
Public Health Risks of Off-Leash Dog Use on Youth Little League Fields in Rollingwood

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To Makayla Rodriguez <mrodriguez@rollingwoodtx.gov>

 4 attachments (4 MB)

2020 - Detection of gastrointestinal parasitism at recreational canine site in the USA.pdf; JEPH2017-5984086.pdf; JVIM-30-1838.pdf; animals-11-01685-with-cover.pdf;

Dear Members of the Rollingwood City Council, Park Commission, and Community Development Corporation-

I am writing as a concerned resident, parent, little league coach, and someone who has owned dogs for most of my childhood and adult life. I want to again bring to your attention a serious public health and safety issue regarding the current policy allowing off-leash dog access to the youth athletic and little league fields during times when baseball and softball games are not scheduled. These fields serve as vital recreational spaces for our community's youngest athletes—boys and girls aged 4, 5, and 6—who participate in T-ball and coach-pitch games and practices. Since the City Council first began allowing dogs off leash on the existing little league fields 3, 4, and 5 in 2017, the off-leash dog use on these field has dramatically increased from a few occasional residents and their dogs to a full blown regional off-leash dog area used by dozens of off leash dogs and their owners daily. Unfortunately, his shared use has led to repeated and unavoidable exposure of these children to dog urine and feces, which contaminates baseballs, cleats, gloves, and playing surfaces. Based upon my first-hand experience, as well as that of several other little league coaches, children frequently handle contaminated equipment and inadvertently touch and likely ingest traces of fecal matter, posing significant risks of zoonotic disease transmission.

While I appreciate efforts to accommodate pet owners, the potential harm to our vulnerable young children outweighs these benefits. Peer-reviewed scientific studies consistently demonstrate that off-leash dog areas, including parks and fields, harbor high levels of pathogens and parasites in feces and soil, many of which are zoonotic (transmissible to humans) and particularly dangerous to young children due to their developing immune systems, hand-to-mouth behaviors, and close contact with the ground. Below, I highlight key findings from several rigorous published and peer reviewed studies:

- A 2017 study in Greater Lisbon, Portugal, analyzed 369 fecal samples and 18 soil samples from three urban dog parks, finding that 33% of fecal samples were positive for parasites, including hookworms (16.5% prevalence), *Cryptosporidium* spp. (11.9%), and *Giardia* spp. (11.4%). Soil contamination with hookworm eggs was detected in all parks (27.8% overall), primarily in grassy areas. These agents pose zoonotic risks such as cutaneous larva migrans from hookworms and visceral larva migrans from *Toxocara* spp., with heightened dangers for children through incidental ingestion or skin contact (Duarte et al., 2017, *BioMed Research International*).
- In a 2021 study of off-leash dog parks in Florence, central Italy, researchers examined fecal, soil, and water samples from 26 parks and 83 dogs. While bacterial pathogens like *Yersinia* spp. (8.4% in feces) and *Listeria* spp. (4.8%) were detected, fungal agents (e.g., *Microsporum* spp. in 61.5% of park soils) and helminths (*Toxocara canis* eggs in 2.4% of feces) were of particular concern. These can cause dermatophytoses (ringworm) and toxocariasis in humans, with children at elevated risk due to environmental persistence (arthrospores viable for over 30 months) and play behaviors (Ebani et al., 2021, *Animals*).
- A 2016 study from three regional dog parks in Northern California tested 300 dogs and detected enteropathogens in 38%, including *Giardia* (9%), *Cryptosporidium* (5.3%), *Campylobacter* (2.7%),

and Salmonella (1%). Notably, 54% of infected dogs showed no symptoms, allowing silent shedding into the environment. Zoonotic strains like Campylobacter and Salmonella were present in nearly 10% of dogs, underscoring transmission risks via contaminated fields, especially to children who may not exhibit immediate symptoms (Menezes et al., 2016, *Journal of Veterinary Diagnostic Investigation*).

- A 2020 DOGPARCS study (Stafford et al., *Parasites & Vectors*) found intestinal parasites in 20.7% of 3,006 dogs and 85% of 288 U.S. off-leash dog parks, including zoonotic Giardia and hookworms that contaminate soil and pose direct transmission risks to children playing on shared fields.

I have attached pdf copies of these 4 scientific studies and ask that they be included in the public meeting materials for the upcoming join City Council RCDC meeting on November 18.

Additional research reinforces these concerns. A 2017 review of off-leash dog parks emphasized that while direct human transmission data is limited, dogs frequently shed zoonotic agents like Giardia, Cryptosporidium, and Toxocara into shared spaces, with children under 15 facing 3–5 times higher bite risks and greater exposure to fecal-oral pathogens. Recommendations include separating dog areas from children's play zones to mitigate these hazards (D'Angelo et al., 2017, *Zoonoses and Public Health*). Similarly, a 2020 U.S. study across southeastern dog parks found gastrointestinal nematodes (e.g., hookworms, roundworms) in 20% of dogs and 85% of parks, highlighting these venues as hotspots for environmental contamination and human infection (Schurer et al., 2020, *Parasites & Vectors*).

The Centers for Disease Control and Prevention (CDC) warns that young children are especially susceptible to zoonoses like toxocariasis, which can lead to vision loss or neurological issues, and cryptosporidiosis, causing severe diarrhea. In Rollingwood, where these fields are central to family and youth athletic activities, allowing off-leash dogs to use little league fields 3, 4, and 5 as an off-leash dog park undermines our commitment to child safety and public health.

Compounding these health concerns is the fact that professional park planners, as part of the 2018 Rollingwood Park Master Plan, explicitly recommended removing off-leash dog use from the little league fields and instead creating a dedicated, separate off-leash dog park area to mitigate conflicts and hazards. This forward-thinking guidance aligns with best practices for balancing community needs while prioritizing child safety and environmental integrity.

I respectfully urge the City Council to act swiftly by prohibiting off-leash dog access to the little league fields and advancing the implementation of a standalone dog park as outlined in the master plan. Such measures would protect our children's health, enhance the usability of our parks, and foster a safer environment for all Rollingwood residents. This would protect our little league participants while honoring our community's love for pets.

I welcome the opportunity to discuss this further and am happy to provide additional documentation.

-dps

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RESEARCH

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Detection of gastrointestinal parasitism at recreational canine sites in the USA: the DOGPARCS study

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Abstract

Background: The rapid growth in off-leash dog parks provides opportunity for canine socialization activities but carries risk of exposure to intestinal parasites. This study assessed the prevalence of these infections in dogs visiting off-leash dog parks.

Methods: Fresh defecations were collected from dogs visiting parks in 30 metropolitan areas across the USA. Samples were analyzed by coproantigen immunoassay (CAI) (Fecal Dx[®] and *Giardia* Test, IDEXX Laboratories, Inc.) and zinc sulfate centrifugal flotation (CF). Owners responded to a questionnaire on their dog's signalment and use of heart-worm/intestinal parasite control medications (HWCM).

Results: Samples were examined from 3006 dogs, 87.9% aged at least 12 months, visiting 288 parks. At least one intestinal parasite was detected in 622 (20.7%) samples, nematodes in 263 (8.8%), with hookworms, whipworms and ascarids in 7.1, 1.9 and 0.6% of samples, respectively. A sample positive for one or more intestinal parasites was found in 245 (85.1%) parks, with nematodes found in 143 (49.7%). Combined, CAI and CF detected 78.4% more intestinal nematode infections than CF alone. Hookworm and whipworm infections were detected in all age groups, but ascarids were only detected in dogs less than 4 years-old. Approximately 42% of dogs aged less than 1 year were positive for nematodes or *Giardia*. Based on owner reports, HWCM was current for 68.8% of dogs, dogs previously diagnosed with intestinal parasitism were more likely to be receiving a HWCM than those without such history, and a significantly lower ($P=0.0003$) proportion of dogs receiving a HWCM were positive for intestinal nematodes compared with those not on such medication.

Conclusions: Intestinal parasites, the most common of which were *Giardia*, *Ancylostoma caninum* and *Trichuris vulpis*, were found in 20% of dogs and 85% of dog parks across the USA. Enhanced detection of canine intestinal parasitism was achieved by combining CF and CAI. Canine intestinal parasites are common across the USA and dog health can be improved by regular testing of fecal samples and routine administration of medications effective against the most common infections.

Keywords: *Ancylostoma*, Centrifugal flotation, Coproantigen, Dog, *Giardia*, Hookworm, Intestinal parasite, Roundworm, *Toxocara*, *Trichuris*, Whipworm

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Background

Canine intestinal parasite infections are often subclinical but can become clinically apparent in puppies and in adults with heavy burdens [1, 2]. Subclinical infections may carry a health cost, and dogs with patent infections shed eggs, oocysts or cysts that can contaminate the environment and act as a source of reinfection, infection of other dogs, and in some cases infection of humans [3, 4]. Stray and shelter dogs have higher rates of patent infections than dogs in the general population and are often rescued and relocated across country and state borders [2, 5–7].

Humane organizations care for and relocate shelter dogs, helping to address the demand for new pets, with pet dog ownership in the USA now the highest since measuring began in 1982 [8]. If newly-homed dogs are harboring intestinal parasites and left untreated, they can be a source of infection for other dogs in their new locations. Additionally, the relationship between dogs and owners has continued to evolve with more people incorporating their dogs into daily activities and travel than in previous generations, exposing the dog to environments potentially frequented by many other dogs [8–10]. Understanding the national risk of canine intestinal parasite infection is therefore important to drive recognition of the need for effective testing and control measures.

Two published reports have provided insights into the national prevalence of intestinal parasite infection of dogs in the USA [2, 5]. In the first report, findings were based on centrifugal sucrose flotation of fecal samples collected from shelter dogs, while in the second the results were based on samples submitted by veterinary practices to a testing laboratory that utilized zinc sulfate centrifugal flotation (CF). Extrapolation of results of the former study to the pet dog population is limited because shelter dogs may not have received anthelmintic treatment, and 84% of sampled dogs were under 3 years of age. Nonetheless, the findings of intestinal parasite infection in those dogs provide an indication of the potential parasite transmission risk associated with the movement of shelter dogs. A limitation of the second study is that samples were submitted by veterinary practices and so would likely have come from dogs receiving consistent high-quality veterinary care.

Both previously conducted national studies used CF, a strategy that is more sensitive than the passive flotation technique commonly used in veterinary practices. However, CF is not able to detect non-patent infections, may fail to identify low intensity infections or those in which particularly dense eggs are shed (e.g. cestodes, trematodes), and can, when high specific gravity solutions are used, collapse or fail to recover *Giardia* cysts. To address these limitations, coproantigen immunoassays (CAI)

have been developed to detect proteins found in *Giardia* cysts or produced by immature and adult nematodes in the intestinal lumen [11–15]. When used with CF, CAI enhances the detection of canine intestinal parasite infections [14–18]. With the interstate relocation of dogs that could be infected with intestinal parasites and the availability of improved testing methodologies, there is opportunity for an up-to-date assessment of intestinal parasitism in dogs in the USA.

Over the last ten years across the USA, the number of off-leash dog parks has increased dramatically, providing ideal locations to sample pet dogs [19]. A national study was initiated with the objective of determining the prevalence of canine intestinal parasite infections in dogs visiting dog parks. Other objectives were to provide insight into the complementary use of CF and CAI testing to diagnose these infections, and to assess the relationship between owner-reported use of heartworm control medications (HWCM) and intestinal parasite infection.

Methods

Fecal samples

Dog parks were selected in 30 major metropolitan areas across the USA (Table 1). Investigators collecting samples were veterinary staff from the College of Veterinary Medicine at Oklahoma State University and veterinary staff of Elanco Animal Health and IDEXX Laboratories, Inc. For each metropolitan area, 10 parks were selected to represent the diversity of geographical, socioeconomic and neighborhood types available in the area, taking into account factors such as safety for those collecting samples and accessibility of the parks. The target was to collect 100 samples from each metropolitan area. Consistent with earlier national surveys, results were divided into four regional areas derived from a previously described segmentation [2, 5].

Table 1 Listing of cities (alphabetical order within region) for sample collection

Southeast	Northeast	Midwest	West
Atlanta	Boston	Chicago	Albuquerque
Austin	New York City	Cleveland	Bakersfield
Charlotte	Philadelphia	Detroit	Boise
Houston	Washington DC	Indianapolis	Denver
Miami/Ft Lauderdale		Kansas City	Los Angeles
Nashville		Minneapolis	Phoenix
New Orleans		St Louis	Portland
Oklahoma City/Tulsa			Sacramento
Raleigh/Durham			Seattle
Tampa			

All dogs from which fecal samples were collected were owned by or under the care of dog park attendees and participation was voluntary. Immediately after defecation, the dog's fecal sample was placed into a plastic bag. No samples were collected from dogs belonging to any employees of the companies supporting the study, their friends or family members, investigators or assistant investigators, or to any staff known to be employed at a veterinary clinic. Dogs brought to the park by professional dog walkers were not eligible. For owners with multiple dogs, only one dog was sampled. The person responsible for the dog had to agree for the feces to be collected and verbally respond to a study questionnaire, which included the dog's signalment and the questions "Is your dog currently on a heartworm/intestinal worm preventive/medication?" and "Has your dog ever been diagnosed with intestinal worms?" Owners responding positively to the former question were asked whether the heartworm/intestinal worm control medication (HWCM) was administered orally, topically or by injection. Brand names were neither asked nor recorded if the owner volunteered the name.

