


Standard Article

J Vet Intern Med 2017;31:1406–1413

Randomized, Controlled, Crossover trial of Prevention of Clindamycin-Induced Gastrointestinal Signs Using a Synbiotic in Healthy Research Cats

J.E. Stokes, J.M. Price, and J.C. Whittemore 

Background: Synbiotics often are prescribed to limit antibiotic-associated gastrointestinal signs (AAGS) in cats, but data to support this recommendation are lacking.

Objective: To determine whether synbiotic co-administration mitigates AAGS in healthy research cats treated with clindamycin.

Animals: 16 healthy research cats.

Methods: A randomized, double-blinded, placebo-controlled, 2-way, 2-period, crossover study with a 6-week washout was performed. Each study period consisted of a 1-week baseline and a 3-week treatment period. Cats received 75 mg clindamycin with food once daily for 3 weeks, followed 1 hour later by either 2 capsules of a synbiotic or placebo. Food consumption, vomiting, fecal score, and completion of treatment were compared using repeated measures split plot or crossover designs with covariates, with $P < 0.05$ considered significant.

Results: Cats that received the synbiotic were more likely to complete treatment in period 1 (100% vs. 50%, $P = 0.04$). Cats vomited less when receiving the synbiotic but this was not significant, but there were significant period effects (F-value = 11.4, $P < 0.01$). Cats had higher food intake while receiving the synbiotic (F-value = 31.1, $P < 0.01$) despite period effects (F-value = 8.6, $P < 0.01$). There was no significant effect of treatment on fecal scores, which significantly increased over time (F-value = 17.9, $P < 0.01$).

Conclusions and Clinical Importance: Administration of a synbiotic 1 hour after clindamycin administration decreased hyporexia and vomiting in healthy cats. Additionally, significant period effects suggest that clinical benefits of synbiotic administration persist for at least 6 weeks after discontinuation, decreasing the severity of AAGS in cats that subsequently received clindamycin with placebo. Unlike in people, synbiotic administration did not decrease antibiotic-associated diarrhea.

Key words: Antibiotic-associated diarrhea; Antibiotic-associated gastrointestinal signs; Diarrhea and vomiting; Probiotic.

Antibiotic-associated gastrointestinal signs (AAGS) occur in 1–44% of people;^{1–3} the prevalence of AAGS varies by the antibiotic administered. Antibiotics that are poorly absorbed from the colon or secreted in bile, including clindamycin, are associated with high rates of antibiotic-associated diarrhea in people,^{1,4} and clindamycin is commonly associated with AAGS in people.¹ Adverse drug effects, including AAGS, are a common cause of noncompliance,^{5–9} resulting in premature antibiotic discontinuation in 6–60% of people and

Abbreviation:AAGS antibiotic-associated gastrointestinal signs

increased hospitalization and complications,^{7,10,11} as well as community-acquired antibiotic resistance.^{12–15} Co-administration of probiotics with antibiotics is associated with up to a 3-fold decrease in AAGS in people.^{2,3,14,16}

Clindamycin and other antibiotics cause AAGS in cats,^{17–19} and the prevalence likely varies by antibiotic. The incidence of noncompliance with antibiotic administration in cats is unknown, but 26% of dog owners failed to administer at least 1 antibiotic dose in 1 study of short-term administration.²⁰ Reasons for noncompliance were not determined, but noncompliance with dosing instructions to administer antibiotics on an empty stomach suggests that 1 possible cause was concerns regarding AAGS. Prevention or mitigation of AAGS in animals might increase owner compliance and, thus, decrease patient morbidity and development of community antibiotic resistance.

High-dose clindamycin (26–44 mg/kg/d) was shown to induce AAGS in 100% of healthy research cats in 1 recent study.^a Vomiting was less common in cats concurrently given a synbiotic^b (a commercial mixture of probiotics and prebiotics), but differences were not statistically significant, potentially because of the antibiotic dosage used, concurrent administration of the antibiotic with the synbiotic, or interindividual variability in susceptibility to AAGS. The purpose of our study was to determine the incidence of AAGS in healthy research

From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine (Stokes, Whittemore); and Office of Information Technology (price) at the University of Tennessee, Knoxville, TN.

All work for this project was performed at the University of Tennessee Veterinary Medical Center, Knoxville, TN.

This project was supported by a grant from Nutramax Laboratories Veterinary Sciences, Inc., Lancaster, SC. The funders had no involvement in the design or performance of the study, writing of the manuscript, or the decision to submit the manuscript for publication.

Corresponding author: J. Whittemore, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996; e-mail: jwhittemore@utk.edu.

Submitted January 18, 2017; Revised May 11, 2017; Accepted June 27, 2017.

Copyright © 2017 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1111/jvim.14795

cats treated with a clinically-relevant dose of clindamycin, followed by either a placebo or synbiotic,^b in a blinded, randomized crossover trial. It was hypothesized that PO administration of clindamycin to healthy cats would cause AAGS, but administration of a synbiotic 1 hour after clindamycin would lessen the severity of, or prevent development of, AAGS.

