Feline Parasitism: Parasite Prevalence and Evaluation of New Immunoassays for *Giardia* and *Cryptosporidium*

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ABSTRACT

Cats are infected with a variety of internal parasites, some of which are zoonotic. Therefore, being able to effectively detect and determine prevalence of internal parasites in cats is important for both feline and human health. Some parasites are easier to detect than others. Diagnosing *Giardia duodenalis* and *Cryptosporidium* spp. can be difficult because cysts and oocysts shed in the feces are small, shed intermittently, and require a trained technician to consistently identify them. As a result, infections with these protozoan parasites can be missed. Fecal immunoassays detect antigens in feces and can have increased sensitivity when compared to traditional microscopic techniques, but still do not detect every infection. The current reference standard is an immunoassay known as the direct immunofluorescent assay, but it requires expensive equipment and a long incubation period. As a result, two prototype lateral flow fecal immunoassays, the *Cryptosporidium EZ VUE* and *Giardia EZ VUE*, designed by TECHLAB® Inc were evaluated for the ability to detect *G. duodenalis* and *Cryptosporidium* spp. infections in cats because they are cheap, easy to use, easy to store and easy to interpret. In addition, samples were examined using a 33% zinc sulfate (ZnSO₄) centrifugal fecal flotation procedure and the MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescent assay (IFA), which served as the reference test. Other internal parasites found on the centrifugal fecal flotation with zinc sulfate were recorded to determine prevalence. Both *EZ VUE* fecal immunoassays demonstrated potential in diagnosing infections in cats when compared to centrifugal fecal flotation and the reference. Additionally, a variety of
other internal parasites were identified. This included several potentially zoonotic species including *Spirometra mansonoides*, *Ancylostoma sp.* and *Toxocara cati*, which was also the most commonly identified species of parasite. Additionally, it was determined that several factors may contribute to higher prevalence of parasites especially in cats with the status of stray or feral.
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GENERAL AUDIENCE ABSTRACT

Internal parasites affect both human and animals. Some parasites do not cause any clinical signs or have a lasting effect, while others can cause damage or contribute to the death of an organism. Certain parasites are zoonotic, meaning they can be transmitted between humans and animals. Several of these zoonotic parasites are found in companion animals, such as cats. This is especially important because cats are a common household pet. How owned animals are treated, especially pets like dogs and cats, has changed over the past few decades. In many of these households they are considered family members. Additionally, there is a large population of free roaming cats, both stray and feral, that have the potential to interact with humans or their pets. Therefore, it is important to be able to effectively diagnose and determine the prevalence of parasites for both feline and human health. Some parasites are harder to diagnose than others due to varying factors and more diagnostic tests are needed to effectively and efficiently detect them. Two of these parasites that can be challenging to detect are *Giardia duodenalis* and *Cryptosporidium* spp. This thesis was aimed at evaluating two prototype diagnostic tests, originally designed for use in humans, for ability to detect these parasites and their potential as diagnostic tests in cats. In addition, this thesis determined the prevalence of other internal parasites found in a large group of cats in Virginia. The obtained results indicated the prototype diagnostic tests had potential. A variety of internal parasites were
also present in Virginia cats, some of which were zoonotic, and showed how important routine veterinary care for maintaining feline and human health
DEDICATION

This thesis is dedicated to friends and family. I would not be here if wasn’t for all their love, support, and guidance.
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CHAPTER 1: Literature Review

SECTION I: GIARDIA DUODENALIS

INTRODUCTION AND IMPORTANCE

*Giardia* spp. are flagellated protozoan enteric parasites that infect a variety of hosts (mammals, amphibians, birds). Currently, the genus *Giardia*, has six recognized species: *G. agilis* in amphibians, *G. muris* in rodents, *G. ardeae* and *G. psittaci* in birds, and *G. microti* in muskrats and voles. The other recognized species is *G. duodenalis*, which is found in humans and many other mammals including companion animals and livestock (Feng and Xiao, 2011). Many infected animals experience limited or no signs at all. Clinical manifestations of giardiasis vary significantly. Signs can include vomiting, weight loss, lethargy, dehydration, and steatorrhea (Adam, 2001; Eckmann, 2003).

Infection with *G. duodenalis* in animals is important for several reasons. First, it affects overall health in a variety of species including companion animals (cats and dogs), livestock, wildlife, and captive animal species. Second, in addition to the health and well-being of the animals, infections in animals could also have an impact on human health because *G. duodenalis* is believed to have zoonotic potential (Sprong et al., 2009). Finally, infections with *G. duodenalis* can also have a financial impact. This can be something on a smaller scale like the cost of treatment at veterinary clinics or on a larger scale, such as the loss of product and decreased income for businesses. For example, Italian investigators determined that an outbreak of giardiasis in lambs caused severe weight loss and mortality, and was linked to economic losses in that geographical area (Aloisio et al., 2006).
ASSEMBLAGES AND ZOONOTIC POTENTIAL IN CATS

*Giardia duodenalis* is considered a species complex, which is a group of closely related individuals that have minimal morphological variation (Andrews et al., 1989; Monis et al., 2003). Within the *G. duodenalis* species complex there are at least eight distinct genetic groups or assemblages, which are labeled A through H (Lasek-Nesselquist et al., 2010; Monis et al., 2003). These eight different assemblages are based on protein or DNA polymorphisms (Cacciò and Ryan, 2008; Monis et al., 2003). In addition, some assemblages have further genetic variation and are broken down into sub-assemblages (AI, AII, AIV, B-I, etc.) (Monis et al., 2003).

The assemblages that can be broken down into sub-assemblages are A and B. Within assemblage A and B there are four sub-assemblages (AI, AII, AIII, AIV, BI, BII, BIII, and BIV). For sub-assemblages of A, humans are infected with AI and AII and animals are infected with AI, AIII and AIV (Monis et al., 2003). Within assemblage B humans are infected with subtypes BIII and BIV and animals are infected with BI and BII (Monis et al., 2003). However, these sub-assemblages within B are not considered true sub-assemblages because of the discrepancies in the literature regarding the validity of these subtypes (Feng and Xiao, 2011).

For *Giardia duodenalis*, assemblages A and B have a broad host range and assemblages C-H generally have a narrower host range and are host adapted (Ballweber et al., 2010; Feng and Xiao, 2011). Assemblages A and B are predominately found in humans (Mayrhofer et al., 1995), but have also been found in nonhuman primates,
livestock, companion animals and some species of wild animals (Appelbee et al., 2005; Cacciò and Ryan, 2008; Farzan et al., 2011; Feng and Xiao, 2011; Geurden et al., 2012; McDowall et al., 2011; Ye et al., 2012). Assemblages C and D have been found predominantly in dogs and other canids (Palmer et al, 2008; Sulaiman et al., 2003). Assemblage E primarily in hoofed livestock (Fayer et al., 2012; Muhid et al., 2007; Thompson et al., 2008), assemblage F primarily in cats (Ballweber et al., 2010; Fayer et al., 2006; Oates et al., 2012), assemblage G in rodents (Feng and Xiao, 2011) and assemblage H is marine mammals (Lasek-Nesselquist et al., 2010).

Certain assemblages of *G. duodenalis* are considered potentially zoonotic. Zoonotic transmission of *G. duodenalis* is not well defined and no published studies currently exist that without a doubt prove it is zoonotic (Feng and Xiao, 2011; Ryan and Cacciò, 2013). Since assemblages A and B have a broad host range and have been found in both humans and a variety of animals it is these assemblages and the overlap humans and animals have within them that have researchers thinking *Giardia duodenalis* could potentially be zoonotic (Feng and Xiao, 2011; Sprong et al., 2009).

Cats are mostly infected with assemblage F and the potentially zoonotic assemblages A and B (Cacciò and Ryan, 2008; Oates et al., 2012; Sprong et al, 2009). However, assemblages C, D, and E have been reported rarely (Jaros et al., 2011; Read et al., 2004; Scorza et al., 2012). For assemblage A, cats have been reported to have sub-assemblages A-1, A-III, and A-IV (Monis et al., 2003; Ponce-Macotela et al., 2002; Vasilopulos et al., 2007). Assemblage B has been identified in cats too, but no sub-assemblages were stated (Oates et al., 2012; Vasilopulos et al., 2007). As a result, cats do
share the sub-assemblage A-1 with humans and assemblage B, which could indicate the potential of zoonotic transmission, but no concrete evidence currently exists.

**LIFE CYCLE**

*G. duodenalis* has a direct life cycle and involves two stages: the trophozoite and the cyst. The trophozoite is the replicative stage and the cyst is the infective stage. Trophozoites are tear drop shaped and 12-17µm x 7-10µm (Zajac and Conboy, 2012). They have a broad anterior, tapering posterior and are bilaterally symmetrical. Each trophozoite has a ventral sucking disk, two nuclei, two median bodies, four pairs of flagella (anterior, caudal, posterior and ventral) (Brugerolle and Lee, 2000). They are rarely shed in the feces and, if passed, do not last long in the environment. Cysts are oval shaped and 9-13µm x 7-9µm (Zajac and Conboy, 2012). They are shed intermittently, immediately infective, and can last for extended periods of time in the environment (Alum et al., 2014; Thompson et al., 2008).

Once the cyst is ingested it passes through the stomach’s acidic environment and enters the small intestine where excystation occurs in response to the change in pH (Adam, 2001; Gardner and Hill, 2001). Each cyst produces two trophozoites, which adhere to enterocytes by the ventral sucking disk and reproduce by binary fission (Adam, 2001; Gallego et al., 2007). Trophozoites are triggered to encyst when the surrounding internal environment of the small intestine changes (DuBois et al., 2008; Midlej and Benchimol, 2009) due to variation in water reabsorption, or chemical, pH or enzyme levels that may occur as *Giardia* travels through the intestinal tract, but this process is not fully understood (Lujan et al., 1998; Midlej and Benchimol, 2009). Once triggered, each trophozoite will then form a cyst that is passed in the feces (Gardner and Hill, 2001).
Animals can become infected by ingesting the cyst through contaminated food, water, or grooming (Payne and Artzer, 2009). Dogs and cats housed or visiting crowded and unsanitary areas tend to have higher incidence of infection with *Giardia*. These can include places like dog parks, kennels, catteries, and animal shelters (Meireless et al., 2008; Mundim et al., 2007; Polak et al., 2014). Infections under these circumstances are more likely because of the increased chance of animals coming into contact with infective cysts before they can be removed. The cysts can also remain on objects that can act as fomites such as cages, toys, food and water bowls (Payne and Artzer, 2009).

