Effect of the Probiotic *Enterococcus faecium* SF68 on Presence of Diarrhea in Cats and Dogs Housed in an Animal Shelter

S.N. Bybee, A.V. Scorza, and M.R. Lappin

Background: Beneficial effects of probiotics have never been analyzed in an animal shelter.

Hypothesis: Dogs and cats housed in an animal shelter and administered a probiotic are less likely to have diarrhea of ≥ 2 days duration than untreated controls.

Animals: Two hundred and seventeen cats and 182 dogs.

Methods: Double blinded and placebo controlled. Shelter dogs and cats were housed in 2 separate rooms for each species. For 4 weeks, animals in 1 room for each species was fed *Enterococcus faecium* SF68 while animals in the other room were fed a placebo. After a 1-week washout period, the treatments by room were switched and the study continued an additional 4 weeks. A standardized fecal score system was applied to feces from each animal every day by a blinded individual. Feces of animals with and without diarrhea were evaluated for enteric parasites. Data were analyzed by a generalized linear mixed model using a binomial distribution with treatment being a fixed effect and the room being a random effect.

Results: The percentage of cats with diarrhea ≥ 2 days was significantly lower ($P = .0297$) in the probiotic group (7.4%) when compared with the placebo group (20.7%). Statistical differences between groups of dogs were not detected but diarrhea was uncommon in both groups of dogs during the study.

Conclusion and Clinical Importance: Cats fed SF68 had fewer episodes of diarrhea of ≥ 2 days when compared with controls suggests the probiotic may have beneficial effects on the gastrointestinal tract.

Key words: Diet; Gastrointestinal tract; Parasites; Stress.

Diarrhea is common in cats and dogs housed in animal shelters and can result from a variety of factors, including stress, diet change, and numerous bacterial, viral, and parasitic agents. For example, in previous work performed in the animal shelter used in the study described here, *Giardia* spp. were detected in approximately 10% of the healthy dogs tested (M.R. Lappin, unpublished data, 2010). Enteric coronaviruses also have the potential to cause diarrhea. These viruses colonize many cats that enter animal shelters or humane societies and are often acquired while the cats are housed in the facility. For example, when cats in a California animal shelter were tested for coronaviruses in feces on entry and on day 7, the prevalence rates were 33% and 60%, respectively.[1] In a different study of cats housed in the animal shelter used in the current study, coronavirus RNA was amplified from 31.8% of cat feces collected on entry.[a] *Clostridium perfringens* is a bacterium harbored in the gastrointestinal tract of many dogs and cats that has the capability to produce enterotoxins and cause diarrhea. In a different study of cats housed in the animal shelter used in the current study, *C. perfringens* enterotoxin was detected in the feces of 18.2% of the cats on entry.[a] In addition, this organism was cultured from feces

From the Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO. This work was performed at a northern Colorado animal shelter. Presented in abstract form at the 2010 ACVIM Forum, Anaheim, CA.

Corresponding author: S. N. Bybee, BS, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, 300 West Drake, Fort Collins, CO 80523-1678; e-mail: shawn.bybee@gmail.com.

Abbreviation:

FS	fecal score

of 58.5% of client-owned dogs in the city where the current study was performed and from 36% of dogs housed in a French animal shelter.[2,3] Each of these organisms can be harbored by healthy animals and then have clinical illness exacerbated by stress, diet change, or other immune-suppressive events.

Regardless of the cause, presence of diarrhea in cats or dogs housed in animal shelters can delay the adoption process, drain limited shelter resources, and in extreme cases, result in euthanasia of the animal. Many animal shelters have adopted methods to provide proper enrichment for their animals as well as decrease environmental stress in attempts to decrease incidence rates of diarrhea. However, additional measures to lessen diarrhea rates in cats and dogs housed in animal shelters are needed.

Probiotics have been defined as live microorganisms that when administered in adequate amounts confer a health effect on the host.[4] These health effects are exerted by a direct inhibition to colonization of pathogenic microorganisms or by immune-enhancing effects on gut-associated lymphoid tissue, thereby increasing immuno-modulating substances.[5–7] *Enterococcus faecium* strain SF68[b] is a PO-administered probiotic with many proven beneficial effects. For example, in studies of dogs, feeding strain SF68 reduced the fecal concentrations of *C. perfringens* but increased levels of the potentially beneficial *Bifidobacteria* spp. ($P < .05$) and *Lactobacilli* spp. ($P < .015$) (G.L. Czarnecki-Maulden and C. Cavadini, unpublished data, 2010).[8] In another study, cats fed a placebo had decreased fecal microbiota diversity while cats fed SF68 maintained their diversity when the cats were

