

A COMPARISON OF THE SNAP[®] *GIARDIA* FECAL ANTIGEN TEST AND THE ZINC
SULFATE DOUBLE CENTRIFUGATION FECAL FLOTATION PROCEDURE TO
DIAGNOSE *GIARDIA INTESTINALIS* INFECTIONS IN TWO POPULATIONS OF
INFECTED DOGS

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Abstract

Giardiasis is a common intestinal protozoal parasitic infection of the pet dog and cat population. Veterinarians often have difficulty correctly diagnosing this parasite. Studies were conducted to compare the zinc sulfate double centrifuge fecal flotation to the SNAP (registered trademark) *Giardia* fecal ELISA test manufactured by IDEXX laboratories Inc. in purpose bred beagles and shelter and commercial kennel dogs. In these evaluations the zinc sulfate double centrifuge fecal flotation and fecal ELISA test performed similarly. Both tests performed better in the shelter and commercial kennel dog population than the chronically infected purpose bred beagles. There was an increase in number of positive animals identified when 3 consecutive daily samples were evaluated as compared to any one individual day for either test method. Post treatment evaluation of the diagnostic tests was performed in 23 laboratory beagles. Each beagle was treated for 3 consecutive days with Drontal plus and then bathed on the last day of treatment and fecal samples were collected from the treated dogs every other day starting one day post treatment for 21 days. It was found that all beagles were negative on zinc sulfate double centrifugation fecal flotation, fecal ELISA and IFA within 24 hours of treatment and nineteen (82.6%) of the beagles did not re-shed cysts during the 21 day post-treatment evaluation period. Four beagles returned to shedding cysts (Flotation or IFA positive) between days 17 and 21. These findings suggest that a positive test within a week of treatment is likely the result of inappropriate treatment. After the prepatent period, positive results may occur due to a return to shedding, reinfection or inappropriate treatment. Chronically infected laboratory beagles may not be a good model for acute *Giardia* infections as these dogs are rarely clinically ill and detection is more difficult.

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Dedication

I would like to dedicate this to my family whose support made it possible to continue my education.

CHAPTER 1 - Literature Review of *Giardia* spp.

HISTORY

Antony van Leeuwenhoek first described *Giardia* in his own stool in 1681.¹ He was a fabric merchant who made many of the first microscopes. He viewed and described many specimens such as bacteria, dog sperm and *Giardia*. In 1859, a Czech physician named Vilem Lambl observed *Giardia* in the stools of children with diarrhea, which he called *Cermomonas intestinalis*. He described and drew the trophozoites and cyst forms. He believed them to be commensal organisms, not pathogens. Raphael Anatole Émile Blanchard renamed the organism *Lambliia intestinalis* in 1888.² Charles Wardell Stiles changed the name to *Giardia lamblia* in 1915.³ He suspected *Giardia* to be a pathogen when he found a correlation between soldiers with diarrhea and the organism. Not until 1954 did the American physician Robert Rendtorff, produced detailed studies linking the parasite with the disease.⁴ The World Health Organization added *Giardia* to the list of parasitic diseases in 1981.²

TAXONOMY

Kingdom – Protista
Subkingdom – Protozoa
Phylum – Sacromastigophora
Subphylum – Mastigophora
Class – Zoomastigophora
Order – Diplomonadida
Family – Hexamitidae
Genus – *Giardia*

Over 50 species of *Giardia* have been described. Previously, the various *Giardia* spp. were given species names and classified by the host from which the organism was isolated and

the phenotypic description.⁵ Recently, the list of species has been condensed based on molecular characteristics. The list now includes:⁵⁻⁷

Table 1.1 *Giardia* species and hosts

Species	Hosts
<i>G. agilis</i>	Amphibians
<i>G. ardeae</i>	Birds
<i>G. microti</i>	Muskrats and Voles
<i>G. muris</i>	Mice
<i>G. psittaci</i>	Birds
<i>G. intestinalis</i>	Wide range of domestic and wild animals including humans, dogs, & cats

Synonyms for *Giardia intestinalis* are *G. duodenalis* and *G. lamblia*. There is considerable diversity within *G. intestinalis*. Groupings called assemblages with further subgroups have been identified. The assemblages are based on PCR techniques using conserved genetic loci including glutamate dehydrogenase (GDH), elongation factor 1- α (efl- α), Triose phosphate isomerase (TPI), and rDNA.^{5,7} These assemblages are as follows:^{5,7,8}

Table 1.2 Assemblages of *Giardia intestinalis* and hosts^{5,7,8}

Assemblages	Hosts
Assemblage A Subgroup I, II	Humans, primates, dogs, cats, cattle, rodents, wild mammals
Assemblage B	Humans, primates, dogs, horses, cattle
Assemblage C	Dogs
Assemblage D	Dogs
Assemblage E	Cattle, sheep, pigs
	Cats
Assemblage G	Domestic rats
Muskrats /Voles	Muskrats /Voles

Recently another taxonomy classification scheme has been suggested. This supports the idea that species specific assemblages may be different *Giardia* species. The proposed renaming is as follows.⁹

Table 1.3 Proposed *Giardia* species, previous assemblage and hosts

Proposed <i>Giardia</i> species	Previous assemblage	Host
<i>G. agilis</i>		Amphibians
<i>G. ardeae</i>		Birds
<i>G. muris</i>		Rodents
<i>G. psittaci</i>		Birds
<i>G. duodenalis</i>	Assemblage A	Humans and other primates, dogs, cats, livestock
<i>G. enterica</i>	Assemblage B	Humans, primates, dogs
<i>G. canis</i>	Assemblage C	Dogs
<i>G. cati</i>	Assemblage F	Cats
<i>G. bovis</i>	Assemblage E	Cattle and other hoofed livestock
<i>G. simondi</i>	Assemblage G	Rats

These suggested taxonomy changes have not been adopted.

MORPHOLOGY

Giardia intestinalis is a biphasic parasite. Trophozoites are the intestinal motile form; cysts are the environmentally more resistant form. Trophozoites are 12 - 15µm long by 5 - 9 µm wide. They are tear-drop shaped which are dorsally ventrally flattened. There are two anteriorly positioned nuclei.¹⁰ The ventral surface is concave with a ventral adhesive disc. There are four pairs of flagella (anterior, posterior, caudal and ventral) which in conjunction with the ventral disc aid in adherence to the host intestinal epithelium. The median bodies are found posterior to the ventral disc and their function is not known. These characteristics give the trophozoites the appearance of a face.^{1, 10, 11}

The cysts are 8 - 12µm long and 5 - 8 µm wide with four nuclei and intracytoplasmic axonemes visible. Cysts contain a mitotically arrested trophozoite.¹⁰ The cyst wall 0.3 to 0.5 µm

thick and composed of two layers. This tough outer covering allows the organism to be environmentally resistant.¹⁰

LIFE CYCLE

Giardia has two life stages; environmentally resistant cysts and the intestinal dwelling replicating trophozoites. The host usually acquires the organism by orally ingesting cysts. Ten to 100 cysts are required to establish an infection in the human host.² As cysts travel through the stomach, the decrease in pH is a stimulus to the parasite to prepare for excystation. Upon entering the duodenum, the environment changes to a more alkaline pH with the presence of pancreatic enzymes trypsin and chymotrypsin. These changes signal for excystation to occur. The presence of a cysteine protease in the parasite's endosome-lysosome like vacuoles aids in triggering the excystation process.¹¹ It has been suggested that the mechanical activity of the flagella may have an important part in triggering the opening of the cyst wall during the excystation process.¹² Separation of the cyst wall allows the organism emerge and divide quickly. The trophozoites numbers increase rapidly by binary fission.

Attachment of the parasite to the host epithelium is a complex process. In vitro it has been shown that it is necessary for both intestinal epithelial cells and the trophozoites to be metabolically active for attachment to take place. Fimbrial extensions of the extracellular matrix of epithelial cells come in contact with the trophozoites. Surface lectins may aid in binding the trophozoite to the epithelial cell. The lateral crest and the ventrolateral flange of the parasite were observed to make direct contact and imprints in the cultured epithelial cells.¹³ The force that maintains the attachment is the negative pressure generated beneath the ventral disk. This force is made by a combination of mechanical and hydrodynamic forces generated by the ventral flagella and contractile proteins of the ventral disk.¹³ Ventral disk tubulin appears to be different from that of the flagella's tubulin. The organism can adhere when flagellar function is abnormal, but if ventral disk tubulin is experimentally altered the organism cannot adhere to the intestinal surface.¹¹ Also, trophozoites have variant surface proteins covering their surface. *Giardia* has approximately 190 variant surface proteins genes in its genome. Only one surface protein is expressed at a time and the surface protein is changed every 6 -13 generations. This antigenic variation may explain how the organism escapes the host's protective mechanisms to establish

repetitive and persistent infections.^{1,5} Two hypotheses have been suggested to explain variant surface proteins. First, changing the surface antigens enables the parasite to evade the infected host's immune system. The host will mount a humoral response to the first surface antigen only later to be faced with a new surface antigen. The second hypothesis states that variable surface proteins are needed to survive in different intestinal environments.^{2,11}

Non-adhered trophozoites travel down the gastrointestinal tract with the ingesta and it is in the lower jejunum that the complex process of encystation begins. The presence of bile salts and a decrease in cholesterol signal the trophozoites to initiate the encystation process.¹² The trophozoites' endoplasmic reticulum produces the building blocks which form the outer cyst wall. The material is packaged into encystation vesicles. The vesicles transport the material to the cell surface where it is released. After the cell wall components are released the vesicle can reseal and may be endocytosed by the parasite or remain empty.¹² The completed cyst wall consists of a thick fibrillar outer cover and two inner cell membranes adjacent to the plasma membrane of the parasite. D-galactosamine polymer, cyst wall protein 1, cyst wall protein 2, and cyst wall protein 3 have been identified as structural components of the cyst wall.² The membrane components are deposited first at the lateral flange area. This causes cell rounding and a depression in the central ventral region of the parasite. Flagella are modified and are internalized by the elongating ventral membrane. The caudal flagella remain external until the last steps of encystation, and then they are retracted into a vacuole within the cysts. These flagella can be observed to be beating within the cyst.¹⁴ This cyst wall is what makes the cysts environmentally resistant.²

As cyst wall production is occurring, the trophozoite partially divides. Trophozoites undergo nuclear replication but not cytokinesis. These nuclei are genetically separate from each other. Daughter cells inherit one copy of each nucleus.^{11, 15} The nuclei can be asymmetrical in their karyotypes and DNA content. It has been proposed that there is at least one copy of each chromosome in each nucleus. To what extent they are different has yet to be discovered.¹¹ When the encystation process is completed, the cyst contains four nuclei, two bi-lobed adhesive disks and doubled flagella which are encapsulated by the cyst wall.¹ Further division of the mitotically arrested trophozoite will occur during excystation. The newly formed cysts are passed into the environment with the feces. Ingestion of cysts by a new host starts the life cycle

over again. The prepatent period, the time from ingestion of cysts to shedding cysts in the feces has been shown to be 5 – 16 days.^{16,18,19}

