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Investigation of Factors Associated with Subclinical Infections of *Giardia duodenalis* and *Cryptosporidium canis* in Kennel-Housed Dogs (*Canis lupus familiaris*)

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***Giardia duodenalis* and *Cryptosporidium* spp. are zoonotic protozoal pathogens, spread by a fecal-oral route, which can infect a wide range of hosts including but not limited to dogs and humans. *Giardia* was recently estimated to be present in 37% to 50% of kennel-housed dogs. *Cryptosporidium* infections in kennel-housed dogs have been reported in 7% to 21% of the population. The goal of this study was to define demographic factors and fecal scores associated with positive screening test cases of *Giardia* and *Cryptosporidium* in kennel-housed laboratory dogs in the state of Texas. Fecal samples were collected from 153 clinically normal laboratory dogs at an academic research facility and a local laboratory dog supplier. We used 3 diagnostic tests evaluated in parallel to determine test positivity to each organism: a human point-of-care coproantigen test, a direct immunofluorescent assay, and an in-house polymerase chain reaction. Dogs were significantly more likely to test positive for *Giardia* (45%) than *Cryptosporidium* (7%) ($P < 0.01$). Dogs that were 18 mo of age or younger had 3 times the odds ($P = 0.009$) of subclinical *Giardia* infection compared with older dogs. We found no significant relationship between age and *Cryptosporidium* prevalence. Dogs with hard feces (fecal score 1-2) at the time of screening had 0.34 times lower odds ($P = 0.049$) of testing positive for *Giardia* than dogs with normal feces, but no statistically significant relationship was found between fecal score and *Cryptosporidium*-positive test status. With these findings, we demonstrated the value of considering age and fecal score when choosing which dogs to screen for subclinical *Giardia*. Additional studies with larger sample sizes should be conducted to determine the relationship between age and fecal score and subclinical *Cryptosporidium* infection.**

Abbreviations and Acronyms: DFA, direct immunofluorescent assay; QC, point-of-care coproantigen test

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Introduction

Giardia duodenalis is a protozoan parasite that can cause both clinical disease and subclinical infection in many species including dogs and humans. Current estimates of infection with *Giardia* in dogs have a wide range. *Giardia* was recently estimated to be present in 37% to 50% of kennel-housed dogs.[17,29] A recent global meta-analysis looked at 127 papers reporting *Giardia* infection in dogs of different ages, housing situations, and geographical regions using testing methods including microscopy, ELISA, direct fluorescent antibody (DFA), and PCR. They found an overall prevalence of 2.61% with a prevalence as low as 0% and as high as 70%.[5] Another recent investigation that sampled 3,022 dogs from 288 dog parks in major cities around the United States evaluated the zinc sulfate centrifugal floatation, coproantigen method (*Giardia* Test, IDEXX Laboratories, Westbrook, ME), or both methods together and found that 13% of dogs were positive for *Giardia* using at least one of these methods.[39] The same study found that 89.8% of positive *Giardia* samples via coproantigen tested negative on fecal floatation.[39] Investigations of kennel-housed laboratory dogs have reported a prevalence of *Giardia* infection ranging from 38.5% to 100.0% depending on housing location.[3,22,32,34,36,42] Factors present in kennel-housed dogs that could potentially lead to higher levels of *Giardia* infection include frequency of cleaning kennels, access to contaminated outdoor spaces, and group housing.

Cryptosporidium infections in dogs are reported less often than *Giardia*. *Cryptosporidium* infections in kennel-housed dogs have been reported in 7% to 21% of the population.[18,19] In 2020, a global meta-analysis of canine *Cryptosporidium* infection revealed an 8% pooled prevalence in studies that conducted various microscopic modalities with and without staining ($n = 76$) and 7% in studies that conducted coproantigenic methods including immunofluorescence assays, ELISA, and enzyme immunoassays ($n = 42$). The pooled prevalence was 6% in studies that used molecular methods including PCR, nested PCR, real-time PCR, and restriction fragment length polymorphism-PCR ($n = 42$).[41]

