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Original Article

Prevalence of enteropathogens in cats with and without diarrhea in four different management models for unowned cats in the southeast United States

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Abstract

The objective of this study was to determine the prevalence of enteropathogens in cats with and without diarrhea in four different models for managing unowned cats: short-term animal shelter, long-term sanctuary, home-based foster care, and trap-neuter-return. Fecal samples from 482 cats, approximately half of the cats with normal fecal consistency and half with diarrhea, were tested by zinc sulfate centrifugation and by real-time PCR for a panel of enteropathogens.

At least one enteropathogen of feline or zoonotic importance was detected in a majority of cats, regardless of management model. For most enteropathogens, the presence or absence of diarrhea was not significantly associated with infection, the exceptions being Trichomonas foetus in sanctuary cats with diarrhea (26%) and normal fecal consistency (10%), respectively (P < 0.04), and feline coronavirus in foster cats (80% and 58%) (P < 0.001). The types of enteropathogens detected were related to the type of management model, e.g., viral and protozoal infections were most common in shelters, sanctuaries, and foster homes (confuption systems), whereas helminth infections were most common in trap-neuter-return programs (free-roaming cats).

These results suggest that management practices for unowned cats are inadequate for control of enteropathogens and that the presence of diarrhea is a poor indicator of enteropathogen carriage. Risk-management strategies to reduce transmission to people and other animals should focus on sanitation, housing, compliance with preventive care guidelines, periodic surveillance, response to specific enteropathogens, humane population management of free-roaming community cats, public health education, and minimizing the duration and number of cats in mass confinement.

Introduction

Millions of relinquished and free-roaming cats are handled by animal welfare organizations each year in the United States. A variety of strategies are used for managing unowned cats, including housing cats individually or in small groups for a limited time (short-term shelters), bringing cats together in long-term free-roaming group enclosures (sanctuaries), using family-based homeless care (foster homes), or avoiding housing cats altogether (trap-neuter-return programs). Some systems bring homeless cats into close contact with people, including children and other individuals who may be uniquely susceptible to disease caused by enteropathogens. The majority of cats are ultimately placed back into the community, either via adoption or returning to their neighborhoods in trap-neuter-return programs.

Diarrhea is common in cats cared for by animal welfare organizations and can be present when cats are initially admitted or develop during confinement. Causes of diarrhea include factors such as stress, dietary change, microbiome dysbiosis (Suchodolski et al., 2015), and infectious enteropathogens. A variety of feline and zoonotic enteropathogens capable of inducing diarrhea have been documented in unowned cats (Hill et al., 2000; Spain et al., 2001; Mekaru et al., 2007; Gw et al., 2009; Weese, 2011; Sabshin et al., 2012; Spada et al., 2013; Raab et al., 2016). Factors that affect
transmission of enteropathogens include single vs. group housing, sanitation practices, facility construction, preventive health protocols, host immunity, and the prevalence of other infectious organisms (Pedersen et al., 2004). However, not all infections are associated with diarrhea, making it difficult to identify and segregate cats that may be shedding enteropathogens based on clinical signs alone (Hill et al., 2000; Vasilopoulos et al., 2006; Gow et al., 2009; Queen et al., 2012; Sabshin et al., 2012; Paris et al., 2014; Silva and Lobato, 2015).

Standardized guidelines exist for many aspects of feline shelter medicine, including vaccination (Holse et al., 2013; Scherk et al., 2013), retroviral management (Levy et al., 2008), spay/neuter surgery (Griffin et al., 2016), infectious disease control (Möstl et al., 2013), housing (Canadian Veterinary Medical Association, 2009), welfare (Newbury et al., 2010; Sparkes et al., 2013), and euthanasia (Leary et al., 2013). However, no such population-level guidelines are available to help shelter managers mitigate the animal and public health risks of enteropathogens. Guidelines developed for care of individually owned cats can be impractical in animal welfare organizations due to limited funding, staffing, and technical expertise. In the absence of expert guidance, shelter managers must devise their own preventive health programs, often in the absence of veterinary input or knowledge of the most important enteropathogens in their specific facilities.

Understanding the epidemiology of infectious enteropathogens of unowned cats is a first step toward the development of practical control programs. The objective of this study was to determine the prevalence of enteropathogens in cats with and without diarrhea in four different models for managing unowned cats and to compare the prevalences across management models.