CYST RESISTANCE

Cysts have been shown to be viable for 56 to 84 days when suspended in lake water in winter conditions.²⁰ Survival of cysts in the environment shows a distinct temperature dependence.²¹ When cysts that were suspended in clean tap water at a pH of 7.6 and were held at 4°C in the laboratory they remained viable for 18 weeks. At 24°C cysts survived up to 14 weeks. Cysts maintained at 37°C were killed in 3 weeks. While at 50°C cysts were considered nonviable within 12 hours. When the temperature was decreased to -20°C, all cysts were killed in 24 hours. Cysts in feces stored at 24°C were no longer viable after 4 weeks. Air drying was also detrimental to cysts, 90% were killed within 24 hours.²¹ An experiment exposing cysts to Sterinol, a quaternary ammonium detergent, showed that at 0.5% concentration of the disinfectant, cidal effects were seen within 10 – 30 minutes. At 0.1% concentration 84% of the cysts were still viable after 30 minutes. The determination of viability was based on the eosin-exclusion method where cysts are exposed to a 1% eosin aqueous solution for 10 minutes. Cysts that became stained were considered nonviable.²¹

Resistance of cysts to supermarket disinfectants has also been evaluated.²² The products evaluated were Ajax and Old Dutch (chlorinated powders), Javex (liquid chlorine), Soft Scrub with bleach (semisolid chlorinated product), Lysol spray, Dettol, Pine Glo and Pine Sol (liquid phenols), ammonia and vinegar. The liquid phenols, ammonia and vinegar were diluted to half strength and the chlorinated products were serially diluted. Inactivation of cysts was achieved with 1 minute exposure to half strength Pine Glo and Dettol, full strength Lysol spray, and chlorinated products (powders, liquids, and semisolids) were diluted to 100 - 166 ppm of free active chlorine (FAC). Ammonia, vinegar and Pine Sol had a reduced ability to decrease cyst viability compared to the other disinfectants. Cyst viability was determined by the eosin-exclusion method, excystation and the ability of cysts to cause a patent infection in mice. The authors of the above study stated effective disinfection could be obtained by surface cleaning and then exposed for one minute to half strength Pine Glo, half strength Dettol, full strength Lysol

spray, Soft Scrub with bleach diluted 1/10 (150 ppm of FAC), Ajax diluted to 1/30 (166 ppm of FAC), or Javex with a dilution of 1/5 (100 ppm of FAC).²²

PATHOLOGY

The degree of symptoms suffered by the patient depends on the virulence of the organism, and on host factors including age, nutritional status, immune status and concurrent disease. The adhesion of the trophozoites can cause apoptotic of the intestinal epithelium increasing the permeability of the barrier. Tight junctions of the epithelium are disrupted adding to the increase in permeability. This increase in intestinal permeability in turn causes an inflammatory response and villous blunting.⁸ The chain of events that occur during infection with *Giardia* may lead to a combination of malabsorption and hypersecretion of electrolytes resulting in diarrhea. Although diarrhea is the most common clinical sign other signs include weight loss, flatulence, and lethargy. A large variability in severity of clinical signs can be observed.⁸

PREVALENCE IN DOGS

Giardia has been documented in animals worldwide. A 2005 study in Japan reported that 40% of cats surveyed were infected with *Giardia* by a fecal ELISA methodology. That study found a higher incidence in cats < 6 months of age and outdoor cats. They found no relationship between the presence of the parasite and clinical signs.²³ A survey of 250 cats in Mississippi and Alabama demonstrated that 13.6% of cats were shedding *Giardia* cysts when feces were evaluated using a centrifugation-flotation technique and a commercially available direct immunofluorescent antibody (IFA) kit. In these cats, only 35% of the positive cats had diarrhea while 6.1% of cats shedding *Giardia* cysts were apparently healthy. Of the *Giardia* infected cats in this study, 73.5% had a concurrent infection with *Cryptosporidium* spp.²⁴

Of the 130 dogs with diarrhea that presented to Colorado State University in 2003, 5.4% were positive for *Giardia* by flotation and an immunofluorescent assay.²⁵ A survey done using the Snap[®] *Giardia* antigen test from IDEXX Laboratories, Inc. Westbrook Maine,^a found an overall prevalence in dogs with gastrointestinal symptoms of 15.6% in the US. Regionally the

prevalence ranged from 19.2% in the northeast to 12.9% in the southeast.²⁶ *Giardia* has been found in many species of animals from beavers, river otters, coyotes, water buffalo and mollusks in all areas of the world.^{7, 27-30}

PREVALENCE IN HUMANS

Giardia spp. has been documented in humans worldwide and the prevalence depends on the geographic location. Voluntary reporting to the CDC shows that annually between 20,075 to 24,225 cases of giardiasis occurred in people within the United States between 1998 and 2003.³¹ The largest numbers of cases were reported in children 1-9 years of age and adults between 30-39 years of age. There also appears to be a summer seasonal peak of cases.^{31, 32} In Australia, in 2002, children were screened for the presence of *Giardia*. Of the 1306 samples evaluated, 7.6% (99 samples) were found to be positive by microscopy. Thirty-five percent of the positive children were positive more than once on subsequent testing. Twenty three isolates were successfully genotyped with 7 being assemblage A and 16 being assemblage B. The children infected with assemblage A were 26 times more likely to have diarrhea, although both groups had asymptomatic children. In that study no asymptomatic children became symptomatic.³³ These results conflict with what was found in 2001 in the Netherlands. A study enrolled 18 patients which had signs of diarrhea and were positive for *Giardia*. They were split into two groups, intermittent diarrhea vs. chronic persistent diarrhea. Their findings showed a strong correlation between assemblage A and intermittent diarrhea. Patients with persistent diarrhea were more often infected with assemblage B.³⁴ The conflicting results of these studies illustrate assemblage and clinical signs may not be linked.

ZOONOSIS

Interestingly epidemiological evidence is sparse supporting the importance of zoonotic transmission of *Giardia*.⁷ In New Zealand, contact with pets was not identified as a risk factor for giardiasis in children or adults.^{7, 16, 43} Similarly, studies in the USA, Canada and UK have not found animal contact a risk factor for human giardiasis.⁷

Studies indicated the majority of human infections occur with assemblage A and B organisms. In the A assemblage, the subtype AII is more common in people while animals infected with assemblage A are more likely to harbor subtype AI. However, infections with both subtypes have been documented in humans as well as many species of animals.⁷

When the aboriginal people of Australia were surveyed it was found that they harbored the *G. intestinalis* assemblage A and B while the indigenous dog population carried assemblage C and D. It was noted that in this community people do not cohabitate with the dogs.⁴⁴ The tea growing community of India has a large population of pet dogs which roam freely and enter the owner's homes. All of the human samples were of assemblage A or B; the dogs also harbored assemblage A and B.⁴⁵ When humans and animals in the Netherlands were sampled, the *Giardia* spp. from people were found to be assemblage A and B, while the dogs were found to have assemblage D, the goats had assemblage E and the deer had assemblage A.⁴⁶ Other studies conducted in central and northern Italy found mixed populations of assemblages in domestic animals. Dogs were found to harbor assemblages C, D, A and A& D, the single cat examined had assemblage F and calves had assemblages A, E and B. One of the isolates from a calf was identical to a human assemblage B isolate.⁶ A Maryland farm found 12% of the ewes and 4% of the lambs to be infected. One ewe carried assemblage A, all the rest harbored Assemblage E.⁴⁷ Portuguese calves were found to have a prevalence rate of 25.4% while 4.5% of adults were shedding. Assemblage E was the predominant type but B and AII were also found.⁴⁸ Seven horses from the state of New York and 2 horses from Australia were identified as carrying assemblage AI, AII, and B.⁴⁹ Recently in Egypt, 34.6% of 52 fecal samples from people evaluated were positive for *G. intestinalis*. Genotyping using the TPI loci was performed. Of the 18 positive samples, 1 was assemblage A, 14 were assemblage B and 3 were assemblage E.⁵⁰

A study took *Giardia* cysts from asymptomatic children in Brazil and grew them in culture. The *Giardia* cysts and trophozoites were orally administered to dogs. All dogs started shedding cysts on day 5 or 6 post inoculation. No dogs had clinical signs of illness. This study shows dogs can become infected when given *Giardia* from a human source. Unfortunately genotyping was not done in this study.¹⁶ These findings illustrate the need for continual surveillance to further understand the epidemiology of this parasite.

TESTING

In veterinary medicine centrifugation fecal flotation method has been traditionally used with zinc sulfate, Sheather's sugar or sodium nitrate flotation solutions for the identification of *Giardia* cysts. Due to the intermittent nature of cyst shedding it has been reported that multiple fecal samples maybe needed to achieve correct diagnosis.³⁵ In addition due to the small size of the cysts, microscopes should be equipped with an ocular micrometer to allow measurement and considerable skill is needed for correct identification of the parasite. This is often a stumbling block for the clinical practitioner and veterinary technician.

Sheather's sugar solution is an excellent solution for routine fecal flotations, but causes the *Giardia* cysts to appear crescent shape due to the disruption of the cyst's internal structures.³⁵ Zinc sulfate solution, while producing less distortion and disruption of the cysts, fails to float heavier parasite eggs such as *Taenia* spp. and *Physaloptera* spp.³⁵ Saline wet mounts from direct fecal smears is an insensitive way to recover many gastrointestinal parasites due to the small sample size evaluated and data on reliability of direct smears for recovery and identification of *Giardia* trophozoites and cysts is lacking.³⁵ To recover trophozoites in a saline wet mount, feces must be fresh and nonrefrigerated. Trophozoites may be observed more commonly in diarrheic feces. Trophozoites die quickly, so immediate evaluation of the slide is needed. Trophozoites have a characteristic "falling leaf" motion that aids in differentiating *Giardia* spp. from *Tritrichomonas foetus* and *Pentatrichomonas hominus*.³⁶

Immunofluorescent antibody (IFA) assays are available commercially. A fluorescent conjugated monoclonal antibody to cyst wall antigen is used to coat the surface of the parasite. The cyst fluoresces when using a light source with wavelength of 490 – 500 to excite it. The fluorescing cysts can then be visualized using a fluorescent.³⁷ While this is a highly specific test, to visualize the fluorescence one needs a fluorescent microscope equipped with the proper filter as select the wavelength of the fluorescent dye. These requirements make this procedure impractical and cost prohibitive for use in private practice.

The polymerase chain reaction (PCR) has revolutionized the field of molecular diagnostics. It allows scientists to amplify an organism's DNA without the time consuming and often unsuccessful need to culture the parasite. PCR techniques have been developed for several *Giardia* spp. gene loci, such as the SSU-rRNA, elongation factor1-alpha, Triosephosphate

isomerase, glutamate dehydrogenase, and β -giardin.³⁸ The technology is highly specific but sensitivity fluctuates. A 2007 study showed that a FTA filter paper method for cyst capture to be the most effective. It was capable of detecting as few as 168 cysts per ml. The SSU-rRNA gene loci using primers RH11/RH4 and GiarF/GiarR was the most sensitive loci to locate.³⁸ While this technology has advanced scientific study greatly, it is not yet ready for mass screening of fecal samples from veterinary practices.

IDEXX Laboratories^a has marketed a *Giardia* fecal antigen test for in-clinic use at veterinary practices. The SNAP[®] test from IDEXX is a lateral flow chromatographic assay designed to detect cyst wall protein. Encystation vesicles release cyst wall proteins when forming the cyst outer wall some escapes in to the feces and this is what the SNAP[®] test is detecting.^{39, 40} According to manufacturer's instructions this test can be performed on fresh feces, previously frozen feces or feces that have been refrigerated at 2°-8°C for 7 days. The SNAP device, reagent and feces must be at room temperature prior to performing the test. This test is easy to run and interrupt the results. The gold standard for comparison is the IFA. In one study comparing the IFA assay and SNAP[®] *Giardia* fecal antigen test, the IFA assay detected 24% of the samples to be positive and 13% of the samples were positive by SNAP[®] test.⁴¹ Another study in cats compared zinc sulfate centrifuged fecal flotation, enzyme immunoassays and an IFA assay. It was found that the zinc centrifugation fecal flotation and IDEXX SNAP[®] test had very comparable results. The float was 85.3% sensitive and 99.7% specific and the IDEXX SNAP[®] test was 85.3% sensitive and 100.0% specific.⁴² A personal observation is that there is no correlation between the coloration of a positive test and a number of cysts being shed.