Infection with *Giardia* or *Cryptosporidium* is also commonly associated with disease in humans.[7,8] *Giardia duodenalis* is the most common gastrointestinal parasite in humans in the United States.[9] *Giardia duodenalis* is currently divided into eight different genetic assemblages, lettered A thru H.[28] Of these assemblages,

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assemblages C/D most commonly affect dogs, assemblages A/B most commonly affect humans, yet dogs can be infected with assemblage A, which can cause disease in humans.[28] Rarely, humans have been reported to contract infection with assemblages C/D as well as other assemblages.[40] *Cryptosporidium* sp. infections are currently on the rise in humans, and the Centers for Disease Control and Prevention has recently implemented CryptoNet, a national reporting database, to enable the collection and molecular characterization of clinical isolates of *Cryptosporidium*.[7] The genus *Cryptospordium* contains many species including *C. parvum*, a zoonotic species that infects cattle and humans; *C. hominis*, a species that infects humans; and *C. canis*, a species that normally infects dogs but has been associated with rare zoonosis.[10,45]

With high levels of *Giardia* infection in kenneled dogs and low, yet consistent, levels of infection of *Cryptosporidium* in these dogs, it is important for veterinarians, researchers, facilities managers, animal rescues, and breeders to understand the factors associated with subclinical infection with either organism. The goal of this study was to identify if fecal score, as determined with the Purina Fecal Score chart, breed, sex, or age was associated with testing positive on a fecal screening test for either *Giardia*, *Cryptosporidium*, or both organisms together.

Materials and Methods

Ethics statement. This study was conducted in accordance with the policies of the Texas A&M Institutional Animal Use and Care Committee. None of the experiments directly influenced the routine activities of the dogs. The committee deemed an animal use protocol unnecessary since all samples were voided voluntarily into the environment and collected during the cleaning of the dogs' standard enclosures.

Animals and sample collection. Fecal samples (n = 170) were collected once from dogs housed at a research facility (n = 96) and a laboratory dog supplier (n = 74) in Texas between March and October 2021.[42] These samples represented a census sample of all dogs in the research facility and a convenience sample of laboratory supplier dogs, with feces collected during daily cleaning activities and routine yearly physical exams. Dogs at the research facility and their associated samples were separated into 4 groups depending on the building or personnel responsible for their care, with the laboratory dog supplier as the fifth and final group. Groups 1 to 4 had characteristics unique to each group including personnel and types of dry dog food fed. All dogs in groups 1 to 4 were housed indoors, some single housed and some group housed, with some dogs having playtime allowed in outdoor grass yards (Table 1). Kennels were cleaned daily with 180 °F (82 °C) water, disinfected chemically with hydrogen peroxide (Peroxigard, Virox, Oakville, ON, Canada) every one to two weeks, depending on the building, and washed down with soap monthly or more often, as needed (Table 1). Dogs included in group 5 were housed in nonclimate-controlled large kennels with concrete flooring and brick walls (Table 1). Group 5 kennels were washed daily with water unless temperatures were too cold to allow for this, in which case they were scraped clean that day. The kennels were chemically disinfected weekly with bleach (Table 1).

The dog's sex, age, breed, location, and fecal score were available for 153 of the samples. A single individual determined fecal scores by visual assessment of the samples, based on the 1 to 7 fecal score metric published by the Purina Institute where 1 is a hard and pellet-like fecal mass and 7 is a watery defecation with no texture (Figure 1).[30] Dog breeds included golden retrievers, Labrador Retrievers, Beagles, and a variety of large hounds and hound mixes. Ages were determined in months based on the date of birth.

Experimental design. This is a secondary data analysis from samples that were tested using the QuickChek point of care rapid membrane enzyme immunoassay (QC; TechLab, Blacksburg, VA), Merifluor *Cryptosporidium/Giardia* direct immunofluorescent assay (DFA; Meridian Bioscience, Cincinnati, OH), and an in-house PCR using previously described primers and sequencing confirmation.[2,16,35,42] All samples were analyzed to determine associations between subclinical *Giardia* infection, *Cryptosporidium* infection, or coinfections with both organisms and age, sex, and fecal score.