The risk of ingesting cysts from the environment as described previously is possible because *Giardia* cysts are environmentally stable and can survive weeks to months in the environment depending on a number of factors that include but are not limited to temperature and type of environment where the cysts reside (Alum et al., 2014; Bingham et al., 1979; DeRegnier et al., 1989). As a result, environmental contamination can lead to infection long after the initial entry of cysts into the environment.

Cysts can last for months in water (Alum et al., 2014). Interestingly the type of water the cysts reside in may play a role in their viability in the environment (DeRegnier et al., 1989). DeRegnier et al. (1989) conducted a study on the viability of cysts in river, lake and tap water. Cysts lost viability in tap water in as little as three days, which differed greatly from cyst viability in river and lake water, where they remained viable up to three months in some cases. The authors credit this to the fact that the river and later water were untreated while chloramine was present in the tap water. The authors could not confirm this was the result of residual chloramine in the tap water, but the pattern is consist with results from another study in which a chemical used in treating water,
chlorine, was associated with decreased cyst viability in a short period of time when compared to cysts in water that did not contain chlorine (Jarroll et al., 1981).

Another major factor that influences cyst viability is temperature. Cysts remain viable in colder temperatures. However, cysts that are frozen and then thawed do not remain viable. Boiling of cysts also results in a loss of viability (Alum et al., 2014; Bingham et al., 1979). Other factors that promote cyst viability besides the presence of water and cold temperature are increased presence of organic material and decreased surface porosity (Alum et al., 2014).

**DIAGNOSIS IN VETERINARY MEDICINE**

Infections with *Giardia* can be diagnosed through several methods: microscopy, fecal immunoassays, and polymerase chain reaction (PCR). Microscopy is the traditional method for *Giardia* detection and requires observation of the cyst or trophozoites. Trophozoites are best identified through direct smears, while centrifugal fecal flotation with 33% zinc sulfate (ZnSO₄) is the best way to recover and identify cysts (Payne et al., 2002; Zajac et al., 2002). However, there are several limitations when using microscopy to identify *G. duodenalis* that account for why the use of microscopy alone tends to underestimate the number of infected patients. The first limitation is that *G. duodenalis* sheds intermittently (Bowman, 2014; Leib and Zajac, 1999). As a result, even though a sample tested negative, the patient could actually be *G. duodenalis* positive. Another limitation of microscopy is that samples need to be fresh. For direct smears, if the sample is not fresh the trophozoites will become less distinguishable due to the loss in their motility. Cysts also degrade in the feces over time making an accurate identification more difficult. However, not identifying the cyst or trophozoites may also be due to
human error. Identifying *Giardia* requires a skilled individual who has been trained to identify the cysts or trophozoites.

Fecal immunoassays have begun to replace or be performed in addition to microscopic examination of feces. This is because fecal immunoassays detect soluble *Giardia* cyst antigens in the feces. The use of fecal immunoassay has allowed for increased test sensitivity and specificity when compared to microscopic examination (Dryden et al., 2006; Mekaru et al., 2007; Olson et al., 2010). However, even though they tend to increase sensitivity and specificity they do not detect every infection. Samples that are positive on fecal flotation can be negative on the fecal immunoassays and vice versa (Payne et al., 2002). In addition, these tests are often species specific and do not have the same results in all animals. For example, the IDEXX SNAP *Giardia* Antigen Test kit for canine and feline feces was found to have 0% sensitivity for *Giardia* in sheep (Wilson and Hankenson, 2010).

There is one fecal immunoassay known as direct immunofluorescent assay (IFA) that was developed for *Giardia* and *Cryptosporidium* spp. detection in humans. This immunoassay detects *Giardia* by using fluorescein isothyocyanate (FITC) labeled monoclonal antibodies against a cell wall antigen in *Giardia* cysts. When present, cysts are bright green when viewed under a fluorescent microscope (Garcia et al., 1987; Garcia et al., 1997). IFA is often used as the reference test in companion animals (Hoopes et al., 2013; Mekaru et al., 2007; Rishniw et al., 2010) because of high sensitivity and specificity in humans (Garcia et al., 1992; Garcia et al., 1997; Johnston et al., 2003), although there is no perfect test (gold standard) in diagnosing *Giardia* infections (Hoopes et al., 2013; Mekaru et al., 2007; Rishniw et al., 2010). However, this test requires long
incubation times and is expensive, which limits its use and is usually used in research studies rather than veterinary clinics.

PCR is also being used to detect *G. duodenalis* through selecting and amplifying a particular gene or genetic marker (Ryan and Cacciò, 2013). PCR improves sensitivity and specificity over microscopy and some believe that is more sensitive than some fecal immunoassays for *G. duodenalis* (Bouzid et al., 2015; McGlade et al., 2003; Tupler et al., 2012). However, like the other diagnostic tests PCR is not perfect. Just like with fecal immunoassays, PCR does not always detect every infection and some samples that are cyst positive do test negative on PCR and vice versa (Tangtrongsup and Scorza, 2010).

**PREVALENCE AND DISTRIBUTION IN CATS**

Reported *G. duodenalis* prevalence varies greatly in the literature. For cats, reported overall prevalence ranges between less than 1% and 80% (De Santis-Kerr et al., 2006; Jaros et al., 2011; McGlade et al., 2003; Papini et al., 2007; Queen et al., 2012). This large variation is tied to a variety of factors including diagnostic technique used, age, lifestyle, and geographical region in which the cats were sampled (Ballweber et al., 2010; Pallant et al., 2015; Papini et al., 2007; Rishniw et al., 2010). For example, McGlade et al. (2003) reported a prevalence of 80%, but PCR was used as the diagnostic method. Palmer et al. (2008) only reported a prevalence of 2% out of 1063 screened samples when microscopy was used. It should be noted that zinc sulfate solution was not used and may have contributed to an even a lower prevalence for *G. duodenalis* because centrifugal fecal flotation with 33% zinc sulfate is the recommended procedure for recovering cysts (Zajac et al., 2002). The age of a cat can also play a role in the
prevalence and increased risk for cats <1 year old is commonly reported (Gates and Nolan, 2009; Itoh et al., 2006; Papini et al., 2007). Cats that have lifestyles involving more time outdoors tend to have higher prevalence than indoor cats (Itoh et al., 2006). However, in some instances cats that spend more time indoors have a higher prevalence (Papini et al., 2007), which is usually associated with multi-cat situations or cats kept in close quarters, like catteries or shelters (Gookin et al, 2004; Polak et al., 2014). For example, Polak et al. (2014) found that *G. duodenalis* in cats from hoarding situations had a prevalence of 56%.

**TREATMENT AND CONTROL**

Cats infected with *Giardia duodenalis* that are asymptomatic usually do not need treatment and many clinical infections are self-limiting. For cats that require treatment there are several drugs used in the treatment of *Giardia duodenalis*. These are metronidazole (Kirkpatrick and Farrell, 1984; Scorza and Lappin, 2004) and a febantel-pyrantel-praziquantel combination (Scorza et al., 2006). In addition to treatment with these drugs bathing is also recommended. Fecal material with the infective cysts can remain on the fur and can cause reinfection when cats groom themselves (Payne and Artzer, 2009). To prevent reinfection or transmission to other cats disinfection of the environment is also advised (Payne and Artzer, 2009; Tysnes et al., 2016).

**SECTION II: CRYPTOSPORIDIUM SPP.**

**INTRODUCTION AND LIFE CYCLE**

*Cryptosporidium* spp. are enteric coccidian parasites and can infect a variety of hosts, including humans, cats, dogs, and livestock (McLauchlin et al., 2000; Pieniazek et
al., 1999; Santin et al., 2006; Xiao and Feng, 2008). In cats, most infections with 
_Cryptosporidium_ spp. are subclinical (Scorza and Tangtrongsup, 2010; Thompson et al., 2008). Clinical signs of the infection include watery diarrhea, anorexia, and weight loss. (Scorza and Tangtrongsup, 2010). The life cycle for _Cryptosporidium_ is direct. In cats, infection occurs via ingestion of the oocyst via contaminated food, water, ingestion of prey, or grooming (Fayer et al., 2000; Scorza and Tangtrongsup, 2010). Once ingested the oocysts undergo excystation and release four sporozoites that invade epithelial cells of the gastrointestinal tract. Within these cells they undergo asexual reproduction (schizogony or merogony). This is then followed by sexual reproduction and the production of macrogamonts and microgamonts that fertilize the macrogamont to form a zygote (Scorza and Tangtrongsup, 2010; Thompson et al., 2008). The zygote then forms thin-walled and thick-walled oocysts with four sporozoites. The thin-walled oocysts usually break open and release the sporozoites in the intestine, causing autoinfection (Fayer et al., 2000). The thick-walled oocysts are passed in the feces and are immediately infective once in the environment (Thompson et al., 2008). Oocysts in the environment are hardy and can last for months in cool, damp environments (Fayer et al., 2000; Thompson et al., 2008). Oocysts will lose viability when frozen, (Fayer and Nerad, 1996; Robertson et al., 1992) at higher temperatures (Harp et al., 1996; Fayer, 1994), and if allowed to dry out (Anderson et al., 1982; Robertson et al., 1992). This is probably why _Cryptosporidium_ spp. last so long in water and why water is such an importance source of contamination (Fayer et al., 2000).

**Zoonotic Potential in Cats**
Cats have been reported to be infected mostly with the species specific Cryptosporidium felis (Bowman and Lucio-Forster, 2010; Santin et al., 2006; Xiao and Fayer, 2008), but C. parvum and C. muris have also been reported (Pavlasek and Ryan, 2007; Santin et al., 2006). Humans are infected with a variety of species (McLauchlin et al., 2000; Pieniazek et al., 1999; Xiao et al., 2001) but are most commonly infected with C. parvum and C. hominis (Xiao and Fayer, 2004; Xiao and Feng, 2008). One of the other species reported is C. felis (Xiao and Feng, 2008). Since there is overlap between humans and cats for the species C. parvum and C. felis it is plausible that there could be some zoonotic potential. Studies document the transmission of Cryptosporidium spp. from infected cattle to people, which is usually attributed to C. parvum (Anderson et al., 1982; Levine et al., 1988). Since there is transmission between humans and cattle involving C. parvum it is possible that this could happen with other animals, such as cats. However, as mentioned before C. parvum is not commonly reported in cats and C. felis is not commonly reported in humans. The zoonotic potential of Cryptosporidium spp. in cats is still being investigated, but it appears that the risk of transmission is minimal (Thompson et al., 2008; Xiao and Fayer, 2008; Xiao and Feng; 2008).

