**Update on Intestinal Giardia and Trichomonas Infections**

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**GIARDIA**

*Giardia duodenalis* (also known as *G. intestinalis, G. lamblia*) is a pear-shaped, binucleated, flagellated protozoan parasite that infects the small intestine, impairs mucosal absorption, and causes diarrhea. There are two forms: the motile trophozoites that inhabit the small intestine, and the nonmotile infective cysts that pass with the feces into the environment where they can infect new hosts.

The numerous species and strains of *Giardia* are grouped by genotype into 7 "assemblages", denoted by letters A through G. Dogs and cats are infected most often by their respective host-specific genotypes (C and D in dogs; F in cats), but they occasionally harbor zoonotic genotypes (A and B) that infect humans (Vasilopulos 2007; Thompson, 2008; Payne 2009; Ballweber 2010; Covacin 2011). *Giardia* has a worldwide distribution. The overall prevalence in most populations of pet dogs and cats is 1% to 5%, but is higher (10 to 20%) in diarrheic animals. The prevalence is highest in young animals and animals confined together in crowded conditions (e.g., kennels, catteries, shelters, pet shops, puppy mills) (Carlin 2006; Payne 2009). Infection rates in group-housed animals can approach 100%.

**Life Cycle**

*Giardia* has a direct life cycle and transmission is feco-oral. The usual source of infection is the ingestion of food or water contaminated with cysts. *Giardia* cysts are environmentally stable and dormant, but upon ingestion by a host they excyst in the duodenum producing two trophozoites from each cyst. The motile trophozoites attach to the brush border surface of the mucosal epithelium by means of ventral cup-shaped adhesion disks, or they swim freely within the adjacent mucus layer. Trophozoites mainly inhabit the duodenum in the dog and the jejunum and ileum in the cat. Clinical signs develop after an incubation period averaging 7 days. As *Giardia* trophozoites pass into the colon they transform into infective cysts, which are the most typical form in the feces. Cyst excretion begins 5 to 16 days post-infection. Trophozoites are occasionally found in severely diarrheic feces. Wild animals are potential reservoirs, and water from contaminated streams and ponds may be a source of infection. In cool wet conditions *Giardia* cysts can remain infectious for months and they are resistant to many disinfectants. Cysts in fecal residue on the haircoat can cause reinfection during self-grooming.

**Clinical Signs**

In most animals *Giardia* causes relatively mild enterocyte injury, so most infections are asymptomatic, especially in healthy mature animals. The presence and severity of clinical signs are determined by the agent (dose and strain virulence), the host (age, stress, nutrition, and immune status), and the environment (crowded unsanitary conditions) (Payne, 2009). Clinically apparent giardiasis occurs most frequently in young dogs and cats and is characterized by intestinal malabsorption with voluminous foul-smelling, light-colored, watery or "cow pie" diarrhea, steatorrhea, and weight loss. Mucoid diarrhea is occasionally seen in cats. *Giardia* diarrhea may be acute or chronic, intermittent or continuous, and self-limiting or persistent. The severity of *Giardia* infection is worsened by concurrent viral, bacterial, protozoal, or helminth infections of the intestines. Corticosteroids can lead to recrudescence of infection in "recovered" animals. *Giardia* does not typically cause anorexia, vomiting, GI bleeding, or fever.

**Diagnosis**
Giardiasis should be considered in any dog or cat with unexplained small bowel diarrhea, especially in young and group-housed animals. The diagnosis depends upon fecal tests for the detection of *Giardia* antigen, cysts, or trophozoites. PCR and sequencing can be used to genotype *Giardia* (Covacin 2011).

Fecal immunoassays for *Giardia* antigen include the in-office SNAP *Giardia* Test (IDEXX) and the microplate ELISA test (ProSpecT/Giardia, Remel), which is offered by commercial labs. Conflicting results have been reported regarding the sensitivity and specificity of the SNAP and microplate ELISA assays for *Giardia* coproantigen (Mekaru 2007; Rimhanen-Finne 2007; Geurden 2008; Rishniw 2010). The SNAP test may be less sensitive, but this is offset by its convenience, ease of use, and lower cost.