All samples were processed at a single laboratory (IDEXX, 401 Industry Rd, Louisville, KY 40208, USA) employing validated CAIs for hookworm, whipworm and ascarids (Fecal Dx® and *Giardia* Test, IDEXX Laboratories, Inc., Westbrook, Maine, USA) and *Giardia* [15–17]. A zinc sulfate CF (specific gravity 1.24) was also used to detect a variety of parasites, including but not limited to nematodes and protozoans [20].

Analysis of results

The proportion of dogs testing positive for each parasite was determined according to signalment, metropolitan area and region in which the dog was sampled, whether the dog's owner reported administering a HWCM, and if the dog had been previously diagnosed with intestinal parasites. A 2-proportion z-test was used to test whether the proportion of dogs returning positive fecal tests for hookworm, whipworm, or ascarids was lower when owners reported the use of a HWCM than when owners reported not using a HWCM. A 2-proportion z-test was also used to test whether dogs reporting a previous intestinal parasite infection were more likely to be currently receiving a HWCM than those without a previous infection.

Dogs were categorized by age grouping in alignment with recent American Animal Hospital Association guidelines, with consistent years applied to each category: puppy, < 1 year-old; young adult, 1 to 3 years-old; mature adult, 4 to 6 years-old; and senior, ≥ 7 years-old [21]. A 4-sample test for equality of proportions was used to test whether the proportion of dogs testing positive

for hookworm, whipworm, ascarid or *Giardia* varied between these age groups.

Holm's multiple comparison correction was used to control the family-wise error rate due to the large number of comparisons being made. *Post-hoc* pairwise comparisons of the proportion of HWCM usage by region were performed using Holm's multiple comparison correction.

Throughout, 95% confidence intervals (CI) were calculated using the modified Wald method, except for the use of HWCMs by age and by region for which a multinomial approach was employed to model the individual probabilities of the response variable (age category × preventive) [22].

Results

Demographics and questionnaire

Samples were collected from 3022 dogs in 288 dog parks during July and August 2019. Sixteen samples were disqualified because they were not accompanied by completed questionnaires or because insufficient feces were available for testing. Thus, fecal testing results and questionnaires were available from 3006 dogs. The most commonly represented age group was young adult (1 to 3 years-old) ($n=1371$, 45.6% of 3006 dogs), followed by senior (≥ 7 years-old) (659, 21.9%), mature adult (4–6 years-old) (613, 20.4%) and puppy (< 12 months) (363, 12.1%). Within the puppy group, 72 dogs (2.4%) were less than 6 months of age. Of the 3006 dogs, 1317 (43.8%) were female, of which 1183 (89.8%) had been spayed while 1689 (56.2%) were male, of which 84.6% had been neutered. As reported by owners, the most commonly represented breeds were: Labrador Retriever (356, 11.8% of sampled dogs), German Shepherd Dog (187, 6.2%), Golden Retriever (137, 4.6%), Australian Shepherd (103, 3.4%), Siberian Husky (102, 3.4%), Chihuahua (83, 2.8%) and Boxer (64, 2.1%). The breeds of 547 dogs (18.2%) were described as mixed or were not specified.

In response to the question on nematode parasite control, 2069 owners (68.8%) stated that they were currently providing a HWCM for their dog. Of those owners, 1847 (89.3%) reported using an oral formulation, 68 (3.3%) a topical formulation and 144 (7.0%) an injection. Ten owners did not know how the HWCM was being administered (Table 2). The proportion of dogs reported to be currently receiving a HWCM was significantly higher ($P<0.0001$) in those previously infected with intestinal parasites (79.2%) (estimated difference of proportions 0.111; 95% CI: 0.075–1), compared with those without known prior intestinal worm infection (68.1%). Regionally, owner-reported HWCM use in the West was significantly lower than in each other region ($P<0.0001$). No other between-region differences were significant. By

Table 2 Number of owners (%) reporting currently using a heartworm/intestinal parasite control medication, by region and formulation

	Southeast (n = 989)	Northeast (n = 400)	Midwest (n = 708)	West (n = 909)	National (N = 3006)
Medication use					
Yes	829 (83.8)	317 (79.3)	570 (80.5)	353 (38.8)	2069 (68.8)
95% CI	81.5–86.1	75.3–83.2	77.6–83.4	35.7–42.0	67.2–70.5
No	14 (1.4)	68 (17.0)	135 (19.1)	536 (59.0)	885 (29.4)
95% CI	12.6–17.0	13.3–20.7	16.2–22.0	55.8–62.2	27.8–31.1
Unknown	14 (1.4)	15 (3.8)	3 (0.4)	20 (2.2)	52 (1.7)
95% CI	0.7–2.2	1.9–5.6	0.1–0.9	1.3–3.2	1.3–2.2
Formulation (n, % of dogs on heartworm/intestinal control medication)					
Oral	728 (87.8)	299 (94.3)	497 (87.2)	323 (91.5)	1847 (89.3)
95% CI	85.6–90.0	91.8–96.9	84.5–89.9	88.6–94.4	87.9–90.6
Topical	23 (2.8)	8 (2.5)	27 (4.7)	10 (2.8)	68 (3.3)
95% CI	1.7–3.9	0.8–4.3	3.0–6.5	1.1–4.6	2.5–4.1
Injectable	74 (8.9)	9 (2.8)	43 (7.5)	18 (5.1)	144 (7.0)
95% CI	7.0–10.9	1.0–4.7	5.4–9.7	2.8–7.4	5.9–8.1

Abbreviation: CI, confidence interval

dog age grouping, the frequency of use of a HWCM was similar at approximately 70% for puppies through mature adult dogs, while 63.6% of owners of senior dogs reported that they were providing a HWCM.

Fecal test results

Using CAI and CF, intestinal parasites were detected in 622 (20.7%) samples, with 8.8% positive for one or more of hookworms, whipworms and ascarids (Table 3). Of the 288 parks, 245 (85.1%) provided samples positive for any intestinal parasites, with 49.7% positive for at least one of the aforementioned nematode groups. The most commonly detected parasite was *Giardia*, while hookworm was the most commonly detected nematode group. Other canine parasites detected on CF included *Cystoisospora* spp. ($n=16$ dogs), *Alaria* sp. ($n=1$), capillariids ($n=2$), *Spirometra* sp. ($n=2$) and a taeniid egg ($n=1$). The spurious parasites *Eimeria* spp. were identified in samples from 37 (1.2%) of dogs.

Use of CF and microscopy allowed identification of hookworm and ascarid ova. Of 110 samples positive for hookworm ova, 108 (98.2%) were *Ancylostoma caninum* and 2 (1.8%) were *Uncinaria stenocephala*. Of 12 samples positive by CF for ascarids, 11 (91.7%) were *Toxocara canis* and 1 (8.3%) was *Toxascaris leonina*. Of the 42 ova with bipolar plugs, two were *Eucoleus aerophilus* and the remainder were *Trichuris vulpis*.

A 4-sample test for equality of proportions of age groups with positive tests for one or more of hookworm, whipworm and ascarids was significant ($P<0.0001$), with

Table 3 Number (%) [95% CI] of dogs and parks with ≥ 1 sample positive for intestinal parasites by coproantigen immunoassay or centrifugal flotation

Parasite species	Dogs n (%) [95% CI] (N = 3006)	Dog parks n (%) [95% CI] (N = 288)
Any parasitic species ^a	622 (20.7) [19.3–22.2]	245 (85.1) [80.5–88.8]
Nematodes ^b and/or <i>Giardia</i>	609 (20.3) [18.9–21.7]	243 (84.4) [79.7–88.1]
<i>Giardia</i> spp.	391 (13.0) [11.9–14.3]	213 (74.0) [68.6–78.7]
Nematodes ^b	263 (8.8) [7.8–9.8]	143 (49.7) [43.9–55.4]
Hookworms	214 (7.1) [6.3–8.1]	125 (43.4) [37.8–49.2]
Whipworms	58 (1.9) [1.5–2.5]	52 (18.1) [14.0–22.9]
<i>Eimeria</i> spp.	37 (1.2) [0.9–1.7]	33 (11.5) [8.2–15.7]
Ascarids	17 (0.6) [0.3–0.8]	16 (5.6) [3.4–8.9]
<i>Cystoisospora</i>	16 (0.5) [0.3–0.9]	16 (5.6) [3.4–8.9]
<i>Alaria</i>	1 (0.0) [0–0.2]	1 (0.4) [0–2.1]
Capillariids	2 (0.1) [0–0.3]	1 (0.4) [0–2.1]
<i>Spirometra</i>	2 (0.1) [0–0.3]	2 (0.7) [0–2.7]
Taeniids	1 (0.0) [0–0.2]	1 (0.4) [0–2.1]

^a Includes all parasitic species of nematodes, as well as *Alaria*, *Cystoisospora* and *Spirometra* and taeniids

^b Nematodes: hookworms, whipworms, ascarids (includes co-infections)

Abbreviation: CI, confidence interval

the highest prevalence in dogs less than 12 months of age. Infection with hookworm, whipworm and *Giardia* was detected regardless of age group, while ascarid infection was only identified in samples from dogs under 4 years of age (Figs. 1, 2; Table 4). Dogs reported to be currently on a HWCM had a significantly lower proportion of positive test results for hookworms, whipworms or ascarids (7.5%) than those not receiving a HWCM (11.4%) ($P=0.0003$; estimated difference of proportions -0.039 ; 95% CI: -1 to -0.018) (Table 5).

Co-infections were detected in 49 (1.6%) dogs. The most common co-infection was hookworm + *Giardia* in 24 dogs (0.8%), followed by hookworm + whipworm co-infection in 12 dogs (0.4%). Prevalence was also calculated by region (Table 6). In each region, less than 1% of tests were positive for *Cystoisospora*. Details of the proportion of infected dogs and parks from which positive samples were collected in each of the 30 metropolitan areas are provided in Additional file 1: Table S1 and Additional file 2: Table S2.

Coproantigen immunoassay and centrifugal flotation

In detection of hookworm, whipworm, or ascarids, 289 infections were found (Fig. 3). Of these, 162 (56.1%) were detected using CF and 244 (84.4%) using CAI. Both methods were in positive agreement in 117 (40.5%) of these infections. The combination of CF and CAI

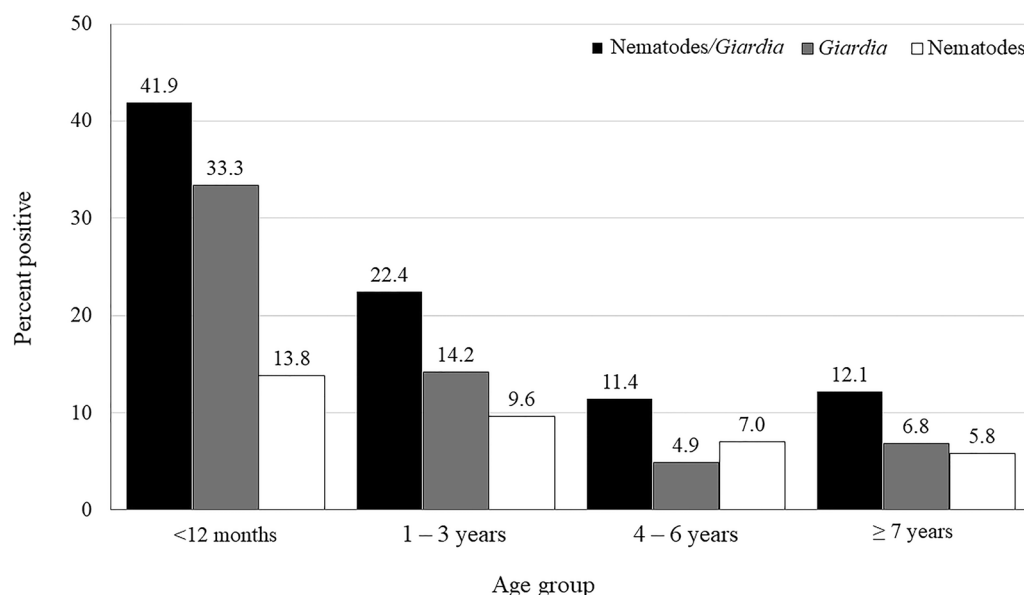


Fig. 1 Percent of dogs in each age group positive for intestinal parasites

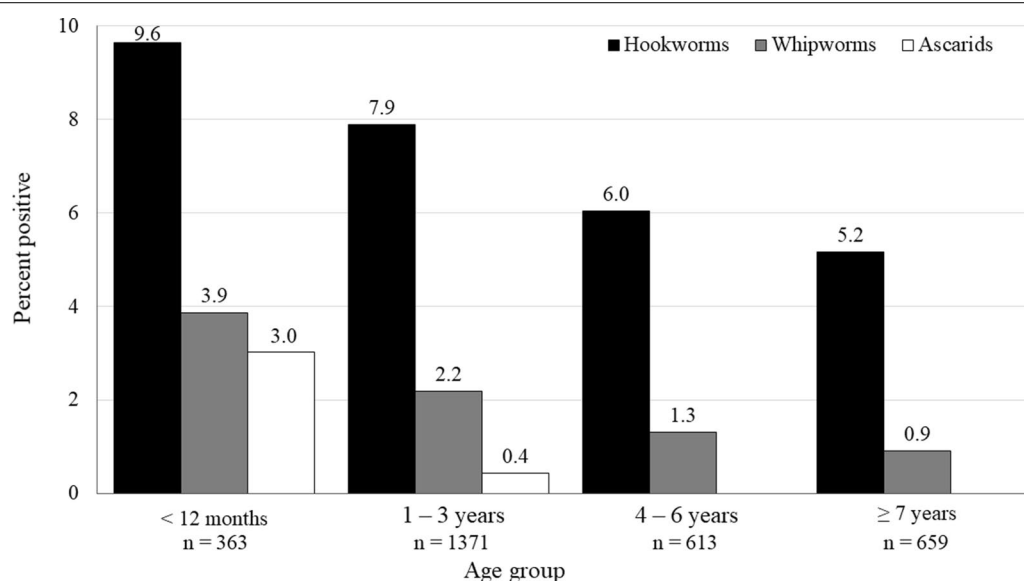


Fig. 2 Percent of dogs in each age group positive for a nematode parasite. Ascarids were not detected in dogs ≥ 4 years of age

detected 78.4% more infections than did CF alone. For hookworm, the methods were in positive concordance for 85 infections (39.7% of detected hookworm infections), CAI detected 104 infections (48.6%) when CF was negative, and for 25 infections (11.7%) the reverse was true. Of the 58 *T. vulpis* infections, the findings for each method were in positive concordance for 22 (37.9%), 18 infections (31.0%) detected by CAI were negative on CF, and 18 (31.0%) infections detected by CF were negative by CAI. For *Giardia*, the methods were in positive

concordance for 38 infections (9.7%), 351 (89.8%) infections detected by CAI were negative on CF, and 2 (0.5%) infections detected by CF were negative on CAI.