**DIAGNOSIS IN VETERINARY MEDICINE**

Infections with Cryptosporidium spp. can be diagnosed through several methods: microscopy, fecal immunoassays, and polymerase chain reaction (PCR). Cryptosporidium spp. can be detected through microscopic examination with different staining methods. Stains that have been used for the detection of Cryptosporidium spp. include Kinyoun, Ziehl-Neelsen, the modified Ziehl-Neelsen and DMSO- carbol fuchsin (Scorza et al., 2012; Thompson et al., 2008; Weber et al., 1991). However, the staining
techniques’ sensitivity and specificity are problematic and results can vary between laboratories (Elliot et al., 1999). Additionally, some staining techniques are not as effective in animal feces (Marks et al., 2004; Weber et al., 1991). A study published by Marks et al. (2004) showed that the modified Ziehl-Neelsen stain only detects about 70% of Cryptosporidium spp. infected cats. Additionally, detection using microscopy can be difficult when oocysts are shed in low numbers and because Cryptosporidium spp. shed intermittently (Thompson et al., 2008).

Fecal immunoassays for identification of Cryptosporidium spp. also allow for increased sensitivity, but can be inconsistent (Marks et al., 2004; Mekaru et al., 2007). One type of fecal immunoassay, known as a direct immunofluorescent assay (IFA), detects Cryptosporidium spp. (and Giardia duodenalis) by using fluorescein isothiocyanate (FITC) labeled monoclonal antibodies against cell wall antigen in oocysts (Garcia et al., 1987; Garcia et al., 1997). Even though it was developed for use in humans, IFA is often used in the diagnosis of companion animals because of high sensitivity and specificity in humans (Garcia et al., 1992; Garcia et al., 1997; Johnston et al., 2003). While there is no perfect test (gold standard) in diagnosing Cryptosporidium spp. infections, IFA has been adopted as the reference standard in diagnosing companion animal infections (Mekaru et al., 2007; Rishniw et al., 2010). However, while IFA is commonly used as the reference test for both Cryptosporidium spp. and Giardia duodenalis in animals, some studies suggest that IFA might not be as effective as a reference standard as it is for Giardia duodenalis in companion animals (Marks et al., 2004; Mekaru et al., 2007). This could be due to the fact the different species of Cryptosporidium spp. infect different hosts and companion animals (dogs and cats) are
more frequently infected with specific Cryptosporidium (Bowman and Lucio-Foster, 2010; Thompson et al., 2008).

PCR can also be used to detect Cryptosporidium spp. by amplifying select DNA from the feces and some studied have reported that is more sensitive than IFA (Scorza et al., 2003; Scorza and Lappin, 2005). PCR is more time consuming and expensive than the other diagnostic techniques. However, one major benefit to using PCR is that the amplification products can be sequenced to determine what species the animal was infected with.

**PREVALENCE AND DISTRIBUTION IN CATS**

The prevalence of Cryptosporidium spp. found in cats ranges from 2.2 % to 12% because of a variety of factors (Ballweber et al., 2009; Fontanarrosa et al., 2006; Hill et al., 2001; Scorza and Lappin, 2005; Spain et al., 2001). Differences in prevalence might be due to diagnostic technique used. The higher reported prevalence of 12% might be due to the fact that Ballweber et al. (2009) added a step to concentrate oocysts before running IFA. The other studies with lower prevalence only used the standard centrifugal fecal flotation procedure (Lucio-Forster and Bowman, 2011; Spain et al., 2001). Cats that are younger have an increased risk for infection with Cryptosporidium spp (Thompson et al., 2008). Additionally younger cats and cats with immunosuppressive conditions have been reported to have more symptomatic infections (diarrhea) (Mtambo et al., 1991; Thompson et al., 2008).

**SECTION III: OTHER INTERNAL PARASITES OF CATS**

**PROTOZOA**
In addition to *Cryptosporidium* spp. and *G. duodenalis* cats can become infected with several other protozoan parasites that can be detected using fecal flotation including *Cystoisospora felis, Cystoisospora rivolta, and Toxoplasma gondii* (Zajac and Conboy, 2012). *Cystoisospora felis* and *Cystoisospora rivolta* are commonly seen on fecal flotation (Gates and Nolan, 2009; Lucio-Forster and Bowman, 2011). *However, T. gondii* is not commonly seen on fecal flotations and fecal prevalence is usually around 1% (Elmore et al., 2010). This is due to the fact that cats only shed oocysts for a short period of time when they are first infected (Dubey, 2010; Elmore et al., 2010). *Toxoplasma gondii* is an important parasite in cats because it is zoonotic. Symptoms of toxoplasmosis in humans range from mild flu like symptoms too more serious symptoms that include seizures and vision impairment. The severe symptoms more commonly appear in immunocompromised people such as children or people with AIDS (Dubey, 2010).

**NEMATODES**

Cats can be infected with a variety of nematodes that can be detected using fecal flotation including *Ancylostoma* spp., *Toxocara cati, Toxascaris leonina, Eucoleus areophilus, Aonchtheca putorii,* and *Aelurostrongylus abstrusus.* *Ancylostoma* spp. are hookworms and cats can be infected with either *Ancylostoma brasiliense, Ancylostoma tubaformae,* or *Ancylostoma ceylanicum* depending on location (Zajac and Conboy, 2012). While *Ancylostoma brasiliense* and *Ancylostoma ceylanicum* are zoonotic, only *Ancylostoma brasiliense* and *Ancylostoma tubaformae* are found in North America and can cause self-limiting cutaneous larva migrans (CLM) in humans in which infective filariform larvae burrow into the skin through contact with infected soil and migrate, causing red and itchy
tracks to appear on the skin (Bowman et al., 2010). *Toxocara cati* and *Toxascaris leonina* are ascarid nematodes. *Toxocara cati* is frequently identified with high prevalence and is one of the most common parasites identified in cats (De Santis-Kerr et al., 2006; Lucio-Forster and Bowman, 2011; Spain et al., 2001), while *Toxascaris leonina* is not as common (Zajac and Conboy, 2012). *Toxocara cati* is also zoonotic and migrating larvae cause ocular larval migrans (OLM) and visceral larva migrans (VLM) (Fisher, 2003; Woodhall et al., 2014). *Eucoleus areophilus*, and *Aelurostrongylus abstrusus* are lungworms. Prevelance ranges from 0.1% to 6.2% for *A. abstrusus* when fecal flotation was used (Lillis, 1967; Lucio-Forster and Bowman, 2011; Rembiesa and Richardson, 2003). However, while *A. abstrusus* can be identified on fecal flotation, it should be noted that the Baermann technique is the preferred method for diagnosis (Zajac and Conboy, 2012).

**CESTODES**

Cats can be infected with a variety of cestodes that can be detected using fecal flotation including *Dipylidium caninum*, *Taenia taeniaeformis*, and *Spirometra* spp. While eggs or egg packets may be seen on fecal flotations it is not the best method for detecting these parasites.

**TREMATODES**

*Alaria* spp., and *Paragonimus kellicotti* are trematodes that can infect cats. However, *Alaria* spp. and *Paragonimus kellicotti* species are not commonly seen when fecal flotation is used (Lucio-Forster and Bowman, 2011).
REFERENCES


CHAPTER 2: Comparison of Diagnostic Tests for the Detection of *Giardia duodenalis* and *Cryptosporidium* spp. in Naturally Occurring Feline Infections

ABSTRACT

*Giardia duodenalis* and *Cryptosporidium* spp. are enteric protozoan parasites that can cause diarrhea in cats. Diagnosis of these parasites can be difficult and currently there is not a prefect diagnostic method available for either. Two prototype lateral flow assays, the *Cryptosporidium EZ VUE* (TECHLAB® Inc) and *Giardia EZ VUE* (TECHLAB® Inc), were evaluated for their ability to detect *G. duodenalis* and *Cryptosporidium* spp. in naturally occurring feline infection in Virginia. In addition, samples were examined using a 33 % zinc sulfate (ZnSO₄) centrifugal fecal flotation procedure and the MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescent assay (IFA), which served as the reference test. Fecal samples from 725 cats were evaluated for *G. duodenalis* and *Cryptosporidium* spp. from July 2016 to March 2017. For *G. duodenalis*, the sensitivity and specificity of centrifugal fecal flotation were 57.1% and 100% when compared to IFA, while the *Giardia EZ VUE* assay had a sensitivity and specificity of 78.1 % and 99.5% when compared to IFA. Statistical analysis detected a significant difference between the sensitivity of these two tests, but there was no difference in specificity. For the *Cryptosporidium EZ VUE* the sensitivity and specificity when compared to IFA were 100% and 98.1%. The results of this study demonstrate that the use of centrifugal fecal flotation can underestimate *G. duodenalis* infection and that the *Giardia EZ VUE* and *Cryptosporidium EZ VUE* assays have potential in feline diagnostics and should be further investigated.
INTRODUCTION

Giardia duodenalis and Cryptosporidium spp. are enteric protozoan parasites. Giardia duodenalis is a species complex and has eight recognized assemblages (A-H) (Cacciò and Ryan, 2008) and infects a wide range of hosts, including cats (Ballweber et al., 2010; Feng and Xiao, 2011; Sprong et al., 2009). The Cryptosporidium species infecting cats is usually C. felis (Bowman and Lucio-Forster, 2010; Thompson et al., 2008), but C. muris and C. parvum have also been reported (Pavlasek and Ryan, 2007; Santin et al., 2006). Infections with G. duodenalis are often subclinical, but clinical infections usually present as acute or chronic fatty diarrhea. Other signs can include vomiting, lethargy, and weight loss (Adam, 2001). Cats become infected with G. duodenalis when they ingest the infective cysts that are shed in feces. The cysts are durable and can last for months in cool moist conditions in the environment (Alum et al., 2014; Bingham et al., 1979). Infective cysts can be ingested though contaminated food, water, or grooming (Payne and Artzer, 2009). Infections with Cryptosporidium spp. in cats are also usually subclinical and have a similar set of clinical sign that include diarrhea, lethargy, and weight loss. However, diarrhea associated with Cryptosporidium spp. is watery, not fatty (Scorza and Tangtrongsup, 2010; Xiao et al., 2004). In addition, similarly to G. duodenalis, cats become infected with Cryptosporidium spp. via the fecal oral route (Thompson et al., 2008). Infective oocysts shed in the feces can survive extreme environmental conditions (Alum et al., 2014), and can be ingested by the feline host in the same ways as G. duodenalis cysts (Thompson et al., 2008).

