

PREVALENCE OF, RISK FACTORS FOR, AND ZOO NOTIC POTENTIAL OF  
*GIARDIA* SPP. INFECTION IN CATS HOUSED IN AN ANIMAL SHELTER

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By

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## ABSTRACT

*Giardia duodenalis* is an intestinal protozoal parasite capable of causing both clinical and subclinical disease in a broad range of species, including humans and cats. The parasite has a ubiquitous distribution and infection occurs worldwide in nearly all mammals. *Giardia* infection in cats may not be associated with any clinical signs, but affected cats most commonly exhibit acute small bowel diarrhea with or without weight loss. Estimates of the prevalence in cats fall within a broad range (between 1-10%), with higher rates generally reported for group housed cats.

Confusion exists regarding the significance of *Giardia* in cats and the need for surveillance and treatment of cats in animal shelters as data regarding the zoonotic potential of *Giardia*-infected cats are lacking. Cysts of the various genotypes are morphologically indistinguishable by light microscopy and advanced molecular techniques are required to determine their assemblage type. Little information exists regarding the differences among the various assemblages, with the principal distinction being host range and possibly geographic location. Assemblages A and B have the widest host range and cause disease in humans. Assemblages C and D are found mainly in dogs, E in livestock, F in cats, and G in rodents. Mixed infections are possible.

The purpose of this study was to estimate the prevalence of *Giardia* in cats available for adoption at an animal shelter in central New York, assess zoonotic risk by determining the assemblage type(s) found in cats shedding *Giardia* cysts, and determine risk factors for infection. Once monthly for 5

months a single fecal sample was collected from all cats available for adoption; samples were tested for the presence of *Giardia* antigen using a commercially available ELISA and all ELISA positive samples were processed for genotype analysis.

There was significant variation in the overall prevalence of *Giardia* from month to month, with more than a two-fold increase between spring (April, May: 6.7%) and summer months (June, July, August: 13%). Stray adult cats and those with outdoor access were more likely to be infected than owner-surrendered cats and those that were kept indoors only. Among adult cats, increasing age was associated with decreasing risk of *Giardia* infection. Cats in colony housing, particularly at high densities and for prolonged periods of time, were also more likely to be shedding *Giardia* cysts.

A total of 61 fecal samples that tested ELISA positive for *Giardia* were submitted for molecular analysis. Both zoonotic and host-adapted assemblages were identified; 75% of samples were the host-adapted assemblage F while 25% of samples (assemblages A and B) had zoonotic potential based on reported host ranges. Cats with potentially zoonotic assemblages were significantly more likely to be adults and to have soft stools or diarrhea than those cats infected with the host-adapted genotype. These results confirm that cats can not be discounted as a potential source of human *Giardia* infection, but identification of cats that pose a risk is not possible without advanced molecular diagnostics. Despite a greater understanding of the molecular epidemiology of the disease, the question as to whether or not

asymptomatic cats found to be shedding *Giardia* cysts should be treated in order to protect public health cannot be answered. Further work is needed with larger numbers of cats, both owned and from animal shelters, from various geographic locations to better understand the importance of various risk factors and to establish the true zoonotic potential of *Giardia*.

## BIOGRAPHICAL SKETCH

Stephanie Janeczko was raised in Wood-Ridge, NJ. She received her Bachelor of Science degree in Biology & Biotechnology from Worcester Polytechnic Institute in 2000 and her DVM from Cornell University in 2004.

Following graduation, Dr. Janeczko spent time in general small animal practice, where she worked with cats, dogs, and small mammals. Her experiences dealing with the animals from shelters and rescue groups strengthened her interest in the field of shelter medicine, and in 2006 Dr. Janeczko became the first resident in shelter medicine at Cornell University.

She has a strong interest in infectious diseases and feline medicine, but enjoys the varied nature of shelter medicine. Dr. Janeczko looks forward to working in the field of shelter medicine following completion of her residency, and hopes to focus on training future veterinarians and providing outreach, consultation, and support for shelters.

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## CHAPTER 1

### LITERATURE REVIEW

#### ***Life Cycle and Pathogenesis***

*Giardia* is an intestinal protozoal parasite capable of causing both clinical and subclinical disease in a broad range of species, including humans and cats. The organism has a simple, direct life cycle, and transmission occurs via the fecal-oral route or through contact with contaminated fomites. Affected animals shed infective cysts, which contain two mitotically arrested trophozoites that emerge in the duodenum of animals following ingestion. The trophozoites then complete mitotic division, multiply, and attach to the microvilli. The exact mechanisms of how they cause disease have not been fully elucidated, but enterocyte apoptosis, villus atrophy, diffuse shortening of microvilli, loss of epithelial barrier function, and increased permeability of enterocytes have all been reported in *Giardia* infections.<sup>1</sup> One study of *Giardia* in kittens revealed that overall loss of the epithelial brush border surface area was associated with experimental infection and 80% (4/5) of those infected developed diarrhea.<sup>2</sup> However, because many infections are subclinical and the pathologic changes can be found in animals with and without clinical signs, it remains unclear as to how such intestinal alterations relate to the manifestation of disease. The development of clinical signs apparently relies on a combination of factors, most notably on the host's immune status.

Both trophozoites and cysts are excreted in the feces of cats, but the former are not infective. To become infective, trophozoites must re-encyst in the gastrointestinal tract (in response to intestinal conditions such as an alkaline

pH and in the presence of bile salts and fatty acids) through the exocytosis of cyst wall antigens that form a filamentous network over their surfaces.<sup>3</sup> These small oval to teardrop shaped cysts are then excreted in the feces, where they are immediately infective and capable of surviving outside of the gastrointestinal tract. Cysts frequently become waterborne and may survive for weeks to months in cool, moist environments but are susceptible to drying.<sup>4</sup> Rapid environmental contamination is likely in animal shelters and catteries where endemic disease is present, and cysts can become well-adhered to the perianal region of cats, facilitating re-infection and complicating control efforts.

### ***Clinical signs***

The prepatent period ranged from 5-16 days with a median of 9.6 days in one study<sup>5</sup> and was reported to average 12 days (range 11-13 days) in another investigation.<sup>6</sup> Many cases of *Giardia* in cats are subclinical,<sup>7</sup> but clinical disease is possible and signs may precede cyst shedding by 1-2 days. Cats developing signs most commonly exhibit acute small bowel diarrhea, with or without weight loss. Disease may be severe in a limited number of cases, and large bowel diarrhea, failure to thrive and even vomiting<sup>8</sup> have been described in cats shedding *Giardia* cysts in their feces. Infection may become chronic, however some cats clear the infection after several weeks without treatment.<sup>6</sup> Cyst shedding is cyclical in nature, and the substantial variation in the intensity of cyst shedding explains why the number of cysts present in a fecal sample does not directly correlate with the severity of infection. Thus, the clinical

significance of finding only a few cysts in a fecal sample may be no different than finding large numbers.<sup>9,10</sup>

Many clinicians believe that infected cats are more likely to be young, have diarrhea, and/or have been recently housed in an animal shelter or cattery, but the available research does not consistently support this clinical impression. Study results have been mixed, with some investigators reporting that cyst shedding is more likely in adults<sup>7</sup> and in cats with normal stools<sup>11,12</sup> while others have found an association with younger cats<sup>11, 13-15</sup> and in those with diarrhea.<sup>16</sup> Cysts can be found in healthy and diarrheic animals, and the presence of *Giardia* cysts on a fecal examination in a cat with consistent clinical signs does not confirm that the clinical disease is a result of *Giardia* infection. The literature suggests that the presence or absence of clinical signs is generally not helpful in predicting the infection status of a cat, and in some studies, a higher prevalence of intestinal parasites (including *Giardia* spp., *Toxocara* spp., *Toxoplasma gondii*, *Salmonella* spp., *Campylobacter jejuni*, and *Cryptosporidium* spp.) has been found in cats without diarrhea.<sup>17</sup>

### ***Geographic Distribution and Prevalence***

*Giardia* infection occurs worldwide in nearly all mammals. Estimates of prevalence in domestic cats are commonly reported to range between 1 and 10%<sup>18</sup> but vary greatly because of subclinical infections, variation in diagnostic methods, and other factors. Prevalence rates for catteries have been reported to be as high as 50%<sup>7</sup> and prevalence estimates have generally been reported to be higher in group-housed animals.

Although it is difficult to compare prevalence rates across studies because of varying sampling approaches and diagnostic methodologies, recent work suggests that the prevalence of *Giardia* infection varies substantially across populations, regardless of the source of cats. There can be substantial geographic variation within and between countries, as evidenced by more than a 30-fold difference in prevalence between areas of the United States in a single study (range 0.1%-3.3% in the mid- and north Atlantic region and Mountain region, respectively).<sup>15</sup>

In the Niagara Region of Canada, 1 of 41 samples (2.4%) collected from cats presented to veterinarians in private practice were positive for *Giardia* when tested with a formalin-ethyl acetate concentration technique at a university laboratory. The same study found a reported prevalence of 0.1% from 5 veterinary practices completing a mail-in survey that asked the clinics to complete a table reporting the frequency of *Giardia* spp, *Cryptosporidium* spp, and *Toxocara* spp in dogs and cats over a specified period of time.<sup>19</sup> Zinc sulfate (ZnSO<sub>4</sub>) fecal flotations without centrifugation performed on 211,105 samples from cats presented to Banfield hospitals across the United States revealed a prevalence of 0.58%.<sup>15</sup> A study of 452 cats presented to a veterinary teaching hospital in Pennsylvania found a prevalence of 3.5% for *Giardia* when samples were evaluated by ZnSO<sub>4</sub> centrifugation flotation.<sup>13</sup> In an urban veterinary clinic in England, 35% (7/20) cats were determined to be shedding *Giardia* cysts based on examination of a single fecal sample with a significantly higher prevalence ( $p = 0.02$ ) in cats less than 3 years of age compared to those 3 years of age and older.<sup>11</sup>

When animals exhibiting clinical signs of gastrointestinal disease were specifically evaluated, overall prevalence rates tended to be higher compared to studies surveying cats regardless of their clinical status. A large national study found a prevalence of 10.3% (513/4978) in cats from all regions of the United States based on a commercially available enzyme-linked immunosorbent assay (ELISA) test (SNAP *Giardia* Test, IDEXX Laboratories, Westbrook, ME.)<sup>3</sup> In Santiago, Chile, investigators found a prevalence of 19% (44/230) based on fecal flotations for cats presented to veterinarians in two private practices.<sup>20</sup>