Discussion

The present study is the first large-scale effort to determine the prevalence of intestinal parasites in dogs visiting dog parks throughout the USA. In 2019, the 100 largest USA cities contained a total of 810 dedicated dog parks [19]. Testing of samples collected from 288 parks

Table 4 Number (%) of dogs positive for intestinal parasites by coproantigen immunoassay or centrifugal flotation by age grouping

Demographic category	Nematodes/ <i>Giardia</i>	Nematodes	Hookworms	Whipworms	Ascarids	<i>Giardia</i>
< 12 months; puppy (<i>n</i> = 363)	152 (41.9)	50 (13.8)	35 (9.6)	14 (3.9)	11 (3.0)	121 (33.3)
95% CI	36.9–47.0	10.6–17.7	7.0–13.1	2.3–6.4	1.6–5.4	28.7–38.3
1–3 years; young adult (<i>n</i> = 1371)	307 (22.4)	132 (9.6)	108 (7.9)	30 (2.2)	6 (0.4)	195 (14.2)
95% CI	20.3–24.7	8.2–11.3	6.6–9.4	1.5–3.1	0.2–1.0	12.5–6.2
4–6 years; mature adult (<i>n</i> = 613)	70 (11.4)	43 (7.0)	37 (6.0)	8 (1.3)	0 (0.0)	30 (4.9)
95% CI	9.1–14.2	5.2–9.3	4.4–8.2	0.6–2.6	0.0–0.8	3.4–6.9
≥ 7 years; senior (<i>n</i> = 659)	80 (12.1)	38 (5.8)	34 (5.2)	6 (0.9)	0 (0.0)	45 (6.8)
95% CI	9.9–14.9	4.2–7.8	3.7–7.1	0.4–2.0	0.0–0.7	5.1–9.0

Note: Percentages are based on the number of positive dogs in that age group as the numerator and total of dogs reported to be in that category as denominator

Abbreviation: CI, confidence interval

Table 5 Number (%) of dogs positive for intestinal parasites by coproantigen immunoassay or centrifugal flotation according to owner-reported current use of a heartworm/intestinal parasite control medication

Demographic characteristic	Nematodes/ <i>Giardia</i>	Nematodes	Hookworms	Whipworms	Ascarids	<i>Giardia</i>
Dogs on medication: Yes (<i>n</i> = 2069)	404 (19.5)	155 (7.5)	125 (6.0)	31 (1.5)	11 (0.5)	276 (13.3)
95% CI	17.9–21.3	6.4–8.7	5.1–7.2	1.1–2.1	0.3–1.0	11.9–14.9
Dogs on medication: No (<i>n</i> = 885)	190 (21.5)	101 (11.4)	84 (9.5)	25 (2.8)	6 (0.7)	106 (12.0)
95% CI	18.9–24.3	9.5–13.7	7.7–11.6	1.9–4.2	0.3–1.5	10.0–14.3
Medication status unknown (<i>n</i> = 52)	15 (28.9)	7 (13.5)	5 (9.6)	2 (3.9)	0 (0.0)	9 (17.3)
95% CI	18.3–42.4	6.4–25.6	3.8–21.0	0.3–13.7	0.0–8.2	9.2–30.0

Note: Percentages are based on the number of positive dogs in that demographic category as the numerator and total of dogs reported to be in that category as denominator

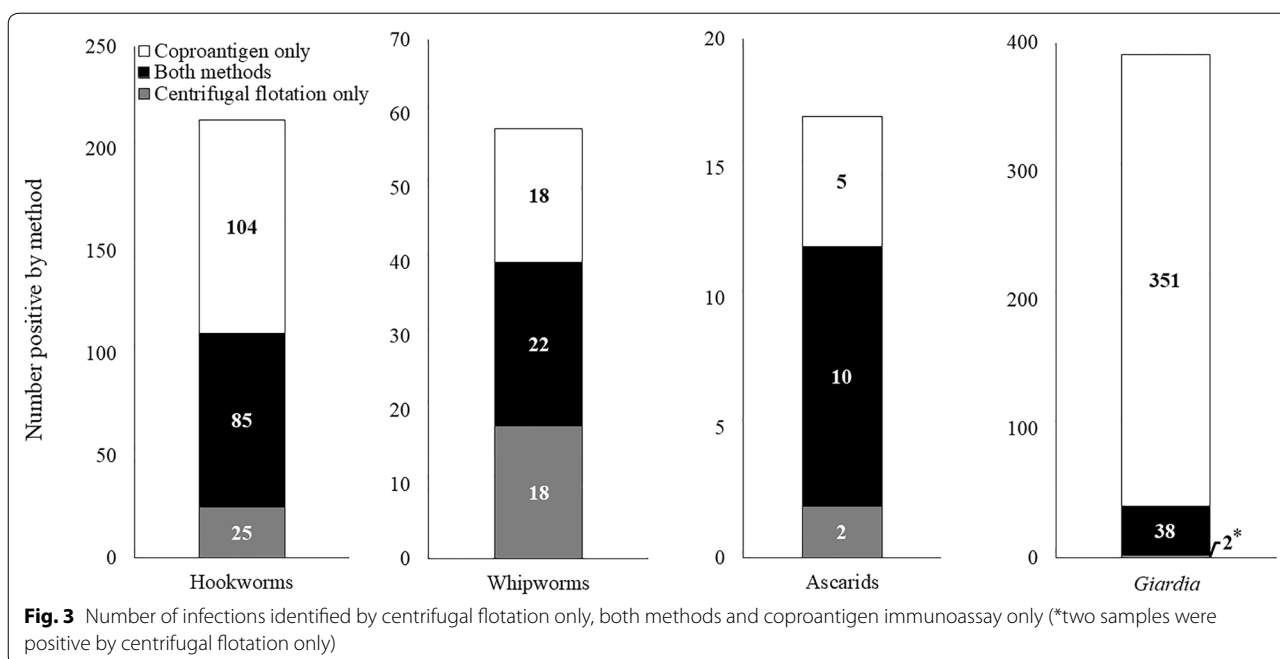
Abbreviation: CI, confidence interval

Table 6 Regional distribution: number (%) of dogs and parks with a positive test (coproantigen immunoassay or centrifugal flotation) for intestinal parasites

Positive tests	Nematodes/ <i>Giardia</i>	Nematodes	Hookworms	Whipworms	Ascarids	<i>Giardia</i>
From dogs						
Southeast (<i>n</i> = 989)	270 (27.3)	169 (17.1)	151 (15.3)	27 (2.7)	5 (0.5)	129 (13.0)
95% CI	24.6–30.2	14.9–19.6	13.2–17.7	1.9–4.0	0.2–1.2	11.1–15.3
Northeast (<i>n</i> = 400)	72 (18.0)	25 (6.3)	21 (5.3)	8 (2.0)	1 (0.3)	48 (12.0)
95% CI	14.5–22.1	4.2–9.1	3.4–7.9	1.0–4.0	0–1.6	9.2–15.6
Midwest (<i>n</i> = 708)	131 (18.5)	44 (6.2)	28 (4.0)	15 (2.1)	6 (0.9)	98 (13.8)
95% CI	15.8–21.5	4.7–8.3	2.7–5.7	1.3–3.5	0.3–1.9	11.5–16.6
West (<i>n</i> = 909)	136 (15.0)	25 (2.8)	14 (1.5)	8 (0.9)	5 (0.6)	116 (12.8)
95% CI	12.8–17.4	1.9–4.1	0.9–2.6	0.4–1.8	0.2–1.3	10.7–15.1
From parks						
Southeast (<i>n</i> = 96)	86 (89.6)	73 (76.0)	69 (71.9)	25 (26.0)	5 (5.2)	70 (72.9)
95% CI	81.7–94.4	66.6–83.5	62.1–79.9	18.3–35.7	2.0–11.9	63.2–80.8
Northeast (<i>n</i> = 39)	31 (79.5)	17 (43.6)	16 (41.0)	7 (18.0)	1 (2.6)	28 (71.8)
95% CI	64.2–89.5	29.3–59.0	27.1–56.6	8.7–33.0	0–14.4	56.1–83.6
Midwest (<i>n</i> = 68)	59 (86.8)	33 (48.5)	26 (38.2)	12 (17.7)	5 (7.4)	50 (73.5)
95% CI	76.5–93.1	37.1–60.2	27.6–50.1	10.2–28.5	2.8–16.5	61.9–82.6
West (<i>n</i> = 85)	67 (78.8)	20 (23.5)	14 (16.5)	8 (9.4)	5 (5.9)	65 (76.5)
95% CI	68.9–86.3	15.7–33.6	10.0–25.9	4.6–17.7	2.2–13.4	66.4–84.3

Note: See Additional file 1: Table S1 and Additional file 2: Table S2 for detailed numbers by metropolitan region

Abbreviation: CI, confidence interval



in 30 of these cities allowed us to document parasites in over 20% of dogs and 85% of parks. The prevalence of parasite infection in dogs in the present study is higher than that seen in pet dogs with fecal samples submitted from veterinary practices to national diagnostic laboratories (12.5%), but lower than that reported from stray dogs upon arrival at municipal shelters (36%). The differences in findings between the pet dog survey and this study may be due to CAI detecting some infections missed by CF, the only method used in the earlier survey, and to the fact that while dogs attending dog parks receive attention from their owners, not all benefit from routine veterinary care [2, 5].

Giardia was the most commonly identified intestinal parasite, both in the present study (13.0%) and in an earlier national report of parasites in pet dogs (4.0%) [2]. In contrast, *Giardia* was only rarely detected (0.6%) in a national survey of shelter dogs in which samples were examined by sugar CF, presumably due to the lower sensitivity of this method for recovering the fragile cysts [5, 12]. Infections with *Giardia* are often subclinical, and cysts or trophozoites are shed intermittently from infected dogs, limiting the diagnostic sensitivity of CF as a stand-alone method. This may have been one factor behind the much higher rate of detection of *Giardia* infection by CAI than by CF, as would the fragility of *Giardia* cysts, leading to their degeneration between collection of fresh samples and CF testing at the laboratory. By combining CAI with CF, the present study may better estimate the true prevalence of infection. An

earlier report using a similar strategy (CF + CAI) found a *Giardia* prevalence of 15.6% among dogs presenting to clinics with diarrhea or vomiting [23]. Dogs in shelters, breeding facilities and kennels are more likely to be infected with *Giardia*, and an increased prevalence among dogs that visit dog parks, compared with those not visiting dog parks, has been reported [24].

Nematodes were also commonly detected, identified in 10% of the samples tested. As in other studies using CF alone, the hookworm *A. caninum* and whipworm *T. vulpis*, which present a risk to canine health throughout all life stages of a dog, were the most common intestinal nematodes identified [14, 25, 26]. The results may underestimate the prevalence of *T. vulpis* and *T. canis*, as samples were collected during July and August, a time when infections with these nematodes may be at their lowest prevalence [27]. Surprisingly, passive flotation remains the most commonly used technique in clinical practice despite multiple studies demonstrating that it fails to detect many infections when compared to CF [12–15]. Combining CAI for nematode antigens with CF in the present study resulted in detection of nearly 80% (78.4%) more nematode infections than CF alone, likely due to the CAI detecting non-patent infections [16, 17]. Detection of parasite ova by CF in instances when CAI was negative could be due to coprophagia or predation, resulting in a positive CF in the absence of infection. In this study, 37 (1.2%) samples tested positive for *Eimeria* spp., supporting the role coprophagy may have played in the discordant results. Another factor could be that a low

intensity infection may not produce sufficient antigen, leading to a negative CAI even though some ova were being shed [14, 17]. These findings reinforce the previously demonstrated complementary value of combining CAI with CF to enhance intestinal nematode detection [28].