TREATMENT AND CONTROL

Medications such as nitroimidazoles, benzimidazoles, furazolidine, nitazoxanide and quiniacrina have all been used successfully in people for the treatment of giardiasis. No medications are approved for the treatment of *Giardia* in animals in the United States. Fenbendazole is approved for the treatment of *Giardia* in dogs in Europe. Veterinary medicine has relied most heavily on metronidazole, fenbendazole and febantel.

Metronidazole has been used successfully for years. Metronidazole at a dose of 25mg/kg every 12 hours for 7 days eliminates the organism in cats experimentally infected with *Giardia* from a human host. This regimen was efficient in eliminating the organism.¹⁷ Metronidazole utilizes the anaerobic metabolic pathways present in the *Giardia* organism. The drug is taken in to the parasite and activated by reduction of the nitro group. The reduced drug form will covalently bind to the parasites DNA. The result is DNA damage and inhibition of trophozoite respiration.⁵¹ Resistance to metronidazole has been induced in vitro.⁵¹

Fenbendazole is in the benzimidazole class of parasiticides. This chemical exerts its toxic effect on the *Giardia* organism by binding to the β -tubulin cytoskeleton. The binding causes both inhibition of cytoskeleton polymerization and impaired glucose uptake. This ultimately causes an inhibition of energy metabolism.⁵¹ Dogs treated with 50mg/kg of fenbendazole orally for 3 days and given a bath after the last treatment, showed reduce shedding of cysts by 90%.^{52,53} Cats infected with *Giardia* from a human host were treated with 50mg/kg/day of fenbendazole orally for 5 days. Cysts shedding fell to non-detectable levels in 4 of the 8 cats. Three cats had reduced shedding and one cat had no shedding for one week then resume shedding cysts. These cats were coinfectd with *Cryptosporidium* sp. and no baths were given after treatment.⁵⁴

A combination product of febantel, praziquantel, and pyrantel (Drontal[®] Plus) is marketed as a broad spectrum dewormer for dogs. Febantel is metabolized to fenbendazole and oxyfenbendazole.⁵³ Drontal has been shown to be effective in dogs after 3 to 5 days of administration with the febantel dosed at 25-35mg/kg.^{53,55} Efficacy of treatment can be increased by giving treated dogs a bath on the last day of treatment.⁵³

Furazolidone undergoes reductive activation in the trophozoite. The antiprotozoal effects cause damage to cellular components including DNA. Resistance to furazolidone is caused by decreased entry of the drug into the parasite or with increased levels of thiol-cycling enzymes, which defend against toxic radicals.⁵¹ Furazolidone is currently not widely used for the treatment of giardiasis in veterinary medicine due to the unwanted side effects of vomiting and decrease in appetite.¹⁷

Nitazoxanide is thought to inhibit the pyruvate:ferredoxin/ferredoxin oxidoreductase enzyme-dependent electron transfer reaction(PFOR system). Nitazoxanide is a noncompetitive inhibitor of the PFOR system in *Giardia*. It has been proposed that nitazoxanide inhibits the first step in the PFOR reaction by interfering with the binding of pyruvate to the thiamine

pyrophosphate cofactor.⁵⁶ This drug is not commonly used and little information about dosing for dogs and cats is available at this time.

Two newer drugs may be effective for use in the treatment in animals. Tinidazole (Tindamax or Fasigyn) has recently been approved for treatment of giardiasis in people in the United States.⁵⁷ It is a second generation nitroimidazole. Its mechanism of action is not clearly understood, but thought to act via production of cytotoxic free radicals.⁵⁸ A similar drug, ronidazole (Ridzol) has been used for treatment of Blackhead in turkeys caused by the protozoa *Histomonas melagris* and recently recently explored as a treatment for *Tritrichomonas foetus* in cats.⁵⁹

Eliminating re-exposure to infective cysts is important to decreasing reinfection. A bath for the animal after the last dose of medication will remove infective cysts that are remaining on the animal's coat. Environmental cleanup is essential in controlling re-exposure.⁵³

VACCINE

In 1999 a vaccine approved for dogs and cats became available from Fort Dodge Animal Health, Fort Dodge, IA. C The vaccine consists of chemically inactivated trophozoites. Initial studies in kittens showed the vaccine decreased number of cysts being shed, a decrease number of trophozoites in the small intestine of infected cats, decrease number of cats with trophozoites in the small intestine and decrease viability of the cysts.⁶⁰ In another study the same author evaluated the vaccine in 13 dogs that were infected with *Giardia* and had not cleared of infection with medication. All dogs had a decrease in their clinical signs and cessation of shedding of cysts after vaccination.⁶¹ Puppies that had been vaccinated showed a significant decrease in number cysts as compared to controls.⁶² Subsequent studies in different laboratories have shown the vaccine did not change the course of the infection in cats, did not significantly decrease cyst shedding in asymptomatic research dogs and did not prevent recurrence of cysts in the feces from research dogs.^{53, 63, 64} The 2006 AAHA canine vaccine guidelines place the *Giardia* vaccine in the not recommended category.⁶⁵

Vaccines may be useful in the future. Research has produced a novel DNA vaccine encoding for *Giardia* cyst wall protein 2 using a *Salmonella typhimurium* bactofection vehicle for use in mice. The vaccine led to a mixed Th1/Th2 cellular immune response as well as IgA and IgG cyst wall protein 2 antibodies found in intestinal secretions of the vaccinated mice. After challenge with *Giardia muris*, vaccinated mice had a 60% reduction in cysts shedding compared to control mice.⁶⁶

CONCLUSION

Giardia is a common intestinal parasite infecting a variety of hosts worldwide. This parasite was once thought to be an evolutionarily simplistic organism; now new research is just starting to unlock some of the secrets about the parasite that affects so many species. New research techniques will help to differentiate the molecular differences between species and assemblages of *Giardia*. This may help to resolve the debate about the zoonotic/anthroponic potential of this parasite. The prevalence of the *Giardia* is not likely to diminish. The more we know the more it becomes obvious that we should not rely solely on pharmaceuticals as the answer for control of this parasite. Novel methods for environmental control and treatment are needed. Currently there is an abundance of information known about this parasite but we are a long way from knowing all there is to know about *Giardia*.

CHAPTER 2 - Comparison of the Snap[®] *Giardia* Fecal Antigen Test and the Zinc Sulfate Double Centrifugation Fecal Flotation to Diagnose *Giardia intestinalis* Infections in Two Populations of Infected Dogs

INTRODUCTION

Giardia is a common intestinal protozoan parasite of pet dogs and cats. This parasite has two morphological forms, trophozoites and cysts. Trophozoites are the motile form found within the intestinal lumen. Trophozoites measure approximately 8 µm wide by 15 µm long. They can be identified by the presence of two anterior nuclei, axonemes present between the nuclei, the median bodies in the posterior third of the organism, and four pairs of flagella.^{10, 11, 67} These structures give the trophozoites their unique face-like appearance. Trophozoites start the encystation process when cues such as the presence of bile salts, fatty acids and a change to an alkaline pH, are detected in the small intestine.⁶⁷ Cysts are the environmentally resistant form of the parasite. Most infections occur by ingestion of cysts which are immediately infective after being passed into the environment. They are approximately 7 µm wide by 12 µm long. Cysts contain two incompletely separated trophozoites. Internal structures that can be visualized are the axonemes, fragments of the ventral disks and nuclei. The cysts protective outer wall allows the organism to survive outside the host.² Cysts can survive long periods of time in cold wet areas but are susceptible to destruction in hot arid environments.²⁰⁻²² Infection typically occurs through ingestion of cysts. Once the cysts are ingested and pass through the stomach, excystation occurs allowing the trophozoite form to proliferate.^{10, 26, 67} The prepatent period of *Giardia* spp. has been found to be 5 – 16 days.^{16, 18, 19}

Veterinarians commonly attempt to diagnose giardiasis in clinic by direct smear saline wet mounts, fecal flotation or the Snap[®] *Giardia* antigen test by IDEXX laboratories^b. When observed using saline wet mounts, the motile trophozoites have a tumbling or falling leaf motion.³⁶ Direct smears are considered a the least sensitive diagnostic technique due to the small amount of feces being evaluated and lack of concentration of the sample.³⁵ Fecal flotation

is used for recovery and identification of cysts. Zinc sulfate is commonly used as the flotation solution because it is effective in floating the cysts with minimal distortion to the internal structures.³⁵ Cysts can also be stained with Lugol's iodine for easier visualization. Sugar flotation solutions used for routine fecal exams can cause distortion of the cysts making them more difficult to identify. Technical skill is needed to correctly identify cysts as they can easily be confused with yeast or other small debris.³⁵ As trophozoites are encysting, cyst wall proteins are made and packaged into encystation vesicles. These vesicles come to the surface of the organism and release the cyst wall proteins that are incorporated in the protective outer covering of the cyst.⁶⁸ The ELISA based technology of the Snap[®] *Giardia* antigen test uses antibodies specific to *Giardia* cyst wall proteins released into the feces during the encystation process. The lateral flow technology allows a blue color to be visualized when antibody binds *Giardia* cyst wall antigen.^{39, 40} Other diagnostic methods such as immunofluorescent assays and PCR are available at diagnostic laboratories but are not suited to be used as an in-house diagnostic test at veterinary clinics.

Due to intermittent cyst shedding it has been shown that flotation of three separate fecal samples from one individual will increase the accuracy of the diagnosis of giardiasis.^{35, 69} However, in most veterinary practices it may be difficult to conduct fecal flotation or Snap[®] tests on three fecal samples.

Although there are several proven therapies for treatment of *Giardia* in dogs; no medications are labeled for the treatment of giardiasis in the dog in the United States. The most common medications used for treatment include metronidazole, fenbendazole, and Drontal[®] Plus containing febantel. Febantel is metabolized to fenbendazole and oxyfenbendazole.^{52, 53, 55} It is also well recognized that a bath on the last day treatment is extremely important to remove infective cysts from the hair coat of the animal.⁵³ Environmental control measures including washing bedding, washing food and water bowls, and immediate clean up of feces with appropriate disposal may help reduce the risk of immediate reinfection.

After a diagnosis, appropriate therapy, and environmental control, the next question for veterinarians is when to retest. While it has been suggested that the IDEXX Snap[®] fecal ELISA test will remain positive for up to two weeks after successful treatment, there is no published data to indicate whether this true.^b

The goals of this study were to compare the IDEXX Snap[®] fecal ELISA test and a zinc sulfate double centrifugation fecal float in detecting *Giardia* infections in dogs. Also, to determine if there is an increase in the diagnostic sensitivity when three fecal samples from one dog were analyzed daily or were combined into a composite sample for evaluation by both methods. Finally, how long after treatment with Drontal[®] Plus and a bath will fecal samples remain positive with the IDEXX Snap[®] fecal ELISA test or zinc sulfate double centrifugation fecal flotation.