Materials and Methods

Animals

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Tennessee, College of Veterinary Medicine (Protocol number 2375). Sample size calculation, performed using data from a previous study,^a was performed for each of 3 variables: food intake, vomiting, and fecal score. These calculations indicated that 7 cats per group would need to complete each study period to detect a 10% difference in food intake, 10% difference in vomiting, or a 1-point difference in fecal score between groups with an alpha of 0.05 and beta of 0.2, in the absence of period effects. To accommodate potential exclusion of cats from enrollment because of potential health conditions and removal as a consequence of severe AAGS during treatment, 19 overtly healthy, purpose-bred, domestic short-haired research cats initially were enrolled in the study. After completion of physical examination and laboratory assessment (CBC, plasma biochemistry, urinalysis), 3 cats were eliminated from the study: 1 each because of aggression, abnormal physical examination findings, and abnormal laboratory test results.

One week before the start of the study, the 16 remaining cats were moved from group housing to individual cages. No other changes were made to the cats' husbandry during the study. Cats were maintained on their individual maintenance diet plans and portion-fed during both individual and group housing. During the week of acclimation, daily food intake was quantitated, cats were monitored daily for the presence of vomiting, and photographs of daily voided feces were taken by an independent observer for scoring using a published fecal scoring system^c by 1 investigator (JCW).²¹ Based on appropriate caloric intake relative to current weight and body condition score, lack of vomiting, and normal fecal scores (median, 2; range, 1–3), all 16 cats were considered healthy and were retained in the study. Based on review of their medical records, 14 cats had received antibiotics during their lifetime: 12 cats received antibiotics >2.5 years before the start of the study and 2 cats within the previous 6 months. Thus, cats were stratified by previous antibiotic use and then randomized to 1 of 2 groups (A or B) with a random number generator.^d

Study Periods

A randomized, double-blinded, placebo-controlled, 2-way, 2-period, crossover study with a 6-week washout was performed. Each study period was 4 weeks long, comprised of a 1-week baseline followed by a 3-week treatment period. For the first 5 weeks of the washout period, cats were returned to group housing, after which they underwent 1 week re-acclimation to individual housing in order to identically match the 2 treatment periods.

Treatments

Each cat received 75 mg clindamycin PO once daily (median dosage, 18.0 mg/kg/d; range, 12.1–22.7 mg/kg/d) after being fed its daily ration of commercial food. One hour after antibiotic administration, each cat received either 2 capsules of the synbiotic^b

or placebo PO. Cats in Group A received placebo in period 1 and synbiotic in period 2. Conversely, cats in Group B received synbiotic in period 1 and placebo in period 2. All cats were syringe fed 2 mL of water after each treatment. If a cat vomited an intact capsule within 1 hour of administration, a new capsule was administered.

Animal Observations

An observer blinded to the treatment group quantitated daily food intake and vomiting (present or absent). Any vomiting, including vomiting of hairballs, was considered true vomiting for the purposes of the study. Feces also were photographed daily by the blinded observer. Body weight was measured every 7 days. Cats were removed from a treatment period if they had <50% of baseline food intake for 3 consecutive days, vomited on 3 consecutive days, or lost $\geq 6\%$ of body weight.

After completion of each treatment period, photographs of daily voided feces were randomized so that investigators were blinded to cat identity, time point, and treatment group. Investigators independently scored photographs of feces with a published fecal scoring system^c after completion of each treatment period, instead of after completion of the study, as a consequence of the performance of an interim analysis after the conclusion of the first treatment period (see Results below).

Statistical Analysis

Descriptive statistics were calculated for each variable. Samples were analyzed for normality using the Shapiro-Wilk test and for the presence of outliers using box-and-whisker plots. Age and weight for the 2 sequence groups were compared using an independent 2-sample Student's *t*-test. Mean percent food intake, percent days of vomiting, and mean fecal scores were determined for each week of each study period (baseline and treatment weeks 1, 2, and 3). Mean food intake for each week in each study period was calculated as a percentage of food intake during the acclimation week. Inter-rater correlation coefficients were calculated for fecal scores. The mean of fecal scores assigned by the 2 investigators was used for all further statistical analyses. Successful completion of treatment was defined as completion of a treatment period (e.g., not being removed from treatment because of excessive hyporexia, vomiting or weight loss). Cats that did not complete a treatment period were censored from data analyses at the point of removal from treatment.

Because there was a marked difference in successful completion of treatment of the first study period between the 2 groups (see Results below), an interim analysis was performed during the washout period to confirm that continuation of the trial was necessary and, thus, ethically appropriate. For the interim analysis, successful completion of treatment, mean food intake, percent days vomiting per week, and mean fecal score were compared between groups using a repeated measures split plot design with covariates. Treatment (A or B), week, and cat were included as categorical variables. Treatment, week, and the treatment-by-week interaction were included as fixed effects. Week was included as a repeated measure with subject as cat. Age, weight, and sex were included as covariates. Cat nested within treatment was included as a random effect. A 1-sided Fisher's exact test was used to compare completion of treatment between groups. Although the treatment completion percentage of sequence Group B was significantly higher than Group A (Group A, 50%; Group B, 100%, $P = 0.04$), no other statistically significant differences were found. The second treatment period therefore was performed.