Materials and methods

Study sites

The study included four different models for managing unowned cats: short-term shelters (STS), long-term sanctuaries (LTS), foster care programs (FCP), and trap-neuter-return programs (TNR). Study participants were agencies from Florida and Georgia that responded to an online survey about their organization and animal health protocols. Enrollment remained open until a minimum of 50 cats with diarrhea and 50 cats without diarrhea from each management model were tested. A traditional STS was defined as a physical building that housed stray, owner surrendered, and/or confiscated cats in single-housing units. Examples of this type of facility included municipal animal control facilities and humane societies. Cats were held in these facilities for a period of time determined by local ordinances, shelter protocols, health, and behavior status. After their respective holding periods, cats were adopted out, transferred to other agencies, reunited with owners, or euthanased. In order to represent typical housing and length of stay in STS, cats selected for the study had been housed individually within the shelter for up to one month at the time of sampling.

Long-term sanctuaries accommodated large numbers of cats in group housing, often with outdoor access, and provided a place for cats to live out the remainder of their lives. Most cats had been living in the sanctuaries for months to years. Some sanctuaries had adoption or transfer programs and accepted cats for intake from other sheltering agencies or directly from the public. Cats selected from LTS were co-housed with multiple unrelated cats using communal litter boxes, so it was not possible to link fecal samples with any individual cat.

Foster care programs used private residences to house cats, with or without resident pets, until they were adopted or transferred to an adoption facility. Agencies used FCP for a variety of reasons including the raising of underaged kittens, medical or behavioral rehabilitation, or for relief of crowding at a main shelter facility. Cats selected from FCP had not been admitted to one of the other sheltering models (STS or LTS) prior to FCP and had been in FCP for up to 6 months. Trap-neuter-return programs included free-roaming, unowned stray and feral cats for neutering. Cats were housed briefly in individual traps prior to and after anesthesia for neutering and vaccination. After recovery, they were returned to the environment from which they were trapped.

Animals

The study was approved by the Institutional Animal Care and Use Committee at the University of Florida (protocol 201207489, approved on 15 June 2012). A convenience sample of cats was selected from multiple agencies within each management model with the objective of testing approximately equal numbers of cats with normal fecal consistency and cats with diarrhea. Normal fecal textures were defined as feces that maintained form when picked up, and diarrhea was defined as feces that was unformed when picked up. When available, the following information was collected on each cat: responsible agency, identification name or number, intake date, estimated age, and sex. In group-housed cats sharing a communal litter box, a single sample was collected and some animal-specific information (e.g., age and sex) was recorded as unknown. In addition, age and sex information on cats missing from some cats in TNR programs when feces were collected from their traps after the cats were removed for surgery. A history of vaccination, parasite medication, or antibiotic treatment did not exclude cats from the study, since these are common procedures in animal care agencies.

Sample collection

Fresh fecal samples from cats in STS, LTS, and FCP were collected from litter boxes or living environments. Fecal samples from TNR programs were collected from the transportation traps or were collected manually from the rectum of anesthetized cats. To avoid cross-contamination of samples, each was collected with a separate set of supplies, including a glove assisted by a wooden tongue depressor or syringe, as needed. Trained study staff collected all samples from STS, LTS, and TNR cats. Foster care programs were provided with a written protocol and supplies to collect fecal samples in the home, and then meet the study personnel at a central location for examination of their cats and packaging of samples. For each sample, two fecal aliquots were collected into new plastic screw-top jars. One jar was selected for PCR assay and placed into its own zipper-lock plastic bag to further reduce the chance of cross-contamination. The second jar was designated for fecal flotation. Samples were transported in a Styrofoam cooler with ice packs and stored at 4°C in a refrigerator pending analysis.

Testing protocol

Fecal samples were shipped on ice within 72 h of collection via overnight courier to a reference laboratory for testing (IDEXX Laboratories, Inc.). Upon arrival, samples were tested by zinc sulfate centrifugation for parasite eggs, cysts, and larvae. Fecal samples were also tested by real-time PCR assay for a panel of potential enteropathogens, including *Trichromonas foetus* 3.85 rRNA gene (*AF339736*), *Cryptosporidium* spp. small-subunit RNA gene (*A093489*), *Giardia* spp. small-subunit RNA gene (*DQ836339*), *Toxoplasma gondii* internal transcribed spacer-1 gene (*L49390*), *Clostridium perfringens* alpha gene (*AM888388*), *Salmonella* invasion *A* gene (*EU434836*), feline panleukopenia virus (FCV) *VP2* gene (*EU252145*), and feline coronavirus (FCoV) *FCoV* 7B gene (*DQ019821*.1) at a reference laboratory within seven days after collection (IDEXX Feline Diarrhea RealPCR Panel, t2627). Real-time PCR was run with seven quality controls, including quantitative PCR-positive controls using synthetic DNA, PCR-negative controls using PCR-grade, nuclease-free water, negative extraction controls using lysis solution only in select position of the 96-well extraction plate, DNA internal sample control targeting the host 18S rRNA gene complex, an internal positive control spiked into the lysis solution, and an environmental contamination monitoring control.