METHODS AND MATERIALS

Assessment of feces

Forty nine purpose-bred research beagles from a facility with a history of chronic *Giardia* infection (Group 1), and eighteen dogs of various breeds from a commercial kennel and twelve dogs from a regional animal shelter (Group 2) had fecal samples collected on three consecutive days. A zinc sulfate double centrifugation flotation was performed on each fecal sample. The zinc sulfate centrifugation technique was conducted using approximately five grams of feces. Twenty mLs of tap water was added to each sample. The samples were suspended in the water and strained into a 40mL centrifuge tube. This suspension was centrifuged for 5 minutes at 280xg. The supernatant was discarded. The sediment was resuspended with a zinc sulfate solution with a specific gravity of 1.18. This solution was poured into a 12 mL centrifuge tube and filled to a slight positive meniscus. An 18mm² cover slip was placed on top of the tube. The samples were centrifuged in a swinging-head centrifuge for 5 minutes at 280x g, and then allowed to stand for 10 minutes. A drop of Lugol's iodine was placed on a slide and the cover slip was transferred to the slide. The slide was examined at 100X magnification by personal in the K-State Veterinary Parasitology Diagnostic Laboratory trained in *Giardia* cyst identification.

Each fecal sample was also evaluated using the Snap[®] *Giardia* antigen test.^a The tests were performed according to product insert directions. The test kits were stored at 2°C (35.6 °F) and were allowed to come to room temperature prior to use. The test kit swab, after being removed from the plastic sleeve, was coated with a thin layer of fecal material from each sample. The plastic sleeve was replaced and the reagent was mixed by breaking the valve stem and pressing the bulb three times. The plastic sleeve was removed and five drops of the

sample/conjugate solution was placed in the sample well of the Snap[®] test device. When the sample solution first appeared in the activation circle of the Snap[®] test device, it was depressed. After eight minutes the test result was read. A blue colored will appear in the sample circle if the test is positive for detecting of *Giardia* cyst wall protein.³⁹

For Group 1, purpose bred research beagles, fecal samples that were positive by both methods were considered diagnostic for *Giardia*. Any fecal sample that was not positive by both diagnostic test methodology (flotation or Snap[®] *Giardia* antigen test), was also evaluated using the MeriFluor[®] *Cryptosporidium/Giardia* immunofluorescent antibody assay (IFA).^c In preparation for the IFA assay a concentrating procedure was conducted to maximize cyst recovery. Two grams of feces from each sample was suspended in 1.5X phosphate buffer saline with EDTA and strained through sterile gauze. The liquid was layered on 7mLs of a sugar solution (specific gravity of 1.13) in a 15mL centrifuge tube. The tubes were centrifuged at 800 x g for 10 minutes in a fixed angle centrifuge. After centrifuging, the interface was pipetted off into a new 15 mL centrifuge tube. PBS-EDTA was added to bring the volume to 12mLs. The sample was centrifuged at 1200 x g for 10 minutes. The supernatant was decanted and resuspended in 8 mLs PBS-EDTA. The tube was then centrifuged for 10 minutes at 1200 x g. Again, the supernatant was decanted. The pellet was resuspended with PBS-EDTA to a volume 1 ml. 15 μ L of sample was placed on one well of the Merifluor slide.⁷⁰ Positive and negative controls were applied to separate wells. The samples were allowed to air dry on to the slides for approximately 30 minutes. The slides were stained according to product directions with a fluorescein isothiocyanate (FITC) labeled monoclonal antibodies to *Giardia* cyst wall antigen. The slides were incubated in a room temperature (21°C/ 69.8°F) chamber in the dark for 30 minutes. After incubation, the slides were rinsed with a buffer wash supplied by the test kit and a drop of mounting medium was applied. A cover slip was placed and the slide was viewed at 100X magnification with a fluorescent microscope with a filter system for FITC with the following parameters: Excitation wavelength consistent of- 490-500 nm, and a barrier filter - 510-530 nm were used to identify the presence of fluorescent *Giardia* cysts.³⁷

To produce a composite sample approximately 2 grams of feces were collected from each of the three daily fecal samples and combined in a cup and thoroughly mixed with a wooden tongue depressor. Using the composite sample, a Snap[®] *Giardia* antigen test and zinc sulfate double centrifugation flotation were performed as previously described. Samples that were

positive by both methods were considered diagnostic for giardiasis. Samples that were not positive by both methodologies had the IFA performed as described using 1 gram of the composite sample. Based on the performance of the Snap[®] *Giardia* antigen test and zinc sulfate double centrifugation in Group 1 purpose-bred research beagles it was decided that for Group 2 dogs the IFA assay would be performed on every fecal sample.

Twenty three beagles (Group1) were available for post treatment diagnostic evaluation. The dogs were treated with Drontal[®] Plus tablets^d (68.0 mg praziquantel, 68.0 mg pyrantel base as pyrantel pamoate and 340.2 mg febantel.) The beagles were administered the label dose of Drontal[®] Plus orally once daily for three consecutive days so they received 25 – 35mg/kg (11.36 – 15.90 mg/lb) febantel daily. On the last day of treatment a bath was performed using a detergent based non-insecticidal pet shampoo. Following bathing the dogs were returned to their cleaned runs. Eleven (11) of the research beagles were housed individually in indoor concrete runs and twelve (12) research beagles were housed individually in in/outdoor concrete runs. The runs were cleaned daily with water. Once to twice weekly the concrete runs were scrubbed with soap and water and sanitized with Coverage Plus NPD^{®e}, a product consisting of four quaternary ammonium compounds. Fecal samples were collected from the treated dogs every other day starting one day post treatment for 21 days. A total of eleven samples from each dog were collected. A zinc sulfate double centrifugation flotation, Snap[®] *Giardia* antigen test and the MeriFluor[®] *Cryptosporidium/Giardia* immunofluorescent assay were performed on each sample. The three tests were conducted as described above.

STATISTICAL ANALYSIS

Detection methods for *Giardia* were initially analyzed separately for Group 1, purpose bred beagles, and Group 2, shelter and commercial kennels, dogs due to differences in the population of dogs. Data for the two groups of dogs were analyzed as follows. In the first analysis, to compare the three methods, samples from the three individual days and the composite samples were all analyzed separately using Cochran's Q (at a significant level of 0.05) because of within dog correlation.⁷¹ When Cochran's Q was significant, pairwise comparisons were done between methods using McNemar's test ⁷¹ with the significant level of

0.05. Group 2 shelter and commercial kennels dogs, this analysis was conducted using all n=30 dogs. However, because the IFA analysis was not complete for all laboratory beagles, sub-analysis were conducted to compare the testing methods. McNemar's test with a significance level of 0.05 was used to compare the fecal flotation and fecal ELISA methods on 37 of the group 1 dogs. McNemar's test with a significance level of 0.05 was also used to compare 32 of the group 1 dogs that had all three methods of testing performed.

An analysis pooling the two groups was a Pearson's Chi-square test of homogeneity to test if the groups differed with respect to *Giardia* status as measured by either fecal flotation or fecal ELISA, separately for the three individual days and the composite sample.

Finally, a pooled proportion of positive testing dogs were calculated for each group and detection method by defining "positive" as dogs that had tested positive on at least one the of three individual days by a specific method and "negative" as dogs that did not test positive on any of the three days by a specific method. The calculated proportion with a 95% confidence interval is reported for each group and method.

RESULTS

Comparison of diagnostics tests

Group 1- Thirty-seven of the 49 dogs in Group1 were found to be shedding *Giardia* cysts on at least one of three fecal samples by either the zinc sulfate double centrifugation fecal flotation, the Snap[®] *Giardia* antigen test, or IFA. (Table 2.1) Thirty-six beagles had at least one fecal sample test positive with the IFA. One beagle was positive by fecal flotation and the Snap[®] *Giardia* antigen test (fecal ELISA test) on all three individual daily fecal samples and the composite sample so an IFA was not performed. Of the beagles identified as shedding cysts on at least one of the 3 days, cysts were recovered in 22 (59.5%), 20 (54.1%) and 19 (51.4 %) of the 37 fecal samples collected on days, 1, 2 & 3, respectively using the zinc sulfate double centrifugation fecal flotation technique. Fecal ELISA tests were positive in 8 (21.6%), 18 (48.6%) and 15 (40.5%) of the 37 fecal samples collected on days, 1, 2 & 3, respectively. The composite samples had cysts recovered by fecal flotation in 23 of the 37 (62.2%) samples and 12 of 37 (32.4%) were fecal ELISA positive.

Table 2.1 Purpose bred beagles (Group 1) positive for *Giardia* by either fecal flotation or Fecal ELISA on daily fecal samples and a composite fecal sample

Fecal Sample ¹	Fecal Flotation positive ³	Fecal ELISA Positive ⁴
Day 1	22/37 (59.5%) ^a	8/37 (21.6%) ^b
Day 2	20/37 (54.1%) ^a	18/37 (48.6%) ^a
Day 3	19/37 (51.4%) ^a	15/37 (40.5%) ^a
Composite sample ²	23/37 (62.2%)	12/37 (32.4%)

¹ Fecal samples were collected daily for 3 days from each beagle

² A 2 gm sample from each of the 3 days was combined to make a composite sample

³ Zinc sulfate double centrifugation fecal flotation stained with Lugol's iodine

⁴ IDEXX Snap[®] *Giardia* cyst antigen test kit

Value within rows with unlike superscripts are significantly different (p>0.05)

In Group 1, thirty-two of the 49 beagles had fecal flotation, fecal ELISA, and fecal IFA done on every fecal sample and cysts were seen on at least one of the 3 days. Cysts were

recovered in 19 (59.4%), 15 (46.9%) and 14 (43.8 %) of the 32 fecal samples collected on days, 1-3 respectively, using the zinc sulfate double centrifugation fecal flotation technique. Fecal ELISA tests were positive in 5 (15.6%), 13 (40.6%) and 11 (34.4%) of the 32 fecal samples collected on days, 1, 2 & 3, respectively. Fecal IFA tests were positive in 23 (71.9%), 21 (65.6%) and 26 (81.3%) of the 32 fecal samples collected on days 1-3 respectively. Composite fecal samples were positive 18/32 (56.3%), 8/32 (25.0%), and 28/32(87.5%) of the time using fecal flotation, fecal ELISA and IFA, respectively.

Table 2.2 Purpose-bred beagles (Group 1) positive for *Giardia* by fecal flotation, fecal ELISA or fecal immunofluorescent assay on daily fecal samples and a composite fecal sample

Fecal Sample ¹	Fecal Flotation positive ³	Fecal ELISA Positive ⁴	Fecal IFA Positive ⁵
Day 1	19/32 (59.4%) ^b	5/32 (15.6%) ^a	23/32 (71.9%) ^b
Day 2	15/32 (46.9%) ^{ab}	13/32 (40.6%) ^a	21/32 (65.6%) ^b
Day 3	14/32 (43.8%) ^a	11/32 (34.4%) ^a	26/32 (81.3%) ^b
Composite sample ²	18/32 (56.3%) ^b	8/32 (25.0%) ^a	28/32 (87.5%) ^c

¹ Fecal samples were collected daily for 3 days from each beagle

² A 2 gm sample from each of the 3 days was combined to make a composite sample

³ Zinc sulfate double centrifugation fecal flotation stained with Lugol's iodine

⁴ IDEXX Snap[®] *Giardia* cyst antigen test kit

⁵ MeriFluor[®] *Cryptosporidium/Giardia* immunofluorescent assay

Value within rows with unlike superscripts are significantly different (p>0.05)

Ten beagles had cysts visualized by fecal flotation in all three samples and 6 beagles were fecal ELISA positive on all three samples. (Table 2.3) Ten beagles had cysts visualized by flotation on two of the three samples and 10 beagles were fecal ELISA positive on two of the three samples. Eleven beagles had cysts visualized by flotation results on only one of the three samples while 3 beagles were fecal ELISA positive on only one of the three samples. Six beagles did not have cysts seen on flotation any of the three days and 18 beagles were fecal ELISA negative all three days. Thirty one of the 37 beagles had at least one day they were shedding cysts that could be identified using the flotation method. Nineteen of the 37 dogs had at least one fecal ELISA that was positive on at least 1 of the three samples.