Cestodes or trematodes were only rarely detected in the present study, even though recent studies in the USA have shown that the prevalence of infection with common tapeworms (e.g. *Dipylidium caninum*, *Taenia* spp.) is greater than that of nematodes in some populations of dogs and cats [14, 29]. Because eggs of cestodes common in dogs are shed in proglottids, and because most cestode and trematode eggs are heavy, recovery by CF is poor [30]. Using a higher specific gravity sugar solution for CF in part addresses this limitation, enhancing recovery of taeniid eggs, but sensitivity remains very low for identifying *Dipylidium caninum* infection [14, 30], and CAI is not yet commercially available for canine cestodes or trematodes. Eggs of *Spirometra* sp. or *Alaria* sp., less common cestodes and trematodes of dogs, respectively, are occasionally detected on CF. In the present study, *Spirometra* sp. eggs were identified in two dogs and *Alaria* sp. eggs in one dog.

As with earlier national reports, although parasites are found in every region, the present study indicates that the highest prevalence of nematode intestinal parasite infection, and in particular *A. caninum* infection, occurs in the Southeast [2, 5]. This factor, together with the very high prevalence of heartworm infection in the southeastern USA, likely explains the common owner-reported use of HWCM in this region. Other canine surveys from the region support that hookworm and whipworm are very common, identifying *A. caninum* in as many as 48% of shelter dogs and 17% of samples from dog parks, and *T. vulpis* in up to 39% of shelter dogs and 8.5% of samples from dog parks [14, 26, 31, 32].

In the present study, more than 15% of dogs visiting dog parks in the Southeast, and 4 to 5.3% of those in the Midwest and Northeast, were infected with hookworm, a finding that is particularly concerning given the recent reports of multiple drug-resistant hookworms in pet dogs, including Greyhounds [33–35]. Although we do not know the resistance status, six of the 12 Greyhounds sampled from dog parks in the present study were positive for hookworm, and five of those six were reported by the owner to be on a HWCM at the time they were sampled, compared to 57.7% of non-Greyhound, hookworm positive dogs that were reportedly receiving a HWCM (data not shown). Hundreds of thousands of stray and rescue dogs, including retired Greyhounds, are commonly relocated from the southern USA to other regions,

a practice that can facilitate movement of parasites, including resistant parasites [7, 36, 37].

Intestinal nematodes, particularly *A. caninum* and *T. vulpis*, but not all intestinal parasites (e.g. *Giardia*, *Cystoisospora* spp.), were less commonly detected in samples from dogs reportedly receiving HWCM in the present study, providing evidence that implementing broad-spectrum parasite control measures reduces infections and limits environmental contamination with eggs. This finding has long been suspected and is supported by other regional surveys [26, 38]. However, hookworm, whipworm, or ascarid infections were still detected in some dogs reportedly receiving HWCM in the present study, perhaps due to the earlier detection afforded by antigen testing, the short (2–3 weeks) pre-patent period of hookworm, and the fact that not all HWCMs are effective against whipworm or other intestinal nematodes [16, 17, 28, 39]. For example, injectable products are not effective against either whipworm or ascarids, and are not FDA-label approved for efficacy against new hookworm infections beyond the time of initial administration [40, 41]. Products containing ivermectin/pyrantel are effective against *A. caninum*, *Ancylostoma braziliense* and *U. stenocephala*, but are not effective against whipworms [42]. Those containing milbemycin oxime are effective against whipworms, ascarids and the common hookworm, *A. caninum*, but not against its relatively scarce and less pathogenic relative, *U. stenocephala* [43]. Topically applied moxidectin is indicated to treat and control *T. vulpis*, *A. caninum*, *U. stenocephala* and ascarids [44]. The hookworm efficacy of all these treatments is based on having demonstrated efficacy prior to reports suggesting the incipient emergence of multi-drug resistant *A. caninum* [33, 35]. Detection of nematode infections and other parasites in dogs reported to be receiving HWCMs in the present study indicates that regular testing is warranted for all dogs even when these medications are used.

Interestingly, a majority (68.8%) of owners in the present study reported current use of a HWCM, similar to other recent papers surveying dog owners in Oklahoma and Florida [26, 45]. This high owner-reported prevalence of use contrasts with other data indicating that, even in areas where heartworm infection is common, only a minority of pet dogs receive a HWCM [46, 47]. Factors that may bias owner-reported use of HWCM include forgetfulness, guilt about not following veterinary recommendations, and confusion about a given product's efficacy for heartworm *versus* external parasites. Additionally, the study was conducted in the summer months, when mosquitoes are most active, a timing that may have resulted in a higher proportion of owners reporting current use of HWCM. Routine

use of HWCM is critically important because many of the products can limit environmental contamination with zoonotic parasites like *A. caninum* and *T. canis* which cause cutaneous *larva migrans* and toxocariasis, respectively. Other strategies such as reducing the number of stray or free-roaming animals, prompt removal of all pet feces, wearing shoes to avoid skin contact with contaminated soil, hand-washing after handling feces or soil, and avoiding geophagy in children can also reduce infection risk [48].

Conclusions

Dog parks and other areas in which dogs are walked (e.g. neighborhood walking paths, apartment complexes) provide valuable human and animal socialization opportunities, but also may increase the risk of exposure to intestinal parasite infection. Maintaining dogs on broad-spectrum parasite control products with efficacy against hookworms, whipworms and ascarids helps mitigate this risk, decreasing the health risks to dogs and the potential for zoonotic infections, particularly as the owner-pet relationship and interaction grow ever closer. Indeed, in the present study reported use of HWCM reduced but did not eliminate infection with intestinal nematodes. Canine cestode infection prevalence remains unclear, but a recent study suggests that tapeworms are common in dogs and that routine treatment for tapeworms may also be warranted [14]. The CAI used in the present study detected more infections than did CF alone, although using the two tests in concert allowed the greatest number of infections to be identified. Regular fecal testing for parasites by CF and CAI is recommended to safeguard canine health by identifying infections early and as a means of monitoring product use and continued efficacy.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13071-020-04147-6>.

Additional file 1: Table S1. Number (%³; 95% confidence interval) of dogs visiting dog parks in each city with a positive test for intestinal parasites by coproantigen immunoassay and/or centrifugal flotation.

Additional file 2: Table S2. Number (%³; 95% confidence interval) of dog parks in each city with at least one positive fecal test (coproantigen immunoassay and/or centrifugal flotation).

Abbreviations

CAI: coproantigen immunoassay; CF: centrifugal flotation; CI: confidence interval; FDA: Food and Drug Administration; HWCM: heartworm/intestinal parasite control medication.

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Authors' contributions

KS, TMK, KTD, SH, TG, CHL, AJR, WGR, SS and SEL participated in the design and implementation of the study. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article and its additional files. Raw data from this study are available for review upon reasonable request to Elanco Animal Health, IDEXX or Oklahoma State University.

Ethics approval and consent to participate

The protocol was approved by Elanco Privacy and Consumer Information Review Board. No animals were handled, and no treatments were administered.

Consent for publication

Not applicable.

Competing interests

KS, TMK, CHL and AJR are employees of Elanco. SH, TG and SS are employees of IDEXX. WGR has received payments for clinical research and consulting fees from Elanco. SL and KTD have received honoraria, expense reimbursement, or research support from several companies that manufacture parasite control and diagnostic products.

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Prevalence of Enteropathogens in Dogs Attending 3 Regional Dog Parks in Northern California

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Background: The prevalence and risk factors for infection with enteropathogens in dogs frequenting dog parks have been poorly documented, and infected dogs can pose a potential zoonotic risk for owners.

Hypothesis/Objectives: To determine the prevalence and risk factors of infection with enteropathogens and zoonotic *Giardia* strains in dogs attending dog parks in Northern California and to compare results of fecal flotation procedures performed at a commercial and university parasitology laboratory.

Animals: Three-hundred dogs attending 3 regional dog parks in Northern California.

Methods: Prospective study. Fresh fecal specimens were collected from all dogs, scored for consistency, and owners completed a questionnaire. Specimens were analyzed by fecal centrifugation flotation, DFA, and PCR for detection of 11 enteropathogens. *Giardia* genotyping was performed for assemblage determination.

Results: Enteropathogens were detected in 114/300 dogs (38%), of which 62 (54%) did not have diarrhea. Frequency of dog park attendance correlated significantly with fecal consistency ($P = .0039$), but did not correlate with enteropathogen detection. Twenty-seven dogs (9%) were infected with *Giardia*, and genotyping revealed nonzoonotic assemblages C and D. The frequency of *Giardia* detection on fecal flotation was significantly lower at the commercial laboratory versus the university laboratory ($P = .013$), and PCR for *Giardia* was negative in 11/27 dogs (41%) that were positive on fecal flotation or DFA.

Conclusions and Clinical Importance: Enteropathogens were commonly detected in dogs frequenting dog parks, and infection with *Giardia* correlated with fecal consistency. PCR detection of *Giardia* had limited diagnostic utility, and detection of *Giardia* cysts by microscopic technique can vary among laboratories.

Key words: Bacteria; Canine; Diarrhea; Infectious; Parasites; Zoonosis.

Dog parks are the fastest growing segment of city parks in the United States and represent a park for dogs to exercise and play off-leash in a controlled environment under the supervision of their owners.¹ These

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Where the work was done: Fecal flotations and direct immunofluorescence were performed in the Parasitology Laboratory, William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis. Fecal flotations and Canine Diarrhea RealPCR™ Panels were performed at IDEXX Laboratories Inc., Sacramento, CA.

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Abbreviations:

CPE	<i>Clostridium perfringens</i> enterotoxin
cpe	<i>Clostridium perfringens</i> enterotoxin gene
DFA	direct fluorescent antibody test
TcdA	<i>Clostridium difficile</i> toxin A
TcdA	<i>Clostridium difficile</i> toxin A gene
TcdB	<i>Clostridium difficile</i> toxin B
TcdB	<i>Clostridium difficile</i> toxin B gene

parks have varying features, although they typically offer a 4' to 6' fence, separate double-gated entry and exit points, adequate drainage, benches for humans, shade for hot days, parking close to the site, water, tools to pick up and dispose of animal waste in covered trash cans, and regular maintenance and cleaning of the grounds. There were 644 off-leash dog parks in the 100 largest US cities in 2015, representing a 20% increase in 5 years,¹ and there are now more American households with dogs than with children.² The health benefits of owning a dog are well documented and include reduced blood pressure, anxiety and depression, increased activity of owners, increased social interactions with other dog owners, and development of a sense of purpose.³ Close contact between dogs and people, however, can pose health risks, particularly in very young, old, and immune-compromised people. Domestic dogs have been identified as potential sources of zoonotic enteric pathogens such as *Salmonella* spp., *Campylobacter jejuni*, *Giardia* spp., and *Cryptosporidium* spp.^{4,5}

Dogs attending dog parks could represent a very different population from those previously studied as they can have off-leash contact with other dogs or their feces, humans other than their owners, and possibly

wildlife, depending on the type of park visited. In addition, infected dogs that visit public parks have opportunities to expose other dogs, as well as humans, to zoonotic bacteria and parasites shed in their feces. *Giardia* spp. and *Cryptosporidium* spp. are well-documented zoonotic parasites in humans and domestic animals;^{6,7} however, little is known about the prevalence of these enteropathogens in dog populations exposed to higher risk environments such as dog parks and their zoonotic potential in these environments. Humans can be exposed to subclinically infected dogs when removing dog feces from public areas,⁸ and infective cysts and oocysts of *Giardia* and *Cryptosporidium*, respectively, can persist for prolonged periods in the environment, posing an increased infection risk in areas where environmental contamination is high, such as public dog parks.^{4,7,8} Dogs that attended a dog park in Fort Collins, Colorado, were significantly more likely to be infected with an intestinal parasite compared to socially active dogs that did not attend dog parks.⁹ A seasonal trend for fecal shedding of *Giardia* in dogs was not demonstrated in a recent study,¹⁰ demonstrating the possibility for year-round risk.

The objectives of this study were 3-fold: (1) to determine the prevalence and risk factors of infection with enteropathogens in a cohort of dogs attending 3 regional dog parks in Northern California; (2) to determine the prevalence of zoonotic *Giardia* strains in dogs attending the regional dog parks; and (3) to compare the performance characteristics of fecal centrifugation flotation procedures performed in a university and veterinary commercial laboratory.

Materials and Methods

Sample Acquisition

This study was approved by the University of California, Davis, Institutional Review Board, and all owners signed an informed consent form. Three Northern California regional public dog parks within Yolo and Sacramento counties were visited between the months of August and November, 2014, by a computerized randomizer^a to determine the schedule for park visits and fecal collection. Fresh fecal samples were collected from all dogs by their owners during the time of park visitation. A comprehensive questionnaire^b was developed to obtain information on each dog's signalment, lifestyle, environment, and medical history, including whether the animal had been dewormed within the past 6 months. The completed questionnaire, informed owner consent form, and contact information were obtained from each dog's owner at the time of sample collection. A modified Nestlé Purina Fecal Scoring chart with color images of different fecal consistencies was utilized by owners to determine their dog's fecal score on a scale of 1–6, with a score of 1 representing a hard, dry fecal specimen, and a score of 6 representing a liquid specimen. Fecal scores ≥ 4 were deemed to be diarrheic. Owners were asked to assess the average consistency of their dog's feces over the previous month before enrolling their dog in the study, as well as on the day of their dog's visit to the dog park. In addition, the investigators determined fecal scores on all but the first 34 fecal specimens at the time of fecal collection. The samples were kept labeled and double-bagged in a temperature-controlled cooler until the end of the 1- to 2-hour park visit, after which they were separated into 2

aliquots for further processing and evaluation. One aliquot was immediately delivered to the Parasitology Laboratory at UC Davis, and the second aliquot on ice packs was delivered via courier to a veterinary commercial reference laboratory^c within 24 hours of collection.