For the final analysis, a 2-treatment, 2-sequence, 2-period AB/BA crossover design with repeated measurements within periods

was performed that included fixed effects of treatment (A or B), order of treatment, week, and treatment-by-week interaction. Age, weight, and sex were included as covariates in the analysis. The repeated measure of time period was accounted for in a repeated statement. Cat nested within sequence group was included as a random effect. A compound symmetry variance/covariance structure was incorporated into each model to account for the inclusion of constant covariates over time (age and weight). The Shapiro-Wilk test of normality of the residuals was evaluated for each marker to confirm that the assumption of normally distributed residuals had been met. Model assumptions regarding equality of variances were verified using Levene's test for equality of variances. Differences in least squares means were determined for markers with significant main effect or interaction terms.

Commercial statistical software packages^{d,e} were used for all analyses. $P < 0.05$ was considered significant.

Results

Animals

There were 5 female spayed (FS) and 3 male castrated (MC) cats in Group A and 2 FS cats and 6 MC cats in Group B. Median age was 7 years (range, 7–10 years) for Group A and 9 years (range, 5–10 years) for Group B. Median weight was 3.9 kg (range, 3.3–6.2 kg) for Group A and 4.3 kg (range, 3.4–5.3 kg) for Group B. There were no significant differences in age ($P = 0.35$) or weight ($P = 0.91$) between sequence groups. Weight did not differ between first and second baseline for cats in either group. No cat vomited any of the administered capsules during the study. Weight, and thus antibiotic dose, was not associated with any of the analyzed outcome variables.

Successful Completion of Treatment

The percentage of cats in each group completing each week of treatment is summarized in Figure 1. During period 1, 4 cats (50%) from Group A were removed from treatment because of vomiting on 3 consecutive days; 1 cat had concurrent hematemesis on the third day of vomiting. In contrast, all cats in Group B

successfully completed treatment. Completion of treatment differed significantly ($P = 0.04$) between groups based on interim analysis, with more cats completing treatment in the group that received the synbiotic.

During period 2, 1 cat in Group A was removed from treatment because of vomiting for 3 consecutive days, and 1 cat in Group B was removed because of weight loss. The cat from Group A that was removed in period 2 was 1 of the cats that failed to complete treatment in period 1. Based on analysis of the crossover data, successful completion of treatment period was not significantly associated with treatment (F-value = 3.0, $P = 0.09$) or any other analyzed variable.

Vomiting

Vomiting increased in both groups with treatment (Table 1) and differed significantly over time (F-value 6.9, $P < 0.01$). Order of treatment was significantly associated with vomiting (F-value 11.4, $P < 0.01$), but treatment (placebo versus synbiotic) was not (F-value 2.1, $P = 0.15$). Increasing age (F-value 10.7, $P < 0.01$) and female sex (F-value 14.6, $P < 0.01$) were significantly associated with vomiting.

Food Intake

Food intake (Table 2) decreased significantly over time (F-value 5.7, $P < 0.01$), and food intake was significantly higher when cats received synbiotic versus placebo (F-value 31.1, $P < 0.01$). There also was a significant effect of period on food intake (F-value = 8.6, $P < 0.01$), primarily reflecting 3 cats that initially received placebo and had markedly higher food intake during the second baseline period.

Fecal Scores

The inter-rater correlation coefficient for fecal scores was 0.72 (95% confidence interval [CI], 0.66–0.77) in period 1 and 0.69 (95% CI, 0.64–0.74) in period 2.

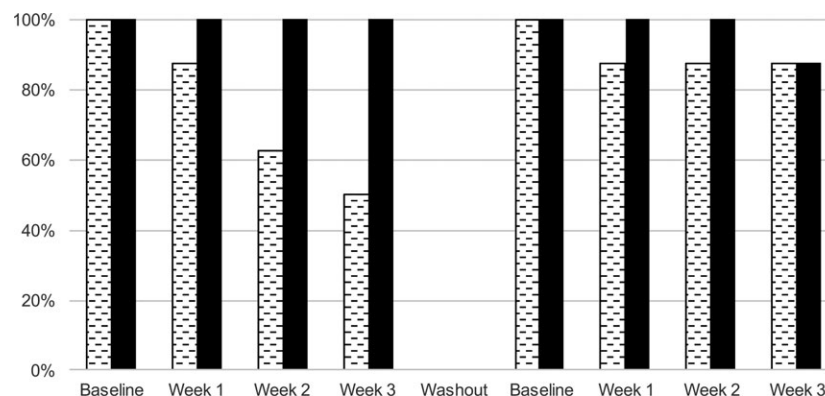


Fig 1. Percentage of 16 healthy cats, 8 per group, that completed treatment with 75 mg clindamycin PO once daily for 21 days, followed 1 hour later by 2 capsules of either placebo or synbiotic PO. Group A: Cats in the column represented by a dashed bar received placebo during period 1 and synbiotic in period 2. Group B: Cats in the column represented by a solid bar received synbiotic during period 1 and placebo during period 2.

Table 1. Mean (\pm standard deviation) percent days vomiting per week for 16 healthy cats, 8 per group, that received 75 mg clindamycin PO once daily for 21 days, followed 1 hour later by 2 capsules of either placebo or synbiotic PO. Cats in Group A received placebo during period 1 and synbiotic in period 2, whereas cats in Group B received synbiotic during period 1 and placebo during period 2.