Table 1. Population descriptive data

Group	Sex	Housing	Diet	Exercise	Kennel disinfection
Group 1 (n = 22)	M = 9 F = 13	Kennel housed with raised flooring indoor, climate controlled, as singles and group	Purina Sport	Free exercised in grassy enclosure outdoors	Cleaned daily with 180 °F (82 °C) water, disinfected chemically with a minimal contact time of 10 min with activated hydrogen peroxide every 2 wk
Group 2 (n = 12)	M = 4 F = 8	Kennel housed with raised flooring indoor, climate controlled, as singles and group	Lab Diet and Purina Sensitive Skin	Free exercised in grassy enclosure outdoors	Cleaned daily with 180 °F (82 °C) water, disinfected chemically with a minimal contact time of 10 min with activated hydrogen peroxide every 2 wk
Group 3 (n = 41)	M = 22 F = 19	Kennel housed with a mixture of raised and float flooring indoor, climate controlled, as singles and group	Lab Diet and Purina Sensitive Skin	Free exercised in grassy and dirt enclosure outdoors	Cleaned daily with 180 °F (82 °C) water, disinfected chemically with a minimal contact time of 10 min with activated hydrogen peroxide every 2 wk
Group 4 (n = 10)	M = 0 F = 10	Kennel housed with raised flooring indoor, climate controlled, as groups	Lab Diet and Purina Sensitive Skin	Free exercised in indoor playroom	Cleaned daily with 180 °F (82 °C) water, disinfected chemically with a minimal contact time of 10 min with activated hydrogen peroxide every week
Group 5 (n = 68)	M = 40 F = 28	Kennel housed outdoors with concrete floors in groups	24/20 Red Diamond all stages of life	Exercised outdoors in grass and woody environment 3 times per week for 5–10 miles each week	Washed daily with water (if temperatures were too cold, they were scraped clean) and chemically disinfected weekly with bleach

M, male; F, female.

NUTRITIONAL AND CLINICAL ASSESSMENT

PURINA FECAL SCORING CHART

Fecal consistency is primarily a function of moisture in stool and can be used to identify changes in colon health and other problems. In a healthy dog or cat, stools ideally should be firm but not hard, pliable, segmented and easy to pick up (Score 2).

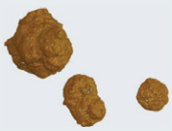
Score	Specimen	Characteristics
1		■ Very hard and dry ■ Often expelled as individual pellets ■ Requires much effort to expel from the body ■ Leaves no surface residue when picked up
2		■ Firm, but not hard; pliable ■ Segmented appearance ■ Leaves little or no surface residue when picked up
3		■ Log shaped; moist surface ■ Little or no visible segmentation ■ Leaves surface residue, but holds form when picked up
4		■ Very moist and soggy ■ Log shaped ■ Leaves surface residue and loses form when picked up
5		■ Very moist, but has a distinct shape ■ Present in piles rather than logs ■ Leaves surface residue and loses form when picked up
6		■ Has texture, but no defined shape ■ Present as piles or spots ■ Leaves surface residue when picked up
7		■ Watery ■ No texture ■ Present in flat puddles

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Figure 1. The Purina Fecal Scoring Chart (Société des Produits Nestlé, Vevey, Switzerland). This metric was used for assessing fecal consistency in this study.

Statistical analysis. All data were analyzed using STATA SE 17.0 (STATA Corp., College Station, TX). The main outcome of this analysis was the subclinical presence of gastrointestinal protozoa, treated as 3 discrete outcomes: *Giardia*, *Cryptosporidium*, and coinfection.

Definitions and handling of variables. *Giardia*, *Cryptosporidium*, and coinfection (presence of both detected) were reported as apparent prevalence. The apparent prevalence of subclinical infection was determined by interpreting the 3 tests, QC, DFA, and PCR in parallel. To be considered positive for either *Giardia* or *Cryptosporidium*, the sample needed to test positive on one of the 3 tests. A dog was considered coinfected with *Giardia* and *Cryptosporidium* if the sample was positive for both pathogens based on the criteria presented above. As a predictor of infection, the variable fecal score was categorized into hard, scores 1 to 2, (n = 28); normal, scores 3 to 4; (n = 108); and soft, scores 5 to 6; (n = 17). Due to the subclinical nature of the population, no samples with a fecal score of 7 were included in the analysis. For descriptive purposes, age was presented as medians with IQR. Previous work in dogs indicated that one component of the immune system needed for defense in parasitic infections, IgA, reaches protective levels by 18 mo of age.[38] Therefore, age was dichotomized based on these biologic characteristics involved in the maturation of the immune system to facilitate appropriate age-based recommendations. These two categories include the following: 0 to 18 mo (n = 53) and greater than 18 mo (n = 100). The variable of breed was collapsed into two groups based on breed standard genetic backgrounds: hounds (n = 72) and retrievers (n = 81).