subjected to stress.[9] Statistically significant increases in mean serum IgA concentrations have been detected in dogs fed SF68 over time when compared with a placebo group (n = 7).[10] Lastly, when administered chronically, this probiotic has been shown to have potential immune-modulating activity in both dogs and cats.[10,11] Anecdotally, many shelters administer this probiotic to their animals because of the potential for decreasing diarrhea. However, this effect has never been analyzed in a blinded and placebo-controlled research study. In this study, we hypothesized that dogs and cats housed in an animal shelter and fed SF68 would be less likely to have diarrhea of ≥2 days duration than untreated controls.

Materials and Methods

Animals

The experimental design was approved by the Institutional Animal Care and Use Committee at Colorado State University and the Humane Society Board of Directors. Dogs and cats included in this study were received, housed, and discharged following the standard operating procedures for the participating shelter. The shelter operations could not be modified for the study, and so the animal population was very dynamic, with dogs and cats being received and removed from the population throughout the course of the study.

The dogs were stray or owner-relinquished and were individually housed in two separate but identical rooms each containing 14 runs. The cats were individually housed in 2 separate rooms, 1 containing 40 cages (stray room) and the other 23 cages (feral room). Cats that were domesticated (owner-relinquished or stray) were housed in the stray room. Cats that were aggressive or caught in feral cat traps were housed in the feral room. The signalment (age, sex, and breed) of each animal included in this study was recorded. However, the history of the animals was mostly unknown; therefore, the exact age, sex, and reproductive status was unknown for the majority. All animals were provided similar enrichment experiences and were fed the same commercial dry maintenance diets[c,d] for the duration of their stay and had water provided ad libitum. The cat cages and dog runs were disinfected daily.

Experiment Design

The study was conducted in a double blind fashion and was divided into 3 periods of 4 weeks duration. Other than feeding all animals in the rooms the standardized diets as described, no treatments were administered in Period 1 to aid in determining the approximate diarrhea rates per room. In Periods 2 and 3, animals in 1 room were administered SF68 in a palatability enhancer with food while the other room was administered the palatability enhancer only with food as a placebo. The daily dose of SF68 was approximately 1 g, which is the label recommendation on the commercial product.[b] The SF68 capsules were analyzed and determined to contain 2.1×10^9 colony-forming units/g. The SF68 and placebo were supplied from the manufacturer in capsules with 1 product marked with a red dot. The investigators were blinded to which capsule contained SF68; the individual scoring the feces was blinded to which rooms were being administered which product over the course of the study. The dog product was stored at 4°C between feedings. Because there was no refrigerator available to store the cat product between feedings, it was stored at 20°C as approved.[b] Treatment was suspended for 7 days between Periods 2 and 3 to allow any SF68 that had potentially contaminated the environment to die. The treatment and placebo rooms were alternated between Periods 2 and 3 to attempt to account for any room effect.

Data Collection

Every day before daily cage cleaning, an individual blinded to the treatment groups assigned a fecal score (FS) to the feces of each animal throughout the study by the Nestle Purina Fecal Scoring System.[e] The scores were distributed as FS 1–3 = normal stool, FS 4–7 = diarrhea. If no stool was recorded for a particular day (FS = 0), the animal was considered to be normal, as those with diarrhea usually have a stool at least once per day. The shelter staff was asked to save all stools classified with a FS of ≥4 for FS assignment by the blinded individual as well as for enteric parasite analysis. To aid in the identification of abnormal stool by the kennel staff, a visual chart of FSs was provided. For each abnormal stool, we attempted to collect a fecal sample from a different animal with a FS≤3 from the same room the same day for enteric parasite analysis. Feces were collected during all 3 periods. Only the 1st stool sample was collected for analysis, even if the animal continued to have diarrhea.