A large study in western Australia screened 418 cats from varying sources for multiple gastrointestinal parasites with fecal flotation techniques and failed to identify any cats that were shedding *Giardia* cysts.<sup>21</sup> However, when a subset of 40 cats from this original sample were tested by ELISA and PCR, 60% and 80% respectively, were found to be positive.<sup>22</sup> Similarly, a prevalence of 40% was found using an ELISA in pet cats in Japan.<sup>23</sup> In the southeastern United States, a prevalence of 13.6% was found in 250 pet cats screened with IFA techniques. Cyst shedding was more common in multiple cat households and households where another cat was shedding cysts, in cats with acute or chronic gastrointestinal complaints, and in cats less than one year of age. Whether the cat was acquired from an animal shelter or not had no effect on prevalence.<sup>8</sup> Investigators in North Carolina found 5% (3/66) of pet cats and 6% (5/87) of feral cats to be shedding *Giardia* cysts as detected by IFA, with no significant difference between the two groups.<sup>23</sup>

Reported prevalence rates were similar in studies that exclusively sampled cats at animal shelters or catteries. In 22 shelters in the Netherlands, only 1%

(3/305) of cats were shedding *Giardia* cysts as detected with a centrifugation-flotation technique,<sup>14</sup> while 9.9% (34/344) of cats in northern California shelters were positive based on a direct immunofluorescence assay.<sup>16</sup> Another study investigating the cause of diarrhea in 89 different catteries found that 36 of 117 (31%) cats were infected with *Giardia* based on ELISA results; at least one cat was infected in 35% (31/89) of the catteries.<sup>24</sup> In studies sampling both owned and shelter cats, similar rates of infection were found for *Giardia*. Using ZnSO<sub>4</sub> centrifugation-flotation, a study in northern Colorado<sup>25</sup> found an overall prevalence of *Giardia* of 2.4% (5/206) in cats with and without diarrhea. Although the shelter cats tended to have higher rates of infection with enteric zoonotic organisms overall, the prevalence of *Giardia* was actually lower in shelter cats (1.3%) compared to owned cats (3.1%) in this study. A similar study of 263 feline fecal samples obtained from privately owned or shelter cats less than 1 year of age in central New York found an overall prevalence of 7.3% (19/263) for *Giardia* using the same diagnostic methods.<sup>17</sup> There was no difference in prevalence for cats from shelters (12/149) compared to those presented to private clinics (7/114), however 6 of the 7 owned cats who were positive for *Giardia* had been adopted from an animal shelter.

Based on this information, it is difficult to conclude whether origin itself is a risk factor for *Giardia* infection in cats. However, conditions that may be found in some shelters (e.g. group housing, high density) and prolonged time living in such conditions have been associated with higher rates of disease. Gookin reported high housing densities, the presence of cats with diarrhea, and contact with non-feline species as risk factors for *Giardia* infection<sup>24</sup> while

Cirak demonstrated that length of residence in shelters was associated with an increased risk of *Giardia* infection.<sup>26</sup> This work suggests that the longer a cat spends in a facility where other cats are infected with *Giardia*, particularly when there is crowding, the greater the risk of infection with *Giardia*.<sup>26</sup> Other studies have found that the main preventive factor against parasitic infection in general was a short stay in the shelter.<sup>14</sup> Additional research is needed in order to better elucidate and define the relationship between shelter conditions and the risk of *Giardia* infection in cats as well as the best practices to limit infection of cats in animal shelters.

### ***Diagnosis***

Even with numerous options for testing, *Giardia* presents a diagnostic challenge and is generally considered to be underdiagnosed. Limitations in diagnosis hamper efforts to better understand the epidemiology of *Giardia* infections, as findings can differ substantially as a result of different methodologies. For example, as previously described, one Australian study reported a prevalence of 0% in cats using fecal flotation, but 80% of those same samples were positive when analyzed by PCR.<sup>22</sup> Similarly, an earlier study of shelter animals in Germany failed to find *Giardia* cysts in the feces of 100 cats, but 22.4% of those same samples were positive when screened with a commercially available ELISA test.<sup>26</sup>

Cysts are small (approximately 12um x 7um) and can be difficult to identify on fecal flotation even when concentrating techniques are used. The cysts can be confused with pseudoparasites such as yeast or plant matter and are easy

to overlook even with careful microscopic inspection of samples. The ZnSO<sub>4</sub> centrifugation-flotation technique (ZNCT) has been considered the gold standard for the diagnosis of *Giardia* when used by trained personnel. However, the shedding of cysts is intermittent and as a result at least three ZNCTs performed on consecutive fecal samples must be negative in order to rule out *Giardia* infection. Furthermore, many veterinary practitioners are unable to successfully identify *Giardia* cysts when they are present; at a major veterinary conference, only 6 of 27 participants in a wet lab were able to identify *Giardia* cysts from a known positive sample.<sup>9,10</sup> While ZnSO<sub>4</sub> centrifugation procedures increase recovery and maintain the integrity of cysts, they do not completely alleviate problems associated with cyst identification and low numbers of organisms.

Although multiple ZNCTs have traditionally been considered to be the gold standard for the diagnosis of *Giardia*, ELISA and IFA evaluations are increasing in use and acceptance. ELISAs, including both commercially available tests for in-house use as well as those performed by diagnostic laboratories, were originally developed for use in humans and are being used with increasing frequency in veterinary practice for cats and dogs. Because they detect an antigen produced by trophozoites that is shed continuously, they theoretically avoid the problem of intermittent cyst shedding and are generally easier to interpret than a fecal flotation. While their specificity is high, reports on the sensitivity of *Giardia* ELISA tests vary substantially, ranging from 68-95%. One author<sup>27</sup> found an in-house ELISA (SNAP® *Giardia* Test, IDEXX Laboratories, Westbrook, ME) to have 90% sensitivity and 96% specificity when compared to immunofluorescence and another investigator

found that antigen was regularly detectable via ELISA in *Giardia*-infected dogs and showed good correlation with ZNCT.<sup>28</sup> Another study evaluating 344 samples from cats in 4 different northern California shelters found that the IDEXX SNAP® *Giardia* test was highly sensitive and specific for diagnosis of cyst shedding in cats with or without diarrhea and was more sensitive than available human test kits.<sup>16</sup> False negatives were most common in samples with low cyst counts and in diarrheic samples, which may be the result of dilution. However, when performed in parallel with fecal flotation the combined sensitivity of the two tests was increased to 97.8% on a single sample.

Direct immunofluorescence (IFA) tests, which use antibodies against *Giardia* to detect and identify cysts, are also available and have been shown to be more sensitive than ZNCT in cattle and sheep, particularly when low numbers of cysts are present.<sup>29</sup> IFA tests are commonly used as the gold standard in prevalence studies if not in clinical practice for the diagnosis of *Giardia* in cats and dogs. Genetic heterogeneity does not appear to significantly alter test results but may be a possible explanation for the diminished sensitivity that is observed when human-validated tests are used in cats. Debate continues over which testing protocol is the most accurate, but most experts agree that some combination of multiple tests on multiple samples is necessary to minimize false negative results. For example, when used in asymptomatic dogs in Finland, false negatives on an ELISA were also found in samples with low cyst counts (less than  $10^4$  cysts/g of feces) when compared to results obtained with IFA testing. One sample, however, seemed to be sufficient in determining infection status based on IFA results (as a very high kappa was noted between results from successive samples).<sup>30</sup> Based on this information, it is

therefore possible that antigen/antibody-based techniques are sensitive enough to allow for a reasonable approximation of prevalence on the basis of a single sample rather than 3 consecutive samples typically considered necessary for ZnSO<sub>4</sub> fecal flotation. This is an important consideration since in many instances it may not be feasible to obtain multiple samples from an individual animal.

### ***Zoonotic Potential***

In addition to the challenge of identifying *Giardia* infections in cats, the public health significance is unknown, raising questions about the need for identifying and treating infected cats. Data on the frequency of zoonotic transmission is lacking and is generally based on circumstantial rather than experimental observations. In one study, investigators were unable to establish persistent, patent infections in kittens (n = 14) after experimental infection with human-source *Giardia* cysts and trophozoites.<sup>31</sup> In another study, investigators evaluated owners and their pets for *Giardia* and found an overall prevalence of 13.7% in people, 7.3% in dogs, and 14% in cats but were unable to demonstrate an association between human and animal infection.<sup>32</sup>

More recently, efforts have focused on identifying human and animal infections using genotyping to improve the understanding of the host specificity and clinical disease produced by various *Giardia* species. Several *Giardia* species are known to have a limited host range. For example, *Giardia agilis*, *argeae*, *psittacci*, *muris*, and *microti* have been shown to infect amphibians, birds, and rodents respectively, but not humans. In contrast, *Giardia duodenalis* (also

known as *Giardia intestinalis* and *Giardia lamblia*) has a wide host range and probably represents a species complex comprised of a variety of genotypes with different host specificities.<sup>33,34</sup> The majority of mammalian infections are thought to be caused by *Giardia duodenalis*, which can be divided into several morphologically identical, but genetically distinct groups with varying host specificities.

A comprehensive phylogenetic study analyzed *Giardia duodenalis* isolates at 4 different genetic loci, confirming the current distinction of seven different lineages known as assemblages.<sup>35</sup> Cysts of these varying genotypes are morphologically indistinguishable by light microscopy and advanced molecular techniques are required to determine their assemblage type. It is important to note that inconsistent nomenclature has been used to describe the various genotypes, complicating the interpretation of the literature. For example, assemblages A and B have also been referred to as Polish, Belgian, and groups 1/2 and 3.<sup>36</sup> Human infections are widely considered to result only from assemblages A or B, which have the widest host specificity, with the former possessing the greatest range. These genotypes can be further broken down into subtypes, with assemblage A divided primarily into subtypes A1 and A2 and assemblage B divided into subtypes B3 and B4. Assemblage A is considered to be the most important genotype with regards to zoonotic transmission. The other 5 assemblages appear to be far more host adapted, with assemblages C and D being found mainly in dogs, E in livestock, F in cats, and G in rodents. Mixed infections are also possible.