Variable analysis. Differences in prevalence between *Giardia* and *Cryptosporidium* overall and by housing group were evaluated using the z test for 2 proportions. Differences in *Giardia* or *Cryptosporidium* prevalence among different housing groups were evaluated with the Fisher exact test. For organisms that were significantly related to the housing group the relationship between prevalence of infection and the different housing groups was determined with exact logistic regression with a multiple comparisons correction adjustment applied to the P value. Due to the small population size, exact logistic regression was then performed, using the outcome variable *Giardia*-positive test status and a grouping variable based on housing group and the independent variable fecal score.[20,27,44] Exact logistic regression was also used to evaluate demographic factors associated with *Giardia* test positivity, with the exposure variables breed, sex, and age. Any independent variable analyzed with a z score P value < 0.2 was included in the final regression model. Results were reported as odds ratios with a 95% CI, and P values less than 0.05 were considered statistically significant. Due to low prevalence, the Fisher exact test was used to determine relationships between *Cryptosporidium* infection or coinfection and fecal score, sex, age, and breed.

Table 3. Univariable analysis of *Giardia* subclinical infection

Variable	Prevalence (%)	OR	[95% CI]	P value
Fecal score				
Hard stool	8/28 (28.57)	0.34	[0.10, 0.99]	0.049*
Normal stool	52/108 (48.15)	(ref)	(ref)	
Soft stool	9/17 (52.94)	1.93	[0.57, 7.01]	0.358
Demographics				
Age				
>18 mo	35/100 (35.00)	(ref)	(ref)	
0–18 mo	34/53 (64.15)	3.36	[1.31, 9.17]	0.009*
Breed				
Hound	34/72 (47.22)	(ref)	(ref)	
Retriever	35/81 (43.21)	1.60	[0.71, 3.67]	0.294
Sex				
Male	35/75 (46.67)	(ref)	(ref)	
Female	34/78 (43.59)	0.73	[0.34, 1.52]	0.453

*Statistically significant.

Results

The overall prevalence was 45.10% [95% CI: 37.34, 53.0] for *Giardia* and 6.54% [95% CI: 3.53, 11.77] for *Cryptosporidium* (Table 2). Group level *Giardia* positivity ranged from 22.72% [95% CI: 9.26, 45.87] in group 1 to 90.00% [95% CI: 45.33, 98.99] in group 4. Housing group was significantly associated with *Giardia* prevalence (P < 0.01) and group level prevalence of *Giardia* infection was significantly greater in group 4 compared with group 1 (P = 0.01). Group level *Cryptosporidium* positivity ranged from no cases in groups 1 and 2 to 11.80% [95% CI: 5.92, 22.03] in group 5. Housing group level prevalence of *Cryptosporidium* infection was not significantly different between the groups (P = 0.18). The overall sample prevalence for *Giardia* was significantly higher than for *Cryptosporidium* (P < 0.01). *Giardia* prevalence was significantly higher than *Cryptosporidium* prevalence in each group.

Fecal score analysis showed that dogs with hard stool (fecal score 1 to 2) had 0.34 [95% CI: 0.1, 0.99] the odds of subclinical infection with *Giardia* compared with normal stool (fecal score 3 to 4) (Table 3). There was no significant difference in the odds of subclinical infection between dogs with soft stool (fecal score 5 to 6) and normal stool. The median age of dogs infected with *Giardia* was 19.0 [IQR: 7.0, 44.0] months. The median age of dogs without *Giardia* was 36.5 [IQR: 19.0, 56.0] months (Table 3). Being