Statistical analysis

Sample sizes (≥50 cats/group) were selected to detect a difference in infection prevalence of ≥28% between cats with and without diarrhea within each management model, with power of 0.80 and level of significance at 0.05. Related parasites were grouped by type as coccidia (*Cystoisospora felis*, *Cystoisospora rivolta*, *Elmeria spp.*), hookworm (*Ankylostoma tubaeforme*, *Ankylostoma braziliense*), roundworm (*Toxocara cati*, *Toxascaris leonina*), and cestode (*Spirometro mansonioides*, *Dipylidium caninum*, *Taenia taeniaformis*, *Mesocestodes spp.*). Prevalence of infection was calculated for cats with and without diarrhea within each management model. The difference in infection prevalence was evaluated by use of the χ² test or Fisher exact test, as appropriate (Epi Info Version 3.5.3, Centers for Disease Control and Prevention). *P* < 0.05 was considered statistically significant.

Results

A total of 482 fecal samples were collected from 13 STS (*n* = 112), 5 LTS (*n* = 121), 24 FCP (*n* = 122), and 5 TNR programs (*n* = 127). Approximately half of the samples from each management model were normal and half were diarrhea (Table 1). A total of 108 cats (96%) were reported to have received
treatment with a medication active against roundworms and hookworms, and 35 (31%) had received a coccidiostat. Cats had been housed in the STS for 7–30 days (median 12 days) at the time of testing. Nearly half of the cats in STS were shedding FCoV in their feces (Table 2). There was no significant difference in the prevalence of any enteropathogen between cats with normal fecal consistency and diarrhea. FPV was identified in 17 cats, 14 of which had been vaccinated against FPV 1–12 days before sample collection, one that was vaccinated 22 days before sample collection, and two that had not been vaccinated. *Aelurostrongylus abstrusus* larvae were observed on the fecal flotation test of one cat with normal feces.

**Long-term sanctuaries**

A total of 121 cats from five LTS in Florida were enrolled in the study (Table 1). The facilities housed anywhere from a few dozen to approximately 700 cats, generally in large free-roaming groups. Most of the cats on the properties appeared to be adults. Since fecal samples were collected from communal litter boxes, it was not possible to characterize the cats or to correlate samples with the administration of parasite control medications. Approximately three-quarters of the samples tested in LTS were PCR-positive for FCoV RNA (Table 2). There was no significant difference in the prevalence of any enteropathogen between normal feces and diarrhea with the exception of *T. foetus* (*P* ≤ 0.04). A majority of the samples with *Capillaria aerophila* (17/19; 89%) were from a single LTS facility, and a majority of the samples with *Cynicilocymes guttulatus* (21/24; 89%) were from 3 LTS facilities. One cat with normal feces was PCR-positive for *Salmonella* spp., and one cat with diarrhea was PCR-positive for *T. gondii*.

**Foster care programs**

A total of 122 cats from 24 FCP in Florida and Georgia were enrolled in the study; 74% were ≤6 months of age (Table 1). A total of 98 cats (80%) were reported to have received treatment with a medication active against roundworms and hookworms, and 12 (10%) had received a coccidiostat. Cats had been housed in FCP for 1–190 days (median 54 days) at the time of testing. A majority of the samples from FCP were PCR-positive for FCoV, with a significantly higher rate of detection in diarrhea than in normal feces (*P* = 0.001) (Table 2). One cat with normal feces was PCR-positive for *Salmonella* spp. FPV was identified in five cats, all of which had been vaccinated against FPV 6–8 days before sample collection.

**Trap-neuter-return programs**

A total of 127 cats from five TNR programs in Florida were enrolled in the study; 23% of the cats with recorded ages were ≤6 months of age (Table 1). More than two-thirds of cats in TNR programs were shedding hookworm ova. There was no significant difference in the prevalence of any enteropathogen between cats with normal feces and cats with diarrhea. Of the cestode infections, 16 of 18 (89%), were identified as *Spirometra mansonioides*. Four cats with normal feces were PCR-positive for *Salmonella* spp.