University Laboratory Tests

Fecal centrifugation flotations were performed on all samples at the University's Parasitology Laboratory. Fresh feces were examined for parasite ova, cysts, and oocysts by use of a zinc sulfate double centrifugation flotation technique as previously described.¹¹ In addition, the parasitologist evaluated the entire slide in a grid pattern evaluating approximately 50 random high-power fields (hpf) to determine the average number of cysts, oocysts, or ova per hpf. A direct fluorescent antibody (DFA) test for detection of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts was also performed at the university laboratory according to the manufacturer's instructions.^d Direct fluorescent antibody testing was only performed on 51 fecal specimens with discordant results for *Giardia* and *Cryptosporidium* (ie, PCR positive at the reference laboratory and fecal flotation negative at the reference laboratory or university laboratory or vice versa). A specimen was considered positive for either protozoa if 1 or more (oo)cysts were observed. Positive slides were further ranked by number of (oo)cysts per slide: 1+ (1–9 (oo)cysts), 2+ (10 to 50 (oo)cysts), and 3+ (>50 (oo)cysts).

Commercial Reference Laboratory Tests

Fecal centrifugation flotations were performed on all samples at the veterinary commercial reference laboratory.^c Fresh feces were examined for parasite ova, cysts, and oocysts by use of a zinc sulfate single centrifugation flotation technique as previously described;¹¹ however, the technician scanned the slide at 10 $\times$ magnification in a grid pattern for approximately 60–120 seconds. A PCR diarrhea panel was performed on each sample for the following 11 enteropathogens and toxin genes: *Cryptosporidium* spp., *Giardia* spp., *Salmonella* spp., *Campylobacter jejuni*, *Campylobacter coli*, canine enteric coronavirus, canine distemper virus, canine parvovirus 2, canine circovirus, *Clostridium difficile* toxin A (*TcdA*) and toxin B (*TcdB*) genes, and *Clostridium perfringens* alpha toxin gene and enterotoxin gene (*cpe*). Fecal samples were processed by a previously validated protocol.^{12,13} Analysis was performed on a Roche LightCycler 480^e and raw data analyzed by the 2nd derivative maximum method to generate crossing points (CP). Real-time PCR was run with 7 quality controls including (1) PCR-positive controls, (2) PCR negative controls, (3) negative extraction controls, (4) DNA pre-analytical quality control targeting the host ssr rRNA (18S rRNA) gene complex, (5) RNA pre-analytical quality control targeting the host ssr rRNA gene complex, (6) an internal positive control spiked into the lysis solution, and (7) an environmental contamination monitoring control.

Extracted DNA from *Giardia*-positive fecal specimens was sent to the Center for Companion Animal Studies, Colorado State University, for genotyping. The PCR assays were performed following published protocols with several modifications described by Scorza et al.^{14–16} In brief, partial regions of 3 genes, including β -giardin (bg), glutamate dehydrogenase (gdh), and triose phosphate isomerase (tpi), were targeted. The DNA sequences were analyzed in both forward and reverse direction with an ABI3100 Genetic Analyzer.^f The nucleotide sequences generated in this study were placed in GenBank under the accession numbers KX164005–KX64017. The DNA sequence data from the *Giardia*-positive isolates were compared by BLAST analysis with sequences from the nucleotide database from GenBank (<http://>

blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment was performed by Geneious R8.1 with the reference strains from a previous publication.¹⁷

Statistical Analysis

A power calculation was used to determine the number of dogs to include in this study. Using a binomial infection prevalence of 15% in the higher socioeconomic status (SES) neighborhood park, and hypothesizing a 2.5-fold increase in the prevalence of intestinal parasites in the lower SES park, a sample size of 100 dogs per park was required to achieve 94% power with a type I error of 5%. Kruskal–Wallis tests were used to compare groups with respect to ordered or continuous variables, and Spearman correlation to compare 1 ordered or continuous variable to a second ordered or continuous variable. McNemar's chi-square tests were used to assess test discordance between laboratories and Fischer's exact test was used to evaluate the association among binary variables. All analyses were performed by Stata/IC 13.1 software.^a A *P*-value <.05 was considered significant.

Results

Three hundred dogs were enrolled in this cross-sectional study: one hundred dogs from each of 3 Northern California regional dog parks. The dog parks all had chain link fences, separate small and large dog sections, water spigots with water bowls, light tree cover, free public access, and benches. The 3 parks were approximately 1.5, 2, and 2.5 acres, respectively. One of the parks had artificial turf, whereas the other 2 had natural grass. The 2.5-acre park with grass would occasionally become flooded in the winter season due to poor drainage; however, fecal specimens were not collected from any of the parks during the winter season. The mean $\pm$ standard deviation (SD) age was 3.9 ± 3.2 years (range 3 months to 17 years) and differed significantly among dog park groups ($P = .049$). The mean $\pm$ SD ages of dogs attending each of the 3 dog parks were 3.5 ± 3.2 years, 3.5 ± 2.8 years, and 4.5 ± 3.5 years, respectively. The majority of dogs were mixed breed (54%). Forty-nine purebred dog breeds were represented, including Labradors (13/300 [4.3%]), Chihuahuas (10/300 [3.3%]), Corgis (7/300 [2.3%]), Boxers (6/300 [2.0%]), Miniature Australian Shepherds (6/300 [2.0%]), and Pit Bull terriers (6/300 [2.0%]), and there were no significant differences in dog breed distributions among the 3 dog parks. All other breeds present had a frequency of 5 or fewer dogs. There were 172 males (57%) and 128 females (43%), and the sex distribution did not differ significantly ($P = .056$) among parks.

Seventy-three of the 266 (27%) scored investigator-fecal specimens were deemed to be diarrheic (fecal score ≥ 4) on the day of sample collection. One or more enteropathogens were detected in 114 of the 300 dogs (38%), and the prevalence of enteropathogens did not differ significantly among dog parks. There was a significant positive association between the presence and absence of 1 or more enteropathogens and increasing fecal score ($P = .0039$), and between the number of enteropathogens detected and fecal score ($\rho_s = 0.17$, $P = .0050$). Dogs previously diagnosed with intestinal parasites were

significantly more likely to have a higher fecal score ($P = .038$). Age was negatively correlated with the number of different enteropathogens ($\rho_s = -0.16$, $P = .0049$) and was also negatively correlated with fecal score ($\rho_s = -0.11$, $P = .066$). Risk factors evaluated including access to water in outdoor locations other than the dog park ($P = .25$), the presence of other household pets having diarrhea ($P = .93$), contact with dogs outside the household, ($P = .58$), attendance of the dog at day-care facilities within the previous 6 weeks ($P = .29$), and frequency of dog park attendance ($P = .099$) were not significantly associated with fecal score or the presence of enteropathogens.

Twenty-six of 300 dogs (8.7%) attended the dog park at least once daily, 51 dogs (17%) attended the dog park 5–7 times weekly, 64 dogs (21%) attended the dog park 3–4 times weekly, 85 dogs (28%) attended the dog park 1–2 times weekly, and 50 dogs (17%) attended the dog park 1–2 times monthly. The frequency with which a dog attended a dog park was not significantly associated with detecting ≥ 1 enteropathogens ($P = .099$); however, there was a significant positive correlation between the frequency of dog park visits and increased fecal score ($\rho_s = 0.12$, $P = .047$).

Parasitic Enteropathogens

Four of 300 dogs (1.3%) tested positive for *Trichuris vulpis* ova on fecal flotation at the commercial reference laboratory, and 3 of the 4 dogs tested positive at the university laboratory. Two of these 4 dogs had diarrhea on the day of collection with no additional enteropathogens detected. One of 300 dogs (0.33%) tested positive for *Toxocara canis* ova at the university laboratory only, and that dog also had diarrhea on the day of collection with no additional enteropathogens detected. Two of 300 dogs (0.67%) tested positive for *Ancylostoma caninum* ova on fecal flotation at the commercial reference laboratory, and 1 of the 2 dogs tested positive at the university laboratory. None of the hookworm-infected dogs had diarrhea on the day of fecal collection. *Cystoisospora* oocysts were detected on fecal flotation in 8 dogs overall (2.7%), 5 of which were detected at the commercial reference laboratory, and 3 of which were detected at the university laboratory (only 1 of the 8 dogs had *Cystoisospora* oocysts detected at both laboratories). Three of the *Cystoisospora*-infected dogs had diarrhea on the day of collection. One of the diarrheic dogs infected with *Cystoisospora* oocysts was co-infected with circovirus, *Cryptosporidium* spp., and *Giardia* spp. The frequency of detection of hookworm ova, *Cystoisospora* oocysts, roundworm ova, or whipworm ova via fecal flotation was not significantly different between the university and commercial reference laboratories.

Twenty-seven of 300 dogs (9%) tested positive on at least 1 or more of the 4 *Giardia* tests: fecal flotation or DFA at the university laboratory and fecal flotation or PCR at the commercial reference laboratory (Table S1). The presence of *Giardia* was not significantly different among dog parks ($P = .20$), although it was associated

with a significantly younger age ($P = .0007$). Twelve of the 27 *Giardia*-infected dogs (44%) had diarrhea on the day of sample collection and a significant positive association between *Giardia* detection and increasing fecal score was found ($P = .0049$). Thirteen of the 27 *Giardia*-infected dogs (48%) were co-infected with up to 3 additional enteropathogens, and *Cryptosporidium* spp. was found in 31% of these co-infected dogs. There was no association between the presence of *Giardia* spp. and the presence of other enteropathogens ($P = .087$). Seventy-nine owners reported that their dogs had been dewormed within the past 6 months, and 217 owners reported that their dog had not been dewormed within this period. Four owners failed to report their dog's deworming status on the questionnaire. Dogs that had been dewormed within the past 6 months were significantly more likely to have an intestinal parasite(s) detected ($P = .039$) compared to dogs that had not been dewormed. Intestinal parasites were detected in 52 dogs, of which 20 had been dewormed with the past 6 months and 32 had not. Intestinal parasites detected in the 20 dogs that had been dewormed included *Giardia* spp. ($n = 11$ dogs); *Cryptosporidium* spp. ($n = 6$ dogs); *Trichuris vulpis* ($n = 3$ dogs); *Ancylostoma caninum* ($n = 1$ dog); and *Cystoisospora* spp. ($n = 1$ dog). One of the dogs was co-infected with *Giardia* spp. and *Cryptosporidium* spp., and 1 other dog was co-infected with *Cryptosporidium* spp. and *Cystoisospora* spp.

The frequency of detection of *Giardia* spp. via fecal flotation was significantly different between the university and commercial reference laboratories ($P = .013$). The university laboratory detected 13 additional positive samples that the commercial reference laboratory did not detect, whereas the commercial reference laboratory detected 3 positive samples that the university laboratory did not detect (Table S1). In addition, 11/27 dogs (41%) that had *Giardia* cysts detected via fecal flotation, DFA, or both were negative on PCR. Five of the 11 dogs (46%) that were infected with *Giardia* and that were negative on PCR were positive on both flotation and DFA. Significant differences were also found when DFA results were compared to the commercial laboratory fecal flotation ($P = .0016$) and to PCR results ($P = .014$).

Sixteen of the 300 dogs (5.3%) were positive for *Cryptosporidium* spp. on DFA or PCR, and 14 of these dogs were positive by PCR detection alone. Three of the 16 dogs did not have a DFA performed, and all dogs positive for *Cryptosporidium* spp. on PCR were negative on DFA. The 2 remaining *Cryptosporidium*-positive dogs were positive on DFA, but negative via PCR. The prevalence of *Cryptosporidium* spp. did not differ significantly among the 3 parks ($P = .13$), and the presence of this protozoan was not significantly associated with age ($P = .37$) or fecal score ($P = .77$).

Genotyping of *Giardia* Isolates

The overall amplification rate was low and was similar in the 3 loci (gdh, bg, and tpi, respectively). Only 7/27 isolates had sufficient DNA for amplification, and all

isolates harbored the dog-adapted assemblages C, D, or both. Accession numbers for the nucleotide sequences can be found in GenBank (KX164005-KX64017).