Although assemblage A possesses the widest host specificity, its subtypes appear to be more restricted. Most human infections involve subtype A2, while

the majority of animal infections involve either subtype A1 or the host-adapted genotypes (e.g. assemblage E in livestock, assemblage F in cats, and so forth), providing little support for the theory that zoonotic transmission among various species is widespread. Although limited, case-control studies have largely failed to identify contact with pets as a risk factor for giardiasis in children and adults.<sup>37</sup> Instead, sanitation and personal hygiene appear to be far more important factors for infection, suggesting that zoonotic transmission is of little, if any, significance. In fact, some authors<sup>1</sup> have proposed that assemblages C, D, E, F, and G be re-categorized as distinct species such as *Giardia canis*, *Giardia felis*, and *Giardia bovis* because of their apparently limited host range. It is possible that zoonotic transmission through contamination of water sources may exist but such contamination is more likely attributable to wildlife (owing to proximity) or livestock (as a result of the volume of manure and number of cysts), rather than companion animals such as cats and dogs. Even the population of feral cats seems unlikely to contaminate water sources given the low prevalence of cyst shedding found by Nutter et al.<sup>23</sup> Most likely, the majority of human cases result from direct or indirect contact with human-origin *Giardia* isolates rather than spread from animals to people.

The presence of genotypes with broad host specificity implies that zoonotic transmission is possible, but neither verifies its occurrence nor the direction of transmission if it occurs. For example, one investigator found a strong association between the prevalence of the same assemblages in humans and *Giardia*-positive dogs in the household, but was unable to determine whether humans were the source of *Giardia* circulating in the dogs or vice-versa.<sup>38</sup>

Other studies have failed to find an association with fecal cyst shedding in pets and cyst shedding in humans, suggesting that transmission is limited at best and of questionable clinical significance.<sup>37</sup>

In order to improve the current understanding of its zoonotic potential, a number of studies have focused on determining the assemblage(s) of *Giardia* spp found in cats. When interpreting these results, however, it is necessary to recognize that genotypic differences are determined by PCR amplification of a particular gene followed by sequence analysis. Since different studies have analyzed different target genes, there can be a significant degree of discrepancy in the assemblage results depending on which genes were selected for sequencing. Indeed, studies employing different genotyping tools can easily yield vastly different results, and it has been suggested that simultaneously screening for several genes would yield more accurate determinations of assemblage type. Targets include ssu-rRNA or any number of genes, including  $\beta$ -giardin, glutamate dehydrogenase, elongation factor 1-alpha, triose phosphate isomerase, GLORF-C4, or inter-genomic rRNA spacer region.<sup>34</sup> These genes vary in their polymorphism as some are much more highly conserved among assemblages and species than others. Furthermore, published primers do not universally amplify *Giardia* DNA, possibly as a result of greater sequence variability in certain genes when compared to the multi-copy nature of ssu-rRNA. Therefore, the assignment of an isolate to one assemblage or another can be unreliable, and isolates typed as “potentially zoonotic” by one marker might be typed as “species specific” by another marker.<sup>34</sup>

Seven studies have attempted to determine which genotypes are found in *Giardia* infected cats to date. Even with the increasing body of scientific literature, assemblage results are only available for a total of 78 cats and some studies had sample sizes as small as 3 individuals. The results have varied substantially among studies, with some investigators finding cats to be exclusively infected with assemblage A,<sup>39</sup> others identifying assemblage F,<sup>40-41</sup> and still others reporting a mixture of assemblages within the populations studied.<sup>40-43</sup> One study even found that sequencing results for 13 of the 14 cat samples were most consistent with a dog-adapted genotype (assemblage D) that had not previously been reported in other species.<sup>22</sup> It is possible that some of these discrepancies are the result of differing methodologies and interpretation of sequencing results, while other variation may be attributable to geographical differences as the work has been conducted in Italy, the United States, Brazil, Columbia, Japan, and Australia.

Because of the small number of cats studied to date and the wide variation in geographical location of the investigations, differences among assemblages regarding risk factors for infection or severity of infection have not yet been identified. Based on descriptive data in the limited body of work available, it appears that assemblage A is more likely to be found in young cats less than 6 months of age and those with outdoor access<sup>39</sup> but further research is needed to determine if these characteristics are truly risk factors for infection.

### ***Treatment and Public Health Recommendations***

Insufficient data have been collected on the assemblage types found in *Giardia* infected cats to make a quantitative, accurate assessment of the public health risk associated with these cats. Despite a greater understanding of the molecular epidemiology of the disease, the question as to whether or not asymptomatic cats found to be shedding *Giardia* cysts should be treated in order to protect public health cannot be answered. Data which suggest that zoonotic transmission of *Giardia* from pets to humans is unlikely to be significant cause of human cases are accumulating.<sup>45</sup> More recent veterinary recommendations reflect this belief and do not call for automatic treatment of asymptomatic cats and dogs found to be shedding cysts. Indeed, current professional guidelines published by the Companion Animal Parasite Council<sup>46</sup> state that dogs and cats should not be treated solely for the purpose of preventing zoonotic transmission and that repeated courses of treatment are not indicated in dogs or cats without clinical signs. However, this belief is not universally accepted and some experts still call for the treatment of all infected companion animals regardless of the presence of clinical disease.<sup>32</sup> In fact, treatment recommendations vary widely among veterinary experts, public health officials, and basic scientists.

As such, many animal shelters and veterinarians remained concerned about the potential liability associated with failing to identify or electing not to treat infected cats. The decision of whether or not to treat is further complicated by the fact that many infections in cats are subclinical and no treatment protocols have been proven to be 100% efficacious. It is unclear whether drug therapy eliminates *Giardia* infections or merely suppresses cyst shedding, and the

efficacy may be further reduced in animals with diarrhea since rapid gastrointestinal transit time may result in decreased drug contact with the trophozoites.<sup>3</sup> Successful clearance of *Giardia* spp. infection from individual cats can thus be difficult, and elimination of endemic disease in feline populations is particularly challenging. Even when initial treatment is successful at stopping cyst shedding, reinfection from a contaminated facility, from other cats, or from a cat's own hair coat while grooming is common.<sup>47</sup> For these reasons, increased sanitation and disinfection are commonly cited as essential factors in eliminating infection from affected facilities. *Giardia* cysts are destroyed with many disinfectants commonly used in animal holding facilities, including sodium hypochlorite (e.g. household bleach) and quaternary-ammonium containing products. Cysts are also susceptible to desiccation, and ensuring a dry environment can be helpful in breaking the cycle of transmission. In addition, bathing cats in order to remove infective cysts from their hair coats and then moving them to uncontaminated enclosures after they have been treated has been recommended to help break the transmission cycle.<sup>4</sup>

There are no approved drugs for the treatment of giardiasis in cats. Available but unapproved drugs (e.g. extra label usage) include metronidazole, albendazole, fenbendazole, and a product containing a combination of febantel, pyrantel, and praziquantal (Drontal Plus<sup>®</sup>, Bayer Animal Health, Shawnee Mission, KS). Several studies have examined the efficacy of these medications in eliminating the shedding of *Giardia* cysts in dogs, but only a few studies have evaluated their efficacy in cats. Reported efficacies in cats range from 50-100% for fenbendazole (n = 8)<sup>48</sup> and 20-60% for Drontal Plus<sup>®</sup>

depending on the dosage (n = 16).<sup>49</sup> Recently, a study found that metronidazole benzoate administered at a dose of 25 mg/kg by mouth twice daily eliminated cyst shedding in 100% (n = 26) of study cats,<sup>50</sup> but the study lacked a control group. A commercially available vaccine has also been studied as a possible treatment for *Giardia* but has neither been demonstrated to be an effective treatment nor preventive.<sup>51</sup> Some authors have suggested concurrent treatment with metronidazole and fenbendazole believing that the combination may be more efficacious than treatment with either drug alone, however no controlled studies have been performed to evaluate such a protocol.<sup>46</sup> In addition, it is imperative that steps are taken to improve an infected cat's overall health and immune system, including elimination or treatment of concurrent diseases and other parasitic infections, reduction of stress, and improved nutrition.

In addition to concerns of efficacy, there also remains a question of safety regarding the treatment protocols for cats. Metronidazole administration has been associated with gastrointestinal and central nervous system toxicity,<sup>52</sup> particularly when used at high doses for long periods of time (e.g. longer than 1 week). Albendazole has been shown to cause bone marrow suppression in some cases.<sup>53</sup>

## **Summary**

*Giardia duodenalis* is a common intestinal protozoal parasite capable of causing both clinical and subclinical disease in a broad range of species, including humans and cats. Cysts of the varying genotypes are

morphologically indistinguishable by light microscopy and advanced molecular techniques are required to determine their assemblage type. Little is known about the differences among various assemblages, but the principal distinction appears to be host range and thus zoonotic potential. Confusion exists regarding the significance of *Giardia* in cats and the need for surveillance and treatment in animal shelters as both the zoonotic assemblage A and host-adapted assemblage F have been isolated from cats. Because most infections are subclinical and there are no conclusive data that *Giardia* infected cats pose a human health risk, treatment may not always be indicated. The decision as to whether or not to treat is further complicated by the fact that there are no approved drugs for the treatment of giardiasis in cats and no treatment protocols have been proven to be 100% efficacious.

The confusion surrounding *Giardia* in cats is particularly evident in animal shelter settings. Concerns regarding public health and legal liability have prompted some shelters to actively screen the cats in their care for *Giardia* infection, but such practices place a tremendous burden on the already limited resources of these organizations and may not be necessary. Research is needed in order to better characterize the host range and zoonotic potential of various assemblages and to determine risk factors for infection associated with them. Such knowledge would then allow for a more quantitative and accurate assessment of the true public health risk associated with *Giardia* in cats and the need for treatment in clinically normal cats. Elimination of endemic infection in feline populations is particularly challenging, and a thorough risk assessment would greatly assist veterinarians and humane organizations in targeting their diagnostic and therapeutic efforts.

This literature review identifies several gaps in existing knowledge regarding *Giardia* infection in shelter cats. For instance, prevalence estimates vary significantly among different populations of cats studied. Although it has been hypothesized that such variation is due, in part, to differences in diagnostic methodology and geographic location, there is a general lack of knowledge regarding risk factors for infection. In addition, there may be clinically significant but still undetermined factors affecting prevalence. The pathogenic mechanisms remain to be fully elucidated, and little is known about the specifics of the host, agent, and environmental factors that determine the clinical course of disease or lack thereof. Optimal testing protocols, based on sensitivity, specificity, ease of use and interpretation and cost have yet to be established for use in individual cats as well as for feline populations. Finally, the true zoonotic potential of *Giardia* has yet to be established. This paucity of knowledge makes evidence-based decision-making difficult if not impossible in the clinical setting. In the context of animal shelters, where large numbers of cats of varying immune status are housed concurrently and where resources for animal care are limited, these challenges are particularly evident.