**Comparison of enteropathogens between management models: Cats with normal fecal consistency**

Several differences were observed in the prevalence of specific enteropathogens among cats with normal feces in the different management models. Cats in STS were more likely to test positive for coccidia than were cats in any other model (*P* ≤ 0.02). Cats from LTS were more likely to test positive for FCoV (*P* ≤ 0.03) than any other model, were more likely to test positive for *T. foetus* than cats in STS (*P* ≤ 0.02) or FCP (*P* ≤ 0.03), and were more likely to test positive for *Giardia* than cats in STS (*P* ≤ 0.03). Cats in LTS and TNR programs were more likely to test positive for ascarios than cats in STS or FCP (*P* ≤ 0.04). Cats from TNR programs were less likely to test positive for FCoV (*P* ≤ 0.03) and were more likely to test positive for hookworms than cats in any other model (*P* < 0.0001).

**Comparison of enteropathogens between management models: Cats with diarrhea**

Cats in LTS with diarrhea were more likely to test positive for *T. foetus* (*P* ≤ 0.02), *Giardia* (*P* ≤ 0.04), roundworms (*P* ≤ 0.01), *C. aerophila* (*P* ≤ 0.007), and *C. guttulatus* (*P* ≤ 0.005) compared to cats in other models. Cats in STS were more likely to test positive for FPV than cats in another model (*P* ≤ 0.01), but less likely to test positive for *C. perfringens* alpha gene than cats in LTS or TNR (*P* ≤ 0.02). Cats in LTS and FCP were more likely to test positive for FCoV than cats in STS or TNR (*P* ≤ 0.004). Cats in LTS and TNR were more likely to test positive for hookworms than cats in STS and FCP (*P* ≤ 0.01).

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Table 1

Demographic information for 482 cats tested for enteropathogens in short-term shelters, long-term sanctuaries, foster care programs, and trap-neuter-return programs.

| Management model and fecal consistency | Total tested | Male | Female | Unknown sex* | Unknown age** |
|---------------------------------------|--------------|------|--------|--------------|---------------|
| **Short-term shelters (n = 13)**      |              |      |        |              |               |
| Diarrhea                              | 60           | 26   | 34     | 57%          | 20%           |
| Normal                                | 52           | 28   | 24     | 46%          | 20%           |
| **Long-term sanctuaries (n = 5)**     |              |      |        |              |               |
| Diarrhea                              | 61           | 31   | 30     | 48%          | 20%           |
| Normal                                | 60           | 30   | 30     | 50%          | 30%           |
| **Foster care programs (n = 24)**     |              |      |        |              |               |
| Diarrhea                              | 60           | 30   | 30     | 50%          | 20%           |
| Normal                                | 62           | 31   | 31     | 51%          | 30%           |
| **Trap-neuter-return programs (n = 5)**|             |      |        |              |               |
| Diarrhea                              | 60           | 30   | 30     | 50%          | 20%           |
| Normal                                | 67           | 33   | 34     | 52%          | 21%           |

* Age and sex were recorded as unknown when a single sample was collected from group-housed cats sharing a litter box. Age and sex were also unknown for some cats in TNR programs when feces were collected from their traps after the cats were removed for surgery.

**Table 2**

Comparison of enteropathogens among cats in short-term shelters, long-term sanctuaries, foster care programs, and trap-neuter-return programs.

| Management model and fecal consistency | Unknown sex* | Unknown age** |
|---------------------------------------|--------------|---------------|
| **Short-term shelters (n = 13)**      |              |               |
| Diarrhea                              | 60           | 20%           |
| Normal                                | 52           | 20%           |
| **Long-term sanctuaries (n = 5)**     |              |               |
| Diarrhea                              | 61           | 20%           |
| Normal                                | 60           | 20%           |
| **Foster care programs (n = 24)**     |              |               |
| Diarrhea                              | 60           | 20%           |
| Normal                                | 62           | 20%           |
| **Trap-neuter-return programs (n = 5)**|             |               |
| Diarrhea                              | 60           | 20%           |
| Normal                                | 67           | 21%           |
Enteropathogen types and co-infections

The vast majority of cats carried at least one detectable enteropathogen, and many carried co-infections (Table 3). For LTS (P = 0.0003) and FCP (P = 0.03) management models, the number of species detected was significantly higher in cats with diarrhea, compared to cats with normal feces (Table 3). When enteropathogens were grouped by type, it was apparent that viral infections were present in a majority of cats in LTS, and FCP, protozoal infections were present in a majority of cats in LTS, and helminth infections were present in a majority of cats in TNR programs (Table 4).