Bacterial Enteropathogens

Eight of 300 dogs (2.7%) tested positive for either *Campylobacter jejuni* or *C. coli* via fecal PCR testing. Four dogs were positive for *C. jejuni*, 3 dogs were positive for *C. coli*, and 1 dog was positive for both *C. jejuni* and *C. coli*. Four of these 8 dogs (50%) were co-infected with 1 to 4 of the following enteropathogens: *C. difficile*, *C. perfringens*, coronavirus, circovirus, and *Cryptosporidium* spp; however, only 1 of the 8 dogs infected with *Campylobacter* spp. had diarrhea on the day of sample collection. None of the 8 dogs were fed a raw diet. Ten dogs were positive for *C. difficile* *TcdA* and *TcdB* genes via PCR. One of the 10 dogs was positive for *C. difficile* *TcdA* gene alone, 1 dog was positive for *TcdB* gene alone, and 8 dogs (2.7%) were positive for both *TcdA* and *TcdB* genes. Detection of *C. difficile* *TcdA* and *TcdB* genes was not associated with fecal score. *Clostridium perfringens* alpha toxin gene or *cpe* was detected in 104 of 300 dogs (35%). Thirty-three of the 104 dogs (32%) had diarrhea on the day of fecal collection, and 12 of these 33 dogs were co-infected with additional enteropathogen(s) (Table S2). *Clostridium perfringens* alpha toxin gene was detected above a threshold of 300,000 gene copies/gram via fecal PCR in 25 of the 300 dogs (8.3%), of which 12 dogs (48%) had diarrhea. *Clostridium perfringens* alpha toxin gene was negative or was detected below threshold in 239 dogs, of which 61 dogs had diarrhea (26%). This difference was significant ($P = .032$). *Clostridium perfringens* *cpe* was detected above threshold in 8 of 264 dogs (3.0%), of which 5 dogs (63%) had diarrhea. Of the 256 dogs in which *C. perfringens* *cpe* was negative or was detected below threshold, 68 dogs (27%) had diarrhea. These differences were significant ($P = .039$). There was a weak correlation found between the quantity of *C. perfringens* alpha toxin gene and fecal score ($\rho_s = 0.13$, $P = .037$) and quantity of *cpe* and fecal score ($\rho_s = 0.12$, $P = .057$). Three of 300 dogs (1%) tested positive on PCR for *Salmonella*. None of these 3 dogs had diarrhea on the day of fecal collection, and none were fed a raw diet. One of the dogs infected with *Salmonella* was co-infected with *Giardia* spp. and *C. perfringens*.

Viral Enteropathogens

Twenty-seven of 300 dogs (9%) were positive for circovirus via PCR, although the presence of this virus was not associated with fecal score ($P = .15$). Fourteen of 300 dogs (4.7%) tested positive for coronavirus via PCR, and 2 of these dogs had diarrhea on the day of collection; however, both of the diarrheic dogs were co-infected with *Giardia*. Seven of these 14 dogs (50%) were co-infected with 1 to 4 other enteropathogens, and 4 of these dogs were co-infected with *Giardia* (Table S2). Three of 300 dogs (1%) tested positive for

parvovirus via PCR. One of these dogs had diarrhea on the day of fecal collection, another was co-infected with *Giardia* spp., and the last dog had a history of diarrhea of 1-day duration within 30 days before fecal collection. Owners of dogs that were PCR positive for parvovirus reported no other abnormal gastrointestinal signs in their dogs. No dogs tested positive for distemper virus via PCR.

Discussion

This study represents the largest completed to date evaluating the prevalence of bacterial, viral, and parasitic enteropathogens in apparently healthy dogs attending dog parks. A similar study investigated the prevalence of intestinal parasites in 129 dogs that attended or did not attend a dog park in Fort Collins, CO.⁹ The overall prevalence of intestinal parasites in that study was 7.0%, and *Giardia* was detected in 3.8% of dogs, compared to 18 and 9.0%, respectively, in this study. The higher prevalence of *Giardia* in our study and other studies^{10,18} might reflect regional differences in the prevalence of *Giardia* spp., differences in the susceptibility of the animals, or differences in testing methods. Veterinary students or staff members at the veterinary university hospital who owned dogs with a lower prevalence of *Giardia* might have been more likely to obtain medical therapies and manage gastrointestinal signs in their dogs more proactively compared to dog owners frequenting dog parks.⁹ Infection with *Giardia* or *Cryptosporidium* was unassociated with diarrhea in 1 study,⁹ in contrast to our study which showed an association between *Giardia* infection and diarrhea. Differences in host factors or *Giardia* virulence factors could have accounted for this difference.

Fecal consistency was significantly associated with both the presence of enteropathogens and the number of enteropathogens detected, and younger dogs were significantly more likely to be infected with enteropathogens. Interestingly, most of the risk factors evaluated were not significantly associated with fecal score or the presence of 1 or more enteropathogens, including the presence of other household pets having diarrhea and the frequency of dog park attendance. Dogs with looser feces were more likely to be infected with *Giardia* spp., and the presence of *C. perfringens* alpha toxin gene and *cpe* above a threshold level of 300,000 gene copies/gram feces was weakly correlated with diarrhea, whereas dogs infected with *C. difficile* did not have altered fecal consistency. Infection with *C. difficile* and *C. perfringens* has been inconsistently associated with diarrhea in dogs;¹⁹ however, both species have been associated with an acute hemorrhagic diarrheal syndrome in dogs.^{20,21} Studies are warranted in healthy dogs to determine the prevalence of recently identified *C. perfringens* spore-forming toxins (netE and netF) associated with hemorrhagic enteritis in dogs.²²

Canine circovirus (DogCV) has been implicated as an emerging pathogen of concern in dogs, and the role of this virus in causing diarrhea in dogs is currently being investigated. Circovirus has been associated with

vasculitis and hemorrhagic gastroenteritis on necropsy; however, coinfection with additional enteropathogens in 68% of diarrheic dogs in 1 study complicates the diagnosis.²³ The prevalence of DogCV detected in this study (9%) was similar to that found in Li et al.'s study (11% and 6.9% in diarrheic and nondiarrheic dogs, respectively). The amplification of DogCV DNA from normal dogs and the lack of association with fecal score suggest that this virus might be nonpathogenic in many dogs.

The prevalence of *Salmonella* reported in this study (1%) was within the previously reported range of 0–2.3%^{20,24} and did not correlate with fecal consistency. In addition, none of the dogs infected with *Salmonella* spp. ingested raw meat diets. The prevalence of *C. jejuni* and *C. coli* in this study (8/300, 2.7%) was slightly higher than previously reported by culture methods.²⁵ A previous study documented a prevalence for *Campylobacter* spp. of 43% via fecal culture in 251 dogs attending dog parks in southwestern Ontario.²⁶ The investigators included detection of *C. upsaliensis* in that study, a nonpathogenic species found in 37% of the dogs.²⁶ Importantly, no association was found between the presence of any bacterial enteropathogen and fecal score with the exception of *C. perfringens*, although potentially zoonotic bacterial enteropathogens detected included *Campylobacter jejuni* and *Salmonella*. *Campylobacter upsaliensis* and *C. helveticus* have been frequently isolated from healthy and diarrheic dogs and cats,^{27,28} however, these relatively nonpathogenic species were not tested for.

The paradoxical results of intestinal parasites being detected significantly more frequently in dogs that had been dewormed compared to dogs that had not been dewormed within the past 6 months were likely a reflection of the type of intestinal parasites most commonly detected. Only 1 of the infected dogs that was dewormed was diagnosed with *Ancylostoma caninum*, whereas all of the other dogs that were dewormed were infected with parasites (*Giardia* spp., *Cryptosporidium* spp., *Trichuris vulpis*, and *Cystoisospora* spp.) that are not susceptible to commonly administered deworming medications such as pyrantel pamoate. The differences in detection of *Giardia* cysts on fecal flotation between the commercial reference and university laboratories could have been associated with known differences in methods for fecal flotation between the 2 laboratories. The commercial laboratory performed a single centrifugation flotation, and technicians scanned the slide at 10× magnification for approximately 60–120 seconds. In contrast, a single parasitologist with over 30 years experience performed all of the flotations at the university laboratory utilizing a double centrifugation flotation technique with evaluation of approximately 50 random high-power fields to determine the average number of cysts, oocysts, or ova per hpf. Interestingly, there were no significant differences between the 2 laboratories in regard to any other parasites detected on fecal flotation. This might be because nematode ova are more readily recognized on a slide because of their size compared to *Giardia* cysts. These results emphasize that if veterinarians recognize discordant results from

different laboratories by similar assays, the laboratories supplying the assays should be alerted to allow for internal investigation into quality assurance.

The relatively high number of dogs with false-negative *Giardia* PCR results at the commercial reference laboratory is concerning and could have occurred for several reasons. The presence of fecal inhibitors is plausible but was deemed unlikely because a positive internal amplification control was used in all samples and confirmed that no significant inhibitory activity remained in the nucleic acid. The internal sample quality control assessed by targeting a housekeeping gene also indicated good nucleic acid quality at both the gDNA and cDNA level. In addition, 2-fold dilutions of a select number of samples resulted in 1 Ct value weaker signals, confirming the absence of PCR inhibition. The high number of false-negative *Giardia* PCR results could have been a consequence of PCR test specificity. The *Giardia* PCR is highly specific for *Giardia duodenalis* based on the primer sets and does not pick up any other *Giardia* species, whereas fecal flotation or antibody-based *Giardia* detection tests are less discriminatory and more likely to detect nonclinically relevant species. In a dog park with a high density of dogs and their feces, combined with a high rodent and bird population, it is conceivable that nonclinical strains of *Giardia* are accumulated and occur in a high frequency of dogs that accidentally ingest these organisms, in the absence of overt infection. The high number of failed *Giardia* assemblage PCR tests, which is also *Giardia duodenalis* specific, could support the specificity aspect. This phenomenon would have to be tested specifically by characterizing *Giardia* strains by sequencing, which was not an objective of this study.

The reasons for the low amplification rates of DNA for *Giardia* genotyping were multifactorial and might have been associated with the lack of freshly extracted DNA, the method of DNA extraction, or the presence of *Giardia* species from birds and rodents that were not *Giardia duodenalis*. It is also plausible that the amount of *Giardia* DNA and the gene abundance (single and multicopy genes) was below the assay's detection limit. The results of *Giardia* genotyping testing of the isolates in this study were in agreement with most previous studies showing assemblage C or D represents the most common assemblages in the dog.^{15,29}

Different amplification rates of the loci tested in the study have also been reported in similar multilocus genotyping studies in dogs.^{15,30,31} Humans are primarily infected with assemblages A and B, and these have also been infrequently isolated from dogs, thus posing a potential zoonotic risk.⁵

Although fecal consistency correlated with both the presence and number of enteropathogens detected, 62/114 dogs (54%) were nondiarrheic. Thus, positive results obtained for any of the enteropathogens do not prove disease causation. In addition, the discordant findings between the university and the commercial reference laboratories in detection of *Giardia* cysts and *Cystoisospora* oocysts via fecal flotation warrant further scrutiny of the methods employed at both

laboratories so the diagnostic yield can be increased. The discrepancy in the detection of *Cryptosporidium* via PCR versus DFA is also concerning because the DFA-positive specimens should have also been PCR positive. These results could be explained by the presence of fecal PCR inhibitors or the incomplete extraction of DNA from oocysts. In addition, all PCR-positive *Cryptosporidium* cases were DFA negative, which raises questions about the utility of DFA and PCR for diagnosing *Cryptosporidium* in dogs. The detection reagent in the commercial *Cryptosporidium* DFA kit utilizes a fluorescein isothiocyanate-labeled monoclonal antibody directed against cell wall antigen of *C. parvum*, and it is plausible that false-negative results could have been obtained if dogs were infected with *C. canis*. Lastly, although companion animals may pose a potential risk for zoonotic infections, the frequency of attending dog parks in this study did not significantly increase the risks of infection with an enteropathogen(s).

There were several limitations to this study, including the lack of *Giardia* ELISA testing that could have helped validate the PCR and fecal flotation results for *Giardia*.⁸ In addition, confirmation testing for PCR assay results was only performed for the dogs that had *Giardia* assemblages determined by genetic sequencing. The direct fluorescent antibody test is the gold standard for diagnosis of *Giardia* in humans;³² however, the test was not performed on all 300 fecal specimens due to its relatively high cost. Detection of *C. perfringens* CPE via ELISA would have also helped improve the diagnosis of *C. perfringens* and would have been helpful to determine the association between CPE detection and the presence of *cpe* above threshold. Future studies should include testing for *C. perfringens* spore-forming toxins (netE and netF) that were unavailable at the time of the study.

In conclusion, dogs diagnosed with ≥ 1 enteropathogens were significantly more likely to have an increased fecal score compared to noninfected dogs; however, most infections were not associated with any specific dog characteristics or risk factors apart from young age. The lack of specific risk factors is similar to the findings of a study that determined the prevalence of enteropathogens in 100 dogs (50 dogs with normal feces and 50 dogs with diarrhea) at an open-admission municipal animal shelter in Florida.³³ Zoonotic enteropathogens were detected in 29 dogs (9.7%) in this study, and most of these dogs had normal feces underscoring the challenges of predicting the risk of infection and zoonotic transmission for individual animals. Pet owners who frequent dog parks should be educated about the potential risks of zoonotic transmission of enteropathogens from dogs, and the fact that a dog with normal feces can pose a risk of zoonotic transmission. Pet owners should also be advised to avoid taking their dog to a dog park if it has diarrhea. This study also highlights important discrepancies in the diagnosis of *Giardia* via fecal centrifugation flotation between a commercial laboratory and university parasitology laboratory, underscoring the potential for underdiagnosing

this enteropathogen at some individual laboratories. The methodology for fecal flotation and improved technician training should be undertaken in an effort to increase the performance characteristics of this test. In addition, the utility of fecal PCR testing for *Giardia* warrants further investigation to determine the reason(s) for the discrepant results with fecal flotation and DFA testing.

Footnotes

^a StataCorp LP, College Station, TX

^b Dog Park Study Questionnaire—Supporting information available online

^c IDEXX Reference Laboratory, Sacramento, CA

^d MERIFLUOR® *Cryptosporidium*/*Giardia*, Meridian Bioscience, Inc., Cincinnati, OH

^e Roche Applied Science, Indianapolis, IN

^f Applied Biosystems, Foster City, CA

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Comparison of results for fecal flotation performed at a commercial reference laboratory versus veterinary university parasitology laboratory in 27 dogs that tested positive for *Giardia* spp. via fecal flotation, fecal PCR, or direct fluorescent antibody testing.