Prevalence estimates of G. duodenalis for cats vary depending on the population studied and the diagnostic techniques used for fecal evaluation. Published prevalence
commonly range between 1% and 15% (De Santis-Kerr et al., 2006; Jaros et al., 2011; Lorenzini et al., 2007; Queen et al., 2012), but a prevalence as high as 80% has been reported when polymerase chain reaction (PCR) was used as the diagnostic method (McGlade et al., 2003). The prevalence of *Cryptosporidium* spp. found in cats ranges from 2.2% to 12% for diagnostic techniques that use fecal evaluation (Ballweber et al., 2009; Fontanarrosa et al., 2006; Hill et al., 2001; Shukla et al., 2006).

Fecal analysis with microscopy, immunoassay and polymerase chain reaction (PCR) can be used to detect infections with both and *G. duodenalis* and *Cryptosporidium* species. Microscopy tends to underestimate the prevalence of *G. duodenalis* because it depends on the visualization of the cyst (Dryden et al., 2006). Dependence on visualizing the cyst is problematic because the cysts are shed intermittently, are small, and require a trained individual to consistently identify them (Bowman, 2014; Leib and Zajac, 1999). Commercial fecal immunoassays do not rely on visualization of the *G. duodenalis* cyst. Instead, they detect *G. duodenalis* cyst wall antigen, and this allows for increased test sensitivity when compared to microscopic examination (Dryden et al., 2006; Olson et al., 2010). However, immunoassays are not perfect and can miss samples that are flotation positive (Payne et al., 2002). Similarly, *Cryptosporidium* oocysts are small, shed intermittently and can be shed in low numbers making identification difficult when using traditional microscopic techniques (Scorza and Tangtrongsup, 2010; Thompson et al., 2008). Fecal immunoassays for identification of *Cryptosporidium* spp. also allow for increased sensitivity, but also may not always agree with the reference standard (Mekaru et al., 2007; Rishniw et al., 2010). PCR is also being used a diagnostic method and detects *G. duodenalis* and *Cryptosporidium* spp. infections through selecting and
amplifying a particular gene or genetic marker (Ryan and Cacciò, 2013; Scorza and Lappin, 2005).

Even though there are a variety of tests for the diagnosis of *G. duodenalis* and *Cryptosporidium* spp., there is no perfect diagnostic test available for detection of these parasites. Currently, the direct immunofluorescence assay (IFA) is used as the reference standard in many studies (Marks et al., 2004; Mekaru et al., 2007; Rishniw et al., 2010). The IFA detects *G. duodenalis* cysts and *Cryptosporidium* oocysts through antibodies generated against soluble cyst and oocyst wall antigens. The antibodies are tagged with fluorescent markers, which allow for visualization under a fluorescent microscope (Garcia et al., 1987; Garcia et al., 1992). While the IFA is considered the best test available, it has its own set of drawbacks. This assay requires more time, training, and equipment than are available in most veterinary clinics and animal shelters. As a result, the need for more sensitive and specific *G. duodenalis* and *Cryptosporidium* spp. diagnostic tests still exists.

TECHLAB® Inc developed two prototype lateral flow fecal immunoassays, the *Giardia EZ VUE* assay and *Cryptosporidium EZ VUE* assay, for the detection of *G. duodenalis* and *Cryptosporidium* spp. in humans as inexpensive alternatives for use outside the United States. These fecal immunoassays are user friendly, easy to store, are inexpensive, do not require extensive training or the use of expensive or specialized equipment. This makes them ideal not only for use in humans in parts of the world with limited resources, but also indicate potential value for veterinary clinics, animal shelters, and field veterinarians and researchers. Therefore, the purpose of this study was to evaluate the ability of the *Giardia EZ VUE* immunoassay to detect *G. duodenalis*
compared to centrifugal fecal flotation with zinc sulfate (ZnSO4) and the MERIFLUOR® Cryptosporidium/Giardia direct immunofluorescence assay (IFA) in cats with naturally occurring infections. In addition, the Cryptosporidium EZ VUE immunoassay’s ability to detect Cryptosporidium spp. was evaluated and compared to IFA. It was expected that for G. duodenalis the Giardia EZ VUE immunoassay would have a sensitivity higher for than microscopy, but lower than the direct immunofluorescence assay. For the Cryptosporidium EZ VUE immunoassay it was expected that it would not be as sensitive as IFA in the detection of Cryptosporidium spp.

MATERIAL AND METHODS

Collection Sites

Fecal samples were collected from cats in four different locations in Virginia from July 2016 through March 2017. These locations were the Prince William County Animal Shelter (Manassas, VA), Operation Catnip (Richmond, VA), Stafford County Animal Shelter (Stafford, VA), and the Fauquier SPCA Animal Shelter (Midland, VA). In addition, one sample came from the Veterinary Medical Teaching Hospital of the Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA (VMTH-VMCVM).

Sample Collection

Animal Shelters (Prince William County, Stafford County and Fauquier SPCA)

Fecal samples were collected from litter pans during routine morning cleaning. Collection was completed during cleaning to avoid additional stress for the cats. Feces was collected within 24 hours of excretion. Each fecal sample was put in a plastic re-sealable bag, placed in a cooler with icepacks for transport, and then refrigerated until
processed. Shelter identification numbers for all animals present in the cage were recorded on the bag. If defecation by a specific cat was observed in a cage with more than one cat that fecal sample was bagged separately and only that animal’s identification number was recorded on the bag.

**Operation Catnip**

Fecal samples from this Trap-Neuter-Return program were collected for another project on *Toxoplasma gondii* in cats. (VT IACUC# 16-106). Fecal samples from this location were collected from the traps or by digital rectal palpation when cats were anesthetized for neutering/spaying. The feces was placed in a re-sealable plastic bag and labeled with an identification number. Samples were then placed in a cooler with ice for transport and refrigerated until processed.

**Sample Duplication**

To the investigator’s knowledge, there was no duplication of samples from individual cats in this study. For this study feces from an individual cat was only tested once and if another fecal sample was collected it was discarded. Since some cats remained in shelters for months before adoption, they might have had their feces collected multiple times. A list of identification numbers was maintained and updated to avoid sample duplication.

**Sample Screening**

Each sample was evaluated with centrifugal fecal flotation with 33% zinc sulfate (*ZnSO₄*), the *Giardia EZ VUE* test (TECHLAB® Inc) and the MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescence assay. In addition, samples were
also screened for *Cryptosporidium* using the *Cryptosporidium EZ VUE* test.

(TECHLAB® Inc).

The MERIFLUOR® *Cryptosporidium/Giardia* Test, Meridian Bioscience, Inc (IFA)
The procedure was performed as described by the manufacturer. Fecal samples (1 to 3 days old) approximately the size of a pea (0.1 grams) were fixed in 600 µL of a 10% buffered formalin solution. Each fixed sample was stored in a labeled 1 mL micro-centrifuge tube. Each sample was applied to a well using a new transfer loop to avoid contamination and allowed to dry completely at room temperature. Each well received a drop of detecting reagent and was then counterstained. The two solutions were mixed and the slides were then incubated in a humidity chamber at room temperature in the dark for 30 minutes. After incubation slides were washed with buffer and mounting medium and a coverslip were applied. Slides were scanned for the presence of *Giardia* cysts and *Cryptosporidium* oocysts at 100x and 200x magnification. Positive and negative control provided in the kit were included for each set of slides that were evaluated.

33% Zinc Sulfate Centrifugal Fecal Flotation (ZnCFF)
The procedure was performed as described by Zajac and Conboy (2012). Twenty mL of a 33% zinc sulfate solution (specific gravity 1.18) was mixed with approximately three grams of feces. In some cases less feces was used due to the limited amount of the sample available. Once thoroughly mixed the feces and zinc sulfate mixture was filtered through a double layer of cheesecloth and poured into a 15 mL disposable centrifuge tube. The tube was filled until a reverse meniscus formed at the top and a glass coverslip was then added. The tube and coverslip were centrifuged for 5 minutes at 200 x g (1500 rpm).
Once finished the coverslip was removed and placed onto a glass slide. The entire slide was scanned for internal parasite eggs, cysts, and oocysts using the 100x magnification. In addition, 200x and 400x magnifications were used to confirm species when identification on the 100x magnification was not definitive.

*Giardia EZ VUE* test, TECHLAB® Inc (*Giardia EZ VUE*/*Cryptosporidium EZ VUE* test, TECHLAB® Inc (*Crypto EZ VUE*)

Test was performed as described by TECHLAB® Inc (Blacksburg, VA). Approximately 50 µL of fresh liquid feces or a small pea sized amount of solid feces was added to a premeasured Specimen Dilution Tube containing diluent and mixed thoroughly together. A premade test strip was then placed into the diluent mixed with feces and incubated for 10 minutes after which the strip was checked for the presence of lines (potentially varying in color from dark red to light pink) and the results were recorded (Figure 2.1)

**Statistical Analysis**

Analysis of data was performed using standard statistical software (JMP®, Version 13. SAS Institute Inc., Cary, NC). Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and 95% confidence interval (CI) for ZnCFF, *Giardia EZ VUE* and *Crypto EZ VUE* tests were evaluated and compared using IFA as the reference test. The McNemar’s chi-square test was used to test for significant difference between the sensitivities and specificities. Statistical significance was set to $p < 0.05$. If the value of a cell in any of the two by two contingency tables was zero, then 0.5 was added to each of the four cells to stabilize the model (Yate’s Correction).
RESULTS

A total of 725 individual cats had their fecal samples collected from July 2016 through March 2017. The distribution of samples from each animal shelter was as follows: 418 from Prince William, 180 from Operation Catnip, 68 from Stafford, and 58 from Fauquier. Additionally, one sample came from VMTH-VMCVM. Of these samples, 90 tested positive for *G. duodenalis* on at least one of the diagnostic tests (ZnCFF, *Giardia EZ VUE*, or IFA) (Table 2.1). However, when using the reference standard (IFA) only 87 cats tested *Giardia* positive. In order for a sample to be considered a true positive, it had to test positive on the reference test, which was the IFA. Twenty samples tested positive for *Cryptosporidium* on either or both the *Crypto EZ VUE* test or IFA (Table 2.1). For the 20 samples that tested positive for *Cryptosporidium* spp. only 6 tested positive on the reference test and were considered true positives.

When compared to the reference test (IFA), the *Giardia EZ VUE* had a sensitivity of 78.1 % and specificity of 99.5%. The centrifugal fecal flotation test had a sensitivity of 57.1 % and specificity of 100% (Table 2.4). The McNemar’s chi square test determined that there was a significant difference between sensitivities (p < 0.0008), but not specificities of these two diagnostic tests. Both tests also had a high positive predictive value (PPV) and negative predictive value (NPV) (Table 2.4). When compared to IFA, the *Crypto EZ VUE* had a sensitivity of 100% and specificity of 98.1% (Table 2.5). The NPV for this test was 100%, but the PPV was only 30%.