Table 2
Prevalence of enteropathogens in 482 cats tested in short-term shelters, long-term sanctuaries, foster care programs, and trap-neuter-return programs.

| Enteropathogen               | Short-term shelters | Long-term sanctuaries | Foster care programs | Trap-neuter-return programs |
|------------------------------|---------------------|-----------------------|----------------------|-----------------------------|
| Cryptosporidium spp.         |                     |                       |                      |                             |
| Diarrhea                     | 3 (5%)              | 9 (15%)               | 5 (8%)               | 5 (8%)                      |
| Normal                       | 5 (10%)             | 12 (20%)              | 6 (10%)              | 9 (13%)                     |
| Giardia spp.                 |                     |                       |                      |                             |
| Diarrhea                     | 4 (7%)              | 19 (31%)              | 9 (15%)              | 4 (7%)                      |
| Normal                       | 3 (6%)              | 13 (22%)              | 9 (15%)              | 6 (9%)                      |
| Trichomonas foetus           |                     |                       |                      |                             |
| Diarrhea                     | 0                   | 16 (26%)*              | 5 (8%)               | 0                           |
| Normal                       | 0                   | 6 (10%)               | 0                    | 1 (1%)                      |
| Coccidia                     |                     |                       |                      |                             |
| Diarrhea                     | 14 (23%)            | 8 (13%)               | 9 (15%)              | 9 (15%)                     |
| Normal                       | 17 (33%)            | 6 (10%)               | 8 (13%)              | 7 (10%)                     |
| Toxoplasma gondii            |                     |                       |                      |                             |
| Diarrhea                     | 0                   | 1 (2%)                | 0                    | 0                           |
| Normal                       | 0                   | 0                     | 0                    | 0                           |
| Salmonella spp.              |                     |                       |                      |                             |
| Diarrhea                     | 0                   | 0                     | 0                    | 0                           |
| Normal                       | 0                   | 1 (2%)                | 1 (2%)               | 4 (6%)                      |
| Clostridium perfringens enterotoxin A | 9 (15%) | 20 (33%) | 16 (27%) | 21 (35%) |
| Normal                       | 11 (21%)            | 11 (18%)              | 16 (26%)             | 21 (31%)                     |
| Feline panleukopenia virus   |                     |                       |                      |                             |
| Diarrhea                     | 12 (20%)            | 0                     | 2 (3%)               | 0                           |
| Normal                       | 5 (10%)             | 0                     | 3 (5%)               | 0                           |
| Feline coronavirus           |                     |                       |                      |                             |
| Diarrhea                     | 28 (47%)            | 45 (74%)              | 48 (80%)*            | 22 (37%)                     |
| Normal                       | 24 (46%)            | 47 (78%)              | 36 (58%)             | 16 (24%)                     |
| Hookworm                     |                     |                       |                      |                             |
| Diarrhea                     | 3 (5%)              | 17 (28%)              | 5 (8%)               | 39 (65%)                     |
| Normal                       | 4 (8%)              | 9 (15%)               | 3 (5%)               | 50 (75%)                     |
| Roundworm                    |                     |                       |                      |                             |
| Diarrhea                     | 3 (5%)              | 18 (30%)              | 5 (8%)               | 6 (10%)                      |
| Normal                       | 3 (6%)              | 14 (23%)              | 3 (5%)               | 10 (15%)                     |
| Cestode                      |                     |                       |                      |                             |
| Diarrhea                     | 1 (2%)              | 1 (2%)                | 1 (2%)               | 7 (12%)                      |
| Normal                       | 4 (8%)              | 3 (5%)                | 0                    | 11 (16%)                     |
| Capillaria aerophila         |                     |                       |                      |                             |
| Diarrhea                     | 0                   | 10 (16%)              | 1 (2%)               | 0                           |
| Normal                       | 0                   | 8 (13%)               | 0                    | 0                           |
| Cyniclomyces guttulatus      |                     |                       |                      |                             |
| Diarrhea                     | 0                   | 14 (23%)              | 1 (2%)               | 0                           |
| Normal                       | 0                   | 7 (12%)               | 2 (3%)               | 0                           |

* Prevalence in cats with diarrhea was significantly greater than in cats with normal fecal consistency within the management model (P < 0.05).

Discussion

This is the first study to compare the prevalence of enteropathogens carried by unowned cats living in different short- and long-term management models. A majority of cats carried at least one enteropathogen of feline or zoonotic importance, regardless of management model. Prevalence of specific enteropathogens varied between management models, likely reflecting differences in preventive healthcare and housing conditions. For example, the TNR cohort consisted of free-roaming unowned cats with exposure to the natural environment and lacking regular preventive care, a possible explanation for the higher prevalence of helminths in this
Cats in FCP shared some of the characteristics of cats in sanctuaries, such as increased likelihood of having one or more enteropathogens, with FCoV in particular. One of the objectives of FCP is to provide a low-risk alternative to high-density shelter housing for vulnerable cats. This commonly includes queens with their nursing litters, under-aged orphan kittens, cats recovering from illness or injury, and shy or feral cats undergoing socialization. Protection against infectious disease transmission is undermined if cats from multiple sources are housed together in FCP, a practice that should be avoided.