*Giardia* cysts (oval; 8–12 μm x 7–10 μm) are present in feces much more consistently than motile trophozoites (pear-shaped; 12–18 μm x 10–12 μm x 2–4 μm). *Giardia* cysts are not usually detected by standard benchtop flotation, so zinc sulfate centrifugal flotation is the preferred method for recovery of *Giardia* cysts from feces (Dryden 2006). For this procedure, 2 to 5 gm of feces are mixed with 10 ml of 33 percent zinc sulfate solution (1.18 specific gravity) and strained, then the mixture is centrifuged and a drop of the surface layer (meniscus) is transferred to a microscope slide and examined. A drop of iodine stain can be used. The pattern of cyst excretion in the feces can be intermittent, so a single fecal specimen may overlook the diagnosis in some cases. To increase sensitivity and maximize the chances of detecting *Giardia* cysts, it is recommended that at least 3 fresh fecal samples be examined over 3 to 5 days.

The most sensitive and specific technique for detecting *Giardia* cysts in feces is the direct immunofluorescence assay (IFA; MeriFluor; Meridian Diagnostics), which is considered to be the "gold standard" for diagnosis of *Giardia* in dogs and cats (Mekaru 2007; Rimhanen-Fenne 2007; Geurden 2008; Rishniw 2010). The IFA is only available through reference labs that have fluorescent microscopy.

Examination of fecal smears for trophozoites is the least sensitive detection method for *Giardia*, so this should only be used in conjunction with one of the other more reliable diagnostic methods. Motile *Giardia* trophozoites can occasionally be identified in wet mounts of fresh diarrheic feces suspended in saline. *Giardia* trophozoites move in a tumbling or "falling-leaf" motion. A drop of Lugol iodine can be added to kill and stain the trophozoites, which resemble a "monkey face" appearance formed by the two nuclei, the axonemes, and the median body. Trophozoites are found more readily in duodenal specimens (endoscopic aspirates, washings, brushings, or mucosal biopsy impression smears). Duodenal sampling is not practical as a routine diagnostic test for *Giardia*, but it might be appropriate in patients undergoing gastroduodenoscopy for other reasons.

PCR testing lacks diagnostic sensitivity and fails to detect 20% of *Giardia* infections. However, PCR with genotyping is useful for assemblage determination, and is available at some labs (Colorado State University Diagnostic Lab; http://dlab.colostate.edu/). Genotyping yields inconsistent results with some single gene assays, so multilocus genotyping is recommended.

None of the diagnostic tests for *Giardia* are 100% reliable and some studies show considerable discordance between assays that detect fecal antigen or cysts (Geurden 2008; Rishniw 2010). Also, the presence of *Giardia* may be masked temporarily by barium, antibiotics, antacids, antidiarrheals, laxatives, and enemas. The chances of detecting *Giardia* are improved when fecal antigen testing (e.g., SNAP or ELISA) is combined with zinc sulfate centrifugal flotation (combined sensitivity of 98%), and when more than one fecal specimen is analyzed. For recommendations of the Companion Animal Parasite Council, see www.capcvet.org. Regardless, some *Giardia* infections escape detection and negative fecal examinations do not exclude a diagnosis of *Giardia*. For this reason, a therapeutic response trial of fenbendazole (Panacur) may be appropriate when diagnostics are negative and "occult" *Giardia* is suspected.

**Treatment**

The safest and most effective treatments for *Giardia* are **fenbendazole** (Panacur; 50 mg/kg PO q24hr for 3 to 5 days) or **febantel-pyrantel-praziquantel** (Drontal Plus; dogs - label dose PO q24h for 3 to 5 days; cats - 56 mg/kg PO q24h for 5 days)(Zajac 1998; Barr 1994; Barr 1998; Payne 2002; Scorza 2006; Montoya 2008; Bowman 2009). Fenbendazole is a benzimidazole that disrupts glucose uptake and energy metabolism by *Giardia* trophozoites. Febantel is metabolized to fenbendazole and oxfendazole.

**Metronidazole** (25 mg/kg PO q12h for 7 days) is sometimes effective, but up to 50% of infections may be metronidazole-resistant. Side effects of metronidazole can include anorexia, vomiting, and reversible neurotoxicity (weakness, ataxia, disorientation, seizures, and blindness). Cats tolerate a liquid formulation of metronidazole benzoate better than bitter-tasting metronidazole USP tablets (Scorza 2004).

