Article

Effects of a perioperative antibiotic and veterinary probiotic on fecal dysbiosis index in dogs

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Abstract

Background

Although widely used, the effects of perioperative antibiotics on the gastrointestinal microbiome are still being researched. The role of probiotics to ameliorate adverse effects of perioperative antibiotics is unclear. The dysbiosis index (DI), based on a quantitative polymerase chain reaction (qPCR) technique, is used to assess gastrointestinal health.

Objective

The DI in dogs receiving perioperative antibiotics and the effects of concurrent probiotics were evaluated in this prospective study.

Animals and procedures

Baseline and 48-hour postoperative fecal DI were evaluated in 20 cliented-owned dogs undergoing hemilaminectomy. Eleven dogs received a probiotic and 9 received placebo.

Results

Preanesthetic DI was not different between treatment groups ($P = 0.378$). One bacterial group, *Blautia,* decreased in the placebo group ($P = 0.002$); however, there was no change in the probiotic group ($P = 0.336$). The DI increased numerically after probiotic administration, but the time × treatment interaction was not significant ($P = 0.996$).

Conclusion and clinical relevance

Administration of a probiotic failed to improve DI. Further investigation is needed to evaluate long-term effects of perioperative antibiotics on the gut microbiome.

Résumé

Effets d'un antibiotique périopératoire et d'un probiotique vétérinaire sur l'indice de dysbiose fécale chez le chien

Contexte

Les antibiotiques périopératoires sont largement utilisés, mais leurs effets sur le microbiome gastro-intestinal sont toujours à l'étude. Le rôle des probiotiques dans l'amélioration des effets indésirables liés aux antibiotiques périopératoires n'est pas clair. L'indice de dysbiose (ID), fondé sur une technique de PCR quantitative, est utilisé pour évaluer la santé gastro-intestinale.

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Objectif
Cette étude prospective a évalué l'ID chez les chiens recevant des antibiotiques périopératoires ainsi que tout effet lié à l'administration d'un probiotique en simultané.

Animaux et protocole
Les valeurs d'ID de référence ainsi que 48 heures après la chirurgie ont été évaluées chez 20 chiens subissant une hémilaminectomie (11 chiens ont reçu un probiotique; 9 ont reçu un placebo).

Résultats
L'ID préanesthésique n'était pas différent entre les deux groupes (P = 0,378). Un groupe bactérien, *Blautia*, a diminué dans le groupe placebo (P = 0,002); il n'y a eu aucun changement dans le groupe probiotique (P = 0,336). L'ID a augmenté quantitativement après l'administration de probiotiques, mais l'interaction « temps × traitement » n'était pas significative (P = 0,996).

Conclusion et portée clinique
L'administration d'un probiotique n'a pas amélioré l'ID. Des recherches supplémentaires sont nécessaires pour évaluer les effets à long terme des antibiotiques périopératoires sur le microbiome intestinal.

Introduction

Perioperative antibiotics are commonly used in veterinary medicine and although antibiotic use can prevent surgical infections, such use is not benign (1–3). Antibiotic use may negatively impact the normal gastrointestinal bacterial microbiome and cause dysbiosis (4), even within only 24 h of usage (5). The gastrointestinal microbiome has an integral role in systemic health and disease (6–8). Disturbances in the composition of the microbiome can affect metabolism, energy homeostasis, immune function, and gut epithelial health (9).

Assessment of the gastrointestinal microbiota may be accomplished by methods such as culture (10), next generation sequencing (11), or fecal PCR analysis, which provide a comprehensive picture of the organisms (pathogenic and nonpathogenic) present in the gastrointestinal tract (12). The dysbiosis index (DI) is an objective measurement of gastrointestinal health (13). This index uses quantitative PCR (qPCR) to detect the population of specific bacterial groups, and the presence and numbers of each species are mathematically combined to generate the DI. A DI < 0 indicates a healthy microbiome, whereas DI ≥ 0 is consistent with dysbiosis. Certain enteropathies in dogs are characterized by an increased DI, and DI has high sensitivity and specificity for discriminating healthy dogs from those with chronic enteropathies (13). The DI increased with antibiotic use in healthy dogs (14) and in dogs with acute diarrhea treated with antibiotics (15). The DI, although a relatively new diagnostic tool, has been used to discern dysbiosis in dogs with disease processes that were not primarily gastrointestinal in origin (e.g., lymphoma), but this requires further investigation, such as the current report, for evidence of utility in dogs with other diseases (16). A PCR-based microbiota analysis using next generation sequencing detected changes in the microbiome of horses after transport, 12 h of fasting, and 24 and 48 h following anesthesia (11). The DI, although a less comprehensive overview of the microbiome, may also be useful as a quantitative measure of change in the gastrointestinal microbiome of dogs undergoing anesthesia and surgery.