Cats in TNR programs had the lowest risk of protozoal and viral infections, possibly as a result of decreased density and their habit of burying feces. However, these cats had the highest risk of helmint infection, particularly with hookworms, which commonly contaminate the sandy soils of the Southeastern US (Anderson et al., 2003; De Santis et al., 2006).

There was little relationship between fecal consistency and the presence of one or more enteropathogens, a finding that has been documented in previous studies (Hill et al., 2000; Vasilopoulos et al., 2006; Gow et al., 2009; Queen et al., 2012; Sabshin et al., 2012; Paris et al., 2014; Little et al., 2015; Silva and Lobato, 2015). Although the pathogens evaluated in this study can be associated with gastrointestinal disease, it is apparent that the mere presence of these agents does not consistently result in diarrhea. Additional co-factors, such as co-infections, stress, immune status, general body condition, genetics, pathogen virulence, and pathogen burden may play a role in the development of disease. Clinicians should proceed cautiously when attributing diarrhea to any detected pathogen, since a majority of cats had one or more pathogens identified regardless of their health status.

Several steps can be taken to minimize the risk of transmission of some enteropathogens to cats and people. The use of personal hygiene, health status, and disease.

### Table 3
Prevalence of multi-pathogen co-infections in 482 cats tested in short-term shelters, long-term sanctuaries, foster care programs, and trap-neuter-return programs.

| Model                          | Fecal consistency | None detected | 1–2 species | 3–4 species | 5–6 species | 7 species |
|-------------------------------|-------------------|---------------|-------------|-------------|-------------|-----------|
| Short-term shelters (n = 13)  | Diarrhea (n = 60) | 16 (27%)      | 36 (60%)    | 8 (13%)     | 0           | 0         |
|                              | Normal (n = 52)   | 16 (31%)      | 26 (50%)    | 6 (12%)     | 3 (6%)      | 1 (2%)    |
| Long-term sanctuaries (n = 5)*| Diarrhea (n = 61) | 1 (2%)        | 18 (30%)    | 35 (57%)    | 7 (11%)     | 0         |
|                              | Normal (n = 60)   | 1 (2%)        | 39 (65%)    | 16 (40%)    | 3 (5%)      | 1 (2%)    |
| Foster care programs (n = 24)*| Diarrhea (n = 60) | 3 (5%)        | 44 (73%)    | 13 (22%)    | 0           | 0         |
|                               | Normal (n = 62)   | 13 (21%)      | 39 (63%)    | 10 (16%)    | 0           | 0         |
| Trap-neuter-return programs (n = 5) | Diarrhea (n = 60) | 7 (12%)       | 35 (60%)    | 18 (30%)    | 0           | 0         |
|                               | Normal (n = 67)   | 8 (12%)       | 36 (52%)    | 20 (31%)    | 3 (4%)      | 0         |

* The number of species detected was significantly higher in cats with diarrhea, compared to cats with normal feces within the management model (P < 0.05).

### Table 4
Prevalence of viral, bacterial, protozoal, and helminth enteropathogens in 482 cats tested in short-term shelters, long-term sanctuaries, foster care programs, and trap-neuter-return programs.

| Model                          | Fecal consistency | None detected | Protozoal | Bacterial | Viral | Helminth |
|-------------------------------|-------------------|---------------|-----------|-----------|-------|----------|
| Short-term shelters (n = 13)  | Diarrhea (n = 60) | 16 (27%)      | 21 (35%)  | 9 (15%)   | 40 (67%) | 7 (12%)  |
|                              | Normal (n = 52)   | 16 (31%)      | 25 (48%)  | 11 (21%)  | 29 (56%) | 10 (19%) |
| Long-term sanctuaries (n = 5) | Diarrhea (n = 61) | 1 (2%)        | 54 (89%)  | 20 (33%)  | 45 (74%) | 36 (59%) |
|                              | Normal (n = 60)   | 1 (2%)        | 37 (62%)  | 12 (20%)  | 47 (78%) | 24 (40%) |
| Foster care programs (n = 24) | Diarrhea (n = 60) | 3 (5%)        | 28 (47%)  | 16 (27%)  | 50 (83%) | 11 (18%) |
|                               | Normal (n = 62)   | 13 (21%)      | 25 (40%)  | 17 (27%)  | 39 (63%) | 6 (10%)  |
| Trap-neuter-return programs (n = 5) | Diarrhea (n = 60) | 6 (10%)       | 19 (32%)  | 21 (35%)  | 22 (37%) | 47 (78%) |
|                               | Normal (n = 67)   | 8 (12%)       | 23 (34%)  | 25 (37%)  | 16 (24%) | 61 (91%) |

* Enteropathogen prevalence in cats with diarrhea was significantly greater than in cats with normal feces within the management model (P < 0.05).