DISCUSSION
Centrifugal fecal flotation with 33% zinc sulfate was not as sensitive for *G. duodenalis* when compared to the *Giardia EZ VUE* test. This is because the centrifugal fecal flotation detected fewer samples considered true positives than the *Giardia EZ VUE* assay. Specificities for both tests were high. (Table 2.4). This is because all the positive *G. duodenalis* samples on ZnCFF were also positive on IFA (100% specificity) and only three samples that tested positive on the *Giardia EZ VUE* did not test positive for *G. duodenalis* on IFA. A lower sensitivity for centrifugal fecal flotation was expected. It is commonly reported that microscopic techniques underestimate the presence of *G. duodenalis* when compared to fecal immunoassays (Dryden et al., 2006; Hoopes et al., 2013). Published sensitivities for microscopic techniques range in the literature (Hoopes et al., 2013; Mekaru et al., 2007; Olson et al., 2010; Rishniw et al., 2010). Hoopes et al., (2013) reported a low sensitivity of 39% in cats using microscopic techniques while another study by Mekaru et al. (2007) reported a high sensitivity of 85.3 % in cats. However, the use of microscopic techniques is influenced by a variety of factors. For instance, the diagnostic test used as the reference, sample size, and the methodology of the technique. The papers published by Hoopes et al., (2013) and Mekaru et al., (2007) used IFA as the reference standard, which is considered the best available in *G. duodenalis* diagnostics. However, the samples in Hoopes et al., (2013) were evaluated using a centrifugal fecal flotation using a sugar solution and Mekaru et al. (2007) added a sedimentation procedure before centrifuging with zinc sulfate and then stained the slides. Sugar solutions are not as effective as using ZnSO₄ for the detection of *G. duodenalis* cysts and may have accounted for the low sensitivity (Zajac et al., 2002). The addition of
the sedimentation step and staining after centrifugation with ZnSO₄ is not standard practice, and could explain the higher sensitivity.

The Cryptosporidium EZ VUE assay had excellent sensitivity and specificity when compared to IFA (Table 2.5). It was expected that this assay would not be as sensitive or specific as IFA. Previous fecal immunoassays used for the detection of Cryptosporidium spp. in cats vary in their effectiveness in the literature depending on factors including varying numbers of oocysts and the immunoassay used (Marks et al., 2004; Mekaru et al., 2007). While sensitivity and specificity were unexpectedly high it should be noted that there was a low number of true positives in this set of samples. This accounts for the broad confidence interval and the low positive predictive value, which is influenced by prevalence. Since there was such a small positive sample, size this test needs further evaluation to obtain a more accurate representation on the diagnostic test’s sensitivity.

The IFA is routinely used as the reference standard for *G. duodenalis* and Cryptosporidium spp. detection (Garcia et al., 1997; Hoopes et al., 2013; Johnston et al., 2003). However, it comes with its own set of drawbacks. It is well documented that *G. duodenalis* cysts and Cryptosporidium spp. oocysts are shed inconsistently (Thompson et al., 2008). Just like other microscopic techniques that require the visualization of the oocysts or cysts, so does IFA. This means that sometimes the samples that tested negative could actually be positive and so-called false positives on other immunoassays could actually be positive since there is the potential that at the time of collection cysts or oocysts were not being shed in the feces. Additionally, IFA is more expensive, requires
specialized equipment, and is time consuming when compared to other diagnostic tests available on the market today.

For *G. duodenalis*, our results are consistent with past findings that show IFA is more sensitive than microscopic techniques and fecal immunoassays in cats (Hoopes et al., 2013). This was especially true for our samples when compared to microscopy which failed to detect *G. duodenalis* in forty two samples that were detected on IFA while the *Giardia EZ VUE* test only missed nineteen. In humans, IFA is routinely used for *Cryptosporidium* spp. because of its high sensitivity and specificity (Garcia et al., 1992; Garcia et al., 1997). Many animal studies involving the diagnosis of *Cryptosporidium* spp., including some involving cats (Mekaru et al., 2007), use this test as a reference because of its high sensitivities and specificities in humans (Garcia et al., 1997).

However, as mentioned before, diagnostic testing in cats is inconsistent (Marks et al., 2004; Mekaru et al., 2007). From the limited amount of published literature on evaluating diagnostic methods for the detection of *Cryptosporidium* spp. in cats there are not a lot studies looking at IFA and its ability to diagnosis infections. Marks et al. (2004) found that IFA did not have the highest sensitivity for detecting *Cryptosporidium* spp. in cats even after they adjusted their procedure to reduce loss of oocysts in the wash step of IFA. In addition, when used as the reference standard by Mekaru et al. (2007) some of diagnostic tests they compared the IFA to had high numbers of false positives. Similarly, our results had a high number of false positives when compared to IFA (more than twice the number of true positives). While IFA may be the best method currently available for diagnosing infections, it still may not be as effective as detecting *G. duodenalis* infections in cats. One possible explanation for this is that cats are usually infected with the species
specific *C. felis*, while humans are traditionally infected with *C. parvum* or *C. hominis* (Bowman and Lucio-Forster, 2010; Thompson et al., 2008). The IFA may be not be as effective at detecting the species specific *C. felis* when compared to the species more commonly identified in humans. However some believe that IFA does detect most *C. felis* (Scorza and Tangtrongsup, 2010). If IFA is not as effective as detecting *Cryptosporidium* spp. in cats then maybe some of the samples that were considered false positives by IFA were actually truly positives. This could be one possible potential explanation for the higher number of false positives found in the study. However, it should be noted that the *Crypto EZ VUE* was designed for use in humans and is supposed to detect *C. parvum* and *C. hominis* and has had high reported sensitivity and specificity in a study using human samples (Fleece et al., 2016). There is no mention of its ability to detect *C. felis*, so there is the potential that the *Crypto EZ VUE* was not effectively detecting the infections in the cat samples that were most likely *C. felis* and that our false positives really were false positives. A potential way to address the higher number of false positives detected by the *Crypto EZ VUE* if further investigation continues would be to add PCR as a diagnostic test to the study since we only compared the *Crypto EZ VUE* to IFA in this study.

In summary, for the detection of *G. duodenalis* the *Giardia EZ VUE* has potential for being an alternative for microscopic diagnostic techniques. For *Cryptosporidium* spp., results indicate that the *Crypto EZ VUE* is 100% sensitive when compared to IFA, but we do not know if the high number of false positives were actually positive or negative. In addition, the number of samples that tested positive for *Cryptosporidium* spp. was very
low. More positive samples are needed to accurately evaluate the potential of the

Crypto EZ VUE in detecting Cryptosporidium spp. infections in cats.
REFERENCES


of *Giardia* detected in cats by PCR. Vet. Parasitol. 110; 197–205.


TABLES AND FIGURES

Figure 2.1 Depiction of *Giardia EZ VUE* (TECHLAB® Inc) or *Cryptosporidium EZ VUE* (TECHLAB® Inc) test outcomes.
Table 2.1 Feline fecal samples testing positive for *G. duodenalis and Cryptosporidium* spp. on two (*Cryptosporidium*) or three (*G. duodenalis*) diagnostic tests. ZnCFF = Centrifugal fecal flotation with zinc sulfate; EZ VUE = *Giardia EZ VUE* test or *CryptoEZ VUE* test; IFA = MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescence assay; (-) centrifugal flotation not performed

<table>
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<th>ZnCFF</th>
<th>EZ VUE</th>
<th>IFA</th>
<th>ZnCFF/ EZ VUE</th>
<th>ZnCFF/IFA</th>
<th>EZ VUE/ IFA</th>
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<td>12</td>
<td>35</td>
<td>33</td>
<td>90</td>
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<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>-</td>
<td>14</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>20</td>
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Table 2.2 Sensitivity, specificity and 95% Confidence Interval of the *Giardia EZ VUE* test and centrifugal fecal flotation in cats compared to IFA. Columns with different superscripts denote significant differences (McNemar’s p < 0.05). EZ VUE: *Giardia EZ VUE* test; ZnSO4: Zinc sulfate centrifugal fecal flotation; IFA: MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescent assay

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<th>Diagnostic Test</th>
<th>95% Confidence Interval</th>
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<td></td>
<td>Sensitivity</td>
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<td>EZ VUE</td>
<td>78.1&lt;sup&gt;a&lt;/sup&gt; (68.3-85.5)</td>
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<tr>
<td>ZnSO4</td>
<td>51.7&lt;sup&gt;b&lt;/sup&gt; (41.3-61.9)</td>
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Table 2.3 Sensitivity, specificity and 95% Confidence Interval of the *Cryptosporidium EZ VUE* test in cats compared to IFA. EZ VUE: *Cryptosporidium EZ VUE* test; IFA: MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescent assay

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<th>Diagnostic Test</th>
<th>95% Confidence Interval</th>
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<tr>
<td></td>
<td>Sensitivity</td>
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CHAPTER 3: Internal Parasite Prevalence and Risk Factors Associated with Infection in Shelter Cats in Eastern Virginia

ABSTRACT

Cats are infected by a variety of internal parasites, some of which are zoonotic. There are a limited number of published surveys of parasite prevalence in cats in the U.S. and there are no extensive surveys of cats in Virginia. Therefore, the aim of this study was to perform a comprehensive survey of cats from animal shelters to determine prevalence of internal parasites in this area. Fecal samples were collected from three different eastern Virginia shelters between August 2016 and March 2017. Samples were examined for parasites using a 33% zinc sulfate centrifugal fecal flotation procedure and the MERIFLUOR® Cryptosporidium/Giardia direct immunofluorescent assay (IFA). We examined 544 fecal samples from individual cats and overall prevalence was calculated for each parasite species found. Parasites identified include Toxocara cati (15%), Giardia duodenalis (10.5% centrifugation flotation and IFA combined), Cystoisospora felis (9.2%), Cystoisospora rivolta (9%), Eucoleus aerophilus (1.5%), Taenia taeniaeformis (1.1%), Aonchotheca putorii (0.9%), Spirometra mansonoides (0.4%), Ancylostoma sp. (0.2%), and Toxascaris leonina (0.2%). Additionally, epidemiological data was collected and evaluated to identify risk factors associated with infection. The results of this survey confirm that a variety of helminth and protozoan parasites are present in Virginia cats and that cat status, sex, and location may be an indicator for infection with internal parasites.