Table 2.3 Purpose-bred beagles (Group 1) positive for *Giardia* on 1, 2, or 3 consecutive daily fecal samples using either fecal flotation or fecal ELISA

Samples ¹	Fecal Flotation ²	Fecal ELISA ³
0 positive samples	6/37 (16.2%)	18/37 (48.6%)
1 positive sample	11/37 (29.7%)	3/37 (8.1%)
2 positive samples	10/37 (27.0%)	10/37 (27.0%)
3 positive samples	10/37 (27.0%)	6/37 (16.2%)
At least 1 of 3 three samples positive	31/37 (83.8%)	19/37 (51.4%)

¹ Fecal samples were collected daily for 3 days from each beagle

² Zinc sulfate double centrifugation fecal flotation stained with Lugol's iodine

³ IDEXX Snap[®] *Giardia* cyst antigen test kit

Group 2 - Three fecal samples were collected daily from 30 shelter/commercial kennel dogs. All of the Group 2 dogs were found to be shedding cysts on at least one of the 3 fecal samples. *Giardia* cysts were identified in 24 (80.0%), of the 30 fecal samples collected on each of days, 1-3 using the zinc sulfate double centrifugation fecal flotation technique, but these were not necessarily always the same dogs (Table 2.4). Fecal ELISA tests were positive on 23 (76.7%), 25 (83.3%) and 22 (73.3%) of the 30 fecal samples collected on days 1-3 respectively. Cysts were observed by fecal IFA on 28 (93.3%), 27 (90.0%) and 29 (96.7%) of the 30 fecal samples collected on days, 1, 2 & 3, respectively. Zinc sulfate double centrifugation fecal flotation of the composite sample found 26 (86.7%) samples to have cysts visualized. Twenty five (83.3%) of these composite samples were fecal ELISA positive. All of the 30 fecal IFA composite samples had cysts visualized.

Table 2.4 Shelter and commercial kennel dogs (Group2) positive for *Giardia* by either fecal flotation, Fecal ELISA or fecal immunofluorescent assay on daily fecal samples and a composite fecal sample.

Fecal Sample ¹	Fecal Flotation positive ³	Fecal ELISA Positive ⁴	IFA ⁵
Day 1	24/30 (80.0%) ^a	23/30 (76.7%) ^a	28/30 (93.3%) ^a
Day 2	24/30 (80.0%) ^a	25/30 (83.3%) ^a	27/30 (90.0%) ^a
Day 3	24/30 (80.0%) ^a	22/30 (73.3%) ^a	29/30 (96.7%) ^b
Composite sample ²	26/30 (86.7%) ^a	25/30 (83.3%) ^a	30/30 (100.0%) ^b

¹ Fecal samples were collected daily for 3 days from each beagle

² A 2 gm sample from each of the 3 days was combined to make a composite sample

³ Zinc sulfate double centrifugation fecal flotation stained with Lugol's iodine

⁴ IDEXX Snap[®] *Giardia* cyst antigen test kit

⁵ MeriFluor[®] *Cryptosporidium/Giardia* immunofluorescent assay

Value within rows with unlike superscripts are significantly different (p>0.05)

All 3 daily fecal samples were positive by zinc sulfate double centrifugation fecal flotation in 19 (63.3%) dogs, by fecal ELISA in 16 (53.3%) dogs and by IFA in 25 (83.3%) dogs (Table 2.5). Two of the three fecal samples were positive by fecal flotation, fecal ELISA and IFA in 6 (20.0%), 9 (30.0%), and 4 (13.3%) dogs, respectively. One of the three fecal samples were positive by fecal flotation, fecal ELISA and IFA in 3 (10.0%), 4 (13.3%) and 1 (3.3%) dog, respectively. Two (6.7%) of the commercial kennel/shelter dogs were never positive by fecal flotation on any of the 3 daily fecal samples and one (3.3%) was never positive by fecal ELISA. Every dog was positive at least once by IFA.

Table 2.5 Shelter and commercial kennel dogs (Group2) positive for *Giardia* on 1,2 or 3 consecutive daily fecal samples using either fecal flotation or fecal ELISA.

Samples ¹	Fecal Flotation ²	Fecal ELISA ³	IFA ⁴
0 positive samples	2/30 (6.7%)	1/30 (3.3%)	0/30 (0%)
1 positive sample	3/30 (10.0%)	4/30 (13.3%)	1/30 (3.3%)
2 positive samples	6/30 (20.0%)	9/30 (30.0%)	4/30 (13.3%)
3 positive samples	19/30 (63.3%)	16/30 (53.3%)	25/30 (83.3%)
At least 1 of 3 positive samples	28/30 (93.3%)	29/30 (96.7%)	30/30 (100%)

¹ Fecal samples were collected daily for 3 days from each beagle

² Zinc sulfate double centrifugation fecal flotation stained with Lugol's iodine

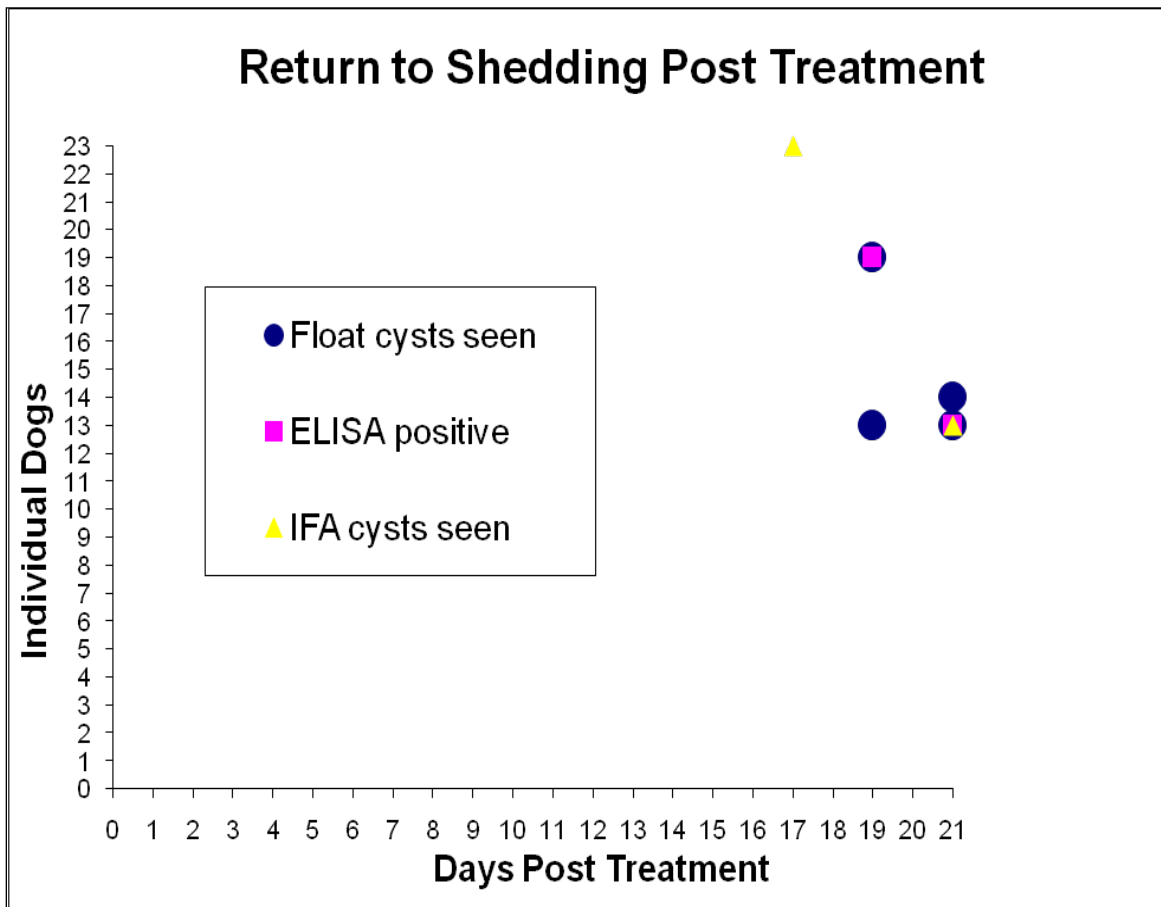
³ IDEXX Snap[®] *Giardia* cyst antigen test kit

⁴ MeriFluor[®] *Cryptosporidium/Giardia* immunofluorescent assay.

Post treatment results

Twenty three of the beagles from Group 1 were available for the treatment phase of the study. Interestingly within one day after the three day treatment protocol with Drontal[®] plus and a bath, all 23 beagles were negative by zinc sulfate double centrifugation fecal flotation, fecal ELISA and IFA. Nineteen (82.6%) of the beagles did not re-shed cysts during the 21 day post-treatment evaluation period. Four beagles had cysts identified by either fecal flotation or IFA between days 17 and 21 post-treatment. Two also had positive fecal ELISA tests. (Figure 2.1)

Figure 2.1 23 Beagles from Group 1 treated with the label dose of Drontal Plus for 3 days with a bath on the last day. Post treatment fecal samples evaluated by fecal flotation, fecal ELISA and IFA performed every other day for eleven samples or 21 days.



DISCUSSION

Giardia is a common intestinal parasite encountered by veterinary practitioners. It is well known that cysts are intermittently shed making accurate diagnosis challenging. Ideally three fecal samples should be collected on separate days and analyzed before a negative diagnosis is made.³⁵ Historically, fecal flotation using 1.18 sp gr zinc sulfate as the flotation solution has been used to identify cysts. Conducting the proper centrifugation technique along with appropriate training of personnel to properly identify the cysts is necessary. Debris and yeast often resemble cysts and the small cysts are easily overlooked. IDEXX laboratories have developed a table top ELISA based test that detects *Giardia* cyst wall antigen that is released by encysting trophozoites.³⁹

When evaluating the day 1 fecal samples from the purpose bred beagles (Group 1), 22 had cysts recovered by zinc sulfate double centrifugation fecal flotation but only 8 were fecal ELISA positive. This disparity was significant. The day 2 and day 3 fecal samples from the purpose bred research beagles (Group 1) and all three samples from kennel/humane society dogs (Group 2) had no significant differences in the number of positive samples identified between the zinc sulfate double centrifugation flotation and the fecal ELISA test methods.

There was no difference in the number of positive samples identified between each individual sampling day and the 3-day composite sample from the purpose bred beagles and kennel/humane society dogs using the zinc sulfate double centrifugation flotation method. There was a difference with the second sample from the purpose bred beagles which had 18 fecal ELISA positive while the composite fecal sample found only 12 fecal ELISA positive. There was no difference between fecal ELISA conducted on the 3-day composite fecal sample and fecal samples collected on days 1 and 3 from purpose bred beagles or all 3 daily fecal samples from kennel/humane society dogs. When making the 3-day composite fecal sample many variables are encountered. The intermittent shedding of cysts may have a dilution effect on the 3-day composite fecal sample and cysts and cyst wall protein may start to degrade over time making it more difficult to obtain a positive diagnosis. The data indicates there is no advantage to performing diagnostic testing on a 3-day fecal composite sample over an individual sample.