* Cats with diarrhea were significantly more likely to have any detectable enteropathogens than cats with normal feces within the management model (P < 0.05).
protective equipment during sanitation procedures, frequent handwashing after handling cats, selection of premise disinfec-
tants effective against common pathogens, installation of imper-
meable surfaces to facilitate cleaning, and frequent sanitation of
litterboxes are recommended standards of practice (Newbury
et al., 2010). Anthelmintics and treatment for external parasites
should be routinely administered to all cats according to
established guidelines1 (Smith et al., 2009; Little et al., 2015).
Intake protocols that include routine treatments against coccidia
(Litster et al., 2014), tapeworms, and fleas (which can transmit
tapeworms) in endemic regions can be more practical and cost-
effective than individualized testing and treatment. Treatments
against some common enteropathogens, such as T. foetus,
Cryptosporidium spp., and Giardia spp., are often impractical
in large populations, or in the case of FCoV, do not exist (Addie
et al., 2009). Such infections can be difficult to eradicate once introduced
into a population. Although guidelines exist for minimizing the risk
of zoonotic transmission from individual cats, particularly to
immunocompromised individuals (Tuzio et al., 2005), no such
guidelines exist for large cat populations. Fortunately, the strains of
Cryptosporidium spp. and Giardia spp. carried by cats are generally
of low zoonotic potential (Palmer et al., 2008; Thompson et al.,
2008).

This study had several limitations. Selection of participating
organizations and the cats that were sampled were selected by
opposition to random selection. Therefore, it was not possible to assure
that the sampled cats were similar to cats not sampled, and caution
should be used when extrapolating the results to broader populations.
Since fecal samples were intentionally collected such that approximately half were diarrhea and
half were of normal consistency, the overall prevalence of diarrhea
and enteropathogens in each sheltering model was not deter-
mined. Most of the cats with positive FPV tests had been recently
vaccinated, and it is possible that the PCR test detected the vaccine
virus rather than natural infection (Truyen et al., 2009). Cats were
tested only once, whereas many pathogens have variable rates of
fecal shedding that may fall below the limit of detection (Mekaru
et al., 2007; Little et al., 2015). In addition, the pathogen screening
panel used in this study did not include all enteropathogens
associated with diarrhea in cats, such as rotavirus (Martella
et al., 2010; German et al., 2015; Otto et al., 2015), calicivirus (Castro
et al., 2015), and astrovirus (Ng et al., 2014). It is likely that more
enteropathogens will be associated with gastrointestinal disease in
cats as they continue to be evaluated (Ng et al., 2014).

Conclusions

Enteropathogens of feline and zoonotic concern were commonly
found in all four systems for managing unowned cats, regardless of whether the cats had normal feces or diarrhea.
Practical options for control are limited by the diverse scope of
enteropathogens, expense and limitations of diagnostic tests, lack
of curative treatment options for many organisms, co-mingling of
cats, and prolonged shedding and environmental persistence of
some organisms. In light of the widespread presence of enter-
opathogens, risk-management strategies should be implemented
to reduce transmission to people and other animals. These should
focus on establishing high standards for housing and sanitation,
routine administration of anthelmintics, compliance with prevent-
tive care guidelines, periodic surveillance, response to specific
enteropathogen patterns, humane population management of
free-roaming community cats, public health education, and most
importantly, minimizing the duration and number of cats in mass
confinement.