The purpose of the study described in the subsequent chapter was to expand the existing body of knowledge pertaining to *Giardia* infections in shelter cats and to specifically address the question of its zoonotic potential in order to provide data that can be used for quantitative risk assessment. Specifically, the study was designed to investigate the following hypotheses:

- (1) The prevalence of *Giardia* infection in shelter cats is within the range reported for owned cats;

(2) Most shelter cats are not a public health threat with regards to transmission of *Giardia* to people;

(3) Conditions that could reasonably be expected to facilitate transmission of *Giardia* (e.g. overcrowding) or increase host susceptibility to infection (e.g. age) are risk factors for infection.

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## CHAPTER 2

### **Prevalence of, risk factors for, and assemblage types of *Giardia* spp. infection in cats housed in an animal shelter**

**Janeczko SD, Griffin B, Barr SC, Santin M, Scarlett JM.**

#### ***Abstract***

**Objective** – To estimate the prevalence and zoonotic potential of *Giardia* in cats housed in an animal shelter and to determine risk factors for infection.

**Design** – Cross-sectional study

**Sample Population** – Cats housed in the adoption ward at an animal shelter in central New York

**Procedures** – Fecal samples were collected once monthly from cats available for adoption for 5 months and tested for the presence of *Giardia* antigen with a SNAP<sup>®</sup> *Giardia* Test. PCR and sequencing were performed on all positive samples to determine the assemblage of each *Giardia* isolate.

**Results** – Monthly prevalence of *Giardia* infection ranged from 6.6% - 14.8% with a significantly higher prevalence in the summer than in the spring ( $p = 0.03$ ). Infected cats were more likely to have entered the shelter as strays and to have been housed with a greater number of

cats than controls. The host-adapted assemblage F was found in 75% of infected cats, and potentially zoonotic assemblages A and B were found in the remaining 25%. The latter were more commonly identified in adults than kittens, and in cats with soft stools or diarrhea.

**Conclusions and Clinical Relevance** – Although the prevalence of *Giardia* in cats in this study was slightly higher than in previous reports, most infections were not associated with clinical signs and did not have zoonotic potential based on reported host ranges. However, the potential for cats to be a source of human *Giardia* infection can not be discounted. Additional studies are needed to better understand the risk factors associated with infection with the various assemblage types and to establish its true zoonotic potential.

## ***Introduction***

*Giardia duodenalis* (also known as *Giardia intestinalis* and *Giardia lamblia*) is an intestinal protozoal parasite capable of causing both clinical and subclinical disease in a broad range of species, including humans and cats. Affected animals shed infective cysts containing two mitotically arrested trophozoites that emerge in the duodenum following ingestion. Cysts are frequently waterborne and may survive for weeks to months in cool, moist environments but are susceptible to drying.<sup>1</sup> *Giardia* has a simple, direct life cycle, and transmission occurs via the fecal-oral route. Rapid environmental contamination is likely in animal shelters and catteries with endemic disease,

and cysts can become well-adhered to the perianal region of cats, facilitating infection and re-infection.

The parasite has a ubiquitous distribution and *Giardia* infection occurs worldwide in nearly all mammals. *Giardia* has a wide host range and may be more accurately described as a species complex comprised of a variety of genotypes or assemblages that have different host specificities.<sup>2,3</sup> Cysts of the various genotypes are morphologically indistinguishable by light microscopy and advanced molecular techniques are required to determine their assemblage type. A comprehensive phylogenetic study analyzed *Giardia* isolates at 4 different genetic loci, confirming the current distinction of seven different lineages.<sup>4</sup> Little information exists regarding the differences among the various assemblages, with the principal distinction being host range and thus zoonotic potential and possibly geographic location. Human infections are usually caused by assemblages A and B. Assemblage A is considered to be the most important genotype with regards to zoonotic transmission and has a wide host range, while assemblage B has a more restricted host range and is not commonly isolated from companion animals. The other 5 assemblages appear to be far more host adapted, with assemblages C and D being found mainly in dogs, E in livestock, F in cats, and G in rodents. Mixed infections are also possible.

Estimates of prevalence in cats fall within a broad range typically between 1-10%, in part because infection does not always result in clinical signs and diagnostic methods vary greatly.<sup>5-23</sup> When clinical signs are present, affected cats most commonly exhibit acute small bowel diarrhea with or without weight loss. Disease may be severe in a limited number of cases, and large bowel

diarrhea, failure to thrive and even vomiting<sup>23</sup> have been described in cats found to be shedding *Giardia* cysts in their feces. However, the presence or absence of clinical signs is generally not helpful in predicting the infection status of a cat; most infections are asymptomatic and in some studies, a higher prevalence of intestinal parasitism has been found in cats without diarrhea compared to those with diarrhea.<sup>10</sup>

Confusion exists regarding the significance of *Giardia* in cats and the need for surveillance and treatment in animal shelters. Data regarding the zoonotic potential of *Giardia*-infected cats is lacking. Both the host-adapted genotype, assemblage F,<sup>24-28</sup> as well as the zoonotic genotype, assemblage A,<sup>26-30</sup> have been found in the populations of cats studied to date. However, insufficient data have been collected to allow for determination of the prevalence of and risk factors for infection with the different assemblages, and thus a quantitative, accurate assessment of the public health risk has not been possible. The purpose of this study was to estimate the prevalence of *Giardia* in cats available for adoption at an animal shelter in central New York, assess zoonotic risk by determining the assemblage type(s) found in cats shedding *Giardia* cysts, and determine risk factors for infection.

## ***Materials and Methods***

### ***Samples and Data Collected***

Once monthly from April 2008-August 2008, a single fecal sample was collected from cats and kittens available for adoption at the Tompkins County SPCA in Ithaca, New York. This facility is the only shelter serving the rural county in which Cornell University is situated, and was an open-admission, adoption guarantee organization at the time of the study. The shelter cared for approximately 1800 cats during 2008, with approximately 70 to 150 cats available for adoption at any given time depending on the month in question. All cats 4 weeks of age and older were vaccinated with a modified-live feline herpesvirus, calicivirus, panleukopenia vaccine and given a dose of oral dewormer (pyrantel pamoate, 10 mg/kg po once). All cats were fed a standard commercially available dry diet as appropriate for their age (Science Diet<sup>®</sup> Adult Cat Original or Science Diet<sup>®</sup> Kitten Healthy Development Original, Hill's<sup>®</sup> Pet Nutrition, Topeka, KS). Cats available for adoption were predominantly group housed in small rooms with several other cats (Figure 1). Room sizes ranged from 16.5 to 170 square feet with 1-18 cats per room. Some cats were housed individually in small rooms or cages as necessary for management of medical conditions (e.g. feline lower urinary tract disease) and/or behavioral reasons (Figure 1).



**Figure 1:** Representative examples of group and individual housing of cats available for adoption at the Tompkins County SPCA. Rooms range in size from 16.5 ft<sup>2</sup> to 75 ft<sup>2</sup>.

A: Smaller room for group housing (32.5 ft<sup>2</sup>)

B: Larger room for group housing (70 ft<sup>2</sup>)

C: Individual housing in cages

Each cat was individually examined by one of two of the authors within 3 days of sample collection in order to determine age, sex, body weight, and body condition score. Cats were assigned a body condition score on a scale of 1 to 9 according to a standardized scoring chart (Purina body condition score chart; Nestle Purina, St. Louis, MO). The following information was collected from the shelter records for each cat, if available: date of entry to the shelter, body weight at time of entry, origin (stray vs. owner surrendered), and outdoor access of surrendered cats (indoor only, predominantly outdoor, or indoor/outdoor). The following information was recorded at the time of sample collection for each cat: date of sample collection, type of housing (individual vs. group), number of other cats in the housing unit (if applicable), housing unit dimensions, and total number of cats in the shelter. The length of stay in the shelter (as determined from the date of entry and the date of sample collection), change in body weight (as determined from the weight at time of entry and weight at time of sampling), square footage per cat (as determined from the housing unit dimensions and number of other cats in the housing unit, if applicable) were calculated for each cat.

An attempt was made to collect a sample from every cat housed in the adoption area over seven days' time during the same week every month; samples were successfully collected from all but approximately 4-10% of cats depending on the month. Some cats were missed because they were removed from the adoption area or because there were an insufficient number of fecal markers available to obtain an individually identifiable sample from each cat housed in larger groups. Samples were collected from cats housed in cages and in colony rooms. In addition to their usual dry food, cats were also offered

canned cat food (Science Diet<sup>®</sup> Adult Optimal Care<sup>™</sup> Liver & Chicken Entrée, Hill's<sup>®</sup> Pet Nutrition, Topeka, KS). Group-housed cats consumed food coloring or glitter once daily in their canned food during the sample collection period according to the methods of Griffin.<sup>31</sup> These served as fecal markers, allowing identification of each cat's feces (Figure 2). All feces were collected from the litter boxes prior to cleaning in the morning for all cats. For group housed cats, fecal samples were individually inspected by two of the authors or trained observers for the presence of a fecal marker. A sample was identified for each cat and scored using a standardized fecal scoring system (Fecal Scoring System, Ralston Purina, St. Louis, MO). Data were collected on a standardized collection form.

Samples were stored at 4°C and tested within 5 days of collection for the presence of *Giardia* antigen using a commercially available ELISA test (SNAP<sup>®</sup> *Giardia* Test, IDEXX Laboratories, Westbrook, ME) in accordance with the manufacturer's instructions. A sample was considered positive if any intensity of color was visible on the *Giardia* sample spot; if a result was in doubt (e.g. very faint color change), the sample was considered positive to minimize any chance of artificially lowering the prevalence and underestimating the public health risk. *Giardia* infection was defined by at least one positive ELISA result and a positive cat was considered a case. Any cat that did not test positive served as a control. Cats that were in the adoption area for multiple months during the study were sampled multiple times. This study was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee.



**Figure 2:** Food coloring and glitter were used as fecal markers for cats that were group housed, allowing identification of each cat's feces.

A: Fecal markers were mixed with a commercially available canned food and offered to cats

B: Most cats readily consumed the colored canned food

C: Fecal markers consistently produced distinctive coloration of feces allowing for easy identification by trained observers. Clockwise from the upper left, individually bagged pink, red, teal, and black samples are seen in bags with cat litter.