The gastrointestinal microbiome may also be modulated by probiotics. Probiotics are formulations of live organisms that confer beneficial effects on the recipient when delivered in adequate amounts (17). Most commonly, probiotics are administered orally to support the gastrointestinal microbiome, stimulating short-chain fatty acid production, displacing pathologic bacteria, and promoting immunomodulation (18–20). Additionally, probiotics can replenish the host's beneficial gastrointestinal microflora and restore balance to a microbiome that has been altered by stress or antimicrobials (11,21,22).

The acute effects of perioperative antibiotics on the microbiome of dogs, with and without probiotic supplementation, has not been reported. The objective of this study was to evaluate the changes in DI in a group of hospitalized dogs that received perioperative antibiotics. An additional objective was to evaluate the effect of a commercially available veterinary probiotic on the observed changes in DI. We hypothesized that perioperative antibiotics result in a worsened DI and that probiotic administration mitigate this change.

Materials and methods

This prospective study took place at the University of Georgia Veterinary Teaching Hospital. Dogs were prospectively enrolled from August 2018 to August 2019. Client-owned dogs undergoing hemilaminectomy were enrolled after informed consent was given by the owner. The study protocol was approved by the clinical research committee and carried out in accordance with institutional animal care and use guidelines (Protocol #CR-524, Jan 26, 2018). Included dogs were undergoing surgery for intervertebral disc disease (IVDD) and had an expected postoperative hospitalization period of at least 48 h. Exclusion criteria included: gastrointestinal disease such as diarrhea in the week prior to enrollment, concurrent disease unrelated to IVDD that may cause diarrhea [e.g., inflammatory bowel disease (IBD), food allergy, liver disease], antibiotic use on admission or outside the immediate perioperative period (defined as $>$ 1 h before or after anesthesia, within 14 d), concurrent administration of an NSAID and steroid without at least a 3-day washout period, administration of any other medication known to cause diarrhea or immunosuppression (defined as $>$ 2 doses of $>$ 2 mg/kg) of prednisone equivalent in the case of corticosteroids, or animals in which administration of oral medications was contraindicated (i.e., those with vomiting, regurgitation, or severe aggression).

An initial power calculation was done, with a change in DI of 2 from a preoperative to a 48-hour postoperative value was deemed to be clinically significant. This suggested a target enrollment of 13 dogs; therefore, 2 groups were planned with a target of 13 dogs per group. A change in DI of 2 was subjectively chosen, as this represented approximately 10% of the range in results used to generate the DI (−9.1 to 9.3) (13); this has also been used previously (23). Enrolled patients were randomized using an online random number generator (random.org) to receive either placebo or probiotic, with the first dose administered ~12 h after recovery from anesthesia and the second given 24 h later. A commercially available veterinary probiotic and placebo (Visbiome; ExeGi Pharma, Rockville, Maryland, USA) were dosed according to package instructions (1 capsule per dose). As per the manufacturer's insert, the probiotic contained at least 112.5×10^9 colony-forming units (CFU) per capsule. The strains of live, lyophilized bacteria included *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis.* Fecal samples were collected by rectal swab using a clean, cotton-tipped applicator before perioperative antibiotic administration; a second sample was collected 48 h after recovery. All dogs received 2 doses of prescribed treatment during the 48-hour study period. All dogs received intraoperative cefazolin and had not received NSAIDs prior to anesthesia. All dogs were fed according to a standardized protocol, offering their normal diet if available first, then a series of prescription gastrointestinal diets, and lastly chicken. Some dogs received peanut butter for administration of the pills. The amount of diet was offered according to attending clinician preference, although the standard of care at the hospital where the study was done is to offer the resting-energy requirement daily. Appetite was typically recorded subjectively (good, fair, poor, none) and the amount of food consumed could not be determined. Animals with poor or no appetite for 1 diet were offered the next diet on the standardized protocol. The time during which food was consumed in relationship to sample collection was not recorded.