Conflict of interest statement

Dr. Christian Leutenegger is the Head of the PCR laboratory at
IDEXX Laboratories, Inc. None of the authors of this paper have a
financial or personal relationship with other people or organi-
sations that could inappropriately influence or bias the content of
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References

Addie, D., Belak, S., Boucouz-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H., et al., 2009. Feline infectious
peritonitis. ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery 11, 594–604.
Anderson, T.C., Foster, G.W., Forrester, D.J., 2003. Hookworms of feline cats in Florida. Veterinary Parasitology 115, 19–24.
Arranz-Solís, D., Pedraza-Díaz, S., Miró, G., Rojo-Montejo, S., Hernández, L., Ortega-Mora, L.M., Collantes-Fernández, E., 2016. Triritchomonas foetus infection in cats with
diarrhea from densely housed origs. Veterinary Parasitology 221, 118–122.
Canadian Veterinary Medical Association, 2009. A Code of Practice for Canadian Catery Operations. Canadian Veterinarian Medical Association, Ottawa, ON, pp.
1–35.
Castro, T.X., Cubel Garcia, R.d.C.N., Fuman, T.M., Costa, E.M., Mello, R., White, P.A., Leite, J.P., 2015. Detection and molecular characterization of calciviruses
(vesivirus and norovirus) in an outbreak of acute diarrhea in kittens from Brazil. The Journal Veterinary 206, 115–117.
Cave, T.A., Golder, M.C., Simpson, J., Addie, D.D., 2004. Risk factors for feline coronavirus seropositivity in cats relinquished to a UK rescue charity. Journal of
Feline Medicine and Surgery 6, 53–58.
De Santis, A.C., Raghavan, M., Caldanaro, R.J., Glickman, N.W., Moore, G.E., Lewis, H.,
B., Schantz, P.M., Glickman, L.T., 2006. Estimated prevalence of nematode parasitism among pet cats in the United States. Journal of the American
Veterinary Medical Association 228, 885–892.
German, A.C., Itrurria-Gómara, M., Dove, W., Sandrasegaram, M., Nakagomi, T.,
Nakagomi, O., Cunliffe, N., Radford, A.D., Morgan, K.L., 2015. Molecular
epidemiology of rotavirus in cats in the United Kingdom. Journal of Clinical
Microbiology 53, 455–464.
Gow, A.G., Gow, D.J., Hall, E.J., Langton, D., Clarke, C., Papasouliotis, K., 2009.
Prevalence of potentially pathogenic enteric organisms in clinically healthy
kittens in the UK. Journal of Feline Medicine and Surgery 11, 655–662.
Goffin, B., Bushby, P.A., McBee, E., White, S.C., Rigdon-Brestle, Y.K., Apell, I.D.,
Makolinski, K.V., Wilford, C.L., Bohling, M.W., Eddleston, S.M., et al., 2016. The Association of Shelter Veterinarians’ 2016 veterinary medical care guidelines for
spay-neuter programs. Journal of the American Veterinary Medical Association
249, 105–188.
Hill, S.L., Cheney, J.M., Taton-Allen, G.F., Reif, J.S., Bruns, C., Lappin, M.R., 2000.
Prevalence of enteric zoonotic organisms in cats. Journal of the American
Veterinary Medical Association 216, 687–692.
Hosie, M.J., Addie, D., Belak, S., Boucouz-Baralon, C., Egberink, H., Frymus, T.,
Gruffydd-Jones, T., Hartmann, K., Lloret, A., Lutz, H., et al., 2013. Matrix
vaccination guidelines: ABCD recommendations for indoor/outdoor cats, rescue
shelter cats and breeding catteries. Journal of Feline Medicine and Surgery 15,
540–544.
Leary, S., Underwood, W., Athony, R., Corey, D., Grandin, T., Greenacre, C., Gwaltney-
Brant, S., McCrackin, M.A., Meyer, R., Miller, D., 2013. AVMA Guidelines for the
Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association,
Schamburg, IL, pp. 1–102.
Levy, J., Crawford, C., Hartmann, K., Hofmann-Lehmann, R., Little, S., Sundahl, E.,
Thayer, V., 2008. 2008 American Association of Feline Practitioners’ feline
retrovirus management guidelines. Journal of Feline Medicine and Surgery 10,
300–316.
Litster, A.L., Nichols, J., Hall, K., Camp, J., Mohamed, A.S., 2014. Use of ponazuril paste
to treat coccidiosis in shelter-housed cats and dogs. Veterinary Parasitology
202, 319–325.
Little, S., Adolph, C., Downie, K., Snider, T., Reichard, M., 2015. High prevalence of
covert infection with gastrointestinal helminths in cats. Journal of the American
Animal Hospital Association 51, 359–364.
Martella, V., Bányai, K., Matthijssens, J., Buonavoglia, C., Carlet, M., 2010. Zoonotic
aspects of rotaviruses. Veterinary Microbiology 140, 246–255.

1 See: Companion Animal Parasite Council, 2017. CAPC recommendations. https://www.capcivet.org/capc-recommendations (accessed 20 May 2017).