### ***Fecal Flotation and Cyst Concentration***

Any fecal sample with a positive result on the ELISA test was processed for genotype analysis. Magnesium sulfate centrifugation-flotation was performed to concentrate the cysts, and samples were examined by light microscopy to estimate the number of *Giardia* cysts per gram of feces. In brief, 5 g of feces were mixed with distilled water, strained using cheese cloth, and allowed to settle at 4°C for up to 90 minutes. The sediment was then centrifuged at 1200g for 5 minutes. The resulting pellet was then resuspended with magnesium sulfate flotation solution (specific gravity 1.30) and centrifuged at 1200g for 5 minutes. The top 15 mL of supernatant was filtered through 25 and 75 micron mesh filters, and the resulting filtrate was mixed with water to a specific gravity of 1.0 and allowed to settle for at least one hour at 4°C. The supernatant was removed and the remaining 50 mL was centrifuged at 1200g for 5 minutes. The supernatant was again removed, leaving a 5 mL pellet that was washed with half-strength phosphate buffered saline solution. The remaining solution was centrifuged at 800g for 5 minutes, the supernatant was decanted, and the pellet was resuspended in 1mL of remaining supernatant. Samples were stored at 4°C and shipped overnight on ice for molecular analysis at the Agricultural Research Service, United States Department of Agriculture, Beltsville, MD.

### ***PCR and Sequencing Analysis***

At the Agricultural Research Service, DNA was extracted from 50 µl of the concentrated cyst suspension, using a DNeasyTissue Kit (Qiagen, Valencia, CA). To maximize recovery of DNA, the nucleic acid was eluted in 100 µl of AE buffer (elution buffer included in DNeasyTissue Kit). For *Giardia*, a fragment of the SSU-rDNA (~292 bp) gene was amplified by PCR as previously described.<sup>24,32</sup> For the primary PCR step, the PCR mixture contained 1x PCR buffer, 1.5mM MgCl<sub>2</sub>, 0.2mM each dNTP, 2 U Taq, 2.5 µl of dimethyl sulfoxide (DMSO), and 0.5mM for each forward and reverse primer in a total of 50 µl reaction volume. A total of 35 cycles, each consisting of 96 °C for 45 s, 58 °C for 30 s, and 72 °C for 45 s, were performed; an initial hot start at 96 °C for 2 min and a final extension step at 72 °C for 4 min were also included. For the secondary PCR step, the PCR mixture was identical. A total of 35 cycles, each consisting of 96 °C for 45 s, 58 °C for 30 s, and 72 °C for 45 s, were performed; an initial hot start at 96 °C for 2 min and a final extension step at 72 °C for 4 min were also included. PCR products were analyzed on 1% agarose gel and visualized by ethidium bromide staining. Exonuclease I/shrimp alkaline phosphatase (Exo-SAP-IT,<sup>®</sup> USB Corporation, Cleveland, OH) was used to purify the PCR products that were then sequenced using the same PCR primers in 10 µl reactions with Big Dye<sup>™</sup> chemistries and an ABI3100 sequencer analyzer (Applied Biosystems, Foster City, CA). After each specimen was sequenced in both directions, chromatograms from each strand were aligned and inspected using Lasergene software (DNASTAR Inc., Madison, WI).

### ***Data and Risk Factor Analysis***

Information collected was entered into a spreadsheet and imported into a commercially available software package for statistical analysis (Statistix 9.0, Analytical Software, Tallahassee, FL). Monthly prevalence of *Giardia* infection was calculated by dividing the number of cats that tested positive on the ELISA by the number of individual cats sampled monthly. The prevalence of infected cats for the 5 month period of the study was estimated by dividing the number of cats that tested positive at least once by the number of cats sampled at least once. Prevalence was expressed as a percentage and 95% confidence intervals were calculated.

Associations between infection status (or assemblage type) and the following variables were evaluated: age, age group (adults > 6 months of age, kittens ≤ 6 months of age), sex, body weight, body condition score, change in body weight since entry to the shelter, origin (stray vs. owner surrender), outdoor access (when provided for owner surrendered cats; any vs. none), season of entry (January – March, April – June, July – September, October – December), length of stay in the shelter, type of housing (individual vs. group), number of other cats in the housing unit, number of cats in the shelter, square footage per cat in the housing unit, number of fecal samples collected, and fecal score.

Associations between potential risk factors and *Giardia* infection status and assemblage type were first evaluated in a univariate analysis (i.e. one factor at a time). In the analyses of factors associated with *Giardia* infection, the characteristics of infected cats were evaluated at the time of the first positive

sample. Since controls may also have been sampled more than once, characteristics from one randomly selected sampling were recorded for each control cat. Therefore, every cat was represented only once in the assessment of risk factors for *Giardia* infection. In the analyses of factors associated with assemblages, the assemblage types were first categorized into those that may be zoonotic (assemblages A and B) and those that are thought not to be zoonotic (assemblage F). Since more than one assemblage could be recovered over time in a cat and characteristics (e.g. weight, number of cats in housing enclosure) could change with time, factors associated with zoonotic vs. non-zoonotic types were evaluated with the sample (rather than cat) as the unit of analysis.

The association between categorical variables (e.g. gender, origin) and *Giardia* infection (or assemblage type) status was assessed using the chi-square test of independence or Fisher's exact test (where expected cell values were less than 5). Differences of continuous factors (e.g. age, body weight) between groups (*Giardia* positive and negative or potentially zoonotic and host-adapted) were evaluated using the Student t test (for Normally distributed data) or the Wilcoxon rank sum test (for non-Normally distributed data). Variables with p values < 0.20 were examined further as risk factors for *Giardia* infection using unconditional logistic regression to examine the joint effect of these factors and to control for confounding. Variables with p values < 0.25 were examined further as risk factors for infection with a particular assemblage with unconditional logistic regression. The model parameters for associations with infection status or assemblage type were obtained by maximum likelihood estimation using the computer program (EGRET<sup>®</sup> version

2.0.3 for Windows, Cytel Inc., Cambridge, MA). The models were constructed using a forward stepwise approach, and significance was determined by evaluating the likelihood ratio chi-square statistic in each step of the fitting process. Variables significant at  $p \leq 0.05$  were retained in the final model. The regression coefficients were exponentiated to obtain adjusted odds ratios, and 95% confidence intervals were calculated.

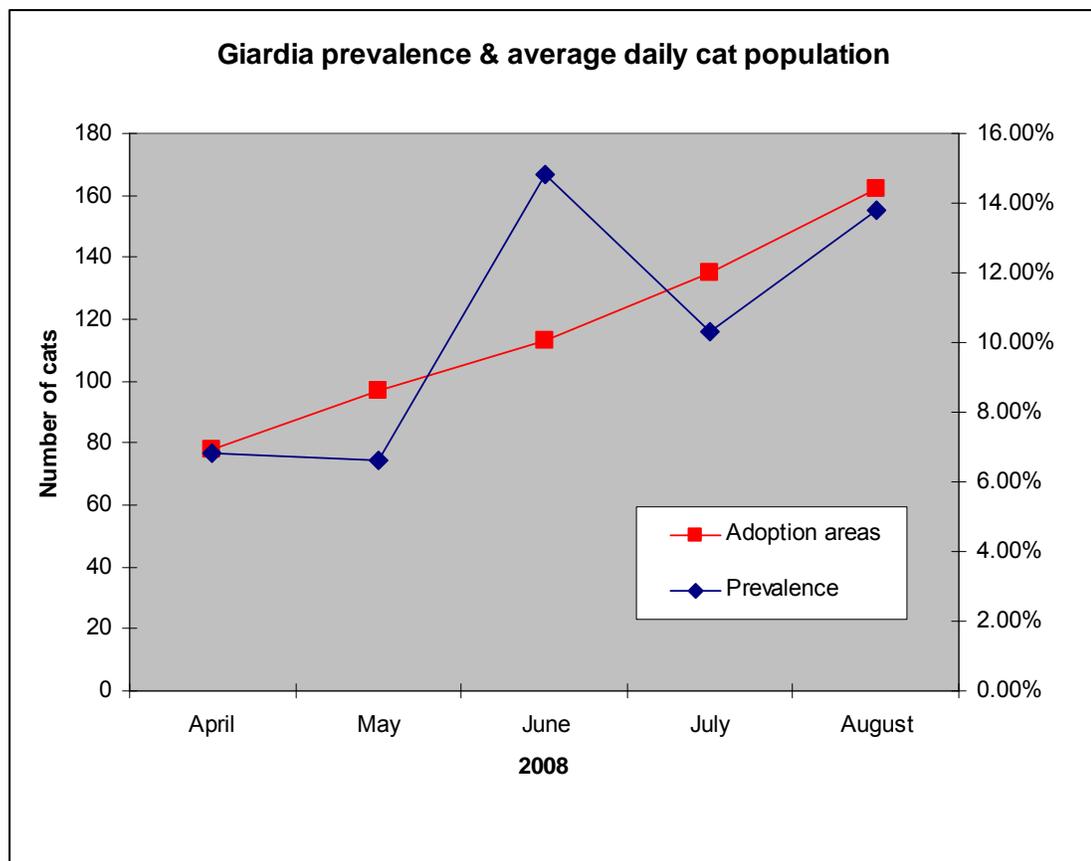
## ***Results***

### ***Prevalence of Giardia Infection***

During the 5 month study period, a total of 554 fecal samples were collected from 302 different cats, and 61 ELISA positive samples were obtained from 49 different cats. The overall prevalence of *Giardia*-positive cats from April-August was 16.2% (49/302); 95% CI: 12.3% - 21.0%. The monthly prevalence ranged from 6.6% to 14.8% (Figure 3 and Table 1), and the number of cats in adoption increased by approximately 20 cats per month over the study ( $p = 0.0004$ ). The prevalence was significantly ( $p = 0.03$ ) higher in the summer (June, July, August) compared to spring (April, May) months.

The number of samples obtained from positive cats was significantly ( $p = 0.01$ ) higher (median 2.2, range 1-5) than among control cats (median 1.7, range 1-5). *Giardia* positive cats had a significantly higher proportion of males than controls ( $p = 0.05$ ), and were significantly younger compared to cats testing negative ( $p = 0.12$ ). Although the median age for both cases and controls was 1.5 years, there was still significant variation between the two groups because

of differences in the distribution. Positive animals were also more likely ( $p = 0.20$ ) to have been strays (60%) compared to negative cats (50%) and if owner-surrendered to have had outdoor access ( $p = 0.10$ ) (Table 2).



**Figure 3:** Monthly prevalence of Giardia infection and average number of cats in the adoption area of the shelter at the time of sampling.

**Table 1:** Monthly prevalence of Giardia infection, average daily population in the shelter and adoption areas, and square footage per cat among cats in the adoption ward of an animal shelter.