Due to the intermittent shedding of cysts it has been previously shown that evaluating three fecal samples by flotation methods increase the sensitivity of finding a positive result up to 94%.^{35,72} In the Group 1 purpose bred research beagles, 31/37 (83.8%) dogs had at least one positive fecal out of the 3 fecal samples collected using the zinc sulfate double centrifuge fecal flotation. In contrast, the individual days results were 22 (59.5%), 20 (54.1%) and 19 (51.4%) out of 37 being positive on days 1, 2, and 3. This was also true for the fecal ELISA results. There were 19/37 (51.4%) dogs that had at least one positive sample out of three collected. This contrasted from the individual days results of 8 (21.6%), 18 (48.6%) and 15 (40.5%) of 37 were positive on days 1, 2, and 3.

In the Group 2 shelter and commercial kennel dogs, 28/30 (93.3%) dogs had at least one sample out of three collected that were positive by double centrifuge fecal flotation. This contrasted from the individual days results of 24/30 (80.0%) were positive on each of days 1, 2, and 3. Using the ELISA test there were 29/30 (96.7%) dogs fecal ELISA positive at least once out of the three samples collected. This contrasts from the individual days results of 23 (76.7%), 25 (83.3%) and 22 (73.3%) out of 30 being positive on days 1, 2, and 3.

Both groups of dogs had cysts detected intermittently. Both groups of dogs had individual animals with intermittently positive fecal ELISA tests. There was a striking difference in the number of times cysts were seen or ELISA's were positive by either method between the two populations of dogs. Group 2 dogs had cysts seen and fecal ELISA's positive more frequently than Group 1 purpose bred research beagles. Group 1 dogs individual fecal samples has cysts seen using the fecal flotation method 22/37 (59.5%), 20/37 (54.1%) and 19/37 (51.4%) for each day's sample. The fecal ELISA tests were positive 8/37 (21.6%), 18/37 (48.6%) and 15/37 (40.5%) for each day's sample. Group 2 shelter and commercial kennel dogs individual fecal samples had cysts seen using the fecal flotation method 24/30 (80%) for each day's samples. The fecal ELISA test was positive 23/30 (76.7%), 25/30 (83.3%), and 22/30 (73.3%) for each day's sample.

The Group 2 shelter and commercial kennel dogs live in a more stressful and changing environment, with variable nutrition, possible parasitism with more than one parasite, and possibly other concurrent diseases. The group 1 purpose bred beagles live in a controlled environment with good nutrition and no other parasites or concurrent diseases. The purpose bred research beagles have limited genetic diversity and may be living with a strain of *Giardia* which

may have been endemic in the research beagles' environment. In this laboratories experience purpose bred research beagles from suppliers are commonly infected with *Giardia* but rarely have diarrhea associated with these infections. It is speculated that these environmental and disease variables play a role in the diagnostic disparity between the purpose bred research beagles and the kennel/humane society dogs. Based on these data the purpose bred beagle that is chronically infected with *Giardia* may not be a good model to use when evaluating *Giardia* diagnostic tests.

The IFA methodology reliably found more cysts than either the double centrifugation flotation or the fecal ELISA in both group 1 and group 2 dogs. The intermittent shedding of cysts makes it impossible for any one test methodology to find all the infected individuals from every sample; but IFA method found more dogs positive on any one day's samples than either of the other two methods.

Twenty-three beagles were used for post treatment diagnostic evaluation. All the beagles were administered the labeled daily dose of Drontal[®] Plus for 3 days and were bathed on the last day of treatment. Surprisingly all 23 treated dogs were negative one day post treatment by the double centrifuge fecal flotation, the fecal ELISA test and IFA methods. Even given the inconsistent results observed with the diagnostic tests during the diagnostic phase of this study finding all 23 dogs negative within 24 hours of the last day of treatment was remarkable. These dogs remained negative for at least 17 days which is past the prepatent period of *Giardia*. We were unable to discern whether the recovery of cysts in 4 purpose bred beagles between days 17 and 21 post-treatment was caused by a treatment induced temporary suppression of cyst/antigen shedding or reinfection. In our study where the dogs were followed for 21 days post-treatment, the treatment and bathing protocol was 82.6% effective. Knowing whether a dog is still shedding cysts and/or is fecal ELISA test positive after therapy before the end of the prepatent period may help a practitioner make treatment decisions. A positive result should have practitioners reevaluate client compliance, and treatment dosing. Resistance to medication can occur but is less likely than the previous two possibilities for failure to eliminate cysts. A positive result after the prepatent period could be caused by failure of the client to comply with treatment recommendations, inappropriate dosing of medication, resistance to medication, temporary suppression of shedding of cysts or reinfection. The recommendation to bath the dog on the last day of treatment and environmental control measures are critical to prevent reinfection.⁵³

Eliminating cysts from the environment can be difficult but immediately picking up feces, washing of all the dogs' bedding, and washing of food and water bowls with hot soapy water is the first step. Owners should consider avoiding places where cyst exposure is likely occur (i.e. dog parks and camping) and find alternative places for exercise. Given that *Giardia* is a common parasite, reinfection is likely very common.

FOOTNOTES

- a) IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, Maine 04092
- b) Leif Lorentzen IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, Maine 04092
personal communication
- c) MeriFluor[®] *Cryptosporidium/Giardia* Test, 3471 River Hills Drive, Cincinnati, OH 45244
- d) Drontal[®] Plus Bayer HealthCare, LLC. Animal Health Division, P.O. Box 390
Shawnee Mission, KS 66201
- e) Coverage Plus NPD[®], STERIS Corporation, 5960 Heisley Road, Mentor, Ohio 44060-1834
USA

CHAPTER 3 - REFERENCES

1. Roberts LS, Janovy J, jr. *Foundations of Parasitology*. seventh ed. 1221 Avenue of the Americas, New York, New York 10020: McGraw-Hill, 2005.
2. Faubert G. Immune response to *Giardia duodenalis*. *Clin Microbiol Rev* 2000;13(1):35-54, table of contents.
3. Ford BJ. The Discovery of *Giardia*. *MICROSCOPE* 2005;53(4):147.
4. Cox FE. History of human parasitology. *Clin Microbiol Rev* 2002;15(4):595-612.
5. Thompson RC. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. *Vet Parasitol* 2004;126(1-2):15-35.
6. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Caccio S. Genetic heterogeneity at the beta - giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. *Int J Parasitol* 2005;35(2):207-213.
7. Xiao L, Fayer R. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *International journal for parasitology* 2008;38(11):1239.
8. Cacciò SM, Thompson RCA, McLauchlin J, Smith AV. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends in parasitology* 2005;21(9):430.
9. Thompson RCA, Palmer CS, O'Hnadley R. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *The veterinary journal* 2008;177(1):18.
10. Adam RD. Biology of *Giardia lamblia*. *Clin Microbiol Rev* 2001;14(3):447-475.
11. Tůmová P, Hofstetrová K, Nohýnková E, Hovorka O, Kral J. Cytogenetic evidence for diversity of two nuclei within a single diplomonad cell of *Giardia*. *Chromosoma* 2007;116(1):65.
12. Sousa MC, Gonçalves CA, Bairos VA, Poiaraes-da-Silva J. Adherence of *Giardia lamblia* trophozoites to Int-407 human intestinal cells. *Clinical and diagnostic laboratory immunology* 2001;8(2):258.
13. Prucca CG, Slavin I, Quiroga R, Elias EV, Rivero FD, Saura A, Carranza PG, Lujan HD. Antigenic variation in *Giardia lamblia* is regulated by RNA interference. *Nature* 2008;456(7223):750.

14. Midlej V, Benchimol M. Giardia lamblia behavior during encystment: How morphological changes in shape occur. *Parasitology international* 2008.
15. Sagolla MS, Dawson SC, Mancuso JJ, Cande WZ. Three-dimensional analysis of mitosis and cytokinesis in the binucleate parasite Giardia intestinalis. *Journal of cell science* 2006;119(Pt 23):4889.
16. Rosa LA, Gomes MA, Mundim AV, Mundim MJS, Pozzer EL, Faria ESM, Viana JC, Cury MC. Infection of dogs by experimental inoculation with human isolates of Giardia duodenalis: clinical and laboratory manifestations. *Vet Parasitol* 2007;145(1-2):37-44.
17. Scorza AV, Lappin MR. Metronidazole for the treatment of feline giardiasis. *J Feline Med Surg* 2004;6(3):157-160.
18. Abbitt B, Huey RL, Eugster AK, Syler J. Treatment of giardiasis in adult Greyhounds, using ipronidazole-medicated water. *J Am Vet Med Assoc* 1986;188(1):67-69.
19. Kirkpatrick CE, Farrell JP. Feline giardiasis: observations on natural and induced infections. *Am J Vet Res* 1984;45(10):2182-2188.
20. deRegnier DP, Cole L, Schupp DG, Erlandsen SL. Viability of Giardia cysts suspended in lake, river, and tap water. *Applied and environmental microbiology* 1989;55(5):1223.
21. Kasprzak W, Mazur T, Wladyslaw M. Resistance of Giardia cysts to physical and chemical agents. *Bulletin of the Institute of Maritime and Tropical Medicine in Gdynia* 1980;31(3-4):239.
22. Lee MB. The effectiveness of commercially available disinfectants upon Giardia lamblia cysts. *Canadian journal of public health* 1992;83(2):171.
23. Itoh N, Muraoka N, Kawamata J, Aoki M, Itagaki T. Prevalence of Giardia intestinalis infection in household cats of Tohoku district in Japan. *J Vet Med Sci* 2006;68(2):161-163.
24. Vasilopoulos RJ, Mackin AJ, Rickard LG, Parr GT, Huston CL. Prevalence and factors associated with fecal shedding of Giardia spp. in domestic cats. *J Am Anim Hosp Assoc* 2006;42(6):424-429.
25. Hackett T, Lappin MR. Prevalence of enteric pathogens in dogs of north-central Colorado. *J Am Anim Hosp Assoc* 2003;39(1):52-56.
26. Carlin EP, Bowman DD, Scarlett JM, Garrett J, Lorentzen L. Prevalence of Giardia in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP Giardia test. *Veterinary Therapeutics* 2006;7(3):199-206.
27. Gomez-Couso H, Freire-Santos F, Amar CF, Grant KA, Williamson K, Ares-Mazas ME, McLaughlin J. Detection of Cryptosporidium and Giardia in molluscan shellfish by multiplexed nested-PCR. *Int J Food Microbiol* 2004;91(3):279-288.

28. Mendez-Hermida F, Gomez-Couso H, Romero-Suances R, Arez-Mazas ME. Cryptosporidium and Giardia in wild otters (*Lutra lutra*). *Vet Parasitol* 2007;144(1-2):153-156.
29. Rinaldi L, Musella V, Condoleo R, Saralli G, Veneziano V, Bruni G, Condoleo RU, Cringoli G. Giardia and Cryptosporidium in water buffaloes (*Bubalus bubalis*). *Parasitol Res* 2007;100(5):1113-1118.
30. Trout JM, Santin M, Fayer R. Giardia and Cryptosporidium species and genotypes in coyotes (*Canis latrans*). *J Zoo Wildl Med* 2006;37(2):141-144.
31. Hlavsa MC, Watson JC, Beach MJ. Giardiasis surveillance--United States, 1998-2002. *MMWR Surveill Summ* 2005;54(1):9-16.
32. Yoder JS. Cryptosporidiosis surveillance--United States, 2003-2005. *Morbidity and mortality weekly report. CDC surveillance summaries* 2007;56(7):1.
33. Thompson A. Human giardiasis: genotype-linked differences in clinical symptomatology. *Trends Parasitol* 2001;17(10):465.
34. Homan WL, Mank TG. Human giardiasis: genotype linked differences in clinical symptomatology. *Int J Parasitol* 2001;31(8):822-826.
35. Dryden MW, Payne PA, Smith V. Accurate diagnosis of Giardia spp and proper fecal examination procedures. *Vet Ther* 2006;7(1):4-14.
36. Leib MS, Zajac AM. Giardiasis in dogs and cats. *Vet Med* 1999;94(9):793-802.
37. merifluor Cryptosporidium/Giardia package insert instruction. Available at: http://www.meridianbioscience.com/product/productCatalog.jsp?content=body_content_prd_specific.jsp&topContentBar=body_top_prd_specific.jsp&catItem=250050. Accessed Nov, 2009.
38. Nantavisai K, Mungthin M, Tan-ariya P, Rangsin R, Naaglor T, Leelayoora S. Evaluation of the sensitivities of DNA extraction and PCR methods for detection of Giardia duodenalis in stool specimens. *J Clin Microbiol* 2007;45(2):581-583.
39. IDEXX Giardia snap test kit package insert. Available at: http://www.idexx.com/pubwebresources/pdf/en_us/smallanimal/snap/giardia/snap-giardia-package-insert.pdf. Accessed Nov. 2009
40. Giardia snap fecal antigen video. Available at: mms://wm.uvision.net/vod/idexx.com/companionanimal/testkits/giardia300_en.wmv. Accessed Nov. 2009.
41. Groat, R., Monn, M., flynn, L., Curato, J. Survey of clinical practices and testing for diagnosis of Giardia infections in dogs and cats. 2003.

