult for the shelter to obtain and submit all samples on every canine that came in as a new intake during the study period. While no adoption samples were submitted to the study, some canines were adopted out of the shelter even before their 7 or 14 day follow up samples were due to be collected. As no adoption samples were submitted, we did not have the ability to determine what percentage of canines were being adopted with a patent infection. As the shelter

administers deworming treatment the day the animal is taken into the facility, ideally the initial sample would be passed and collected the first day in the shelter, the same day of deworming. However, not all canines passed a sample their first day in the shelter, so some initial samples are one or more days after deworming. Furthermore, many of the follow up samples were not collected within +/- 12 hours of seven or fourteen days after deworming treatment. Rather, some of the follow up samples were collected either a day or more early or late.

Summary

Currently in the literature, there is a lack of uniformity in fecal egg counting protocols as there are numerous testing methodologies and various lab specific modifications applied that can also affect test results. In addition to the lack of uniformity regarding testing technologies, much of the literature is focused on monitoring livestock parasite populations and resistance, rather than parasites and resistance associated with companion animal parasites. Over the last century, the relationship between humans and their pets has evolved tremendously as pets that were once kept strictly outdoors, have been welcomed into houses and bedrooms. The proximity in which humans live with their companion animals calls to attention the need to control and evaluate companion animal parasite populations. Animal shelters are at constant and consistent risk for parasite transmission among their colony population while maintaining higher parasite prevalence rates than pet populations. Animal shelters take in canines of an unknown exposure and treatment history. Canines being brought into the facility may immediately begin to shed parasitic ova in the environment and facilitate the spread of G.I. parasites or may enter the shelter harboring a prepatent infection and begin to contaminate the environment days to weeks after intake. It is not only of an animal welfare concern to keep shelter animals healthy and free of

disease but is also of a public health concern as many of the parasites discussed have zoonotic potential. Even more concerning is the possibility of anthelmintic resistant zoonotic parasite species infecting the public. To proactively control and monitor these concerns, having a reliable and sensitive method for monitoring parasite control programs is essential.

The purpose of this study was to compare the performance of the three following fecal egg counting technologies; Mini-FLOTAC method, a Modified McMaster method, and Modified Wisconsin method. The Mini-FLOTAC produced significantly different averages for total EPG, total EPG level, and hookworm EPG. While other comparisons yielded no statistically significant difference it is worth considering the clinical significance. In addition to being a sensitive FEC method, the Mini-FLOTAC offers convivence as it does not require centrifugation and comes with the Fill-FLOTAC to prepare the sample. While both the Modified McMasters and Modified Wisconsin technique required extra steps for preparation and a centrifuge. As animal shelters are often working with low budgets, a sensitive test that is convenient and requires little equipment is ideal. Implementing a parasite control protocol utilizing a sensitive FEC method to preform FERT on canines entering a shelter would provide a quantitative analysis of the efficacy of the shelter's parasite control program.

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APPENDICES

Appendix A: Research Compliance

Protocol #2021-05 IACUC approved 4/12/2021

Missouri State University

ANIMAL CARE & USE APPLICATION

INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

v. July 2019

All Animal Care & Use Applications should be submitted electronically to IACUC@missouristate.edu.

A. Investigator Information		
Principal Investigator: Lacy Sukovaty	Department: Animal Science	Office Address: Karls Hall 206
Office Phone: 417-836-6491	Emergency Phone: 417-770-1770	Email: hobbs7@missouristate.edu

B. Project Information

Project Title: Prevalence and Treatment Efficacy of Intestinal Parasites in a Rural Shelter Dog Population through FEC and FECRT

Protocol Action:	**Protocol Type:**	**Protocol Class:**
☒ New Proposal	☒ Research	☒ Agricultural
☐ Pilot Study	☐ Teaching	☐ Behavioral
☐ Renewal (due to protocol expiration)		☐ Biomedical
☐ Review for Exemption		☐ Wildlife/Conservation

Is this project externally funded and/or do you anticipate future funding? ☒ **Yes** ☐ **No**
If Yes, what is the name of the Funding Agency and grant number/title?

C. Previous Approved Protocol

For work that is similar to a previously approved protocol, provide the original protocol number and approval date. On the remainder of the forms, indicate changes to the originally approved protocol in bold font.

Original Protocol Number:	Approval Date:
2021-05	04/09/2021

D. Investigator Assurances

1. The information provided herein is accurate and, to the best of my knowledge, conforms to all applicable University, PHS, and USDA policies on the use of animals in research and teaching.
2. I affirm that all procedures involving vertebrate animals will be performed only by personnel trained in the humane care, handling, and use of animals and that all personnel will abide by the recommendations of the University's Occupational Health and Safety Program.
3. I agree not to proceed with any portion of this project until I receive written approval from the Missouri State University Institutional Animal Care and Use Committee.
4. I agree any changes in the procedures contained in this protocol will be promptly forwarded to the IACUC for review and approval prior to performing any revised procedures.
5. I agree to provide proper, current documentation (e.g., licenses, permits and additional approval forms), when applicable, to the Office of Research Compliance throughout the course of this project.
6. I agree to allow inspection of my research facilities by members of the IACUC, including the veterinarian, and to comply promptly if informed of any violations of the Missouri State University's Animal Care and Use Policy.
7. I have taken into consideration the three "Rs" (replacement, reduction, and refinement) for my study and provided adequate justification for the animal model chosen, animal numbers requested, and procedures to reduce pain and distress.
8. I have conducted a literature search to ensure that I am not unnecessarily duplicating previous experiments.

Lacy Sukovaty	2/26/2021
Signature of Principal Investigator	Date

Appendix B: Intake Sheet Submitted by the Shelter with Each Canine's Sample

Shelter ID # _____

Breed _____

Age _____

Date of Fecal Collection_____ Time of Collection _____

Owner Questionaire: Please complete the following questions to the best of your ability for an intestinal parasite study. Place an 'X' in the appropriate answer field

How old is the dog? □ Under 6 months □ 6 months to 1 year □ Over 1 year

Is the dog? □ Male __Intact __Neutered or Spayed __Unknown __Pregnant
□ Female

When was the dog last dewormed?
□ Within the last 14 days
□ Within the last months
□ Less than 6 months ago
□ More than 6 months ago
□ Unknown

If dewormed, please list product used if known. _____

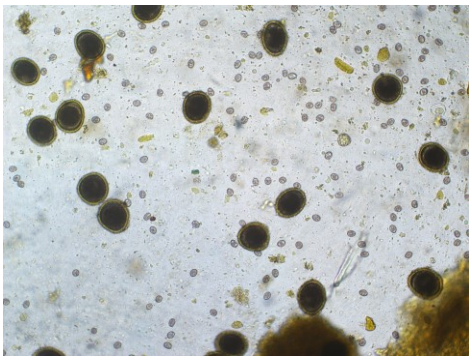
Has the dog received flea/tick preventative? □ Yes, date applied _____ □ No
Name of Product _____

Does the dog visit any of the following □ Dog Parks □ City Parks □ Boarding

How has the dog been housed?
□ Mostly Indoor
□ Indoor with large amounts of outdoor time
□ Completely outdoor
□ Only dog
□ Multi-dog Household, Number dogs in household _____

Appendix C: Reference Images for Ova Identification

Reference Image 1. Roundworm ova and Coccidia Cysts



Reference Image 2. Hookworm ova