	Period 1				Period 2			
	Baseline	Treatment			Baseline	Treatment		
		Week 1	Week 2	Week 3		Week 1	Week 2	Week 3
Group A	8.9 \pm 13.0	23.7 \pm 26.2	38.4 \pm 30.6	29.2 \pm 26.3	1.8 \pm 5.0	26.8 \pm 33.7	10.2 \pm 10.8	2.0 \pm 5.4
Group B	5.4 \pm 10.6	21.4 \pm 21.6	16.1 \pm 11.9	16.1 \pm 14.2	1.8 \pm 5.0	17.9 \pm 18.3	8.9 \pm 10.6	7.1 \pm 7.6

Table 2. Mean (\pm standard deviation) percent food intake per week for 16 healthy cats, 8 per group, that received 75 mg clindamycin PO once daily for 21 days, followed 1 hour later by 2 capsules of either placebo or synbiotic PO. Cats in Group A received placebo during period 1 and synbiotic in period 2, whereas cats in Group B received synbiotic during period 1 and placebo during period 2.

	Period 1				Period 2			
	Baseline	Treatment			Baseline	Treatment		
		Week 1	Week 2	Week 3		Week 1	Week 2	Week 3
Group A	92.5 \pm 12.7	80.7 \pm 16.1	75.1 \pm 20.0	74.5 \pm 17.5	112.1 \pm 21.8	109.4 \pm 23.4	97.4 \pm 24.8	94.5 \pm 29.6
Group B	91.8 \pm 8.9	92.3 \pm 15.3	98.0 \pm 10.6	82.4 \pm 16.5	89.4 \pm 15.0	92.0 \pm 15.5	82.1 \pm 15.5	72.9 \pm 26.8

Fecal scores (Table 3) significantly increased over time (F-value = 17.9, $P < 0.01$). There was no significant effect of treatment group ($P = 0.37$) or period ($P = 0.10$) on fecal scores. Three cats that initially received placebo had mean fecal scores >4 during the second baseline. Interestingly, these cats were not the same cats that had markedly increased food intake during the same time period.

Discussion

Veterinarians often recommend prophylactic administration of probiotics to minimize AAGS in cats and dogs receiving antibiotics, although objective data to support this recommendation are lacking in veterinary medicine. In our study, administration of a synbiotic 1 hour after antibiotic administration significantly decreased hyporexia in healthy cats receiving clindamycin, although it did not prevent development of diarrhea. Synbiotic administration was associated with increased likelihood of completion of antibiotic

treatment as a result of decreased frequency of vomiting on multiple sequential days based on interim analysis, although statistical significance was not maintained after completion of the crossover. Cats initially treated with the synbiotic had a significantly lower frequency of vomiting when treated with antibiotics in conjunction with placebo, compared to cats that initially received the placebo, despite a prolonged washout period. If decreased vomiting reflected habituation to repeated antibiotic administration, then both groups should have experienced equivalent decreases in the frequency of vomiting during the second period of the study. Thus, the presence of significant period effects suggests a prolonged protective effect of prior synbiotic administration against future development of AAGS, even in the absence of repeated synbiotic administration. This finding likely confounded the analysis of successful completion of treatment. Review of the data indicated significantly increased food intake in the absence of weight change 6 weeks after discontinuation of antibiotics for 37.5% (3/8) of cats that initially received

Table 3. Mean (\pm standard deviation) fecal score per week for 16 healthy cats, 8 per group, that received 75 mg clindamycin PO once daily for 21 days, followed 1 hour later by 2 capsules of either placebo or synbiotic PO. Cats in Group A received placebo during period 1 and synbiotic in period 2, whereas cats in Group B received synbiotic during period 1 and placebo during period 2.

	Period 1				Period 2			
	Baseline	Treatment			Baseline	Treatment		
		Week 1	Week 2	Week 3		Week 1	Week 2	Week 3
Group A	1.7 \pm 0.3	3.5 \pm 1.1	4.5 \pm 1.3	4.8 \pm 0.9	3.2 \pm 1.3	2.6 \pm 1.0	4.0 \pm 1.8	3.6 \pm 1.6
Group B	1.7 \pm 0.3	3.6 \pm 1.2	4.4 \pm 1.6	3.3 \pm 1.5	2.1 \pm 1.0	2.6 \pm 1.0	3.3 \pm 1.9	3.1 \pm 1.9

placebo, but not for cats that initially were treated with the synbiotic. Fecal scores also were increased during the second baseline for cats that initially received the placebo compared to those for cats treated with synbiotic (although this difference did not reach significance), with 37.5% (3/8) of them having fecal scores >4 . In total, changes consistent with chronic enteropathy were noted during the second baseline in 75% (6/8) of cats that initially received the placebo. These results suggest development of antibiotic-induced chronic enteropathy in cats treated with the placebo, but not in cats that initially received the synbiotic. These results are consistent with results of a recently completed study that identified persistent antibiotic-induced dysbiosis in cats treated with clindamycin.^a