Table S2. Parasitic, Bacterial, and Viral Enteropathogens Detected in 114 of 300 dogs Frequenting 3 Regional Dog Parks in Northern California.

Data S1. Dog Park Study Questionnaire.

Research Article

Urban Dog Parks as Sources of Canine Parasites: Contamination Rates and Pet Owner Behaviours in Lisbon, Portugal

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Dog parks represent a recent trend in western countries, enabling owners to spend quality time with their pets in a controlled environment. Despite their growing popularity, few studies have been performed to date on these parks to investigate dog intestinal parasitic infections and soil contamination. The present study examined 369 faecal and 18 soil samples collected from 3 dog parks in Greater Lisbon, Portugal. Additionally, 102 interviews were performed with dog owners to assess dog-walking behaviours and parasite risk. In total, 33% of the faecal dog samples were infected with at least one parasitic agent: hookworms (16.5%), *Cryptosporidium* spp. (11.9%), *Giardia* spp. (11.4%), *Toxascaris leonina* (1.1%), *Cystoisospora* spp. (1.1%), *Toxocara* spp. (0.5%), and *Sarcocystis* sp. (0.3%). The soil of all the parks was contaminated with hookworm eggs. This is the first study performed in a European urban area to assess canine faecal contamination and parasitic agents in dog parks. Our results highlight the potential of these parks as a source of transmission for canine parasites, including some with zoonotic potential. Public awareness and effective preventive measures should be promoted to minimise the health-risk impact to both animals and humans, under the scope of environmental and public health.

1. Introduction

In modern-day society, the human-animal bond has become stronger with pets playing an important role as a source of companionship, emotional support, and recreation. Dogs encourage easier social interactions between people and promote the physical and psychological health of their owners [1, 2]. Despite the positive effects that pets can have on people's lives, this close bond may also compromise human health due to allergic reactions, trauma, and infectious diseases [2]. Dogs have been implicated in the transmission of more than 60 zoonotic infectious diseases [1], some of which are due to canine intestinal parasitic infections and are of serious concern. For instance, *Toxocara* spp. are responsible for visceral larva migrans (VLM), ocular larva migrans (OLM), covert toxocariasis, and neurological and atopic signs [3]. Some

hookworms can cause cutaneous larva migrans (CLM) and eosinophilic enteritis [4]. Assemblages A and B in the protozoan genus *Giardia* are considered to have a zoonotic potential [5], and the risk of human infection by *Cryptosporidium* sp. from dogs, though limited, has not been excluded [6]. Until now, children, pregnant women, elderly, and immunocompromised people are all at a higher risk of disease resulting from parasitic zoonoses [7].

Dog parks (i.e., enclosed areas for domesticated dogs to play off-leash and socialise with other canines in a controlled environment) enable owners to spend quality time with their dogs. These areas promote social interactions among dogs and offer a safe setting for regular exercise in a controlled environment under the supervision of their owners [8]. Over the last decades, dog parks became very popular in urban areas and are one of the fastest growing segments of city parks.

However, these parks may pose an increased risk for the transmission of parasitic zoonotic agents, via faecal and soil contact, among dogs, humans, and wildlife [8, 9]. Despite this, few studies investigating soil contamination and intestinal parasitic infections in dog parks have been carried out thus far, including one in Colorado, USA [10], and a second in Calgary, Alberta, Canada [8]. No studies have been performed in urban dog parks in Europe to date.

In order to assess faecal environmental contamination and intestinal parasites in frequenting purpose-built dog parks, faecal and soil samples were collected from such parks located in urban areas of Lisbon, Portugal. Interviews were conducted to assess owners' behaviour, veterinary care, and the owner-pet relationship.

2. Materials and Methods

2.1. Sampling Area. Three purpose-built dog parks located in Greater Lisbon were chosen, in order to represent this type of facility located in densely populated areas distributed throughout the city: (A) Algés, a 350 km² parish with 48,665 inhabitants and 1,693 licensed dogs; (B) Benfica, a 465 km² parish with 36,821 inhabitants and approximately 400 licensed dogs; and (C) Campo Grande, a 1,120 km² parish with 31,813 inhabitants and about 1,700 licensed dogs (Figure 1). The dog parks were fenced with a double-gated entry and had shades, drainage, water sources, and covered garbage cans. All parks were regularly maintained, including ground cleaning. This study was conducted from October to December 2015. For a better representation of the visiting population, fresh faecal samples randomly distributed throughout the parks were collected every 15 days, at different periods of the day (morning, midday, and afternoon). Once a month over the same period, soil samples were collected from five distinct spots with a gardening spade and subsequently pooled (approximately 250 grams). Samples were obtained from a depth of between 0 and 5 cm of grass (3 samples/park) and gravel (3 samples/park). Although cleaning and disinfection activities are implemented on a regular basis, the authors were unaware of any of them performed on the surveyed parks immediately prior to sampling dates.

Over the same period, 102 owners walking their dogs in the three dog parks were interviewed.

2.2. Coprological Analysis

2.2.1. Parasite Egg Isolation and Identification. A Centrifugal Sedimentation Flotation (CSF) technique was used [11]. Briefly, 3–5 g of each faecal sample was homogenised in 55 ml of distilled water and sieved through a tea strainer into a tube. Tubes were centrifuged (3 minutes at 3000 rpm) and the supernatant was discarded. A third of the tube was filled with sucrose solution (specific density: 1.3 g ml⁻¹), vortexed, filled again with sucrose solution, and centrifuged (3 minutes at 3000 rpm). Tubes were then filled with sucrose solution until a convex meniscus was formed and a coverslip was then placed immediately on the top. After 25 minutes, the coverslip was placed on a slide for observation using an optical microscope at 100x–400x magnification. Eggs, oocysts,

and cysts were identified morphologically according to published guides [12–14]. Names of the parasites of genus *Cystoisospora* followed a recent taxonomic revision [15].

2.2.2. Faecal Smears. A faecal smear, stained by the modified Ziehl-Neelsen technique [16], was performed on each sample. Briefly, a small amount of faeces was spread over a slide to form a thin layer, using a stirring rod. After drying, smears were fixated with methanol for 1 minute, covered with fuchsin for 10 minutes, and washed under running water. They were subsequently washed with 1% hydrochloric alcohol to remove excess fuchsin and washed again with running water. Slides were then covered with 0.4% malachite green for 30 seconds, washed again with running water, and finally left to air-dry. Smears were observed at 1000x magnification for the detection of *Giardia* sp. cysts, as transparent oval bodies with 4 nuclei, and *Cryptosporidium* sp. oocysts, as round oocysts frequently containing typical crescent shaped sporozoites stained with a pink-reddish colour. A minimum of 50 fields were observed per slide. Genotyping of *Giardia* spp. and *Cryptosporidium* spp. isolates was not possible to perform.

2.3. Soil Analysis. Soil samples were analysed using a modified Sieving and Centrifugal Sedimentation Flotation (CSF) technique [17, 18]. For each sample, one hundred grams of soil was weighed, mixed with 100 mL of 5% Tween-20 solution, and then homogenised for 10 minutes and allowed to stand overnight. The contents were then sieved (diameters 1,000 mm, 0,500 mm, 0,250 mm, 0,150 mm, 0,063 mm, and 0,020 mm) and washed under running water for 30 minutes. The sediment present in the 0,063 mm and 0,020 mm sieves was resuspended in distilled water and allowed to stand overnight. The sediment was then resuspended with distilled water and the tubes were centrifuged for 3 minutes at 2000 ×g. The supernatant was discarded and the parasite eggs were collected from sediment and identified as mentioned above.

2.4. Interviews. Multiple-choice questionnaires were completed as oral face-to-face interviews conducted with 102 dog owners walking their dogs in each one of the three dog parks. Overall, 34 questionnaires were performed per park. Owners were asked several questions intended to assess dog-walking behaviours, including park visitation frequency, animal healthcare, and dog-owner habits.

2.5. Statistical Analysis. Statistical analysis was performed using R, version 3.1.3, and the extension R Commander (the R Foundation for Statistical Computing, 2013). Chi-square or Fisher's exact tests were used to compare proportions and a probability *p* value < 0.05 was considered as statistically significant. Exact binomial 95% confidence intervals (CI) were established for proportions.

3. Results

3.1. Faecal Samples. In total, 369 faecal samples were collected: 125 from Algés (A), 124 from Benfica (B), and 120 from Campo Grande (C). A total of 18 soil samples were also collected, 6 from each park (3 from grass and 3 from gravel surfaces). The overall prevalence of various parasites in the



FIGURE 1: Map highlighting the three dog parks assessed in Greater Lisbon, Portugal.

TABLE 1: Prevalence of the parasites detected in faecal samples collected in three dog parks (A, B, and C) of Greater Lisbon, Portugal.

	A (n = 125) (95% CI)	B (n = 124) (95% CI)	C (n = 120) (95% CI)	Total (n = 369) (95% CI)
Hookworms	14.4% (9.0–22.1)	18.5% (12.4–26.7)	16.7% (10.7–24.8)	16.5% (13.0–20.8)
<i>Cryptosporidium</i> spp.	12.0% (7.1–19.3)	15.3% (9.7–23.2)	8.3% (4.3–15.2)	11.9% (8.9–15.8)
<i>Giardia</i> spp.	16.0% (10.3–23.9)	6.5% (3.0–12.7)	11.7% (6.8–19.1)	11.4% (8.4–15.2)
<i>Cystoisospora</i> spp.	0.8% (0.0–5.0)	1.6% (0.3–6.3)	0.8% (0.0–5.2)	1.1% (0.4–2.9)
<i>Toxascaris leonina</i>	0	0	3.3% (1.1–8.8)	1.1% (0.4–2.9)
<i>Toxocara</i> spp.	0.8% (0.0–5.0)	0.8% (0.0–5.1)	0	0.5% (0.1–2.2)
<i>Sarcocystis</i> sp.	0.8% (0.0–5.0)	0	0	0.3% (0.0–1.7)
Total of positive samples	35.2% (27.0–44.3)	31.5% (23.6–40.5)	32.5% (24.4–41.7)	33.1% (28.3–38.2)

faecal samples from the three different parks is presented in Table 1.

Hookworms were the most prevalent group of parasites detected. *Cryptosporidium* spp., *Giardia* spp., and *Cystoisospora* spp. were also identified in all three parks whereas *Toxocara* spp. were detected in only two parks and *Toxascaris leonina* and *Sarcocystis* sp. were detected in only one. Dog park A showed a greater biodiversity in its parasitic fauna with 6 of the 7 parasite groups diagnosed, whereas dog parks B and C had only 5 different types of parasites (Figure 2). No significant statistical differences were detected between parks ($p = 0.81$).

3.2. Soil Samples. In total, 18 soil samples were collected, 6 per each park (3 from grass and 3 from gravel). Five of the 9 samples (55.6%) from grassed areas contained hookworm

eggs (Ancylostomatidae) whereas 0/9 samples from gravel areas had hookworm eggs, showing a significant statistical difference between grassed and gravel areas ($p = 0.03$). Overall, 27.8% (5/18) of the soil samples (all from grassed areas) were contaminated with hookworm eggs, in the three assessed parks: 33.3% (CI 6.0–75.9%) from A, 16.7% (CI 0.9–63.5%) from B, and 33.3% (CI 6.0–75.9%) from C. Eggs were only found in grassed areas. Regarding soil samples, no significant statistical differences were detected between the parks ($p = 1$).