Stool Analysis

To evaluate presence of enteric parasitism as a confounder in the final statistical analysis, each stool sample collected was transported to Colorado State University for performance of sugar solution centrifugation with commercially available reagents.[12,f] The sample was then examined microscopically at 100× for the presence of parasite eggs, oocysts, or cysts. Each fecal sample was also evaluated for the presence of *Giardia* spp. cysts or oocysts and *Cryptosporidium* spp. oocysts by use of a commercially available immunofluorescence kit following manufacturer's instructions.[g]

Statistical Evaluation

For the statistical evaluation, treatment was defined to be a fixed effect and room was defined to be a random effect. Before the investigators becoming unmasked, the data were stratified to include all animals that were housed for 2–7 days. The 1st day of data were excluded from the statistical analysis, as it is unknown what time on a particular day each animal entered the shelter and it is unlikely that a treatment would have noticeable gastrointestinal effect within the first 24 hours. The number of days each animal was observed to have diarrhea during their stay was determined and analyzed as categorical outcomes by percentages. From this dataset, the percentage of animals with diarrhea of ≥2 days duration was calculated. A generalized linear mixed model using a binomial distribution was used to assess for statistical differences between treatment groups. Mean and median of housing duration were calculated. If indicated, results of the fecal assays were to be used as a covariate in the statistical analysis. Prevalence rates for parasitism between rooms and prevalence rates of cats and dogs with FS≥4 and FS≤3 were compared by Fisher's exact test. Significance was defined as $P < .05$ in all analyses.

Results

Cats

A fecal sample was available from a total of 222 cats (119 cats with a FS≥4 and 103 cats with a FS≤3) over the course of the study (Table 1). Based on fecal examinations by centrifugal flotation and IFA, 14.9% (33 of 222) were positive for at least 1 infectious agent and 45.5% (15 of 33) of those positive cats had a normal FS (Table 1). In the feral room, 1 cat was concurrently infected with *Cystoisospora felis*, *Toxocara cati*, and *Giardia* spp. and 1 cat was concurrently infected with *C. felis*, *Giardia* spp., and

Table 1. Distribution of fecal examination results in cats housed in 2 rooms of an animal shelter and fed either *Enterococcus faecium* SF68[b] or a placebo.

Room/FS	Cats Positive for 1 or More Parasites (%)	Organism			
		Cystoisospora felis	*Taenia* spp.	*Toxocara cati*	Other
Feral room[a]					
Total: 91	21 (23.1)	9	6	4	6 *Trichuris* spp. (1) Hookworms (1) *Cryptosporidium* (2) *Giardia* (2)
FS ≤ 3: 40	9 (22.5)	4	3	0	2 *Trichuris* spp. (1) Hookworms (1)
FS ≥ 4: 51[a]	12 (23.5)	5	3	4	4
Stray room[b]					
Total: 131	12 (9.2)	5	5	0	4 *Giardia* (2) *Cryptosporidium* (2)
FS ≤ 3: 63[b]	6 (9.5)	3	1	0	4
FS ≥ 4: 68	6 (8.8)	2	4	0	0
Combined					
Total: 222	33 (14.9)	14	11	4	10
FS ≤ 3: 103	15 (14.6)	7	4	0	6
FS ≥ 4: 119	18 (15.1)	7	7	4	4

Fecal flotation by sugar centrifugation[f] and a commercially available immunofluorescent antibody assay[g] that detects *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts were utilized. There was no correlation between diarrhea and presence of intestinal parasitism.

FS, fecal score.

[a]In the feral room: 1 cat was concurrently infected with *Cystoisospora felis*, *Toxocara cati*, and *Giardia* spp., and 1 cat was concurrently infected with *Cystoisospora felis*, *Giardia* spp., and *Cryptosporidium* spp.

[b]Two cats from the stray room were concurrently infected with *Giardia* spp. and *Cryptosporidium* spp.

Cryptosporidium spp. Two cats from the stray room were coinfected with *Giardia* spp. and *Cryptosporidium* spp. The parasite prevalence rates varied between rooms (23.1% in the feral room; 9.2% in the stray room; $P = .0066$) but the presence of parasites was not correlated with the presence of diarrhea.

Of the cats administered SF68 over the course of the study, 130 cats were housed in the shelter for 2–7 days and were included in the data analysis. Of these, 82 cats were stray and 48 cats were feral. Sex was not recorded for 55 cats, 34 cats were male, and 41 cats were female. The mean and median durations of housing for these cats were 4.1 and 5 days, respectively. Of the cats administered the placebo over the course of the study, 87 cats were housed in the shelter for 2–7 days. Of these, 61 cats were stray and 26 cats were feral. Sex was not recorded for 32 cats, 23 cats were male, and 32 cats were female. The mean and median duration of housing for these cats was 3.9 and 4 days, respectively.