**Tinidazole** compounded into capsules (dogs - 44 mg/kg PO q24h for 6 days; cats - 30 mg/kg for 7–10 days) has been effective in some refractory *Giardia* cases. Albendazole is effective (Barr 1993), but is not recommended because it has been associated with severe bone marrow toxicity. Secnidazole (30 mg/kg PO, given once) had 100% efficacy in cats, but needs further study (DaSilva 2011). **Furazolidone** suspension and quinacrine have also been used to treat *Giardia*, but are not recommended because they are less effective than other drugs and have a high incidence of side effects (anorexia, lethargy, vomiting). In 20 dogs with *Giardia*, *Enterococcus faecium* SF68 (Fortiflora) had no beneficial effect on fecal cyst excretion or antigen load (Simpson 2009).

**Prevention**

Reexposure and recurrence of infection is often mistaken for failure to respond to treatment, especially in groups of animals confined together. Cysts remaining in the environment and on the haircoat in treated animals can be a source of reinfection. Control measures should include 1) treatment of all contact animals that are housed together; 2) cleaning and decontamination of the environment, including steam cleaning or disinfection with quaternary ammonium (Roccal), 3) trimming hair from the perianal area and cleaning daily with baby wipes, and 4) bathing to remove cysts from the haircoat. The previously available *Giardia* vaccine was not recommended because it failed to cure or prevent infection and did not reliably lessen cyst shedding (Stein 2003; Anderson 2004).

Zoonotic transmission of *Giardia* from pets to humans is possible but considered uncommon. Surveys have shown that some dogs and cats in the U.S. harbor zoonotic genotypes of *Giardia* (A and B), and that some of these animals are asymptomatic shedders (Vasilopoulos 2007; Ballweber 2010; Covacin 2011).

**TRITRICHOMONAS INFECTION IN CATS**

*Tririchomonas foetus* has emerged as an important cause of infectious large intestinal diarrhea and chronic colitis in cats (Gookin 1999; Gookin 2001; Levy 2003; Bell 2010). Trichomonads are pear-shaped, flagellated protozoa with a characteristic undulating membrane along their body. They are similar in size to *Giardia*, but they lack a cyst stage and are transmitted directly between hosts as trophozoites. Moist, warm, anaerobic conditions are optimal for trichomonads. In addition to colonizing the colon and distal ileum in cats, *T. foetus* causes venereal infection in cattle, but bovine and feline isolates differ in infectivity and pathogenicity. The prevalence of *T. foetus* infection is highest in densely housed young cats in crowded catteries and shelters. In one survey a prevalence in pet cats was 10% (17 of 173) (Stockdale 2009). In a study of purebred show cats, infection was identified in 31% of 117 cats from 89 catteries (Gookin 2004).

**Clinical Signs**

*Tririchomonas foetus* causes mild to severe lymphoplasmacytic and neutrophilic colitis associated with waxing and waning large bowel diarrhea, which is typically semiformal or “cow pie” in consistency and foul smelling (Gookin 1999). Diarrhea sometimes contains fresh blood or mucus. Severely affected kittens sometimes develop painful anal irritation with dribbling of feces or rectal prolapse. The diarrhea often improves transiently in response to antibiotics. Affected cats generally remain otherwise healthy and in good body condition. Diarrhea is often exacerbated by concurrent enteric infections or parasites, especially *Giardia* and *Cryptosporidium* (Gookin 2004).
Diagnosis

The diagnosis of *Trichomonas foetus* infection can be confirmed by direct fecal microscopy, fecal culture, fecal polymerase chain reaction (PCR) assay, or colonic mucosal biopsy. Testing is most reliable in cats that have been off antibiotics for 2 weeks or more. This is because antibiotics can temporarily decrease the number *T. foetus* and cause false negative test results. Feces should be freshly voided and litter free or collected by colonic flush.

Motile trophozoites *T. foetus* of can be identified in fresh wet smears of diarrheic feces taken directly from the rectum in about 14% of cases. A drop of feces mixed with a drop of saline is coverslipped and examined under low light at 200X to 400X magnification. The likelihood of detecting trophozoites is lower in formed or dried feces, and in cats recently treated with antibiotics. Trichomonads, which are similar in size and shape to *Giardia*, are identified by their distinctive undulating membrane and rapid, jerky, “jitterbug” motility compared with the “falling leaf” motility of *Giardia*.