42. Mekaru SR, Marks SL, Felley AJ, Chouicha N, Kass PH. Comparison of direct immunofluorescence, immunoassays, and fecal flotation for detection of *Cryptosporidium* spp. and *Giardia* spp. in naturally exposed cats in 4 Northern California animal shelters. *J Vet Intern Med* 2007;21(5):959-965.
43. Hoque ME. Risk of giardiasis in Aucklanders: a case-control study. *International journal of infectious diseases* 2002;6(3):191.
44. Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RC. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. *J Parasitol* 1997;83(1):44-51.
45. Traub RJ, Monis PT, Robertson I, Irwin P, Mencke N, Thompson RC. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. *Parasitology* 2004;128(Pt 3):253-262.
46. van der Giessen JW, de Vries A, Roos M, Wielinga P, Kortbeek LM, Mank TG. Genotyping of *Giardia* in Dutch patients and animals: a phylogenetic analysis of human and animal isolates. *Int J Parasitol* 2006;36(7):849-858.
47. Santin M, Trout JM, Fayer R. Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* species and genotypes in sheep in Maryland. *Vet Parasitol* 2007;146(1-2):17-24.
48. Mendonca C, Almeida A, Castro A, deLurdes DM, Soares S, da Costa JM. Molecular characterization of *Cryptosporidium* and *Giardia* isolates from cattle from Portugal. *Vet Parasitol* 2007; Jun 20;147(1-2):47-50
49. Traub R, Wade S, Read C, Thompson A, Mohammed H. Molecular characterization of potentially zoonotic isolates of *Giardia duodenalis* in horses. *Vet Parasitol* 2005;130(3-4):317-321.
50. Foronda P, Bargues MD, Abreu-Acosta N, Periago MV, Valero MA, Valladres B, Mas-Coma S. Identification of genotypes of *Giardia intestinalis* of human isolates in Egypt. *Parasitology research* 2008 2008 Oct;103(5):1177-81.
51. Gardner TB, Hill DR. Treatment of giardiasis. *Clin Microbiol Rev* 2001;14(1):114-128.
52. Zajac AM, LaBranche TP, Donoghue AR, Chu TC. Efficacy of fenbendazole in the treatment of experimental *Giardia* infection in dogs. *Am J Vet Res* 1998;59(1):61-63.
53. Payne PA, Ridley RK, Dryden MW, Bathgate C, Milliken G, Stewart P. Efficacy of a combination febantel-praziquantel-pyrantel product, with or without vaccination with a commercial *Giardia* vaccine, for treatment of dogs with naturally occurring giardiasis. *J Am Vet Med Assoc* 2002;220(3):330-333.

54. Keith CL, Radecki SV, Lappin MR. Evaluation of fenbendazole for treatment of Giardia infection in cats concurrently infected with Cryptosporidium parvum. *Am J Vet Res* 2003;64(8):1027-1029.
55. Barr SC, Bowman DD, Frongillo MF, Joseph S. Efficacy of a drug combination of praziquantel, pyrantel pamoate, and febantel against giardiasis in dogs. *Am J Vet Res* 1998;59(9):1134-1136.
56. Anderson VR. Nitazoxanide: a review of its use in the treatment of gastrointestinal infections. *Drugs* 2007;67(13):1947.
57. Newly approved Tinidamax. Available at: <http://www.centerwatch.com/patient/drugs/dru873.html>. Accessed 05/21, 2007.
58. Tinidmax tablets. Available at: http://www.fda.gov/cder/foi/label/2004/21618_tindamax_lbl.pdf. Accessed 05/21, 2007.
59. Gookin JL, Copple CN, Papich MG, Poore MF, Stauffer SH, Birkenheuer AJ, Twedt DC, Levy MG. Efficacy of ronidazole for treatment of feline Tritrichomonas foetus infection. *J Vet Intern Med* 2006;20(3):536-543.
60. Olson ME, Morck DW, Ceri H. The efficacy of a Giardia lamblia vaccine in kittens. *Canadian journal of veterinary research* 1996;60(4):249.
61. Olson ME, Hannigan CJ, Gaviller PF, Fulton LA. The use of a Giardia vaccine as an immunotherapeutic agent in dogs. *The Canadian veterinary journal* 2001;42(11):865.
62. Olson ME, Morck DW, Ceri H. Preliminary data on the efficacy of a Giardia vaccine in puppies. *The Canadian veterinary journal* 1997;38(12):777.
63. Stein JE, Radecki SV, Lappin MR. Efficacy of Giardia vaccination in the treatment of giardiasis in cats. *J Am Vet Med Assoc* 2003;222(11):1548-1551.
64. Anderson KA, Brooks AS, Morrison AL, Reid-Smith RJ, Martin SW, Benn DM, Peregrine AS. Impact of Giardia vaccination on asymptomatic Giardia infections in dogs at a research facility. *Can Vet J* 2004;45(11):924-930.
65. Paul MA, Carmichael LE, Childers H, Cotter S, Davidoson A, Ford R, Hurley KF, Roth JA, Schultz RD, Thacker E, Welborn L. 2006 AAHA Canine Vaccine Guidelines. *J Am Anim Hosp Assoc* 2006;42(2):80-89.
66. Abdul-Wahid A, Faubert G. Mucosal delivery of a transmission-blocking DNA vaccine encoding Giardia lamblia CWP2 by Salmonella typhimurium bacteriofection vehicle. *Vaccine* 2007;25(50):8372.

67. Barr SC. Enteric Protozoal Infections. In: Greene CE. *Infectious diseases of the dog and cat*. third ed. St. Louis Missouri: Saunders, 2006;736.
68. Chavez-Munguia B, Omana-Molina M, Gonzalez-Lazaro M, Gonzales-Robles A, Cedillo-Rivera R, Bonilla P, Martinez-Palomo A. Ultrastructure of cyst differentiation in parasitic protozoa. *Parasitol Res* 2007;100(6):1169-1175.
69. Zimmer JF, Burrington DB. Comparison of Four Techniques of Fecal Examination for Detecting Canine Giardiasis. *JAAHA* 1986;22(March/April):161.
70. O'Handley RM, Olsen ME, Fraser D, Adams P, Thompson RC. Prevalence and genotypic characterisation of Giardia in dairy calves from Western Australia and Western Canada. *Veterinary parasitology* 2000;90(3):193.
71. Agresti A. In; Categorical data analysis. New York, John Wiley and Sons Inc. 1990:558.
72. Dryden MW, Payne PA, Ridley R, Smith V. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Vet Ther* 2005;6(1):15-28.

Appendix A - Project Photographs

Figure A.1. The materials needed to perform a centrifuged fecal flotation; swinging head centrifuge, zinc sulfate solution (specific gravity 1.18), glass microscope slide, 12 ml centrifuge tube with fecal sample for flotation and 18mm² glass coverslip.



Figure A.2 A centrifuged fecal sample is on the microscope slide with a coverslip and a drop of Lugol's iodine.

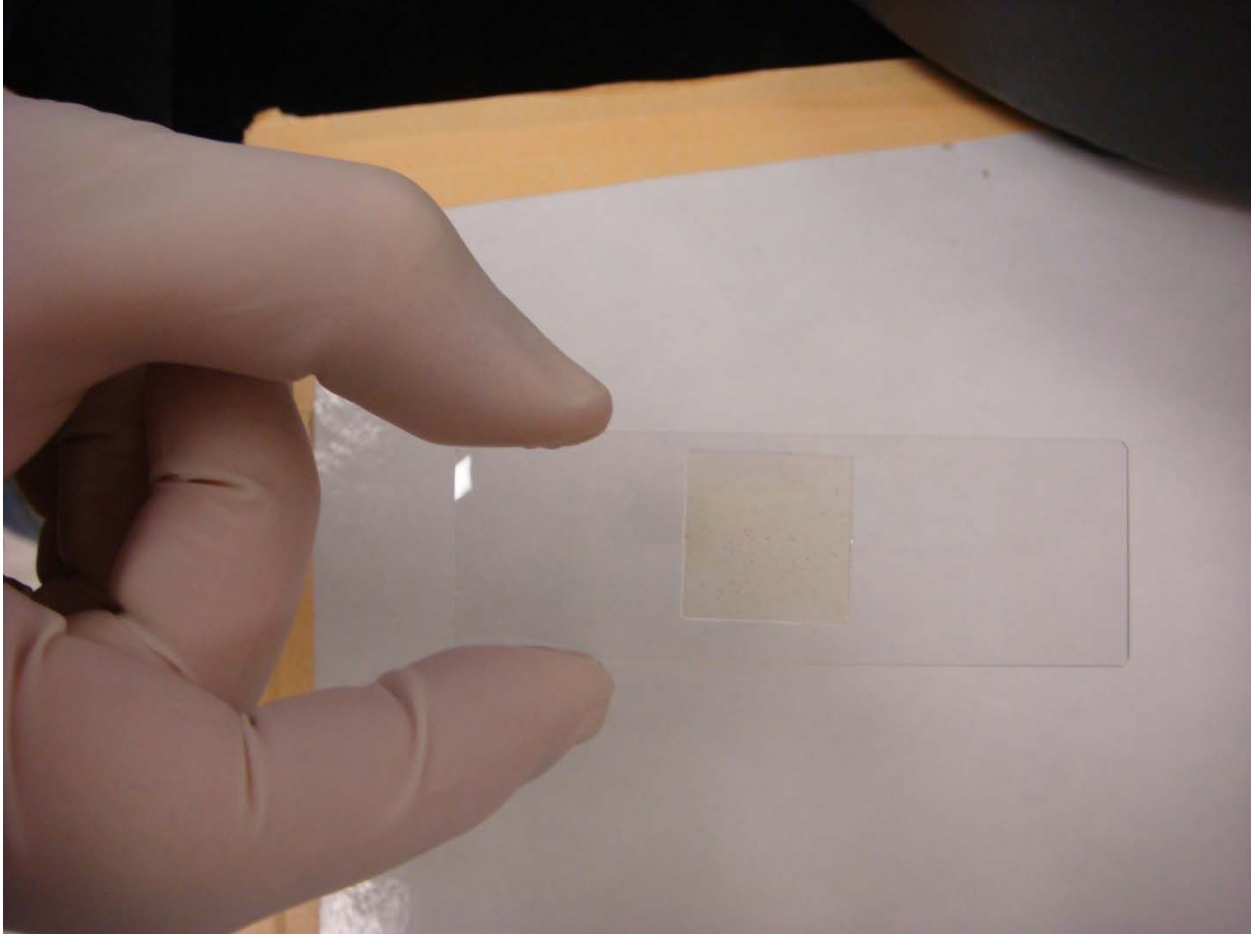


Figure A.3. *Giardia* cysts visualized after floated in zinc sulfate solution stained with Lugol's iodine magnified 400X.

