A cute hemorrhagic diarrhea syndrome (AHDS) in dogs presents with sudden onset of hemorrhagic, watery diarrhea, often accompanied by vomiting, anorexia, and lethargy. Treatment primarily involves fluid therapy, analgesics, and antiemetics. Rapid clinical improvement is observed in most cases without the need for antibiotics. Histopathological examinations have revealed necrotizing enterocolitis and layers of Clostridium perfringens strains adherent to the necrotic surface, and fecal real-time PCR (qPCR) analysis has been found to show an increase in the abundances of C perfringens in dogs with AHDS compared to healthy dogs. Based on a summary of all current information, it seems very likely that overgrowth by C perfringens type A strains associated with the release of NetF toxins, possibly in combination with other toxins, is responsible for the necrotic enterocolitis in dogs with AHDS. Clostridial strains, such as C perfringens and Clostridioides difficile, have also been associated with the disease.
with some gastrointestinal diseases in human medicine.\textsuperscript{3,10} For example, \textit{C difficile} infections can lead to severe colitis in humans. In contrast, many bacteria of the Clostridia class, including \textit{C perfringens}, are considered as components of the normal microbiota of humans and animals.\textsuperscript{11–13} The clinical role of different \textit{C perfringens} (eg, enterotoxin-producing strains) and \textit{C difficile} strains, such as enteropathogens, is highly controversial in dogs, and a recent study\textsuperscript{14} has shown that the presence of \textit{C difficile} is strongly associated with an increased dysbiosis index (DI) and a decreased abundance of bile-acid–converting \textit{Peptacetobacter (Clostridium) hiranonis} (\textit{P hiranonis}). \textit{P hiranonis} is an important commensal of the microbiome due to its role in bile acid conversion, and a decrease in \textit{P hiranonis} abundance has been associated with overgrowth of \textit{C difficile} and other clostridial organisms.\textsuperscript{14,15} A previous study\textsuperscript{16} reported a low prevalence of \textit{C difficile} in dogs with AHDS.

Currently, fecal microbiota transplantation (FMT), which involves the transfer of fecal material from a healthy donor to a recipient with an imbalanced gut microbiota, is being explored as a potential treatment option for various gastrointestinal disorders in dogs and cats.\textsuperscript{17} Dogs with AHDS show similar histopathological findings as humans with \textit{C difficile}–induced colitis and dogs with parvovirus. Necrotizing enteritis is present in these diseases and is associated with intestinal dysbiosis.\textsuperscript{3} Fecal microbiota transplantation has shown remarkable success rates in treating recurrent \textit{C difficile} infections.\textsuperscript{17–19} In a study\textsuperscript{20} of dogs with parvovirus, FMT resulted in a significantly faster resolution of diarrhea and significantly shorter hospitalization time. So far, it’s unclear whether FMT could effectively lead to a faster resolution of clinical signs in dogs with AHDS by reducing toxigenic \textit{C perfringens} strains and preventing harmful bacterial colonization. Additionally, FMT may aid in restoring intestinal barrier integrity via a mechanism like mucin secretion, potentially hastening clinical recovery by improving metabolic function and mucosal immune response.\textsuperscript{20,21} The changes in the microbiome and the effects of FMT are often determined by use of the dysbiosis index (DI). This index is based on a mathematical algorithm and has been shown to accurately correlate with general microbiome shifts as assessed by metagenomic sequencing.\textsuperscript{22–24} The goals of this study were to compare the clinical course, changes of potentially enteropathogenic bacterial strains, and DI between dogs with AHDS that were treated with FMT and symptomatic treatment (ST) versus ST alone. It was hypothesized that the use of FMT in dogs with AHDS would result in a faster improvement of clinical signs and intestinal dysbiosis.

**Methods**

**Study design**

This prospective, double-anonymized, randomized case-control study was approved by the Ethics Committee of the Center for Clinical Veterinary Medicine at Ludwig Maximilian University in Munich, Germany (approval No. 128-10-06-2018). Owners were informed of the purpose of the study and signed a written informed consent form prior to study enrollment. All dogs were client-owned and presented between October 2020 and March 2022. The dogs were randomized into 2 groups, generated by an online randomizer in September 2020 (Research Randomizer; Randomizer.org). Dogs receiving antimicrobials were retrospectively grouped into a separate category, especially to assess the (long-term) influence on the intestinal microbiome in comparison to dogs with standard therapy or standard therapy plus FMT.

**Dogs with AHDS**

Dogs of any breed, both sexes, and over 1 year of age were included in the study if the hemorrhagic diarrhea lasted <3 days. Dogs receiving mucosal irritants (such as NSAIDs, doxycycline, and/or corticosteroids) within 1 week prior to diagnosis or dogs with an underlying disease that could also cause acute hemorrhagic diarrhea (such as acute pancreatitis, exocrine pancreatic insufficiency, acute renal and/or liver failure, mechanical obstruction, focal intestinal disorder, or hypoadrenocorticism) were excluded. Therefore, a CBC, serum biochemistry profile, abdominal ultrasound, and fecal examination (floation and SNAP \textit{Giardia} Test; Idexx Laboratories Inc) were consequently performed in all dogs and basal cortisol in 13 dogs. All dogs received ST during hospitalization consisting of a high-fiber gastrointestinal diet (Gastrointestinal Biome; Hill’s Pet Nutrition GmbH), fluid therapy (crystalloids, fluid volume depending on dehydration grade, ongoing losses, and maintenance requirements), antiemetics (metoclopramide, 60 µg/kg/h, IV), and analgesics (buprenorphine, 0.01 mg/kg, IV, q 6 to 8 h; or metamizole, 50 mg/kg, IV, q 8 h). To the best of our knowledge, no medication or diet (eg, raw food) with significant impact on the intestinal microbiota was given. However, before entry in the study, 2 dogs received omeprazole (ST and antibiotic treatment [AT] groups) and 3 dogs may have received a single dose of probiotics administered by clinicians from the emergency service (2 dogs from the ST group and 1 dog from the FMT treatment [FMTT] group).