Overall, 26% (34 of 130) of cats in the SF68 group and 32% (28 of 87) of cats in the placebo group had at least 1 episode of diarrhea. The distribution frequency for the number of days of diarrhea stratified by treatment groups is shown in Figure 1. When all outcomes are included in the analysis (0–5 days with diarrhea), there was no significant difference between groups ($P = .5295$). However, the percentage of cats that had ≥2 days of diarrhea was significantly lower ($P = .0297$) in the SF68

group (7.7%) when compared with the placebo group (20.7%). Of the 15 cats in the placebo group with ≥2 days of diarrhea, only 2 cats (1 feral cat and 1 stray cat) were positive for intestinal parasites. The feral cat was concurrently infected with *Toxocara* spp., *Cystoisospora* spp., and *Giardia* spp. and the stray cat was infected with *Taenia* spp. Of the 9 cats in the SF68 group with ≥2 days of diarrhea, only 1 feral cat was positive for an intestinal parasite (*Cryptosporidium* spp.).
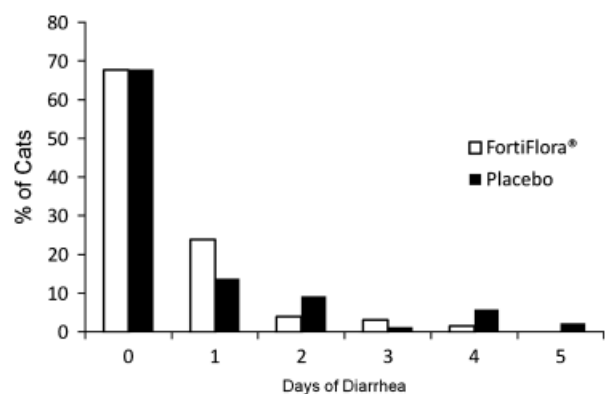
Fig 1. Distribution frequency of diarrhea (fecal score ≥ 4) based on treatment group over the study period in cats. There were no overall differences between groups ($P = .5295$).

Dogs

A fecal sample was available from a total of 91 dogs (44 dogs with a FS≥4 and 47 dogs with a FS≤3) over the course of the study (Table 2). Based on fecal examinations by centrifugal flotation and IFA, 15.4% (14 of 91) were positive for at least 1 infectious agent and 71.4% (10 of 14) of those positive had a normal FS (Table 2). In room 2, 1 dog was concurrently infected with *Taenia* spp. and *Trichuris* spp. and 1 dog was concurrently infected with *Giardia* spp. and *Cryptosporidium* spp. The parasite prevalence rate did not vary between rooms. While dogs with a FS≤3 had a higher prevalence of intestinal parasitism (21.3%) than dogs with a FS≥4 (9.1%), the results were not statistically different ($P = .1481$).

Of the dogs administered SF68 over the course of the study, 102 dogs were housed in the shelter for 2–7 days and were included in the data analysis. The mean and median durations of housing for these dogs were 2.9 and 2 days, respectively. Of the dogs administered the placebo over the course of the study, 80 dogs were housed in the shelter for 2–7 days. The mean and median of housing for these dogs was 3.1 and 3 days, respectively.

Overall, 9.8% (10 of 102) in the SF68 group had at least 1 episode of diarrhea and 12.5% (10 of 80) of dogs in the placebo group had at least 1 episode of diarrhea. However, only 1 dog in each group had diarrhea for ≥2 days and statistical differences were not detected ($P > .05$).

Discussion

The dogs and cats of this study readily ate the SF68 or the placebo and neither substance led to any obvious ill effects. The percentage of abnormal stools varied numerically among cat treatment groups on days 1 and 3 of housing but the results were not statistically different. This finding might reflect a lag time in potential response to probiotic administration. Based on the statistical analysis, cats fed SF68 were less likely to have diarrhea for ≥2 days duration when compared with the control group and thus the results support the hypothesis.

A potential limitation to the cat study was the use of 2 separate populations of cats and rooms. The majority of cats in the stray room had behavioral evidence of domestication and the majority of the cats in the feral room did not. While objective measures were not applied, the investigators and shelter staff overall believe that these behavioral differences could be perceived as greater stress in cats in the feral room. In addition, the differences in the parasite prevalence rates (23.1% in the feral room; 9.2% in the stray room; $P = .0066$) emphasize the differences in case management before entering the shelter. However, we believe that by dividing the study into 2 periods where the SF68 supplemented room and placebo supplemented rooms were switched and inclusion of the parasitism rates in the analysis should have adequately accounted for these variables. Although no studies have been done to demonstrate the stability or sensitivity of SF68 in the environment after gastrointestinal transit, the cages were cleaned and disinfected daily and so the 7-day period should have been adequate to allow any residual organism to die.