Table 2. Positivity of *Giardia* and *Cryptosporidium*

Group	*Giardia* test positive	*Giardia* (%) [95% CI]	*Cryptosporidium* test positive	*Cryptosporidium* (%) [95% CI]	P value
Overall (n = 153)	69	45.10 [37.34, 53.10]*	10	6.54 [3.53, 11.77]	<0.01
Group 1 (n = 22)	5	22.72 [9.26, 45.87]*	0	No cases detected	0.02
Group 2 (n = 12)	5	41.67 [16.44, 72.16]*	0	No cases detected	0.01
Group 3 (n = 41)	15	36.59 [23.05, 52.63]*	1	2.39 [0.32, 16.21]	<0.01
Group 4 (n = 10)	9	90.00 [45.33, 98.99]*,†	1	9.49 [1.01, 5.47]	<0.01
Group 5 (n = 68)	35	51.47 [39.52, 63.25]*	8	11.8 [5.92, 22.03]	<0.01

**Giardia* prevalence significantly greater than *Cryptosporidium* with P value < 0.05.
†*Giardia* prevalence in group 4 significantly greater than group 1 (P value = 0.01).

Table 4. Univariable analysis of *Cryptosporidium* subclinical infection

Variable	Prevalence	Percentage (%)	*P* value
Fecal score			0.666
Hard stool	1/28	3.57	
Normal stool	9/108	8.33	
Soft stool	0/17	0	
Demographics			
Age			0.120
>18 mo	4/100	4.00	
0–18 mo	6/53	11.32	
Breed			0.301
Hound	6/72	8.33	
Retriever	4/81	4.94	
Sex			0.348
Male	6/75	8.00	
Female	4/78	5.13	

18 mo of age or younger was associated with 3.36 [95% CI: 1.31, 9.17] times the odds of having a subclinical *Giardia* infection compared with older dogs (Table 3). Because dog age was the only statistically significant demographic variable, multivariable regression was not performed.

There was no statistically significant relationship detected between infection with *Cryptosporidium* and fecal score (Table 4). The median age of dogs infected with *Cryptosporidium* was 7.0 [IQR: 5.0, 46.0] months and 29.0 [IQR: 7.0, 50.0] months for those not infected. There was no statistically significant association between *Cryptosporidium* infection and age, sex, or breed (Table 4).

Only 4 of the 153 dogs sampled had coinfections with *Giardia* and *Cryptosporidium*. The median age of dogs with and without coinfection was 5.5 [IQR: 3.5, 17] and 29 [IQR: 7, 50] months, respectively. Coinfection was not significantly related to breed ($P = 1.0$), fecal score ($P = 1.0$), or age ($P = 0.12$).

Discussion

Understanding the relationship between variables associated with subclinical infection of parasites, such as *Giardia* and *Cryptosporidium*, is important for veterinarians, breeders, researchers, and any professional who maintains large populations of dogs in a colony, breeding, performance, or other kennel setting. This study looked at a population of kennel-housed dogs and evaluated the associations between commonly available variables, including fecal score, age, breed, and infection with either *Giardia*, *Cryptosporidium*, or coinfection with both organisms.

There are several fecal scoring metrics available to animal care professionals. For this study we chose the widely used Purina 7-point fecal scoring system. A recent study compared the Purina metric and the Waltham 5-point fecal scoring scale on 126 canine bowel movements. The Purina metric resulted in a κ of 0.40 to 0.77, and the Waltham metric resulted in a κ of 0.54 to 0.61 when scored by 3 sets of evaluators.[6] The κ statistic is a method for evaluation of interrater agreement, and these values indicate that there could be more variability of scoring with the Purina Scale than the Waltham Scale, which is reasonable due to a greater number of rating options in that metric. Despite this difference in agreement, we chose the Purina Scale due to its wide availability and the familiarity of veterinarians with the scale.