INTRODUCTION
Cat are commonly found throughout most of the world. In the United States, approximately 35% of households own one or more cats, making up a population of about 85.8 million with approximately 80 million additional stray and feral cats (ASPCA, www.aspca.org/animal-homelessness/shelter-intake-and-surrender/pet-statistics, accessed July 9, 2017). Any cat can be infected with a variety of internal parasites including nematodes, cestodes, trematodes, and protozoa. Some of these parasites do not cause any clinical signs or have a lasting effect, while others can cause damage or contribute to the death of the cat (Zajac and Conboy, 2012). In addition, some of these parasites are zoonotic, including *Toxoplasma gondii*, *Toxocara cati*, and *Giardia duodenalis*. The way pet owners view cats and other companion animals has changed significantly over the past few decades. They are now viewed more as part of the family and spend more time in the house and in closer contact with their owners. The close interactions of owners and pets and the large population of free roaming cats make it important to be aware of potential health risks. Therefore, it is important to know the prevalence of parasites for both the health of cats and humans.

Currently, there are a limited number of prevalence surveys available for different areas of the United States (DeSantis-Kerr et al., 2006; Gates and Nolan, 2009; Rembiesa and Richardson, 2003; Sabshin et al., 2012). Reported prevalence depends on a variety of factors and the locations represented in published surveys may not accurately reflect the situation in other areas. Factors that can affect prevalence in these surveys include location in the U.S., demographics of the population sampled such as age and lifestyle, and diagnostic test used (Ballweber et al., 2010; Carlin et al., 2006; De Santis-Kerr et al., 2006; Itoh et al., 2006; McGlade et al., 2003). For example, the prevalence of *G.*
*duodenalis* tends to be higher in studies with younger animals (Pallant et al., 2015) and in crowded or multi-cat situations such as hoarding situations or catteries (Gookin et al., 2004; Polak et al., 2014). Pallant et al. (2015) and Papini et al. (2007) both reported that cats less than a year old had a higher prevalence of *G. duodenalis* when compared to older cats. Gookin et al. (2004) reported a prevalence of 56% in cats sampled from a large scale hoarding investigation, which is high compared to other reports of *G. duodenalis* prevalence in cats.

For Virginia specifically, there are no extensive surveys available. However, there are some published studies that focus on individual parasites such as *Toxoplasma gondii* and *G. duodenalis* (Carlin et al., 2006; Lilly, 2013). Prevalence data for Virginia has been published by the Companion Animal Parasite Council, but is limited to roundworms (3.85%), hookworms (0.42%), and *Giardia duodenalis* (1.88%) (Companion Animal Parasite Council, http://www.petsandparasites.org/parasite-prevalence-maps/, accessed August 20, 2017). However, the CAPC data comes only from veterinarian submitted samples and may not accurately represent the prevalence of these parasites in Virginia. Therefore, the aim of this study was to perform a survey in a diverse population of cats to identify feline internal parasites in eastern Virginia and to evaluate any reported epidemiological data to see if there are any risk factors associated with infection.

**MATERIALS AND METHODS**

**Collection Sites**

Fecal samples were collected from cats in three different locations in Virginia from August 2016 through March 2017. These locations were the Prince William County
Animal Shelter (Manassas, VA), Stafford County Animal Shelter (Stafford, VA), and the Fauquier SPCA Animal Shelter (Midland, VA) (Figure 1).

**Sample Collection**

Fecal samples were collected from litter pans during routine morning cleaning. Collection was completed during cleaning to avoid additional stress for the cats. Feces was collected within 24 hours of excretion. Each fecal sample was put in a plastic resealable bag, placed in a cooler with icepacks for transport, and then refrigerated until processed. Shelter identification numbers for all animals present in the cage were recorded on the bag. If defecation by a specific cat was observed in a cage with more than one cat that fecal sample was bagged separately and only that animal’s identification number was recorded on the bag.

**Deworming**

Each of the three animal shelters had a different deworming protocol in place and some of the samples included in the study came from dewormed cats. Staff at the Prince William County animal shelter dewormed with pyrantel when a cat was seen by one of the shelter veterinarians and allocated for adoption or if parasites were seen in the feces by staff. Staff at the Stafford County Animal Shelter dewormed with pyrantel when parasites were seen in the feces or if the cat was approximately a year or younger. There was no way to determine which of the cats received pyrantel at the Prince William and Stafford County shelters prior to sample collections. We did not know which cats were dewormed before collection at Prince William and Stafford County shelters. All cats
housed at the Fauquier SPCA Animal Shelter received pyrantel when they first arrived at
the shelter and were also treated with a moxidectin/imidacloprid combination as well.

Sample Duplication

To the investigator’s knowledge, there was no duplication of samples from individual
cats in this study. For this study feces from an individual cat was only tested once and if
another fecal sample was collected it was discarded. Since some cats remained in shelters
for months before adoption, they might have had their feces collected multiple times. A
list of identification numbers was maintained and updated to avoid sample duplication.

Sample Screening

Samples were evaluated with centrifugal fecal flotation with 33% zinc sulfate (ZnSO₄)
and the MERIFLUOR® Cryptosporidium/Giardia direct immunofluorescence assay.

The MERIFLUOR® Cryptosporidium/Giardia Test, Meridian Bioscience, Inc (IFA)

The procedure was performed as described by the manufacturer. Fecal samples (1 to 3
days old) approximately the size of a pea (0.1 grams) were fixed in 600 µL of a 10%
buffered formalin solution. Each fixed sample was stored in a labeled 1 mL micro-
centrifuge tube. Each sample was applied to a well using a new transfer loop to avoid
contamination and allowed to dry completely at room temperature. Each well received a
drop of detecting reagent and was then counterstained. The two solutions were mixed
and the slides were then incubated in a humidity chamber at room temperature in the dark
for 30 minutes. After incubation slides were washed with buffer and mounting medium
and a coverslip was applied. Slides were scanned for the presence of Giardia cysts and
Cryptosporidium oocysts at 100x and 200x magnification. Positive and negative controls provided in the kit were included for each set of slides that was evaluated.

Centrifugal Fecal Flotation

The procedure was performed as described by Zajac and Conboy (2012). Twenty mL of a 33% zinc sulfate solution (specific gravity 1.18) was mixed with approximately three grams of feces. In some cases less feces was used due to the limited amount of the sample available. Once thoroughly mixed the feces and zinc sulfate mixture was filtered through a double layer of cheesecloth and poured into a 15 mL disposable centrifuge tube. The tube was filled until a reverse meniscus formed at the top and a glass coverslip was then added. The tube and coverslip were centrifuged for 5 minutes at 200 x g (1500 rpm). Once finished the coverslip was removed and placed onto a glass slide. The entire slide was scanned for internal parasite eggs, cysts, and oocysts using the 100x magnification. In addition, 200x and 400x magnifications were used to confirm species when identification on the 100x magnification was not definitive.

Epidemiological Data

In addition to recording the parasites present in the fecal samples, additional data was collected for each cat, when available, to evaluate whether there were any potential risk factors associated with internal parasite infection. This data included sex, approximate age, fecal grading score, cat status, and location. For sex, there were four different classifications: female (F), male (M), neutered male (MN), spayed female (FS). Age was recorded in years and for fecal grading score every fecal sample was ranked using the Virginia Maryland College of Veterinary Medicine (VMCVM) teaching hospital scoring
system. This system utilizes scores of 1-5, with 1 being liquid feces and 5 being solid, formed feces. For status, cats were classified as owner surrender, stray, or feral and location refers to which animal shelter the cat came from.

**Statistical Analysis**

Analysis of data was performed using standard statistical software (JMP®, Version 13. SAS Institute Inc., Cary, NC). Associations between outcomes and potential risk factors were assessed using Fisher’s exact test (p < 0.05). Associations were assessed for samples that were *G. duodenalis* positive and separately for samples that were positive for at least one internal parasite. *G. duodenalis* positive infection was analyzed separately because infections with this parasite can sometimes be difficult to detect and we wanted to see if we could identify some risk factors that would be useful for veterinarians and shelters. Potential risk factors assessed for these outcomes were the cat’s sex, fecal score, age, status, and location. The sex, fecal score, and age data were arranged and evaluated several ways for each outcome. Any potential risk factors that were independently determined to be significant using Fisher’s exact test were further analyzed and included in a multivariable logistic model (p < 0.05) for predicting the two different outcomes.

**RESULTS**

A total of 544 samples were collected from the three animal shelters from August 2016 through March 2017. Parasites identified were *Toxocara cati, Giardia duodenalis, Cystoisospora felis, Cystoisospora rivolta, Eucoleus aerophilus, Taenia taeniaeformis, Aonchotheca putorii, Spirometra mansonoides, Ancylostoma sp.*, and *Toxascaris leonina.*
Toxocara cati was the most common nematode observed and the most common parasite seen overall. It was the most common at each individual location as well, ranging from 12% in Fauquier County up to 24% in Stafford County. *Giardia duodenalis* was the most common protozoan parasite (Table 3.1). At least one parasite was present in 36% of the samples overall. The Fauquier SPCA shelter had at least one parasite present in 29% of the samples, while the shelters in Prince William County and Stafford County had at least one parasite present in 35% and 50% of the fecal samples collected, respectively.

The potential risk factors were sex (M, MN, F, FS), age (in years), fecal grading score (1-5), cat status (feral, stray, owner surrender), location (Prince William County, Stafford County, and Fauquier County). The first outcome assessed was *G. duodenalis* positive samples and no statistically significant risk factors associated with *G. duodenalis* infection were identified (Table 3.2). When the data was evaluated for factors associated with infections with at least one parasite, sex (p = 0.045), cat status (p = 0.01), and location (p = 0.03) were statistically significant (Table 3.3). Sex was tested several different ways and was significantly different when female, spayed females, males, and neutered males were compared (M vs MN vs F vs FS (p = 0.045). The three risk factors were further analyzed in a multivariable logistic model for predicting the outcome of being parasitized with at least one internal parasite. However, when this model was run only cat status remained significant. The odds of having at least one parasite present was 1.6 times greater (Odds ratio 1.6, 95% CI 1.1-2.4 and p = 0.011) in feral or stray cats compared to owner surrenders.