2008 Month	Average Shelter Population (Cats)	Average Adoption Population (Cats)	Average Ft <sup>2</sup> per Cat (Controls)	Average Ft <sup>2</sup> per Cat (Giardia positive)	Number of Positive Results	Number of Cats Tested	Prevalence (95% CI)
April	186	78	13.3 (range: 6.7-20)	7.2 (range: 6-8.3)	5	74	6.8% (2.5-15.7%)
May	245	97	9.1 (range: 7.2-10.9)	16.2 (range: 0-44.4)	6	91	6.6% (2.7-14.3%)
Spring	216	88	11.2	11.7	11	165	6.7% (3.5-11.9%)
June	338	113	8.7 (range: 6.5-10.8)	9.8 (range: 2-17.7)	16	108	14.8% (9.0-23.2%)
July	430	135	5.9 (range: 5.4-6.4)	4.5 (range: 4-5.1)	13	126	10.3% (5.8-17.3%)
August	447	162	5.7 (range: 4.8-6.5)	5.3 (range: 3.9-6.8)	20	145	13.8% (8.8-20.7%)
Summer	402	137	6.8	6.5	49	379	13.0% (9.8-16.8%)

**Table 2:** Host characteristics and potential risk factors for *Giardia* infection in cats in an animal shelter in New York state.

Variable	Description	Cases		Controls		p value
Age (years)	Median	1.5		1.5		0.12
	Range	0.2-8.0		0.1-13.0		
Body condition score	Median	5.0		5.0		NS
	Range	4.0-9.0		3.0-9.0		
Body weight at intake (kg)	Median	3.4		3.5		NS
	Range	0.4-8.9		0.3-9.0		
Body weight at sample collection (kg)	Median	3.3		3.5		NS
	Range	0.9-9.1		0.7-8.8		
Number of fecal samples collected	Median	2.2		1.7		0.01
	Range	1-5		1-5		
Length of stay (days)	Median	54		48		NS
	Range	3-264		1-383		
Total # cats in shelter	Median	370		427		NS
	Range	165 - 448		168-448		
Total # cats in adoption	Median	113		135		NS
	Range	78-162		78-162		
# cats in housing unit	Median	6		4		0.10
	Range	1-17		1-18		
Sq. footage per cat	Median	5.7		6.3		NS
	Range	3.0-56.7		3.0-170.0		
		Cases		Controls		
Variable	Description	No.	%	No.	%	p value
Sex	Female	20	40.8	137	56.4	0.05
	Male	29	59.2	106	43.6	
Origin	Surrendered	21	40.0	126	50.0	0.20
	Stray	30	60.0	121	50.0	
Outdoor access	Yes	4	80.0	12	40.0	0.10
	No	1	20.0	18	60.0	
Housing in shelter	Individual	4	7.8	44	17.7	NS
	Group	47	92.2	204	82.3	
Weight loss	Any	7	15.9	72	36.9	0.007
	None	37	84.1	123	63.1	

NS – not significant

### ***Potential Risk Factors for Giardia Infection in All Cats***

More than 80% of all cats sampled were housed with other cats (range 1 – 17 other cats). *Giardia* positive cats were housed with a greater number of cats ( $p = 0.08$ ) than negative cats. Although body weight at the time of intake and time of sampling and body condition score were not associated with *Giardia* status, uninfected cats were nearly twice as likely to have lost weight during their stay in the shelter than *Giardia* positive cats ( $p = 0.007$ ). The length of stay in the shelter, total number of cats in the adoption area, square footage per cat, and type of housing (individual vs. group) did not differ significantly between *Giardia* positive and *Giardia* negative cats.

### ***Potential Risk Factors for Giardia Infection by Age Group***

Since risk factors differed by age group at the time of sampling, data were also analyzed separately for adult cats and kittens (Table 3).

Adult cats: Among adult cats greater than 6 months of age, *Giardia*-positive cats were significantly younger than controls ( $p = 0.08$ ). Similarly, adult male cats were overrepresented among positive cats (52.9% among cases and 38.5% among controls) ( $p = 0.11$ ).

*Giardia* positive adults were more likely to have been found as strays (75.8%) than *Giardia* negative cats (52.8%) ( $p = 0.05$ ). Information regarding outdoor access was available for a small number ( $n = 35$ ) of adult, owner-surrendered cats; 80% (4/5) of *Giardia* positive cats had outdoor access compared to only 40% (12/30) of control cases ( $p = 0.10$ ).

**Table 3:** Host characteristics and potential risk factors for *Giardia* infection in adult cats in an animal shelter in New York state

Variable	Description	Cases		Controls		p value
Age (years)	Median	2.0		2.5		0.08
	Range	0.2 – 8.0		0.2 – 13.0		
Body condition score	Median	6.0		6.0		NS
	Range	4.0 – 9.0		3.0 – 9.0		
Body weight at intake (kg)	Median	4.1		4.0		NS
	Range	0.7 – 8.9		0.3 – 9.0		
Body weight at sample collection (kg)	Median	5.0		4.2		0.16
	Range	1.1 – 9.1		1.1 – 8.8		
Number of fecal Samples collected	Median	3.0		1.0		0.004
	Range	1.0 – 5.0		1.0 – 5.0		
Length of stay (days)	Median	58		60		NS
	Range	4 – 264		1 – 383		
Total # cats in shelter	Median	335		341		NS
	Range	165 – 448		168 – 448		
Total # cats in adoption	Median	113		113		NS
	Range	78 – 162		78 – 162		
# cats in housing unit	Median	6		4		0.02
	Range	1 – 17		1 – 18		
Sq. footage per cat	Median	5.9		7.0		NS
	Range	3.4 – 56.7		3.0 – 170.0		
		Cases		Controls		
Variable	Description	No.	%	No.	%	p value
Sex	Female	16	47.1	112	61.5	0.11
	Male	18	52.9	70	38.5	
Origin	Surrendered	8	24.2	84	47.2	0.05
	Stray	25	75.8	94	52.8	
Outdoor access	Yes	4	80	12	40	0.10
	No	1	20	18	60	
Housing in shelter	Individual	3	8.8	40	21.7	0.08
	Group	31	91.2	144	82.3	

NS – not significant

Among adult cats, there was also a significant association between *Giardia* cyst shedding and being housed with a greater number of cats ( $p = 0.02$ ). Control cats were housed in groups with a median size of 4 cats compared to groups with a median size of 6 cats for cases. Infection status was associated with group housing in that 91.2% (31/34) of *Giardia* positive cats were group housed compared to 78.3% (144/184) of *Giardia* negative adults ( $p = 0.08$ ).

Kittens: When data for kittens 6 months of age and younger were analyzed, none of the previously mentioned associations were statistically significant. Mean body weight at the time of sample collection was greater for *Giardia* positive kittens than for controls, with control kittens having a median weight of 1.4 kilograms compared to 1.8 kilograms ( $p = 0.023$ ) among infected kittens. Weight change was also associated with infection status; no infected kittens lost weight during their time at the shelter (0/15) compared to 16.4% (51/61) of control kittens ( $p = 0.09$ ). However, there was no difference in body condition scores between infected and uninfected kittens.

Multivariate analysis: For adults, age and origin (e.g. owner surrendered or stray) were found to be significant risk factors for *Giardia* infection in the multivariate model (Table 4). The odds ratio decreased by approximately 18% for each 1 year increase in age. Stray cats were 2.69 times more likely to be infected than owner-surrendered cats. For kittens, body weight at the time of sample collection was the only factor found to be significant in the regression analysis. An increase of 1 pound in body weight at the time of sample collection increased the odds of being positive on the SNAP<sup>®</sup> *Giardia* test by 72% (OR 1.72; 95% CI 1.02-2.78).

**Table 4.** Multivariate (logistic regression) models of risk factors associated with *Giardia* infection among cats available for adoption at an animal shelter.

<b>Model I: <i>Giardia</i> infection in Adult Cats</b>		
<b>Risk Factor:</b>	<b>Odds Ratio</b>	<b>95% CI</b>
• Age	0.82	0.66 – 1.04
• Origin		
Owner-surrendered	1.00	
Stray	2.66	1.14 – 6.34

### ***Prevalence of Assemblage Types***

Prevalence: A total of 61 fecal samples that tested ELISA positive for *Giardia* were submitted for molecular analysis, and assemblage type was determined for 72% (44/61) of the samples. Those samples for which an assemblage type could not be identified (i.e. PCR negative) were excluded from the analysis. Because of the small sample size, the assemblage types were collapsed into two categories for the purpose of analysis: those characterized as potentially zoonotic and those that are not zoonotic. Both zoonotic and host-adapted assemblages were identified (Table 5); 75% (33/44) of the PCR positive samples were the host-adapted assemblage F, while 25% (11/44) of samples had zoonotic potential based on reported host ranges (assemblages A, B, and mixed A/F infections). Cyst counts did not differ significantly among cats

identified as having potentially zoonotic, host-adapted, or PCR negative assemblage results. Demographic information is presented for all cats on the basis on infection type (e.g. potentially zoonotic or host-adapted assemblages) in Table 5.

**Table 5.** *Giardia* assemblage types found in ELISA positive fecal samples collected from cats available for adoption at an animal shelter.

<b>Assemblage type</b>	<b>Reported host range<sup>2</sup></b>	<b>Number identified</b>	<b>Zoonotic potential?</b>
PCR negative	N/A	17 (27.9%)	N/A
A	Humans, livestock, cats, dogs, beavers, guinea pig, slow loris	2 (3.3%)	Yes
B	Humans, slow loris, chinchillas, dogs, beavers, rats, siamang	6 (9.8%)	Yes
F	Cats	33 (54.1%)	No
Mixed A & F	N/A	3 (4.9%)	Yes

N/A not applicable

**Table 6:** Host characteristics and potential risk factors for assemblage type among *Giardia* infected cats in an animal shelter in New York state

Variable	Description	Assemblage A, B or mixed		Assemblage F		p value
		No.	%	No.	%	
<b>Age (years)</b>	Median	1.5		1.5		NS
	Range	0.4-3.0		0.2-6.0		
<b>Body condition Score</b>	Median	6.0		5.0		0.16
	Range	5.0-9.0		4.0-7.0		
<b>Body weight At intake (kg)</b>	Median	4.3		3.3		0.11
	Range	0.7-8.9		0.6-6.7		
<b>Body weight at sampling (kg)</b>	Median	4.4		3.5		0.14
	Range	2.0-9.1		0.9-6.4		
<b>Days in shelter</b>	Median	46		66.5		NS
	Range	12-119		3-264		
<b>Total # cats in Shelter</b>	Median	435		399		NS
	Range	245-448		165-448		
<b>Total # cats in Adoption</b>	Median	135		124		NS
	Range	97-162		78-162		
<b># cats in housing unit</b>	Median	6.5		5.0		NS
	Range	1-18		1-18		
<b>Sq. footage per cat</b>	Median	5.9		5.7		NS
	Range	3.9-12.2		3.0-56.7		
		Assemblage A, B or mixed		Assemblage F		
Variable	Description	No.	%	No.	%	p value
<b>Sex</b>	<b>Male</b>	7	63.6	18	54.5	NS
	<b>Female</b>	4	36.4	15	45.5	
<b>Origin</b>	<b>Surrender</b>	3	30.0	11	32.4	NS
	<b>Stray</b>	7	70.0	23	67.6	
<b>Outdoor access</b>	<b>Yes</b>	1	100	4	80.0	NS
	<b>No</b>	0	0	1	20.0	
<b>Housing in shelter</b>	<b>Individual</b>	1	10.0	1	2.9	NS
	<b>Group</b>	9	90.0	33	97.1	
<b>Age group</b>	<b>Kitten</b>	1	8.3	11	91.7	0.24
	<b>Adult</b>	10	31.2	22	68.8	

NS – not significant

### ***Potential Risk Factors for Zoonotic Assemblage Types***

Univariate analysis: Most infections were not of zoonotic potential, but adults were more frequently infected with potentially zoonotic assemblages ( $p = 0.24$ ) than kittens were. More than 90% of samples obtained from kittens were assemblage F (11/12 samples) compared to approximately 69% of samples obtained from adults. There was a striking seasonal trend with regards to assemblage type. Every cat infected with a potentially zoonotic genotype entered the shelter between the months of April and June, compared to only 64.7% of cats infected with assemblage F ( $p = 0.04$ ).