There is disagreement in the literature regarding the effect of subclinical infection with either *Giardia* or *Cryptosporidium* on fecal consistency. However, it is biologically plausible that dogs harboring either organism would have softer feces than those without the organism. One study reported that a positive test status for *Giardia*, but not *Cryptosporidium*, was significantly associated with development of loose stool in sled dogs during racing.[26] The study was limited by small sample size with only 5 of 53 dogs testing positive for *Giardia* before racing and 5 of 67 testing positive during the race. The same study only found 1 of 53 dogs positive for *Cryptosporidium* before the race and 2 of 67 positive during the race. Chronic subclinical infection has been associated with dysbiosis, with a recent study reporting an enrichment in proinflammatory bacterial species and opportunistic pathogens in *Giardia*-positive dogs.[4] Unlike subclinical *Giardia* infection, subclinical *Cryptosporidium* has not been associated with fecal consistency, with no clear link to soft stools in subclinical animals.[1,34,46]

In the present study, age was related to infection with *Giardia*. This fits with our understanding of the immune system and its responses to *Giardia* infection. The host's response to *Giardia* has been demonstrated to depend on IL-17A, Th17 cells, and production of IgA.[14,24] IL-17, secreted by the CD4+ Th17 cells, has been shown to upregulate complement and play a role in the secretion of parasite-specific IgA antibodies.[14,24] We know that these cells, cytokines, and immunoglobulins take time to fully develop and increase during the maturation process.[31] A study in Beagles revealed that IgA showed a steady age-dependent increase over 10 to 20 mo.[38,43] Furthermore, future research could assess the involvement of long-term stimulation of the Th17 cells and the IL-17A response in parasitic infections including the predisposition to autoimmune conditions such as immune-mediated hemolytic anemia, irritable bowel disease, asthma, and idiopathic epilepsy.[13,21,25,31,37]

There were more dogs 18 mo of age or younger that were positive for *Cryptosporidium* than dogs older than 18 mo, although this relationship was not statistically significant. The relationship of age to *Cryptosporidium* infection susceptibility is biologically plausible, as recent investigations have led to a deeper understanding of the immune response to *Cryptosporidium* infections. There are several components essential to the immune response to this organism, including intestinal epithelial cells, innate and adaptive immunity, chemokines, cytokines, and antimicrobial peptides.[23,33] While some components of the immune system are viable early in a dog's life, it is accepted that full functionality and serum immunoglobulin levels do not reach those of adults until 12 mo of age.[12] A recent study found that CCL20, a chemokine needed for T-lymphocyte recruitment, was downregulated during *Cryptosporidium* infection in neonatal mice, likely making them susceptible to infection.[15] When researchers supplemented mice with recombinant CCL20, they found that the number of *Cryptosporidium* oocysts significantly decreased compared with control mice. This is an area for further research in dogs and may provide a potential model for human cryptosporidiosis.

The size of our study limits the degree to which we can assess significant relationships between specific breeds, genetics, and fecal scores. Due to this limitation, breed was dichotomized. Despite this limitation, this study provides valuable management insight that can be built on in the future. This study adds to the literature as we show a potential relationship between fecal score and *Giardia* infection as well as between age and *Giardia* infection. With these findings, we hope to provide managers of canine facilities with the information needed to refine evidence-based

standard operating procedures. Studies using larger cohorts of both clinical and subclinical dogs to assess the viability of using fecal scores as a predictor for infection with either of these organisms are needed. Future studies that focus on age-related biologic factors and their association with subclinical infection will help researchers and clinical veterinarians better manage kennel-housed dog colonies.

In conclusion, we found that kennel-housed dogs 18 mo of age or younger were at 3.4 times the odds of *Giardia* infection compared with older dogs and that hard stool was associated with negative test status for *Giardia* in the stool. Based on these findings, dogs 18 mo of age or younger should be screened for *Giardia*. Given the increased odds of *Giardia* infection, screening should be as robust as the recommended combination of testing methods.[11,36] This study demonstrates that coinfections with *Giardia* and *Cryptosporidium* can occur in kennel-housed groups of dogs. These data suggest that there is no clear link between sex or breed and *Giardia* or *Cryptosporidium* infections in kennel-housed dogs. While there were more young dogs (that is, 18 mo or younger) with *Cryptosporidium* infections, the relationship was not significant and there was no clear link between fecal score and *Cryptosporidium* infection. Future studies with larger numbers of kennel-housed dogs are recommended to fully understand the relationship between fecal score and *Giardia* infection and fecal score, age, and *Cryptosporidium* infection.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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