Fecal samples were frozen at −80°C until batch analysis at a university veterinary teaching hospital laboratory (TAMU Gastrointestinal Laboratory, College Station, Texas, USA). Aside from the method of sample collection (swab), DI was determined as described (13), DNA was extracted from each fecal sample, and 7 bacterial taxa were amplified using qPCR. The qPCR data were described as the log amount of DNA (fg) for each bacterial group per 10 ng isolated DNA in total. LogDNA was expressed for each bacterial group *(Faecalibacterium, Turicibacter, Streptococcus, Escherichia coli, Blautia, Fusobacterium* and *Clostridium hiranonis)*, and then combined using a mathematical algorithm to generate the DI. A DI < 0 indicates a healthy microbiome, whereas a DI ≥ 0 indicates fecal dysbiosis.

Statistical methods

Statistical analyses were performed using SAS 9.4 software (Cary, North Carolina, USA). Descriptive statistics were calculated as mean ± standard deviation. Data were assessed for normality using a Shapiro-Wilk test. The qPCR data were expressed as the log amount of DNA (log SQ) for each bacterial group/10 ng of isolated total DNA. The DI between groups (placebo and probiotic), the log SQ for individual species, and comparisons between pre- and postanesthetic DI in all dogs were performed using linear mixed models. The full model for each measurement included fixed factors for treatment group, time (pre- or post-) and a treatment group by time interaction effect. Additionally, a random intercept for each dog was included to account for within dog correlation. Histograms and Q-Q plots of conditional model residuals were examined to evaluate the assumption of normality. Plots of conditional residuals *versus* predicted values of measurements were examined to evaluate the assumption of homogeneity of variances. Multiple comparisons were corrected using the Hochberg correction. Satterthwaite degrees of freedom method and REML estimation were used. Baseline comparisons between placebo and probiotic groups was performed with an unpaired Student's *t*-test. *P*-values < 0.05 were considered significant.

Results

Twenty dogs were enrolled in the study. Breeds included 15 Dachshunds (75%), 2 shih tzus (10%), 2 mixed breed dogs (10%), and 1 Xoloitzcuintli (5%). The mean age was 6.45 ± 2.60 y (range: 2.7 to 12.1 y). Mean body weight was 6.92 ± 2.42 kg (range: 4.6 to 15.8 kg). Nine dogs were spayed females (45%) and 11 dogs were neutered males (55%). At admission, 7 dogs (35%) received prednisone, 4 (20%) received methocarbamol, 6 (30%) received gabapentin, 2 (10%) received tramadol, and 1 (5%) received trazodone, phenobarbital and diphenhydramine aside from monthly preventatives and daily nutraceuticals (exact doses and manufacturers unknown, therapy instituted by referring veterinarian). The mean duration of anesthesia for MRI and hemilaminectomy was 267 ± 70 min (range: 164 to 467 min). There was no difference in duration of anesthesia between dogs receiving placebo and probiotic ($P = 0.41$). All dogs received a standard dose of cefazolin (22 mg/kg) every 90 min. Otherwise, the anesthetic protocol for pre-medication, induction, and maintenance was at the discretion of the anesthesiologist, generally including a preanesthetic agent with either hydromorphone or methadone, combined with midazolam, induction of anesthesia with propofol, with or without ketamine, and maintenance of anesthesia using isoflurane. Analgesia was supplemented with fentanyl in 9 dogs (45%) or lidocaine in 6 dogs (30%). All dogs received a fentanyl constant rate infusion (CRI) for analgesia for the first day after surgery. Eleven dogs (55%) were given atropine during anesthesia. Eighty-five percent of dogs ($n = 17$) experienced a systolic blood pressure < 90 mmHg at some point during anesthesia.

qPCR analysis and dysbiosis index

A total of 40 paired fecal samples were collected from 20 dogs for qPCR analysis. Eleven dogs received probiotic treatment and 9 received placebo (Figures 1, 2). Treatment was well-tolerated in all dogs. Preanesthetic DI was not different between treatment groups ($P = 0.378$). The only bacterial group with a significant time × treatment interaction was *Blautia,* which decreased in