**DISCUSSION**
*Toxocara cati* (15%) was the most commonly detected parasite. This is most likely due to the fact that cats can be infected in a number of way including ingestion of eggs via the fecal – oral route (Overgaauw, 1997), transmammary transmission (Swerczek et al., 1971) or ingestion of a paratenic host (Strube et al, 2013). Additionally, this parasite is a prolific eggs shedder, which would increase the chance of contact with the eggs in the environment (Overgaauw, 1997). Compared to other surveys our prevalence was lower than expected involving shelter cats in the United States (Lucio-Forster and Bowman, 2011; Spain et al., 2001). However, the lower prevalence of *T. cati* can be explained by the use of pyrantel at the shelters in Stafford, Fauquier, and Prince William and the additional use of a moxidectin/imidicloprid combination at the shelter in Fauquier. Pyrantel will remove infections with *Toxocara cati*, *T. leonina* and *Ancylostoma* spp., as will the moxidectin/imidicloprid combination. The moxidectin/imidicloprid combination would probably have also removed other nematodes including capillarids (*Eucoleus aerophilus* and *Aonchotheca putorii*). Our prevalence for *Toxocara* was higher than the CAPC data for Virginia (3.85%) (Accessed August 20, 2017) and may be due the fact that the fecal samples were submitted to veterinarians, while our samples came from shelter cats that came from of variety of different background (owner surrender, stray, or feral). Cats visiting vets are more likely to be screened and treated for parasites than cats that are not owned and free roaming. De Santis et al. (2006) also looked at samples submitted to veterinary clinics owned by Banfield in 2003 and reported a *Toxocara* prevalence of 2.92%.

The other ascarid detected in this survey was *Toxascaris leonina* (0.2%). It was not unexpected that there was a low prevalence of *T. leonina* since it is not as routinely
found as *T. cati* (Zajac and Conboy, 2012). This could be due to the difference in the life cycles of these ascarids. The life cycle of *T. leonina* is simpler than the life cycle of *T. cati*. *Toxocara leonina* cannot be passed through the transmammary route like *T. cati* and larvae do not migrate throughout the tissues of the cats (Epe, 2009). The literature contains reports of *Toxascaris leonina* in states near Virginia (Pennsylvania and New Jersey) with prevalences of 10% and lower (Gates and Nolan, 2009; Mann and Fratta, 1952; Mann, 1955). One of the studies had a very low prevalence like our survey. Nolan and Gates (2009) reported that two (0.1%) *T. leonina* infections were diagnosed out of 1,566 cat samples that were examined.

*Ancylostoma* spp. eggs were detected in only 1 out of the 544 samples screened. In Virginia the egg could have been either *A. braziliense* or *A. tubaeforme* (Bowman et al., 2002). However, it was most likely *A. tubaeforme* because although *A. braziliense* can occur in Virginia, it appears to be uncommon (D. Bowan, personal communication). Our prevalence seemed low at 0.2%, but when compared to other surveys in both shelter cats and veterinarian submitted samples was not surprising. Lucio-Forster and Bowman (2011) reported a prevalence of 2.2% in shelter cats in New York, while a prevalence of 0.63% and 0.5% were reported in several studies involving samples submitted to veterinary hospitals (DeSantis et al., 2006; Gates and Nolan, 2009). While our prevalence was lower than previous reports for shelter cats, the difference in percentage was small and could be attributed to the fact some cats were dewormed. Similarly, *Aonchotheca putorii* (0.9%) and *Eucoleus aerophilus* (1.5%) prevalence could have been affected by treatment with moxidectin at the Faquier shelter since prevalences were lower than previously reported (Lillis, 1967; Lucio-Forster and Bowman, 2011).
The three protozoan parasites identified were *Cystoisospora felis* (9.2%), *Cystoisospora rivolta* (9%), and *Giardia duodenalis* (10.5% centrifugation flotation and IFA). *Cystoisospora felis* and *Cystoisospora rivolta* prevalences were within previously reported ranges in the literature and similar to another study conducted in New York that sampled a similar population (Lucio-Forster and Bowman, 2011). *G. duodenalis* prevalence was higher than expected. The Companion Animal Parasite Council (CAPC) prevalence for Virginia as a whole is 1.88% and ranges from 1.68% to 2.5% for the three counties sampled (Accessed August 20, 2017). However, Carlin et al. (2006) reported a prevalence of 12% - 16% in Virginia for veterinary submitted samples. The difference could be due to the fact that a fecal immunoassay, the IDEXX SNAP *Giardia* test, was used. The higher prevalence in this survey could be due to the fact that both IFA and centrifugal fecal flotation were used. Using centrifugal fecal flotation and other standard microscopic techniques tends to underestimate the prevalence of *G. duodenalis* (Dryden et al., 2006). While IFA is not a perfect diagnostic test for *G. duodenalis* either, it is considered one of the best available diagnostic tests and is often used as the reference standard (Mekaru et al., 2007; Rishniw et al., 2010). In this case the IFA detected all samples that were also positive on centrifugal fecal flotation and detected twenty-five additional samples that the zinc sulfate centrifugal fecal flotation missed.

The two cestode species identified were *Taenia taeniaeformis* (1.1%) and *Spirometra mansonioides* (0.4%). *Spirometra mansonioides* has been reported in low prevalence in the United States (Lillis and Burrows, 1964; Lucio-Forster and Bowman, 2011; Sabshin et al., 2012). Additionally, when reported, *S. mansonioides* seems to be more commonly identified in the southeastern and Gulf coast states (Little et al., 2000).
One study in the Northeast with similar study parameters reported the same prevalence of 0.4% (Lucio-Forster and Bowman, 2011). The low prevalence of S. mansonoides reported in the literature is probably due to its life cycle that utilizes multiple hosts and the fact that part of its life cycle requires water. For cats to become infected they ingest reptiles, amphibians, birds, or small mammals infected with the plerocercoid larva (Mueller, 1974). Low prevalence of T. taeniaeformis was expected because it is well known that the prevalence of tapeworms determined by fecal flotation underestimates true prevalence. A recent study by Little et al. (2015) showed that T. taeniaeformis and Dipylidium caninum were in 30 (25.9%) and 40 (34.5%) cats, respectively, out of 116 examined by necropsy. However, when a centrifugal fecal flotation with sugar solution was used on the same 116 cats’ feces only 8 samples tested positive for T. taeniaeformis and zero samples tested positive for D. caninum. As a result, it is likely that our population actually had a higher prevalence rate for T. taeniaeformis and that some cats were infected with D. caninum even though no eggs or egg packets were seen on the centrifugal fecal flotation.

The absence of Paragonimus kellicoti, Physaloptera spp., and Aelurostrongylus abstrusus in our study was unexpected. These three parasites have previously been reported in states throughout the southeast (Lucio-Forster and Bowman, 2011; Rembiesa et al., 2003; Rochat et al., 1990). It was unexpected to not see any with such a large and diverse sample size, although none of these parasites are common. The absence of P. kellicoti and Physaloptera spp. may be due to the use of zinc sulfate as the fecal flotation solution. Eggs of these parasites eggs are denser than other common parasite eggs and might not have floated as well in the zinc sulfate solution (sp. 1.18) as they may have
done in a higher density fecal flotation solution. Also, pyrantel and moxidectin may have treated any *Physaloptera* spp. Although the preferred diagnostic test for *A. abstrusus* is a Baermann exam, fecal flotation can be used as a less sensitive test. Other surveys using fecal flotation have found prevalence of this lungworm up 6.2% (Lucio-Forster and Bowman, 2011). We were surprised by the absence of any *A. abstrusus* larvae in the flotations since they are in Virginia and can be recovered with zinc sulfate centrifugal fecal flotations (Lillis, 1967; Lucio-Forster and Bowman, 2011; Rembiesa and Richardson, 2003). While moxidectin treatment would have removed *Aelurostongylus* infection, pyrantel treatment would have been ineffective. *Cryptosporidium* spp. were also not detected. The use of zinc sulfate centrifugal fecal flotation is not recommended method for the detection of *Cryptosporidium* spp. and would have contributed to not detecting any oocysts in the feces. However, IFA was also used, which is considered one of the better methods available for detection (Garcia and Shimizu, 1997; Johnston et al., 2003). Even with the use of IFA no *Cryptosporidium* spp. oocysts were detected. However, *Cryptosporidium* spp. can be difficult to accurately diagnose because it is shed intermittently and is not as common in cats as some other protozoan parasites (Mekaru et al., 2007). Another parasite seen not seen in this survey was *Toxoplasma gondii*. However, this was not entirely unexpected. Cats only shed *Toxoplasma gondii* oocysts for about one week when they are first infected (Dubey, 2010; Elmore et al., 2010). Therefore, cats could have been infected with this parasite, but were not shedding the oocysts when the feces was collected.

Some of the parasites identified in this survey are zoonotic or have zoonotic potential. These parasites are *G. duodenalis, T. cati, S. mansonoides, and Ancylostoma*
spp. *Giardia duodenalis* is potentially zoonotic because it has multiple assemblages (A-H), some of which are found in both humans and cats (Feng and Xiao, 2011; Lasek-Nesselquist et al., 2010; Monis et al., 2003). This overlap of assemblages A and B might indicate that cats and humans can pass the parasite between one another. However, there is currently no published study in the literature confirming zoonotic transmission from cats (Feng and Xiao, 2011). *Toxocara cati* can cause self-limiting visceral or ocular larva migrans in humans when they accidentally ingest the infective egg. Once ingested the larva will hatch and penetrate the intestinal wall and migrate to various body tissues (Fisher, 2003; Woodhall et al., 2014). *Ancylostoma braziliense*, may cause self-limiting cutaneous larva migrans where infective filariform larvae burrow into the skin through contact with infected soil (Bowman et al., 2000). The last zoonotic parasite identified was *S. mansonoides*, which can cause sparganosis, which refers to spargana (larvae of the *Spirometra* genus). This occurs when humans accidentally ingest sparganum, which can migrate all through the body including the brain and eyes (Liu et al., 2015).

Epidemiological data was statistically analyzed for different outcomes to determine if there were any risk factors associated with infection with *G. duodenalis* infections. None of the potential risk factors were determined to be significant. Not finding any significant associations between infections with *G. duodenalis* and sex, fecal grading score (symptomatic vs asymptomatic), and location were not surprising and similar to findings of Papini et al (2007) in Italy. However, no significant association or trend with age was unexpected. In the literature, younger animals tend to have higher *G. duodenalis* infection rates (Ballweber et al, 2010; Pallant et al., 2015; Papini et al., 2007). Additionally, while not significant, stray and feral cats had a slightly higher prevalence...
for *G. duodenalis* than owner surrenders. This was expected because cats that are strays or feral are most likely outside more and have a higher chance of being exposed to *G. duodenalis* cysts than the average owner surrender, unless the owner surrender was a barn cat or lived mostly outdoors. However, it should be noted that some indoor cats were found to actually be more likely to be infected with *G. duodenalis* (Papini et al., 2007). Those infections were usually associated with crowded or multi-cat situations like with hoarding situations or catteries (Gookin et al., 2004; Polak et al., 2014).