Although concomitant administration of the synbiotic significantly decreased hyporexia and increased the likelihood of successful completion of antibiotic therapy, it did not prevent development of diarrhea. The observed lack of mitigation of diarrhea after synbiotic administration differs from most previous reports in cats.^{21–23} Probiotic administration was associated with a significant decrease in diarrhea in cats admitted to an animal shelter in 1 double-blind study,²² and mean fecal scores significantly decreased after synbiotic administration to cats with idiopathic chronic diarrhea in 1 open-label trial.²¹ Similarly, probiotic administration resulted in increased weight gain and decreased diarrhea (a common cause of death) in juvenile cheetahs.²³ In contrast, no difference was found in incidence of diarrhea in weanling kittens treated with probiotics in 1 recent placebo-controlled study.^f Although similar studies have not been reported for cats, time to resolution of acute idiopathic diarrhea is significantly shorter in dogs treated with probiotics.^{24,25} These studies all assessed the response of naturally occurring gastrointestinal disease to probiotic or synbiotic administration, not prevention of AAGS. Thus, the discordance in findings could reflect different mechanisms of action. Different results also could reflect use of different probiotic or synbiotic formulations, although 1 of the previous studies used the same product as used in our investigation.²¹ Clindamycin previously has been shown to stimulate the motilin receptor in the small intestine,⁴ an effect that might not be attenuated by synbiotic administration. To the authors' knowledge, it is unknown which AAGS are most likely to affect client compliance. Interestingly, some cats in the study had sporadic frequency of defecation (once every 2 days) in the face of marked diarrhea (fecal scores ≥ 6), suggesting high fecal score might not necessarily equate with defecation urgency. Because privately owned cats typically have free access to either a litterbox or the outdoors for defecation, a cat's fecal score might be less likely to impact client compliance than vomiting, hyporexia, or weight loss in the absence of inappropriate elimination. Because dogs do not necessarily have the same access for appropriate defecation in case of urgent need, potential beneficial effects of synbiotic administration on client compliance should be extrapolated to that species with caution.

The assumed mechanism for AAGS is an imbalance in the intestinal microbiome.^{26,27} Positive effects of

probiotics on the intestinal microbiome of healthy cats have been demonstrated previously in 2 studies, as were decreased fecal pH (which can inhibit bacterial enteropathogenic species), fecal ammonia concentrations, plasma endotoxin concentrations, and red blood cell fragility (which could be caused by decreased circulating toxins).^{28,29} Because no studies had been published regarding the effects of different antibiotics on the microbiome of cats at the time of study development, the washout period was chosen after review of the literature regarding antibiotic effects on the microbiome of dogs, as well as the effects of pro- and synbiotics on markers of gastrointestinal health and immune function in healthy cats and dogs. In 1 study, healthy dogs treated for 4 to 7 days with amoxicillin had changes to the microbiome and antibiotic resistant patterns that lasted up to 14 days after antibiotic discontinuation.³⁰ Similarly, administration of tylosin for 14 days to healthy dogs resulted in persistent changes to the microbiome 14 days after discontinuation.³⁰ Conflicting data exist regarding the effects of probiotics on immune function. Previous studies in healthy cats found improved fecal ammonia concentrations²⁹ and immunomodulatory factors²⁸ 10 and 28 days after discontinuation of probiotic supplementation, respectively. Additionally, histologic and immunohistochemical changes consistent with decreased inflammation recently were reported for cats with chronic megacolon treated for 90 days with a probiotic, but not for cats treated with a placebo.^g Conversely, no changes were identified in the immune function of healthy cats and dogs after 21 days of administration of the synbiotic used in our study.³¹ Furthermore, clearance of the probiotic bacteria was demonstrated 3 days after discontinuation of supplementation in that study. Based on potentially prolonged immunomodulatory effects after probiotic administration, we elected to use an extended washout period compared to that used in previous studies. Despite this, significant period effects were identified. Cats treated with the synbiotic in period 1 were significantly less likely to have AAGS when receiving placebo in the second period, compared to cats that received the placebo initially. Additionally, 75% of cats that received placebo in period 1 developed evidence of antibiotic-induced chronic enteropathy, which was not anticipated. Prior antibiotic exposure increases the risk of AAGS in people,³² suggesting that AAGS should have been more common or severe during the second treatment period. As a result, our study likely underestimates the beneficial effects of synbiotic administration on prevention of AAGS.

Evidence-based guidelines regarding probiotic use for prevention of AAGS are lacking in veterinary medicine. The ideal bacterial strains, their relative proportions, overall colony-forming units (cfu), and benefits of concurrent prebiotic inclusion likely vary by indication, and some effects appear to be dose-dependent.^{32–34} Higher dosages of probiotics currently are recommended for management of active gastrointestinal disease in animals.^{35,g} Therefore, we elected to use a dose of 2 capsules of synbiotic per cat. It is unknown

whether similar results would be obtained using lower cfu or a different balance of pro- and prebiotics.