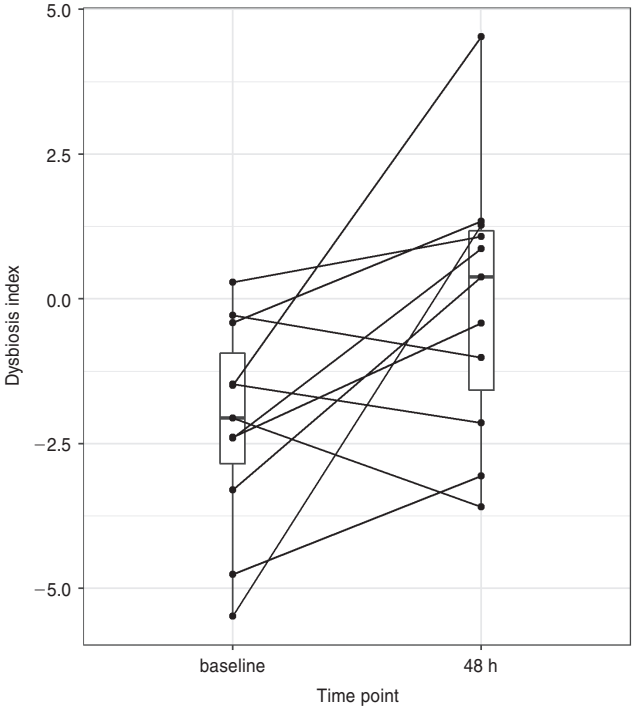

Figure 1. Dysbiosis index for 11 dogs receiving probiotic. The DI was more positive at the 48-hour time point; however, the time × treatment interaction was not significant for either group ($P = 0.996$). Dots represent individual patient data, with lines showing change in DI. The baseline DI before administration of anesthesia or antibiotics is displayed on the left and the 48-hour post-surgery for hemilaminectomy on the right. The bold lines indicate the median and the box indicates the 50th percentile.

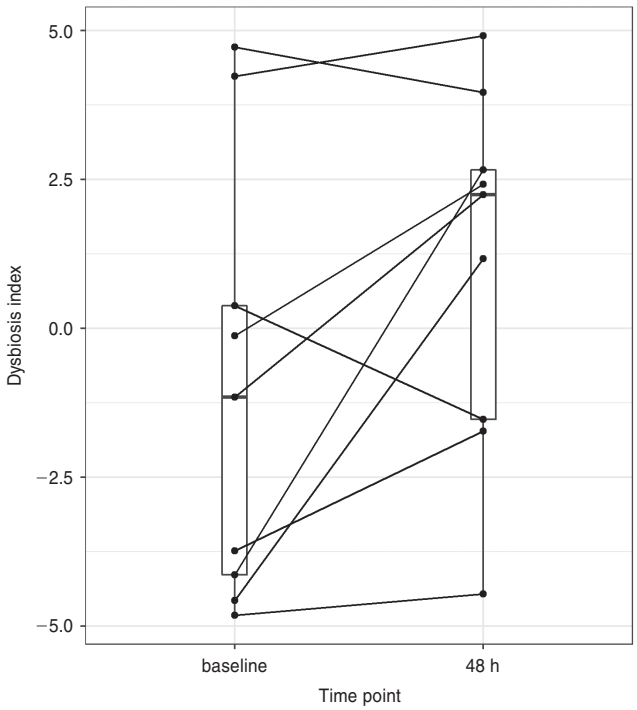

Figure 2. Dysbiosis index for 9 dogs receiving placebo. The DI was more positive at the 48-hour time point; however, the time × treatment interaction was not significant for either group ($P = 0.996$). Dots represent individual patient data, with lines showing change in DI. The baseline DI before administration of anesthesia or antibiotics is displayed on the left and the 48-hour post-surgery for hemilaminectomy on the right. The bold lines indicate the median and the box indicates the 50th percentile.

the placebo-treated group ($P = 0.002$), but did not change in the group receiving probiotics ($P = 0.336$; Table 1). Although DI was more positive at the 48-hour time point, the time × treatment interaction was not significant ($P = 0.996$). The abundance of each bacterial taxon and the DI were compared between the post-anesthetic placebo and probiotic groups and there were no differences (Table 1).

Discussion

In contrast to our hypothesis, there was no change in DI in dogs following anesthesia and surgery, including the administration of perioperative antibiotics. There are several possible explanations for these findings. Most importantly, the time course of the investigation was relatively short. Paired samples were acquired at baseline and 48 h later, primarily to ensure that postoperative fecal samples were collected before patient discharge from the hospital. Whereas most prior studies utilizing DI evaluated single time points in a target group *versus* a comparison group (13) and not over time, 1 study evaluated DI following 7 d of treatment with a probiotic (24). There is evidence to support alterations of the gastrointestinal microbiome even with short-term antimicrobial administration (5,25); however, some studies reported no difference in DI during treatment with certain antibiotics (23). Additionally, the sample collection method used in this study (clean swab) instead of that used in the creation and validation of the DI (100 mg of naturally passed sample) (13) may have introduced a bias into the results by limiting the number of bacteria for analysis.