3.3. Interviews. It was found that 40.2% of the dogs present in the parks live in a home/dwelling with at least one other animal (most with other dogs and cats and a minority with birds, rabbits, or guinea pigs). Regarding daily walking, 82.3% were walked both on the streets and in parks, 16.7% only in

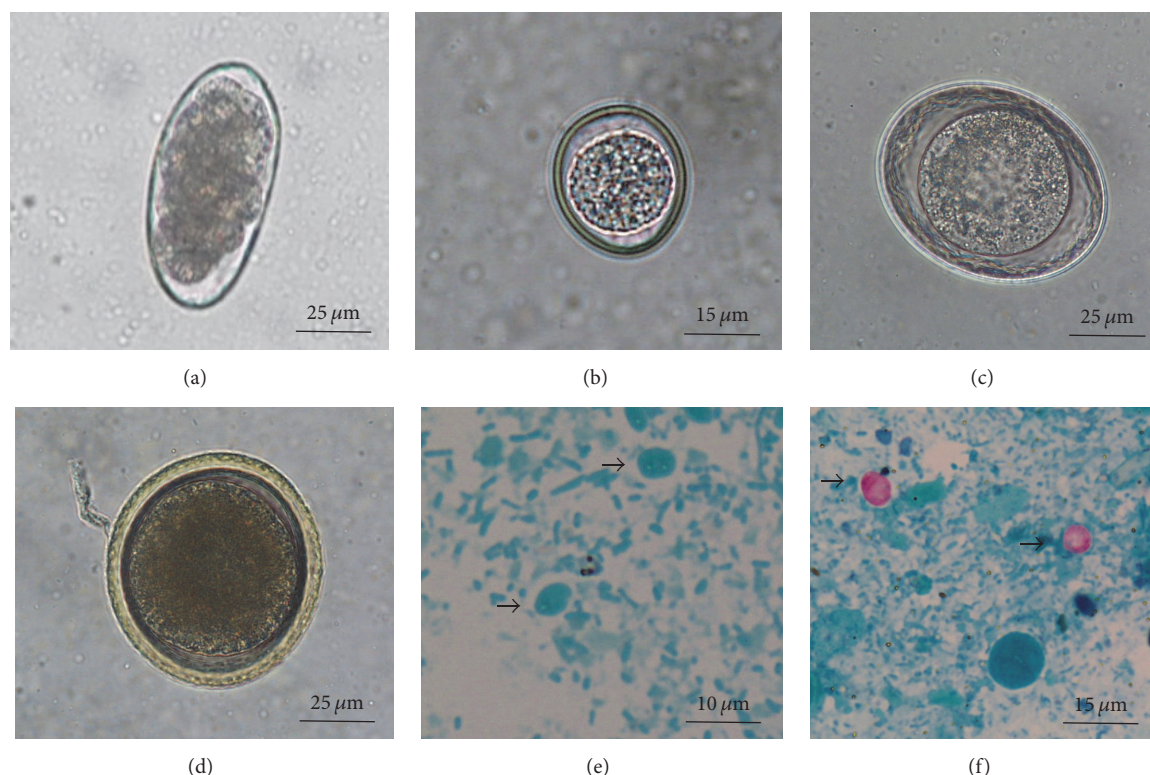


FIGURE 2: Some of the eggs, cysts, and oocysts detected in fresh faecal samples collected from dog parks using Centrifugal Sedimentation Flotation technique and faecal smears stained by the modified Ziehl-Neelsen technique. (a) Morulated hookworm egg; (b) *Cystoisospora* spp. unsporulated oocyst; (c) nonembryonated *Toxascaris leonina* egg; (d) nonembryonated *Toxocara canis* egg; (e) *Giardia* spp. cysts in faecal smear (arrows); (f) *Cryptosporidium* spp. oocysts in faecal smear (arrows).

parks, and 1.0% in parks and open field. In addition, 41.2% of the respondents mentioned visiting with their dogs more than one of the dog parks located in Lisbon.

Of the total respondents, 50.0% visited the park daily, 29.4% at least once a week, and 20.6% less than 1–3 times a month. Most dog owners walked their dogs off-leash (57.8%), 17.6% on-leash, and 24.5% both (82.3% with off-leash activity, overall). Almost all owners, 94.1%, claimed to collect their dog's faeces.

Regarding animal healthcare, 93.1% of dog owners answered to have taken their dog to a veterinarian consultation in the previous 12 months. Concerning anthelmintic treatments, 89.9% of the owners stated to have internally dewormed their dogs in the previous six months. However, when asked regarding its specific frequency, 14.5% answered three times a year, 41.0% twice a year, 13.3% once a year, and only 27.7% at least four times a year.

The most commonly used anthelmintic drug (72.7% of respondents) was the combination of praziquantel-pyrantel embonate with a third molecule (febantel, oxfantel, or fenbendazole).

In 82.4% of the households, the dog was allowed to visit the owners' bedroom, 75.5% were allowed to lick their owners' faces, and 43.1% were allowed to sleep with the owners in their beds.

4. Discussion

This is the first study performed to assess canine faecal contamination and parasitic agents in urban dog parks and dogs frequenting such parks in Europe. The three parks had similar rates of contamination, with one-third of dog faecal samples positive for at least one parasite. Hookworms were the parasite group detected with the highest prevalence (16.5%) in all sampled parks. In Europe, there are two main species of hookworms: *Ancylostoma caninum* (the potentially zoonotic helminth responsible for cutaneous larva migrans) and *Uncinaria stenocephala* (nonzoonotic). As faecal culture and larvae examination were not performed, these two species were not differentiated and zoonotic potential could not be assessed. Although faecal samples were fresh, they were directly exposed to sunlight and warm temperatures for some hours until collection. Indeed, according to Anderson (2000) [19], the embryo formation of *A. caninum* eggs easily takes place and greatly varies with environmental temperature, ranging from 6–12 days at 12°C to 10–12 hours at 30°C. Similarly, *U. stenocephala* eggs can hatch within 12 hours at 20°C. Considering the high temperatures registered in Lisbon during the study sampling period, this fact explains why the authors found several embryonated eggs, despite working with fresh collected samples.

Concerning the percentage of hookworm eggs contaminating the herbage (55.6%), it mirrors the results found also by other authors concerning both domestic and wild canids. Hookworm infections had been commonly reported in household, hunting, kennel, and farm canids from north and south of Portugal, being the predominant helminth eggs detected [20, 21]. Although free-living nematodes may also be found when sampling herbage, they were distinguished by the characteristics of the adult stages (e.g., rhabditids) and by their eggs which are smaller and more transparent. Additionally, hookworm samples were found in parks where neither rabbits nor rodents were found, given the regular cleaning measures and rodent control program performed by municipal city services in these areas. In the present study, hookworm eggs were the only parasites found in soil samples, which suggests recent contamination, as these eggs do not generally persist for long periods in the environment [22]. Additionally, only grass samples were positive for parasite ova. The lack of ova in gravel samples is possibly explained by the large size of the gravel grains that do not retain parasitic elements, or by the fact that dogs prefer to defaecate on grass rather than on gravel. Furthermore, grass areas protect more the eggs from direct sun exposure in comparison with sandy areas, where eggs may have been destroyed by desiccation after direct sunlight exposure.

Protozoa were also found in all sampled parks, being more prevalent than nematodes. Indeed, a declining trend in the prevalence of intestinal helminths has been observed in certain countries over the last few decades, possibly explained by owner's increased awareness and consequent application of routine preventive anthelmintic treatments [7]. Such anthelmintics usually do not have label claims that include intestinal protozoa [7].

In the present study, oocysts of *Cryptosporidium* spp. were found in 11.9% of the faecal samples. This prevalence is in between the 4.8% of park-attending dogs from Colorado, United States of America [10], and the 14.7% detected in park-attending dogs from Calgary, Canada [8]. Lower prevalence was detected in other European countries, such as 0% in Belgium [23] and 2.6% in France [24]. *Cryptosporidium* infection in dogs is mainly caused by *Cryptosporidium canis* whereas in humans it is mainly due to *Cryptosporidium hominis*. In fact, the risk of humans acquiring the infection from dogs seems to be minimal, mostly limited to immunocompromised individuals, although zoonotic potential has not been conclusively ruled out by the scientific community [6].

Giardia spp. were found in 11.4% of the faecal samples, again an intermediate value between the 7.6% in park-attending dogs from Colorado [10] and the 24.7% from Calgary [8], performed with direct immunofluorescence assay, a more sensitive method of detection. *Giardia* spp. trophozoites and cysts were searched using CSF and Ziehl-Neelsen staining of faecal smears. Although the latter technique is not much referred for detection of *Giardia* spp., it was chosen in this study because of its common use for the diagnosis of *Cryptosporidium* spp., allowing the simultaneous detection of both agents. This is an easy and well-suited detection method for general practice [25]. Similar studies conducted in other

areas of Portugal using zinc sulphate showed prevalence of 7.4% in asymptomatic dogs and 15.5% in symptomatic dogs from Oporto [26], and 1.3% in household dogs and 61.2% in kennel dogs from Évora [21].

In fact, *Cryptosporidium* spp. and *Giardia* spp. are frequently associated with waterborne outbreaks. In a study conducted in Lisbon to assess the presence of *Cryptosporidium* and *Giardia* in raw and treated water by immunofluorescence (IFA) microscopy and PCR, *Cryptosporidium* spp. oocysts were found in 53.6% of untreated and in 41.5% of treated water samples, whereas *Giardia* spp. cysts were detected in 58.0% of untreated and in 25.6% of treated water samples [27].

Although there is only one species of *Toxocara* in the dog (*Toxocara canis*), as dogs may also shed eggs of *Toxocara cati* due to coprophagy and as morphological distinction between *T. canis* and *T. cati* eggs is very difficult with light microscopy, the authors only indicated the genus. The prevalence of dogs shedding *Toxocara* eggs in the present study was low, particularly when compared to the results found by Otero et al. [28] who detected 63.2% of soil and 15.8% of faecal samples positive for *Toxocara* spp. in urban public parks and children playground sandpits of Lisbon. A higher prevalence of dogs shedding *Toxocara* eggs was detected in a study performed in Oporto, Portugal, using zinc sulphate, where 5.1% of asymptomatic dogs and 7.8% of gastrointestinal symptomatic dogs admitted to a veterinary hospital tested positive [26]. Nevertheless, the prevalence detected in the present study might be underestimated as several parasites (in particular *Toxocara canis*) affect mainly puppies, which are not taken to public places or spaces of canine socialisation, because they have not yet been fully vaccinated. This might also be the justification for the low prevalence (1.1%) of samples positive for *Cystoisospora* spp., a protozoan that is mostly found in puppies, usually not taken to public places or dog parks. A higher prevalence of *Cystoisospora* spp. was found in Oporto (13.5%) in dogs presenting gastrointestinal signs [26]. Other studies performed in Europe reveal higher prevalence, such as central Italy (7.5%) [29] and Spain (10.2%) [30].

T. leonina was also found in 1.1% of the samples, a similar prevalence to the 0.5% detected in dogs with gastrointestinal signs from Oporto [26].

Sporocysts of *Sarcocystis* spp. were found in only one sample (0.3%), a very low prevalence, possibly explained by the indirect life cycle of this parasite. This parasite was also diagnosed in domestic canids in other researches carried out in Northern Portugal and its prevalence rates were also low [31].

Regarding *Trichuris vulpis*, heavy infections tend to be geographically localised or to occur mostly in kennels [32], which might explain the lack of positive results for this parasite in the present study.

The high prevalence of detected helminths generally covered by regular deworming products suggests that few dogs are internally dewormed with the recommended schedule (minimum quarterly) [32] despite the frequent contact with other animals. Indeed, the percentage of dewormed dogs in this study is in agreement with Matos et al. [33], who observed that although the majority of Portuguese pet owners give antiparasitic drugs to their pets, most of them do not follow

the manufacturer's recommendations and veterinary advice, deworming at irregular intervals.

According to Smith et al. [8], infection with enteric parasites is positively associated with off-leash activity, park visitation frequency, and visiting more than one park. In the present study, 82.3% had off-leash activity, 50.0% of the dogs visited dog parks daily, and 41.2% frequented other parks. Additionally, approximately 40% of the surveyed dogs shared the house with other animals and less than one-third were dewormed according to the recommended regimen. Although 94.1% of the owners stated that they collect their pet's faeces, 5 to 10 faecal samples were spotted by the authors in each dog park, every sampling date (Ana Ferreira, personal communication). In the study of Matos et al. [33], 63.3% of the Portuguese dog owners affirmed collecting their dogs' faeces in public areas, 95.6% whenever this occurs on a city path or pavement and 82.9% whenever this occurs in city parks. These results match the 94.1% of the owners who stated that they collect their pet's faeces in the present study. Nevertheless, it could be possible that the percentage found in our study may be overestimated, not reflecting owners' real behaviour, as this is a sensitive matter and data was not collected anonymously. Still, this measure should be encouraged, as it is an extremely important and easy way to reduce environmental contamination to safeguard public and animal health.

Despite the prevalence of the various parasites detected in these dog parks, the present results should be interpreted with caution, as some limitations should be pointed out. Multiple sampling of the same animal(s) cannot be excluded because the source of each faecal sample was not known. In addition, it was not possible to pair survey findings to faecal samples on an individual basis, which hampers the capacity to assess the risk factors of this population. Larvae examination after faecal culture, assessment of *Toxocara* spp. egg infection ability, and genotyping would have been particularly relevant to determine the hookworm species, the zoonotic potential of *Toxocara* eggs, and the genotypes of *Giardia* spp. and *Cryptosporidium* spp. isolates and, consequently, the potential zoonotic impact of these parasites. The sample size was small regarding the number of samples and geographic distribution, hindering an inference to the whole area of Lisbon. However, one-third of faecal samples with at least one parasite, using the above-mentioned techniques, must be considered a relevant finding for a supposedly well-controlled dog population regarding canine gastrointestinal parasitic diseases, according to the owner's answers. For this reason, further studies are needed involving larger samples and other geographic areas in Portugal, to better understand the potential of dog parks as a transmission source of parasitic diseases for animals and humans.

5. Conclusions

In conclusion, soil contamination with the potentially zoonotic hookworm eggs was present in all the parks assessed. In addition, one-third of dog faecal samples contained detectable parasites, including two nematodes with potential zoonotic impact (hookworms and *Toxocara* spp.) and two

potentially zoonotic genera of protozoa (*Cryptosporidium* spp. and *Giardia* spp.). Further studies are needed to assess if such risks are present in other dog parks, located in other cities in our country, and all over Europe. Despite being considered for many owners as a destination of excellence for their dogs, these results highlight the potential of dog parks as a source of transmission of several parasitic diseases, especially when considering the high level of human and canine movement in such confined areas. This is particularly likely when appropriate cleaning measures and effective deworming practices are lacking. Additionally, the close physical contact and some behavioural practices reported by several owners not only show a lack of knowledge regarding animal and public health issues but also can pose an increased risk for the transmission of zoonotic diseases. Public awareness and effective preventive measures should be promoted, to minimise the health-risk impact to both animals and humans, under the scope of environmental and public health.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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