The same set of potential risk factors was looked at in association with infection with at least one internal parasite. It was determined that cat status, sex, and location were statistically significant. The fact that cat status was significant was not unexpected. Cats that are strays or feral are at higher chance of being exposed to *G. duodenalis* cysts through the environment and their diet (contaminated food or water) than the average owner surrender (Itoh et al., 2006). Additionally, feral and stray cats are not likely receiving routine veterinary care, which would include screening for and removing any parasites identified. The association between location (the different counties) and infection was not expected to be significant. There are reports in the literature of regional differences (DeSantis-Kerr et al., 2006), but all our locations were in three adjacent counties where the animal shelters we collected significant differences on such a small scale are not usually reported. The most likely reason for this difference is the fact that each shelter had a different deworming protocol. The shelter in Fauquier County had the most comprehensive deworming protocol, followed by Prince William County, and then Stafford County. Interestingly, the shelter in Fauquier had the lowest prevalence of internal parasites while Stafford had the highest. In addition to location, all the potential
risk factors evaluated for this outcome could have been affected since every deworming with pyrantel would have cleared infections with *Toxocara cati*, *T. leonina* and *Ancylostoma* spp. and the moxidectin/imidicloprid combination is effective against a wide range of nematodes.

Another potential reason for this significance is the location of the cats within in the different counties before they were brought to the shelters. All three counties have dense urban areas, suburbs, and farmland within the county. If cats came from different parts of the counties it may contribute to the difference, especially since differences in parasite prevalence in cats from urban and suburban have been documented (Itoh et al., 2006).

Fecal score and age were not significantly associated with parasitism, but both had trends (Table 3.3). Cats with a fecal score of three or less (soft or diarrheic) and younger cats (< 1 year old) had higher prevalences of internal parasites, which is in agreement with reports from other authors (DeSantis-Kerr et al., 2006; Gates and Nolan, 2009; Itoh et al., 2006). The factors that were determined to be statistically significant were further analyzed and included in a multivariable logistic model to see if there was a relationship between those three factors specifically and the chance of being infected with at least one internal parasite, but only cat status remained significant. The odds of having at least one parasite present was 1.6 times greater in cats that were identified as feral or stray when compared to those labeled as owner surrenders. Once again this can be linked back to their higher chance of exposure in the environment and diet.

This survey confirms that there are a variety of internal parasites in Virginia cats, some of which are zoonotic. The results fell within ranges previously reported in
literature even though some of the cats were dewormed and it should be noted our survey may underestimate the true prevalence of the various nematode species found in Virginia. This survey also lends support to the fact that some factors may contribute to higher prevalence of parasites present, which was especially true for cats with the status of stray or feral. Overall, it is important to be aware of internal parasites in the environment and how we can limit contact with them in both cats and humans and that routine veterinary care is vital in maintaining cat and human health.
REFERENCES


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Figure 3.1 Map of Virginia. Counties where feces was collected in are outlined in red and shown in the inset. The counties surveyed were Prince William, Stafford, and Fauquier.
Table 3.1 Internal parasites detected in cats at three animal shelters and overall and individual site prevalence.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Number of positive cats and prevalence (%)</th>
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<tbody>
<tr>
<td></td>
<td>Overall (n = 544)</td>
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<tr>
<td><strong>Protozoa</strong></td>
<td></td>
</tr>
<tr>
<td><em>Giardia duodenalis</em></td>
<td>57 (10.5%)*</td>
</tr>
<tr>
<td><em>Cystoisospora felis</em></td>
<td>50 (9.2 %)</td>
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<tr>
<td><em>Cystoisospora rivolta</em></td>
<td>48 (9 %)</td>
</tr>
<tr>
<td><strong>Nematodes</strong></td>
<td></td>
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<tr>
<td><em>Toxocara cati</em></td>
<td>82 (15%)</td>
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<tr>
<td><em>Aonchotheca putorii</em></td>
<td>5 (0.9 %)</td>
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<tr>
<td><em>Eucoleus aerophilus</em></td>
<td>8 (1.5%)</td>
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<tr>
<td><em>Ancylostoma tubaeformae</em></td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td><em>Toxascaris leonina</em></td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td><strong>Cestodes</strong></td>
<td></td>
</tr>
<tr>
<td><em>Taenia taeniaeformis</em></td>
<td>6 (1.1%)</td>
</tr>
<tr>
<td><em>Spirometra mansonoides</em></td>
<td>2 (0.4%)</td>
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</table>

* Positive on IFA or 33% zinc sulfate centrifugal fecal flotation

Table 3.2 Results of Fisher’s exact test. Associations between being infected *Giardia duodenalis* and potential risk factors were assessed for statistical significance (p < 0.05).

<table>
<thead>
<tr>
<th>Risk Factors for Infection with Giardia Duodenalis</th>
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<tbody>
<tr>
<td>Outcome</td>
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<tr>
<td>Predictor Category</td>
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<tr>
<td>Number Positive Negative P Value</td>
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<tr>
<td>N (%) N (%)</td>
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<tr>
<td>G. duodenalis positive Sex1 F+FS 259 23 (8.9) 236 (91.1) 0.2463</td>
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<tr>
<td>M+MN 245 30 (12.2) 215 (87.8)</td>
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<tr>
<td>Sex2 F 126 11 (8.7) 115 (91.3) 0.6434</td>
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<tr>
<td>FS 133 12 (9.0) 121 (91.0)</td>
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<tr>
<td>M 123 14 (11.4) 109 (88.6)*</td>
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<tr>
<td>MN 122 16 106</td>
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<td><strong>Age1</strong></td>
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<td><strong>Age2</strong></td>
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<td><strong>Age3</strong></td>
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<tr>
<td><strong>Cat Status</strong></td>
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<td><strong>Location</strong></td>
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|                |                          |       |   |       |       |       |
| **Fecal Score1**| 3 or less              | 90    | 11 | (12.2)| 79    | (87.8) |
|                | 4                        | 157   | 20 | (12.7)| 137   | (87.3) |
|                | 5                        | 297   | 26 | (8.8) | 271   | (91.2) |
| **Fecal Score2**| 3,2 or 1               | 90    | 11 | (12.2)| 79    | (87.8) |
|                | 4 or 5                   | 454   | 46 | (10.1)| 408   | (89.9) |

|                |                          |       |   |       |       |       |
| **Location**  | FC                        | 58    | 6 | (10.3)| 52    | (89.7) |
|                | PWC                       | 418   | 45| (10.8)| 373   | (89.2) |
Table 3.3 Results of Fisher’s exact test. Associations between being infected with at least one internal parasite and potential risk factors were assessed for statistical significance (p < 0.05). Significant p-values are denoted with *.

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<th>Predictor</th>
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<th>Number</th>
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<th>Negative</th>
<th>P Value</th>
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<td></td>
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<td>N (%)</td>
<td>N (%)</td>
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<tr>
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<td>22 (57.9)</td>
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<tr>
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<td>Age3 &lt; 1 year</td>
<td>Age3 =&gt; 1 year</td>
<td>Cat Status OTI</td>
<td>Cat Status S</td>
<td>Location FC</td>
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<tr>
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<td>229</td>
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</tr>
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</table>

F: female; FS: female spayed; M: Male; MN: Male Neutered; OTI: Owner-Turn-In; S: Stray; FC: Fauquier County; PWC: Prince William County; SC: Stafford County
CHAPTER 4: CONCLUSIONS

*Giardia duodenalis* and *Cryptosporidium* spp. are enteric protozoan parasites of cats. Diagnosing infections can be difficult and the current reference standard (IFA) for both requires specialized and expensive equipment, long incubation times, and is not feasible for most veterinary practitioners. I was able to show that two prototype lateral flow assays created by TECHLAB® Inc, the *Cryptosporidium EZ VUE* and *Giardia EZ VUE*, designed for humans may have potential in detecting feline infections. There is already a commercially available point of care fecal immunoassay (IDEXX SNAP Giardia Antigen Test) for detecting *G. duodenalis* in cats, but it is not perfect and not as sensitive or specific when compared to IFA. For *Cryptosporidium* spp. there are no point of care lateral flow assays currently available on the market for use in cats. These immunoassays could provide a cheap, effective, easy to use alternative that should be further investigated to see if they could compete with what is already currently available for the detection of *Cryptosporidium* spp and *G. duodenalis*. Future work would include screening more samples to obtain additional *G. duodenalis* and *Cryptosporidium* spp. positive samples. While having negatives is important and collections from shelters would continue, I would also actively seek out positive samples from other sources. Additionally, I would add PCR to the comparison for both *Cryptosporidium* spp. and *G. duodenalis*. The addition of PCR for each parasite would help to determine if samples positive on either the *Cryptosporidium EZ VUE* or *Giardia EZ VUE* immunoassay, but not on IFA were false or true positives. While it would not without a doubt prove the samples were false positives or true positives it would least be able to give us some indication. Both EZ VUE tests are prototypes designed for use in humans and with no
published data in animals and in this study I did not have an effective way to support that
the samples were either false positives or actually positive. For *G. duodenalis* I could
have said a sample positive on the *Giardia EZ VUE* and zinc sulfate centrifugal fecal
flotation, but not IFA was actually positive since I would have seen cysts. For
*Cryptosporidium* spp. I only compared the *Cryptosporidium EZ VUE* to the IFA and had
no way of determining if the samples only positive on the *Cryptosporidium EZ VUE* were
actually positive.

To my knowledge this was the first survey specific for Virginia of this scale and
helps provide important prevalence data that is lacking for this part of the United States.
We were able to confirm that Virginia cats are infected with a variety of parasites, some
of which are zoonotic, and that certain epidemiological factors may contribute to
infection, especially the lifestyle of the cats (feral, stray, owner surrender). Establishing
that a variety of internal parasites infect cats supports the need for routine veterinary care
in maintaining both human and feline health and that feral and stray cats are important
reservoirs for internal parasites. Besides education, this data could also be useful to
shelters and trap - neuter- release programs and help them optimize the use of deworming
protocols, especially since funds are often limited at these facilities and often cannot treat
every cat that is brought to them. Further work for this study would include continuing
collection from a variety of different cats including ones that varied in age, lifestyle, fecal
score, and sex. If possible, I would want to find new locations that did not deworm so I
could remove that variable entirely or at least try to find out if cats were dewormed
before collection from here on so I more accurately access the effect that deworming had
on the nematode prevalence. Additionally, I would try to add other organizations such as

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rescues from the counties already included in the study and from other surrounding counties. This would help give a more robust set of internal parasite prevalence data for the counties than we already have and increase the amount of area sampled in Virginia to help see a more complete picture of the prevalence of different internal parasites in Virginia cats.