Cats with potentially zoonotic assemblages were significantly more likely to have higher fecal scores (e.g. soft stools or diarrhea) than those cats infected with the host-adapted genotype ( $p = 0.03$ ). For cats with potentially zoonotic assemblages, 40% (4/10) had soft stools and 20% (2/10) had diarrhea. For cats with assemblage F, 28% (9/32) had soft stools and 3% (1/31) had diarrhea. Other gastrointestinal parasites (*Isospora* spp.  $n = 20$ , *Toxocara* spp.  $n = 4$ , *Aelurostrongylus* spp.  $n = 1$ , *Ancylostoma* spp.  $n = 1$ ) were identified in 39% (24/61) of SNAP<sup>®</sup> positive fecal samples. Cats with potentially zoonotic assemblages were just as likely (3/11) to be co-infected with other parasites as cats with assemblage F (12/33) ( $p = 0.58$ ).

Cats infected with potentially zoonotic assemblages tended to be of higher body weight both at entry to the shelter ( $p = 0.14$ ) and at the time of sample collection ( $p = 0.11$ ) than those with assemblage F, likely due to confounding by age (e.g. adults and older kittens weighed more than younger kittens).

Cats with potentially zoonotic assemblages also had higher body condition scores ( $p = 0.16$ ) than those cats infected with the host-adapted isolates.

Among adult cats, there was no difference between body weight at time of entry to the shelter or at the time of sample collection or with body condition score between cats infected with the different assemblages. Cats infected with potentially zoonotic strains had been in the shelter for a significantly ( $p = 0.04$ ) shorter period of time (median 44 days) than those cats infected with the host-adapted assemblage F (median 78 days). When data for kittens were analyzed, none of the previously mentioned associations were statistically significant.

Multivariate analysis: Since too few kittens were infected potentially zoonotic assemblages, multivariate modeling was not attempted and risk factor analysis was performed using logistic regression for adults only. Among adult cats, higher fecal scores (e.g. soft stools, diarrhea) were a significant risk factor for infection with a potentially zoonotic assemblage. For each 1 unit increase in fecal score, the odds of being infected with a potentially zoonotic assemblage increased by a factor of 1.96 (95% CI: 1.00 – 3.85) compared to the odds of being infected with the host-adapted assemblage F.

## ***Discussion***

The overall prevalence of *Giardia*-positive cats from April-August was 16.2%, which is within the range or slightly higher than that reported in other studies.<sup>5,6,9-12,14-22,27</sup> Investigators have failed to consistently find a higher prevalence of *Giardia* in shelter cats compared to owned cats, but it is generally accepted that prevalence rates are higher in group-housed animals.<sup>1,13,33</sup> It has been hypothesized that cats housed in animal shelters are at greater risk of infection because of increased direct contact with other cats and their feces, the immunosuppressive effects of stress, and contact with a potentially contaminated environment.<sup>11,12</sup> Because most of the cats available for adoption in this shelter were group housed, their exposure to other cats and their feces was probably higher than for cats in animal shelters with traditional individual housing. Although previous studies involving cats from animal shelters did not specify the type of housing, it is possible that the relatively high prevalence reported in this study is due to group housing rather than being in a shelter setting per se. Although neither association was significant ( $p = 0.10$ ) in the final multivariate model, a higher proportion of *Giardia* positive cats were group housed than controls and they were housed with a greater number of cats. The lack of significance may be due to a lack of statistical power rather than an absence of association as less than 20% of cats in the adoption areas of this shelter were housed in individual cages.

There was a significant association for adult cats between *Giardia* cyst shedding and being housed with a greater number of cats in the univariate analysis, but this factor was not retained in the final multivariate model. Control cats were in smaller groups (median size 4 cats) than infected cats

(median size 6 cats), suggesting that an undetermined factor associated with group size may increase the risk of being infected with *Giardia*. However, it is not possible to use this data to determine a specific number of cats above which a housing unit is too crowded or the rate of contact becomes too high. The success of group housing depends greatly on the space and quality of the environment as well as its management, including the provision of an adequate number of litter boxes and diligent maintenance. Group housing is not appropriate for all cats (e.g. “bully” cats) and ideally cats should be introduced slowly to minimize stress and disruption of the social structure. Because of these many factors, the point at which an area becomes overcrowded is dependent on specific circumstances. Although colony housing itself was not significantly associated with infection, this may also be due to low power to detect a difference as only 8.8% of *Giardia* infected cats were individually housed.

The higher prevalence in this shelter in summer months is not surprising because of the high numbers of cats and kittens entering the shelter at that time of year. The amount of space available for each cat housed in a group was reduced as the number of cats housed in the shelter increased each month (Table 1). One might expect to find a consistent linear trend between the number of infected cats and the total number of cats in the shelter or the square footage per cat. However, neither square footage per cat nor the total number of cats in the shelter or the adoption areas were correlated significantly with continually increasing prevalence (Figure 3). This may have been the result of on-going removal of infected cats from the population for treatment. According to the shelter’s policy, positive cats were isolated in a

separate area of the shelter and treated with metronidazole (25 mg/kg po q12 hours for 5 days) and fenbendazole (50 mg/kg po q24 hours for 5 days). These cats were not returned to the general population until treatment had been deemed successful on the basis of fecal flotation. Removal of the infected cats from the adoption population would have reduced the spread of disease that may have occurred had these cats not been identified and/or remained within the population, particularly given the group housing structure.

Other factors that may be responsible for the discrepancy in overall prevalence between this and other studies include geographic location, study population (e.g. healthy cats awaiting adoption in an animal shelter), and differing diagnostic methods. However, the prevalence reported here may still be an underestimate of the true prevalence on *Giardia* infection in cats in this shelter. Because cysts are shed intermittently, it is possible that some cats were incorrectly identified as being uninfected on the basis of a single fecal sample. The ZnSO<sub>4</sub> centrifugation-flotation technique (ZNCT) has been considered the gold standard for the diagnosis of *Giardia* when used by trained personnel, but at least three ZNCTs performed on consecutive fecal samples must be negative in order to rule out *Giardia* infection.<sup>1,34-36</sup>

However, ELISA and IFA evaluations are increasing in use and acceptance. Because the ELISA tests detect an antigen produced by the trophozoites that is shed continuously, they theoretically avoid the problems of intermittent cyst shedding and the need for multiple fecal samples. Thus, their use may result in comparable prevalence estimates to those obtained using multiple ZNCTs. The sensitivity and specificity of the SNAP<sup>®</sup> *Giardia* Test range from 90-95% and 96-100% respectively, and independent studies have shown that these

tests have high sensitivities and specificities when used in cats.<sup>1,20,37</sup> For these reasons, and because collection of multiple fecal samples from individual cats was neither technically nor economically feasible in this study, diagnosis was based on a positive ELISA result in a single sample using an in-house kit. It would be possible to correct the apparent prevalence estimates to true prevalence estimates using the sensitivity and specificity of the ELISA; because these are so high, the apparent prevalence would change a nominal amount and such calculations were not done in this study.

Although many cases were tested only once and many controls were tested every month during the study period, A higher median number of fecal samples were collected from *Giardia* positive cats than from controls. This may have biased the estimates upwards because of the enhanced sensitivity gained from testing multiple samples. Nonetheless, diagnosis may have been improved by using a more sensitive screening test than the ELISA. For instance, one study found a 0% prevalence of *Giardia* using fecal flotation and microscopic evaluation,<sup>11</sup> but an 80% prevalence using PCR on the same samples.<sup>12</sup> These results, while limited, suggest that false negatives are unlikely to occur with PCR. Although false positives are possible with such screening techniques, PCR analysis of all fecal samples may have increased sensitivity and would have allowed for the determination of assemblage type for those cats falsely testing negative with the SNAP<sup>®</sup> *Giardia* Test. Insufficient data exists to conclusively determine whether the ELISA tests in general, and the SNAP<sup>®</sup> *Giardia* Test in particular, are able to detect all genotypes of *Giardia duodenalis* with equal sensitivity and specificity. Moreover, it is possible but unknown whether there is an association between

assemblage type and intensity of cyst shedding, although we were unable to find any association between PCR results and cyst counts in this study. However, it is possible that PCR may be able to detect *Giardia* in samples with antigen levels below the detection threshold of the in-house ELISA kits.

Risk Factors: Cats greater than 6 months of age and those 6 months old or younger had different risk factors associated with testing positive for *Giardia* in this study. Although few risk factors were identified for kittens, several risk factors for infection in adult cats were identified that are unique to cats in animal shelters and may also pertain to cats in other group-housing settings such as catteries. Unfortunately, despite having more than 500 fecal samples from 302 different cats, the low number of infected cats and kittens greatly reduced the power to find risk factors for infection. We failed to find a higher rate of infection in kittens less than 6 months of age compared to older cats. Because kittens are considered highly desirable by the general public, they tend to spend only a short period of time in the shelter before adoption unless they are too young to be available for immediate adoption (e.g. less than 8 weeks of age). As a result, the majority of cats sampled during the study period were greater than 6 months of age. Only mean body weight at the time of sample collection was greater for *Giardia* positive kittens than for controls, suggesting that these kittens had been in the shelter for a longer period of time than those that were uninfected and/or were older when they entered the shelter.