Protozoal fecal culture is more sensitive than microscopy for diagnosis of *T. foetus* (Gookin 2003). Culture requires 0.05 g (size of rice grain) of freshly voided feces inoculated into commercially available protozoal media (Feline In Pouch TF™; Biomed Diagnostics) and incubated at 37°C for 48 hr or at room temperature (25°C) for up to 12 days. The pouch should be examined daily with a microscope to avoid missing a positive result. The sensitivity (detection limit) is 1000 or more trichomonads per sample. Use the wettest part of the stool to obtain viable trichomonads. If voided stool is dry or contaminated with litter, collect a rectal specimen with loop or swab. Rectal mucus on a swab is sufficient for culture, but not PCR. An excessively large inoculum of feces into the pouch can promote overgrowth of bacteria, which impairs performance of the culture system. Clouding of the liquid media and the formation of gas bubbles indicate interfering bacterial overgrowth in the culture. Do not refrigerate specimens as temperatures below 60°F rapidly kill *T. foetus*.

Fecal PCR assay is the most accurate test (high sensitivity and specificity) for detecting *T. foetus* (Gookin 2002). Feces for PCR (180 to 220 mg) should be free of litter and is best preserved in 3 to 5 ml of isopropyl rubbing alcohol for shipping at room temperature. The sensitivity limit of PCR is 10 trichomonads per 200 mg fecal sample.

In colonic mucosal biopsies from infected cats, trichomonads may be identified in the superficial mucus and mucosal crypts, accompanied by an infiltrate of lymphocytes, plasma cells, and neutrophils (Yaeger 2005). Fluorescence and chromogenic in situ hybridization assays (FISH; CISH) can also identify *T. foetus* in formalin-fixed and paraffin-embedded tissue samples (Gookin 2010).

Treatment

Treatment of feline trichomoniasis is often unsuccessful. In untreated cats the long-term prognosis is good based on the finding that diarrhea resolves spontaneously in 88% of infected cats within 2 years (median, 9 months; range 5 months to 2 years) (Foster 2004). However, subclinical infection persists for years in over half of the cats after resolution of the diarrhea (median, 3 years; range 2 to 5 years (Foster 2004). Some cats seemingly remain infected for life. Clinical disease may be prolonged in cats living under dense housing conditions, possibly attributable stress. Treatment with diet changes and conventional antibiotics may prolong clinical signs and delay spontaneous resolution in some cats.

*Trichomonas foetus* is resistant to most antibiotics and is extremely difficult to eradicate (Gookin 2001). Numerous antibiotics have been evaluated. Some antibiotics reduce the number of organisms and improve the diarrhea without eliminating the infection, so diarrhea relapses whenever antibiotics are stopped. Diarrhea is typically refractory to corticosteroids.

The most successful treatment for eliminating *T. foetus* is **ronidazole** (30 mg/kg PO, once daily for 14 days). Ronidazole is a nitroimidazole related to metronidazole (Gookin 2006). The side effects in some cats include lethargy, decreased appetite, and neurotoxicity (agitation,
trembling, weakness, hyperaesthesia, ataxia, seizures) (Rosado 2007). Cats with neurotoxic signs usually improve when the drug is stopped, but recovery can take 1 to 4 weeks. Ronidazole should not be used in pregnant and nursing queens or in very young kittens. Ronidazole is not approved for veterinary or human use, but some pharmacies compound chemical grade ronidazole for veterinary use. Because of its bitter taste, ronidazole compounded in gel caps is better tolerated than flavored suspension. When prescribing ronidazole obtain informed consent and instruct owners to wear protective gloves when handling it.

Ronidazole is not effective in every case and resistant T. foetus isolates have been documented (Gookin 2010). Relapse of infection can occur up to 20 weeks after completion of treatment; thus, follow-up PCR testing is recommended to confirm infection is eliminated at 1 to 2 weeks after completion of treatment and again after 20 weeks. A cure can only be proven by repeated negative PCR tests for 20 weeks or more after treatment.

Infected cats should be isolated during treatment to prevent reinfection, which is a common problem in catteries. To prevent auto-reinfection during treatment the litter should be replaced frequently and the box disinfected. In infected catteries, control of T. foetus requires repeated testing to identify infected cats, which can then be isolated and treated. Other measures include reducing housing density, reducing stress, improving diet, and treating concurrent infections such as Giardia or Cryptosporidium. Concurrent infection with Giardia is common, so T. foetus-infected cats should be routinely tested for Giardia and even treated empirically with fenbendazole to eliminate “occult” Giardia and helminth co-infections (Gookin 2004). In catteries, older cats can be asymptomatic carriers and infect younger cats.

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