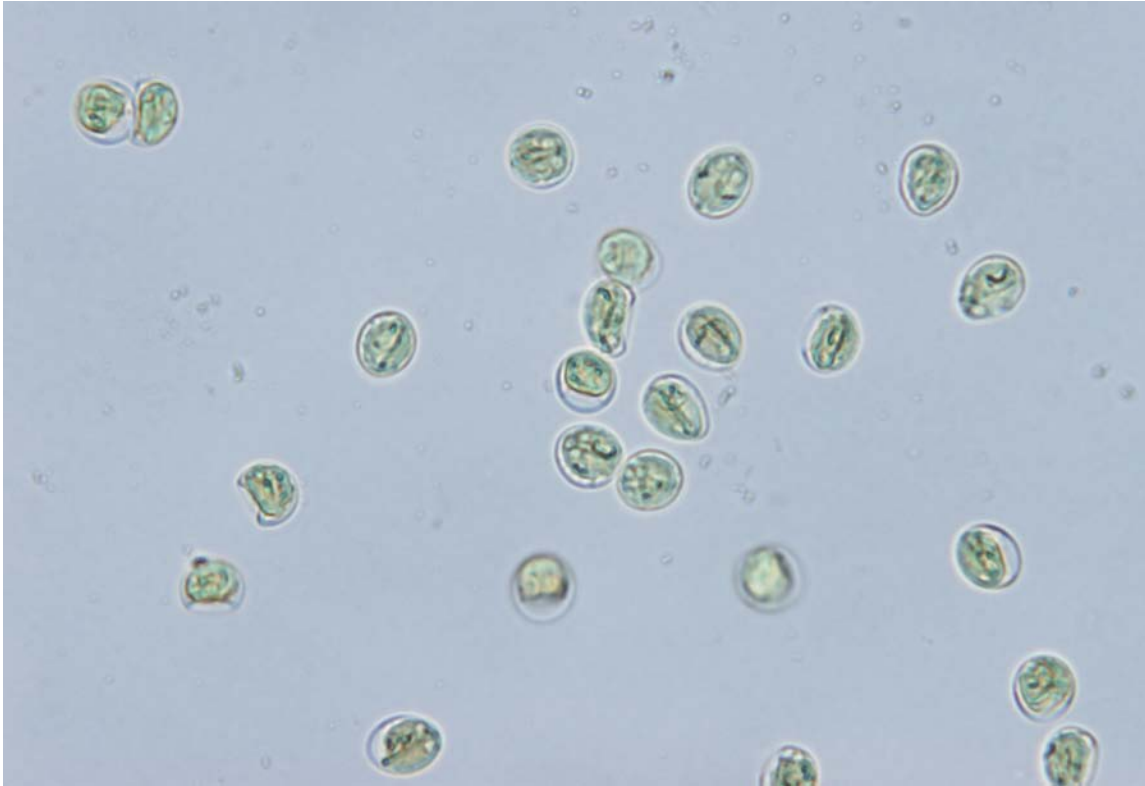


Figure A.4. A SNAP® *Giardia* antigen test kit manufactured by IDEXX and a fecal sample.

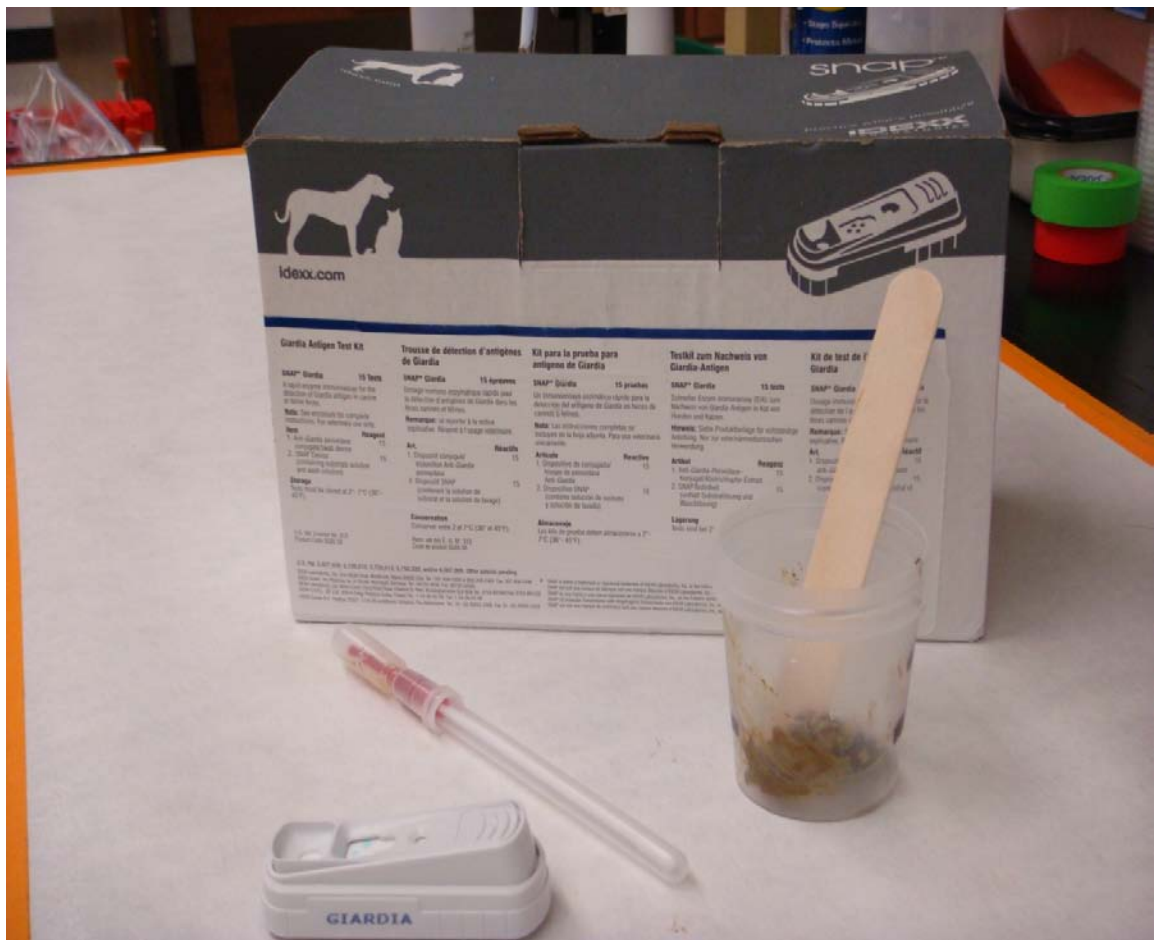


Figure A.5. A positive and negative SNAP[®] *Giardia* antigen test is seen below.



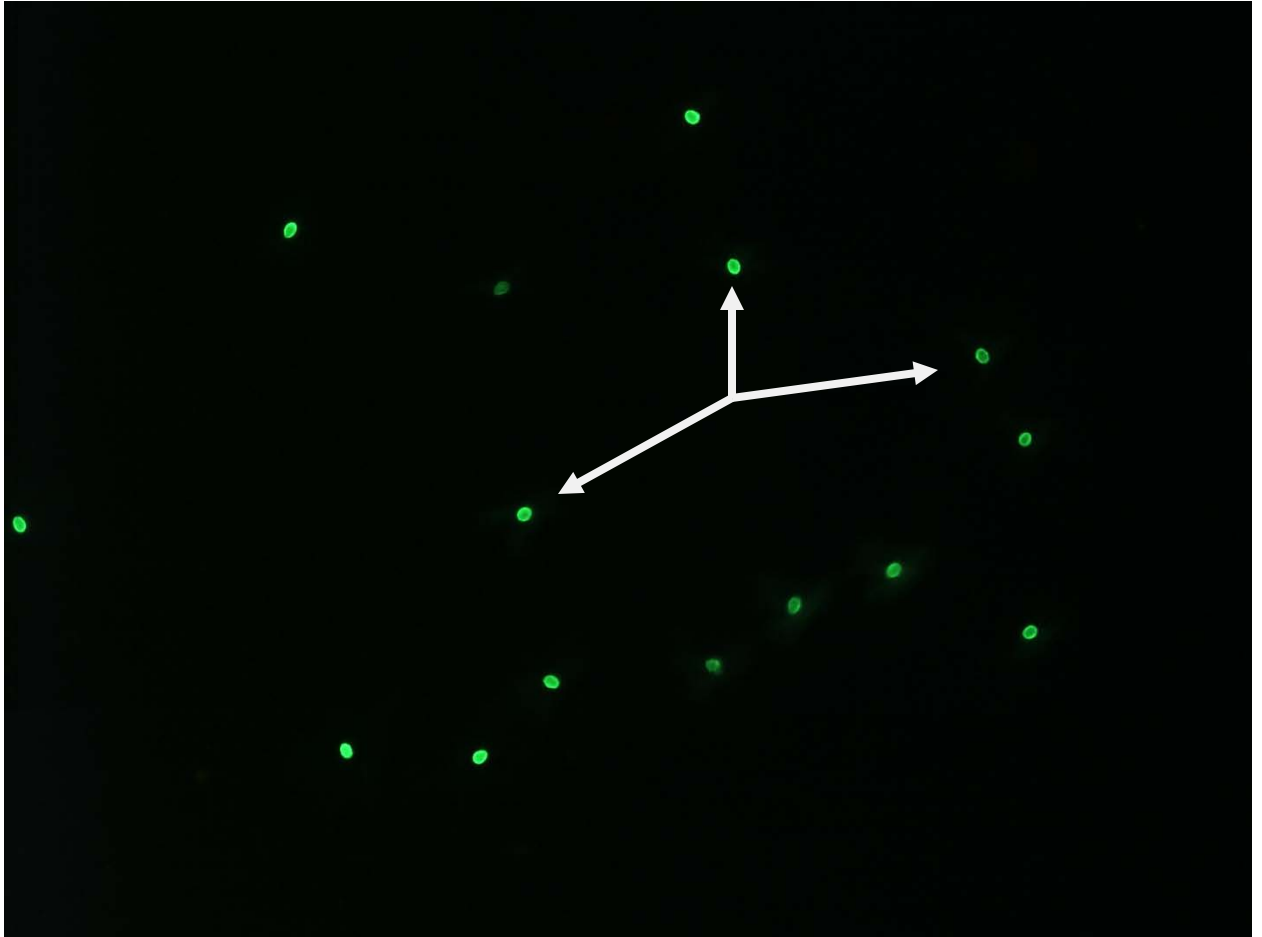
POSITIVE

NEGATIVE

Figure A.6 Materials needed to perform the MeriFluor® *Cryptosporidium*/*Giardia* direct immunofluorescent assay; a fixed head centrifuge, PBS-EDTA solution, 15 ml conical centrifuge tube, and MeriFluor® *Cryptosporidium*/*Giardia* direct immunofluorescent assay microscope slide (rest of test kit not shown).



Figure A.7 *Giardia* cysts identified by the MeriFluor® *Cryptosporidium/Giardia* direct immunofluorescent test.



Arrows are pointed to *Giardia* cysts which can be visualized due to fluorescent antibody coating the surface of the parasite.