Conflicting positions have been taken regarding the benefits versus risk of inclusion of antibiotic-resistant bacterial strains in probiotics.^{36–39} Antibiotic-resistant bacteria might improve protection against AAGS,^{36,37} but they also could serve as a source of antibiotic-resistant genes for normal flora.^{38,39} As a result, the use of resistant strains in probiotics generally is discouraged, and it is explicitly banned by the European Food Safety Authority.^{38,39} Given this information, results of the prior study using high-dose clindamycin,^a and anecdotal experience with improved efficacy by a staged administration protocol (JCW, personal experience), we chose to administer the synbiotic 1 hour after clindamycin administration. Although the synbiotic was administered PO to assure complete ingestion, many probiotics and synbiotics are formulated to allow administration on or mixed with food without compromising efficacy, eliminating the need for pet owners to directly administer such medications.

As has been found in people, the incidence and severity of AAGS have been shown to vary by antibiotic in dogs.^{30,40,h} Adverse gastrointestinal effects were not noted during or after amoxicillin or tylosin therapy in healthy dogs,^{30,40} but a high incidence of diarrhea (56%) was noted after metronidazole administration in another recent report.^h The incidence of AAGS in cats similarly might vary for different antibiotics. Clindamycin was chosen for our study because it seems to be associated with a high incidence of AAGS in cats, as has been documented in people.^{4,26} Clindamycin is available in liquid and capsule formulations. Because many cats tolerate the taste of the liquid formulation poorly, commercial capsules were used for our study. Variability in the size of subjects resulted in a wide dosing range, but weight (and, thus, antibiotic dosage) was not significantly associated with the frequency of AAGS. However, the value of synbiotic administration in mitigating AAGS might differ for cats receiving antibiotics other than clindamycin.

A few other limitations to this study should be noted. The first was the use of healthy cats as subjects. Although cats in our study had fewer AAGS while receiving clindamycin during or after synbiotic administration, results might differ in systemically ill cats. Additionally, most of the cats had received antibiotics previously, although only 2 within the previous 2.5 years. Administration of antibiotics, such as macrolides, can cause dysbiosis that persists for up to at least 4 years in people,⁴¹ and we recently have demonstrated persistence of microbiome alterations >600 days after clindamycin administration in cats.^a Given associations between prior antibiotic administration and increased risk of AAGS in people, AAGS might have been more severe for cats in our study than would be identified in antibiotic-naïve cats. Age was positively associated with AAGS, but no interaction between age and response to treatment was identified. Significant differences previously have been demonstrated between the microbiome of older cats (ages 8–15 years) compared to kittens.^{42,43}

Some studies^{11,44} have found that probiotics do not decrease AAGS in older people (>65 years), although other studies suggest strong benefits⁴⁵ and several meta-analyses have confirmed their efficacy in adults and children.^{16,32,33,46} The lack of collinearity between age and treatment effect in our study suggests that synbiotics have prophylactic effects independent of age in cats. Confirmation of these findings in a study population with a greater percentage of geriatric cats is warranted, however, because the majority of the cats in our study were middle-aged, with a median age of 9 years. The association between female sex and vomiting also warrants further scrutiny. No association was found between sex and AAGS in a previous study performed in cats.^a The majority of studies of AAGS in people employ sex-matching. However, 1 prospective study in which patients were not sex-matched to controls found no association between sex and development of AAGS.⁴⁷ Human females, however, have been found to be at increased risk of community-acquired, but not hospital-acquired, *C. difficile* infection.⁴⁸ Patients with community-acquired infection were less likely to have prior antibiotic exposure (78%) than those with hospital-acquired infection (94%), although the prevalence of antibiotic exposure was extremely high in both groups. Finally, cats were maintained on their individual maintenance diets, instead of being placed on 1 or more standardized diets. Although this design improves the applicability of results to clinical practice in which there is marked heterogeneity in patient diets, it is unknown whether results would differ in cats that received concomitant alterations in their diets.

In conclusion, concurrent synbiotic administration significantly increased the likelihood of completion of a 3-week course of clindamycin in healthy cats, and it was associated with improved food intake and decreased frequency of vomiting during antibiotic administration. Initial administration of clindamycin with placebo instead of the synbiotic was associated with persistence of clindamycin-induced chronic enteropathy 6 weeks after antibiotic discontinuation in 75% (6/8) of cats. Mitigation of AAGS has the potential to improve patient outcome and decrease community-acquired antibiotic resistance by increasing owner compliance in completing antibiotic treatment. Further evaluation of the impact of synbiotic administration in ameliorating AAGS in clinically ill cats and dogs receiving antibiotics is warranted.

Footnotes

^a Whittemore JC, Stokes JE, Laia N, Price JM, Suchodolski J. Short and long-term effects of a synbiotic on clinical signs, fecal microbial diversity and metabolomic profiles in healthy research cats receiving clindamycin: a randomized, controlled trial. 2017 ACVIM Forum Research Abstract Program. *J Vet Intern Med*, 31. <https://doi.org/10.1111/jvim.14778>