The larger number of adult cats sampled allowed for a better assessment of potential risk factors for infection. *Giardia* positive adults were more likely to have been found as strays (75.8%) with an odds ratio of 2.41 for stray cats

compared to owner-surrendered cats. Information regarding outdoor access was available for a small number (n = 35) of adult, owner-surrendered cats. Because of this small sample size, there was low power to detect a significant difference, but 80% (4/5) of *Giardia* positive cats had outdoor access compared to only 40% (12/30) of control cases. This is not surprising, given that stray cats and those with outdoor access are likely to be at greater risk of exposure to parasitic and infectious diseases.

There was a trend for *Giardia* positive cats to be younger than controls; although not statistically significant in this study overall, infected adult cats were significantly younger than adult controls. Younger animals have been reported to be at higher risk of *Giardia* infection in other studies and this is biologically plausible given the naïve immune system of young animals.<sup>6,7,15,21</sup> Alternatively, it has been suggested that older cats may become refractory to *Giardia* infection because of acquired immunity.<sup>11,12</sup> Among adults, the odds of being *Giardia* positive decreased by approximately 27% with each increasing year of age. In other words, increasing age was associated with decreasing risk of *Giardia* infection. Although there was a tendency for males to be underrepresented among control cats, this factor was not retained in the final model.

A sex predilection has not been previously reported in humans or animals, and current understanding of the epidemiology and pathophysiology of *Giardia* infection does not suggest a basis for such a predisposition. It is likely that this trend is spurious.

Assemblage data: Both potentially zoonotic and non-zoonotic assemblages were detected in the fecal samples of cats in this animal shelter. Although cats are well-recognized to be susceptible to infection with assemblages A and F, to the authors' knowledge, this is only the second report of the detection of assemblage B in cats. Interestingly, the only previous report of assemblage B was also from cats sampled in the same geographic region as the present study.<sup>29</sup> In that study, 3 of 9 cats sampled were identified with assemblage B while the other 6 cats were identified as being infected with assemblage A. However, demographic information for the cats in the previous study was not presented, and thus no assessment of differences in assemblage type could be made. Some investigations have found the study population to be exclusively infected with assemblage A<sup>30</sup> or assemblage F,<sup>24,25</sup> while other studies have found the population of cats in question to be infected with both assemblages A and F alone or in combination (e.g. mixed infections.)<sup>26-28</sup> Rarely, infection with other "host-adapted" assemblages (e.g. assemblages C and D) has been reported in cats.<sup>12</sup> Despite a growing body of scientific literature, little data exists regarding differences in assemblage types other than host specificity and possible geographic distribution.<sup>2,3</sup> Although assemblages A and B have a wide host range (including humans), this only suggests a potential for zoonotic spread, but neither guarantees its occurrence nor specifies the direction of transmission if it occurs.

While not statistically significant, adults were more likely than kittens to be infected with potentially zoonotic assemblages. More than 90% (11/12) of samples obtained from kittens were assemblage F. Although it was not specifically investigated, it seems likely that cats who become infected with

*Giardia* while in the shelter are more likely to be infected with the host-adapted assemblage F. This is supported by the fact that more than 80% (18/22) of cats that tested positive after being in the shelter 60 days or longer were infected with assemblage F. On average, these 18 cats had tested negative on two occasions (range 0-4 previous tests) prior to the point at which infection with assemblage F was detected. Given the target (e.g. cyst wall antigen) of the ELISA tests and their high sensitivities, it is improbable that these cats would have had multiple false negative results on the earlier tests. Screening at the time of entry to the shelter would have been necessary to definitively establish that cats were not infected at the time of arrival but acquired the infection in the shelter; unfortunately this was not possible in this study.

Interestingly, all cats infected with a zoonotic assemblage entered the shelter between the months of April and June. It was not possible to determine if all of these cats came from a common location that would suggest a point source exposure, but intake dates varied by as much as 3 months within this time frame making such exposure very unlikely. It may be that cats were more likely to come in contact with assemblage A and B *Giardia* isolates during the months preceding their entry (e.g. February and March) to the shelter, perhaps as a result of environmental factors that are more conducive to transmission of *Giardia* in these months. With seasonal rain and snow melt, the cool and moist environment favors the persistence of cysts<sup>1</sup>. Unfortunately, it was not possible in this study to collect data throughout the entire year to better assess any possible influence of season on *Giardia* infection.

For cats greater than 6 months of age, higher fecal scores (e.g. soft stools or diarrhea) were a significant risk factor for infection with a potentially zoonotic

assemblage. Due to the small sample size, there was insufficient power to detect a significant difference between fecal scores when they were collapsed into more intuitive categories of “normal” or “diarrhea” but the highly significant association with the ordinal variable of fecal score suggests that the risk of infection with a potentially zoonotic strain is greater for cats with soft stools or diarrhea. The presence and severity of clinical signs associated with infection are determined by many host and parasite factors. We were unable to find an association between potentially zoonotic assemblages and concurrent parasitism, suggesting that parasite genotype may be an important determinant of the severity of disease. It is known that infection with different assemblages can cause varying clinical signs in humans,<sup>39,40</sup> but this has not been investigated in cats. However, it is reasonable to expect that the host adapted assemblages would not cause as severe clinical disease and the results of this study support that hypothesis.

Interestingly, cats infected with potentially zoonotic strains had been in the shelter for a significantly shorter period of time than those cats infected with the host-adapted assemblage F. Although this factor was not retained in the final multivariate model, it raises the question of immunity to and persistence of *Giardia* infection in cats and warrants further study. It is unknown whether infection with one assemblage imparts cross protection against infection with other assemblages or if the immunity is genotype-specific, and no data exist for cats regarding the persistence of infection with respect to assemblage type. Recent studies have found that dogs in household settings are equally likely to be infected with assemblage A as their own host-adapted assemblages C and D,<sup>41</sup> but are predominantly infected with the host-adapted genotypes in areas

that are highly endemic among animals for *Giardia*.<sup>32</sup> As a result of these findings, it has been suggested that competitive exclusion occurs in dogs in environments with high infection pressure, whereby the host-adapted assemblages “out-compete” the other assemblages.<sup>28</sup> It is unknown if this occurs in cats.

Some cats in this study that were sampled multiple times tested positive for *Giardia* on more than one occasion (n = 7). In these cats, the same assemblage type may or may not have been found in subsequent samples. One cat was initially infected with assemblage A and then found to be infected with assemblage B one month later. Two cats were found to be infected with assemblage F on two separate occasions one month apart, and another cat was initially found to have mixed infection (assemblages A and F) and then found to be infected only with assemblage F one month later. In all other cases (n = 3) where multiple positive results were obtained for a single cat using the ELISA, assemblage results were not obtainable on subsequent samples (e.g. PCR negative). This occurred with both host-adapted and potentially zoonotic assemblages and may be due to false positive results that have been reported to occur with the SNAP<sup>®</sup> *Giardia* Test following treatment (personal communication, IDEXX technical services). Alternatively, this may have been due to problems with the PCR (e.g. false negatives) rather than with the ELISA (e.g. false positives). Historically it has been difficult to amplify DNA extracted from fecal material, due to insufficient quantity or poor quality of the DNA in combination with the presence of inhibitors.<sup>27</sup> Although it was not possible to determine whether the negative PCR results were accurate, cats for which assemblage was not determined were excluded from the

analysis out of necessity. Because the inability to determine the assemblage types seemed to be random, we believe that we did not introduce bias to the analysis by deleting these missing results, but it is possible that one or more of the risk factors were directly influenced by this decision.

It is possible that other risk factors for *Giardia* infection or differences in assemblage type exist but were not detected. One reason for this may be due to selection bias, in that only cats available for adoption in one shelter were sampled. Cats in this facility spend a varying amount of time (one day to several months) in other areas of the shelter and may have been treated for a number of conditions, including *Giardia*, prior to being made available for adoption. Sampling these cats as they entered the shelter may have resulted in the identification of different risk factors for infection, but cats available to the public for adoption were chosen for this study as we considered them to be the best population in which to investigate potential for public health risk. Additionally, there may be different risk factors for cats in other animal shelters with shorter lengths of stay or different housing. All attempts were made to sample as many cats as possible, but the sample size coupled with a relatively low prevalence reduced the power to detect significant differences. Because of the cross-sectional nature of the study, the prevalence estimates reported here represent only detection of *Giardia* at the point of sampling and do not provide any indication of duration or persistence of infection.

## *Conclusion*

The monthly prevalence of *Giardia* infection reported in this study is comparable to that reported by other investigators, with variations depending on the population studied and the diagnostic methods used. Stray adult cats and those with outdoor access were more likely to be infected than owner-surrendered cats and those that were kept indoors only. Cats in colony housing, particularly at high densities and for prolonged periods of time, may also be more likely to be shedding *Giardia* cysts although these did not retain their significance in the multivariable model. Although group housing may increase shelter cats' exposure to parasitic and infectious disease in certain cases, the benefits of stress reduction and improved physical and behavioral health may outweigh such risks. Furthermore, shelters can reduce that risk with disease surveillance systems and prompt removal and isolation of any cat with signs of an infectious disease. Further research is needed to better understand the impact housing style has on *Giardia* infection in shelter cats.

The majority of cats were not infected with assemblages that are thought to have zoonotic potential. Kittens were particularly unlikely to be infected with the assemblages A and B. However, 25% of cats were found to have an assemblage A, B, or a mixed A/F infection and these cats were more likely to have soft stools or diarrhea than cats infected with assemblage F. Thus, the potential that cats may be a source of human *Giardia* infection can not be discounted. Unfortunately, identification of cats that could pose a risk is not possible without advanced molecular diagnostics. To date, insufficient data has been collected on the assemblage types found in *Giardia* infected cats to make an accurate, quantitative assessment of the public health risk associated

with these cats. Despite a greater understanding of the molecular epidemiology of the disease, the question as to whether or not asymptomatic cats found to be shedding *Giardia* cysts should be treated in order to protect public health cannot be answered. This paucity of knowledge makes evidence-based decision-making difficult if not impossible in the clinical setting. In the context of animal shelters, where large numbers of cats of varying immune status are housed concurrently and where resources for animal care are limited, these challenges are particularly evident. Further work is needed with larger numbers of cats, both owned and from animal shelters, and from various geographic locations to better understand the importance of various risk factors and to establish the true zoonotic potential of *Giardia*.

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