The 1 significant change was a decrease in the presence of *Blautia* spp. in the group that was not treated with probiotic. *Blautia* is a member of the *Lachnospiraceae,* and is decreased from normal population prevalence in dogs with acute hemorrhagic diarrhea and in those with chronic enteropathy and inflammatory bowel disease (26). *Blautia* spp. apparently contribute to intestinal health through formation and excretion of short-chain fatty acids (e.g., butyrate) and other metabolites that can support intestinal mucosal health (27). The degree of decrease in prevalence of *Blautia* spp. in this population was relatively small (log SQ decrease from a mean value of 10.2 to 9.3), and there was no difference between the treated and placebo dogs in the 48-hour prevalence. Perhaps collection of samples from a larger number of dogs would further elucidate this change in population, and longer periods of observation may provide insight into the implications of decreases in this species prevalence.

The worsening of the DI in the group receiving a probiotic following 48 h of therapy, despite the lack of a significant time × treatment interaction, was unexpected. One possible cause of this increase is the presence of *Streptococcus* in the probiotic used, as this particular bacterium is included in calculation of the dysbiosis index. Additionally, the predominant genera present in the probiotic used in this study were *Lactobacillus* and

Table 1. Analysis of selected bacterial groups (qPCR) in dogs pre- and 48 h post-anesthesia and comparison between placebo and probiotic treatment groups. Pre- *versus* post-anesthesia *P*-value provided for *Blautia;* other pre- *versus* post-48 h *P*-values are not listed, as the interaction statistic revealed no statistical significance.

Bacterial group	Interaction statistic (time × treatment)	Group	Time point	Mean (log DNA)	Standard deviation	Median	*P*-value (pre- *versus* post-)
Dysbiosis index	0.996	placebo	pre	−1	3.7	−1.2	
			48 h	1.1	3	2.2	
		probiotic	pre	−2.2	1.8	−2.1	
			48 h	−0.1	2.3	0.4	
Blautia	0.042	placebo	pre	10.2	0.4	10.3	
			48 h	9.3	0.9	9.1	0.002
		probiotic	pre	9.9	0.6	10.1	
			48 h	9.6	0.8	10.1	0.336
Escherichia coli	0.633	placebo	pre	5	2.1	5.4	
			48 h	5.2	1.8	5.4	
		probiotic	pre	4.7	1.7	5	
			48 h	4.5	1.9	5.1	
Faecalibacterium	0.729	placebo	pre	5.8	1.1	6.3	
			48 h	5.1	0.7	5.2	
		probiotic	pre	5.8	0.9	5.6	
			48 h	5.2	0.9	5.4	
Fusobacterium	0.227	placebo	pre	8.9	0.8	8.9	
			48 h	7.5	1.1	7.4	
		probiotic	pre	9.1	0.6	9.3	
			48 h	8.4	1.2	8.2	
Clostridium hiranonis	0.694	placebo	pre	4.9	2.3	5.9	
			48 h	3.7	2.3	3.8	
		probiotic	pre	6	0.8	6.1	
			48 h	5.1	1.8	5.3	
Streptococcus	0.139	placebo	pre	5.6	1.4	5.1	
			48 h	5.5	1.1	6	
		probiotic	pre	5.4	0.6	5.2	
			48 h	6.4	1.2	6.1	
Turicibacter	0.291	placebo	pre	5.7	0.6	5.7	
			48 h	4.8	0.7	4.6	
		probiotic	pre	5.8	0.6	5.5	
			48 h	5.3	0.9	5.5	
Universal	0.259	placebo	pre	10.8	0.4	10.9	
			48 h	10.1	0.6	10.3	
		probiotic	pre	10.6	0.5	10.8	
			48 h	10.3	0.7	10.4	

Bifidobacterium. These genera are not included in the 7 primary taxa used to form the dysbiosis index, so the specific effect of the probiotic (i.e., introducing or promoting growth of favorable bacteria) may have been underestimated. The taxa chosen for the DI were those with the best performance for discriminating dogs with chronic enteropathies; dogs with chronic gastrointestinal diseases were excluded in this study, which may have made it difficult to interpret the true effect in this population of dogs (13). Perhaps more prolonged treatment with a probiotic may be required to show beneficial alterations in DI, but the lack of significant change in the DI of these dogs following anesthesia and perioperative antimicrobials raises the question as to whether this therapy is necessary in dogs undergoing similar procedures with similar perioperative management and duration of hospitalization.