^b Provable-DC, Nutramax Laboratories Veterinary Sciences, Inc., Lancaster, SC

- ^c Greco DS. Diagnosis and dietary management of gastrointestinal disease. Available at: https://www.purinaveterinarydiets.com/media/1202/gi_quick_reference_guide.pdf. Accessed July 11, 2016
- ^d MedCalc 15.8 MedCalc Software, Ostend, Belgium
- ^e SAS 9.4 release TS1M3, SAS Institute Inc., Cary, NC
- ^f Marks S, McDonnell S, Smith C, et al. Effect of the probiotic *Enterococcus faecium* SF 68 on presence of diarrhea in weanling kittens. 2015 ACVIM Forum Research Abstract Program. J Vet Intern Med, 29. <https://doi.org/10.1111/jvim.12609>
- ^g Rossi G, Jergens A, Cerquetella M, et al. The effect of the probiotic Sivoy on clinical and histopathological parameters in cats with chronic idiopathic constipation and megacolon. 2015 ACVIM Forum Research Abstract Program. J Vet Intern Med, 29. <https://doi.org/10.1111/jvim.12609>
- ^h Olson E, Honneffer J, Waddle M, et al. Evaluation of the effects of a 2 week treatment with metronidazole on the fecal microbiome of healthy dogs. 2015 ACVIM Forum Research Abstract Program. J Vet Intern Med, 29. <https://doi.org/10.1111/jvim.12609>

Acknowledgments

Conflict of Interest Declaration: Dr. Whittemore has received honoraria from Nutramax Laboratories for development of educational materials and public speaking.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials in this study.

References

- McFarland LV. Antibiotic-associated diarrhea: Epidemiology, trends, and treatment. *Future Microbiol* 2008;3:563–578.
- Lenoir-Wijnkoop I, Nuijten MJ, Craig J, et al. Nutrition economic evaluation of a probiotic in the prevention of antibiotic-associated diarrhea. *Front Pharmacol* 2014;5:13.
- Hempel S, Newberry SJ, Maher AR, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: A systematic review and meta-analysis. *J Am Med Assoc* 2012;307:1959–1969.
- Bartlett JG. Antibiotic-associated diarrhea. *New Engl J Med* 2002;346:334–339.
- Llor C, Hernandez S, Bayona C, et al. A study of adherence to antibiotic treatment in ambulatory respiratory infections. *Int J Infect Dis* 2013;17:e168–e172.
- Pechere JC, Hughes D, Kardas P, et al. Non-compliance with antibiotic therapy for acute community infections: A global survey. *Int J Antimicrob Agents* 2007;29:245–253.
- Chan YH, Fan MM, Fok CM, et al. Antibiotics nonadherence and knowledge in a community with the world's leading prevalence of antibiotics resistance: Implications for public health intervention. *Am J Infect Control* 2012;40:113–117.
- Muñoz EB, Dorado MF, Guerrero JE, et al. The effect of an educational intervention to improve patient antibiotic adherence during dispensing in a community pharmacy. *Aten Primaria* 2014;46:367–375.
- Kardas P, Devine S, Golembesky A, et al. A systematic review and meta-analysis of misuse of antibiotic therapies in the community. *Int J Antimicrob Agents* 2005;26:106–113.
- Jefferds MD, Laserson K, Fry AM, et al. Adherence to antimicrobial inhalational anthrax prophylaxis among postal workers, Washington, D.C., 2001. *Emerg Infect Dis* 2002;8:1138–1144.
- Jafarnejad S, Shab-Bidar S, Speakman JR, et al. Probiotics reduce the risk of antibiotic-associated diarrhea in adults (18–64 years) but not the elderly (>65 years): A meta-analysis. *Nutr Clin Pract* 2016;31:502–513.
- Perez-Gorricho B, Ripoll M, Group PS. Does short-course antibiotic therapy better meet patient expectations? *Int J Antimicrob Agents* 2003;21:222–228.
- Larson E. Community factors in the development of antibiotic resistance. *Annu Rev Public Health* 2007;28:435–447.
- Selinger CP, Bell A, Cairns A, et al. Probiotic VSL#3 prevents antibiotic-associated diarrhoea in a double-blind, randomized, placebo-controlled clinical trial. *J Hosp Infect* 2013;84:159–165.
- Omotosho BA, Adebayo AM, Adeniyi BO, et al. Tuberculosis treatment outcomes and interruption among patients assessing DOTS regimen in a tertiary hospital in semi-urban area of south-western Nigeria. *Niger J Med* 2014;23:51–56.
- Goldenberg JZ, Lytvyn L, Steurich J, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea (Review). *Cochrane Database Syst Rev* 2015;(12):CD004827.
- Suspected drug adverse reactions reported to the Bureau of Veterinary Drugs. *Can Vet J* 1995;36:246–249.
- Hunter RP, Lynch MJ, Ericson JF, et al. Pharmacokinetics, oral bioavailability and tissue distribution of azithromycin in cats. *J Vet Pharmacol Ther* 1995;18:38–46.
- Albarellos GA, Landoni MF. Current concepts on the use of antimicrobials in cats. *Vet J* 2009;180:304–316.
- Adams VJ, Campbell JR, Waldner CL, et al. Evaluation of client compliance with short-term administration of antimicrobials to dogs. *J Am Vet Med Assoc* 2005;226:567–574.
- Hart ML, Suchodolski JS, Steiner JM, et al. Open-label trial of a multi-strain synbiotic in cats with chronic diarrhea. *J Feline Med Surg* 2012;14:240–245.
- Bybee SN, Scorza AV, Lappin MR. Effect of the probiotic *Enterococcus faecium* SF68 on presence of diarrhea in cats and dogs housed in an animal shelter. *J Vet Intern Med* 2011;25:856–860.
- Koeppel KN, Bertschinger H, van Vuuren M, et al. The use of a probiotic in captive cheetahs (*Acinonyx jubatus*). *J S Afr Vet Assoc* 2006;77:127–130.
- Herstad HK, Nesheim BB, L'Abée-Lund T, et al. Effects of a probiotic intervention in acute canine gastroenteritis – a controlled clinical trial. *J Small Anim Pract* 2010;51:34–38.
- Kelley RL, Minikhiem D, Kiely B, et al. Clinical benefits of probiotic canine-derived *Bifidobacterium animalis* strain AHC7 in dogs with acute idiopathic diarrhea. *Vet Ther* 2009;10:121–130.
- McFarland LV. Evidence-based review of probiotics for antibiotic-associated diarrhea and *Clostridium difficile* infections. *Anaerobe* 2009;15:274–280.
- Vidlock EJ, Cremonini F. Meta-analysis: Probiotics in antibiotic-associated diarrhea. *Aliment Pharmacol Ther* 2012;35:1355–1369.
- Marshall-Jones ZV, Baillon ML, Croft JM, et al. Effects of *Lactobacillus acidophilus* DSM13241 as a probiotic in healthy adult cats. *Am J Vet Res* 2006;67:1005–1012.
- Biagi G, Cipollini I, Bonaldo A, et al. Effect of feeding a selected combination of galacto-oligosaccharides and a strain of *Bifidobacterium pseudocatenulatum* on the intestinal microbiota of cats. *Am J Vet Res* 2013;74:90–95.
- Grønvold AM, L'Abée-Lund TM, Sørsum H, et al. Changes in fecal microbiota of healthy dogs administered amoxicillin. *FEMS Microbiol Ecol* 2010;71:313–326.
- Garcia-Mazcorro JF, Lanerie DJ, Dowd SE, et al. Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. *FEMS Microbiol Ecol* 2011;78:542–554.