In the dogs, statistical differences were not noted but this may have merely related to the low number of dogs that had diarrhea for ≥2 days over the course of the study (1 in each group). As stress diarrhea is common in dogs that are boarded, this finding was unexpected. The median duration of stay for the SF68 and placebo groups was 2 and 3 days, respectively. Therefore, the opportunity

Table 2. Distribution of fecal examination results in dogs housed in 2 rooms of an animal shelter and fed either *Enterococcus faecium* SF68[b] or a placebo.

Room/Fecal Score (FS)	Dogs Positive for 1 or More Parasites (%)	Organism			
		Cystoisospora canis	*Giardia* spp.	*Trichuris* spp.	Other
Room 1					
Total: 51	6 (11.8)	1	3	2	0
FS ≤ 3: 28	4 (14.3)	0	3	1	0
FS ≥ 4: 23	2 (8.7)	1	0	1	0
Room 2[a,b]					
Total: 40	8 (20.0)	2	3	1	4
					Sarcocystis spp. (1)
					Cryptosporidium (1)
					Taenia spp. (1)
					Toxocara canis (1)
FS ≤ 3: 19[a,b]	6 (31.6)	1	2	1	4
FS ≥ 4: 21	2 (9.5)	1	1	0	0
Combined					
Total: 91	14 (15.4)	3	6	3	4
FS ≤ 3: 47	10 (21.3)	1	5	2	4
FS ≥ 4: 44	4 (9.1)	2	1	1	0

See the Table 1 legend for a description of the methods.
[a]One dog in Room 2 was coinfected with *Taenia* spp. and *Trichuris* spp.
[b]One dog in Room 2 was coinfected with *Giardia* spp. and *Cryptosporidium* spp.

to detect diarrhea that continued for ≥2 days in the dog population was low. The shelter staff was also requested to collect all abnormal feces (FS≥4) from any animal included in this study because the dogs defecated at irregular times during the day. As there were several shelter employees that cleaned the runs during a 24-hour period, it is possible that some diarrhea samples were discarded unintentionally. Other reasons for this low occurrence of diarrhea are purely speculative. It is possible that stress to dogs housed in these 2 rooms was minimal as there are only 14 runs per room and the runs are large. The shelter staff anecdotally believes that the dog diarrhea rate in the study period was lower than in previous years. The major change compared with previous years is that in this study, a consistent ration was fed. In previous years, food donations from multiple sources were used and so the dog rooms were rarely fed the same diet consistently and some diets donated may have been of low quality. A larger study in dogs will be required to further evaluate for effects of SF68 on diarrhea rates in shelters or other stressful environments.

There have been several other studies of SF68 administration to dogs or cats housed in research facilities that suggested this probiotic has potential immune-enhancing effects. In 1 study, puppies fed SF68 were compared with a placebo group and were shown to have statistically greater total plasma IgA concentrations ($P \leq .05$), numerically greater fecal IgA concentrations ($P = .056$), and increased canine distemper virus-specific plasma IgG and IgA concentrations over time after vaccination.[10] Healthy cats fed SF68 had greater CD4+ lymphocytes counts compared with cats fed a placebo on week 22 of the study.[11] Lastly, cats with chronic feline herpesvirus-1 infection fed SF68 had less conjunctivitis over time than cats fed a placebo.[9] However, in each of these studies, the positive effects were only evident after long-term supplementation of the probiotic. The maximum amount of time an individual cat in the study described herein was fed the probiotic was 6 days. Thus, we believe that the decreased diarrhea rate in the SF68 group was likely from colonization inhibition effects of the probiotic on the gastrointestinal tract rather than systemic immune-enhancing effects.

The presence of gastrointestinal parasites did not correlate with diarrhea in either dogs or cats of this study. However, that result was not unexpected as each of the parasites detected is known to colonize healthy animals and some of the parasites detected are only rarely associated with diarrhea (*Toxocara* spp.; *Taenia* spp.). In addition, intestinal parasites can shed eggs, cysts, or oocysts intermittently even when diarrhea is present which could have affected the results of the study.

Most dogs or cats housed in animal shelters will not be placed on the adoption floor with diarrhea. Thus, decreasing diarrhea should indirectly save the shelter time and money as well as lessen animal suffering. Whether this potential financial savings is greater than the cost of administering the probiotic to all animals would have to be determined by each shelter or other facilities that house animals and have problems with diarrhea.

Footnotes

Acknowledgment

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