Another reason for the lack of overall change in the DI may be because the DI measures quantitative DNA present. Therefore, even if gut bacteria were killed by perioperative antibiotics, if DNA from killed organisms remained in the colon at the time of sampling, it might be analyzed as present, resulting in a false negative. Perhaps a longer interval between collection of samples would demonstrate an effect. In this study population, 80% of patients had a baseline DI < 0, indicating a healthy microbiome, at the time of enrollment. It is possible that antibiotics and probiotics may not have as profound an impact on this specific patient population compared to a population

with pre-existing dysbiosis. Alternatively, the exclusion criteria may have been insufficient to exclude all dogs with pre-existing dysbiosis that would be detected by DI. Many antibiotics can impact the microbiome for months to years (28), and validation of the DI was performed on dogs not receiving antibiotics for at least 3 mo (13). Some dogs enrolled in this study may have received antibiotics within 3 mo, and it is unclear how this impacted the DI and results of this study. Attempts were made to minimize the impact through randomization and there was no difference in preanesthetic DI between groups.

The impact of a specific antibiotic on the gastrointestinal microbiome differs within antimicrobial class (29). Patients in this study received perioperative cefazolin. This antibiotic is efficacious against Gram-positive bacteria, which includes many of the groups in the DI. Other types and classes of antibiotics may have a different effect on the DI when used in this context.

There were several limitations of this study, not least of which included a small sample size and short time course of investigation. There was no control group for the primary objective of this study, as it was considered outside of the hospital standard of care to withhold antibiotics from dogs undergoing spinal surgery. Additionally, evaluation of a group of dogs undergoing anesthesia alone (MRI only) to control for the effects of surgery was not done. This was determined to be outside the goal of the study, which was to evaluate the factors of antibiotics and probiotics on the DI of dogs undergoing both surgery and anesthesia. Future studies are warranted to investigate the effect of anesthesia alone on DI (30). Although statistical significance was achieved only in the placebo group, we did not meet our recruitment targets for either group, decreasing the power of analysis in the placebo group (power of a 1-tailed test with alpha = 0.050 was 0.64). There is some evidence that not all neurosurgeries require perioperative antibiotics, so future studies may be designed with a comparator group that isolates the role of antibiotics in the observed changes (31). These results should be interpreted considering the significant limitations. Separate studies for more finite control of every variable, with large sample sizes, could be considered to address these concerns.

There was no prescribed anesthetic protocol for the dogs in this study, although most dogs received the same medications (generally premedication with an opioid and benzodiazepine, induction of anesthesia with propofol, and maintenance of anesthesia with isoflurane). The minor variability amongst protocols may have had unknown effects on the microbiome, and the use of medications known to affect intestinal motility was not restricted. Several dogs received atropine and/or lidocaine during anesthesia and all dogs received postoperative opioid analgesia, which could have altered intestinal motility and affected the ability to obtain fecal samples that were fully representative of all microbiome changes throughout the GI tract. Additionally, no physiologic data that may have helped clinically corroborate DI information, such as fecal scores or the development of diarrhea, were obtained due to low fecal output following anesthesia in most patients (i.e., many samples were obtained by swab).

As DI is a relatively new tool in the investigation of the gastrointestinal microbiome, there are some limitations associated with its use in a clinical research setting. In this study, there was no comparison of the results to previously validated methods of microbiome analysis, such as next-generation sequencing. However, research evaluating the impact of metronidazole on microbiota had good agreement between 16S sequencing and DI (32). The use of a different diagnostic modality might confirm results or provide insight into the utility of different testing methodologies in the investigation of perianesthetic dysbiosis in a clinical population.

All enrolled patients were fed according to protocol, but the desired food was not always the same. Diet, particularly fiber content, can impact the composition of the gastrointestinal microbiome and may have affected our results (33). The length of time each patient was hospitalized before surgery (at our institution or at the referring veterinarian) was also not standardized, which may have affected patient stress levels, intestinal motility, and magnitude of diet change from the patient's normal diet. Due to the nature of this particular study population, dogs may have had surgery on the day of admission or several days later, depending on their neurologic function.

In this small population, there was no significant effect on DI in dogs undergoing hemilaminectomy and receiving perioperative cefazolin and a placebo or probiotic medication. Change in prevalence was only noted in 1 bacterial species, and this is of unknown clinical relevance. Future studies are indicated to further investigate the long-term effects of perioperative antibiotics, as well as perioperative probiotic administration, on the canine gastrointestinal microbiome.

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