32. Ouwehand AC, Forssten S, Hibberd AA, et al. Probiotic approach to prevent antibiotic resistance. *Ann Med* 2016;48:246–255.
33. Szajewska H, Konarska Z, Kołodziej M. Probiotic bacterial and fungal strains: Claims with evidence. *Dig Dis* 2016;34:251–259.
34. Pagnini C, Saeed R, Bamias G, et al. Probiotics promote gut health through stimulation of epithelial innate immunity. *Proc Natl Acad Sci USA* 2010;107:454–459.
35. Rossi G, Pengo G, Caldin M, et al. Comparison of microbiological, histological, and immunomodulatory parameters in response to treatment with either combination therapy with prednisone and metronidazole or probiotic VSL#3 strains in dogs with idiopathic inflammatory bowel disease. *PLoS ONE* 2014;9:e94699.
36. Stropfová V, Lauková A, Ouwehand AC. Selection of enterococci for potential canine probiotic additives. *Vet Microbiol* 2004;100:107–114.
37. Tian P, Xu B, Sun H, et al. Isolation and gut microbiota modulation of antibiotic-resistant probiotics from human feces. *Diagn Microbiol Infect Dis* 2014;79:405–412.
38. EFSA Panel on Additives and Products or Substances used in Animal Feed Scientific. Opinion on the safety and efficacy of Proctora Max (*Bifidobacterium animalis*) as a feed additive for dogs. *EFSA Journal* 2012;10:2964–2978.
39. Sharma P, Tomar SK, Goswami P, et al. Antibiotic resistance among commercially available probiotics. *Food Res Int* 2014;57:176–195.
40. Suchodolski JS, Dowd SE, Westermarck E, et al. The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. *Microbiology* 2009;9:210.
41. Jakobsson HE, Jernberg C, Andersson AF, et al. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS ONE* 2010;5:e9836.
42. Jia J, Frantz N, Khoo C, et al. Investigation of the faecal microbiota of geriatric cats. *Lett Appl Microbiol* 2011;53:288–293.
43. Jia J, Frantz N, Khoo C, et al. Investigation of the faecal microbiota of kittens: Monitoring bacterial succession and effect of diet. *FEMS Microbiol Ecol* 2011;78:395–404.
44. Wright K, Wright H, Murray M. Probiotic treatment for the prevention of antibiotic-associated diarrhoea in geriatric patients: A multicentre randomised controlled pilot study. *Australas J Ageing* 2015;34:38–42.
45. Hickson M, D'Souza AL, Muthu N, et al. Use of probiotic *Lactobacillus* preparation to prevent diarrhea associated with antibiotics: Randomized double blind placebo controlled trial. *BMJ* 2007;335:80–85.
46. Lau CSM, Chamberlain RS. Probiotics are effective at preventing *Clostridium difficile*-associated diarrhea: A systematic review and meta-analysis. *Int J Gen Med* 2016;9:27–37.
47. Wiström J, Norrby SR, Myhre EB, et al. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: A prospective study. *J Antimicrob Chemother* 2001;47:43–50.
48. Khanna S, Pardi DS, Aronson SL, et al. The epidemiology of community-acquired *Clostridium difficile* infection: A population-based study. *Am J Gastroenterol* 2012;107:89–95.