**Fecal microbiota transplantation**

A single donor was used for the FMTT group: an 11-year-old 18-kg neutered male mixed-breed dog, assessed as healthy based on history, normal physical examination, CBC, serum biochemistry parameters, and T4, vitamin B12, and folic acid levels. The dog was fasted exclusively 1 diet (Gastrointestinal Biome; Hill’s Pet Nutrition GmbH) during the study period and did not receive any supplements or probiotics. Fecal testing revealed a normal dysbiosis index (~3.5) and negative results for endoparasites (floation and SNAP \textit{Giardia} Test; Idexx Laboratories Inc) and enteropathogens (\textit{Salmonella} spp, canine parvovirus, \textit{Campylobacter} spp, \textit{NetF}–toxin–producing \textit{C perfringens}). Donor feces were processed for a maximum of 4 hours after collection. To 10 g of feces, 6 mL of 0.9%
sodium chloride solution and 2.5 mL of 85% glycerol were added, mixed with a blender, and then filled into 100-mL syringes. These syringes were then stored at −80 °C and kept for a maximum of 3 months. The FMT procedure was conducted with a rectal enema on the day of presentation and again after 48 hours. Dogs of the FMTT group received 6 g of feces per kg of body weight as a rectal enema without anesthesia.17,26 The prepared syringes were then carefully defrosted in a warm water bath 1 hour before FMT. Each FMT was performed by the same clinician who was not involved in the clinical care for the dog. After the FMT, the dogs were kept in their kennels and were not allowed to walk for at least 3 hours.

**Antibiotic treatment**

The dogs with AHDS were randomized into 2 groups (FMTT and ST). Based on the decision of the primary responsible clinician, a subpopulation of dogs from both the ST and FMTT groups received antibiotics. These dogs were retrospectively classified as a third study group. The decision as to whether or not dogs received antibiotics was made by clinicians based on the systemic inflammatory response syndrome criteria (Table 1).1,26,27 All dogs that received antibiotics showed at least 2 of these criteria.

**Table 1—Criteria for inflammatory response syndrome (SIRS) for evaluating disease severity in dogs as based on studies by Chu et al,26 Hauptman et al,27 and Dupont et al.**

<table>
<thead>
<tr>
<th>Clinical SIRS criteria</th>
<th>Hypo- or hyperthermia</th>
<th>&lt; 37.5 or &gt; 39.3 °C &lt; 99.5 or &gt; 102.74 °F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tachycardia</td>
<td>&gt; 140 beats/min</td>
<td></td>
</tr>
<tr>
<td>Tachypnea</td>
<td>&gt; 40 breaths/min</td>
<td></td>
</tr>
<tr>
<td>Laboratory SIRS criteria</td>
<td>Leukopenia or leukocytosis</td>
<td>&lt; 6 or &gt; 25 G/L</td>
</tr>
<tr>
<td></td>
<td>Band neutrophils</td>
<td>&gt; 3%</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemia</td>
<td>&lt; 70.2 mg/dL (&lt; 3.9 mmol/L)</td>
</tr>
</tbody>
</table>

**Evaluation of disease severity**

The AHDS index was used to determine clinical severity.2,16,28 For better visualization, this index is shown as a table in the supplementary material (Supplementary Table S1). This score was evaluated daily by a veterinarian during the hospitalization period. The AHDS index of the individual dogs was determined by the same veterinarian every day, with the exception of weekends, when the index was determined by the veterinarian on duty. A posthospitalization diary was completed by the owner to determine the AHDS index 2 times per week over a period of 2 weeks. Both the primary veterinarian and the owner were masked regarding the treatment group. For better objectivity, the Purina fecal scoring system for dogs was used to determine fecal consistency.28 At some time points, the AHDS index could not be determined in all dogs. The AHDS index was available for the following numbers of dogs: day 1, 12 of 12 (ST group), 12 of 12 (FMTT group), and 8 of 8 (AT group); day 2, 12 of 12 (ST group), 12 of 12 (FMTT group), and 8 of 8 (AT group); day 3, 10 of 12 (ST group), 11 of 12 (FMTT group), and 8 of 8 (AT group); week 1, 10 of 12 (ST group), 11 of 12 (FMTT group), and 7 of 8 (AT group); and week 2, 10 of 12 (ST group), 11 of 12 (FMTT group), and 7 of 8 (AT group). For better understanding, this is also shown in a table in the supplementary material (Supplementary Table S2). Dogs were discharged when they presented a good general condition and when the diarrhea was nonhemorrhagic and the frequency was < 3 times per day, so that fluid losses did not have to be compensated by IV fluid therapy.

**Assessment of the intestinal microbiome and clostridial organisms**

Fecal samples were collected on day 1 of the study and by owners at home on days 7, 21, and 42. Fecal samples were frozen immediately after collection during hospitalization or by the owners at home. All dog owners brought the frozen samples for long-term storage at −80 °C on days 42 to 104. The fecal samples were stored at −80 °C for a period between 5 and 550 days. Fecal samples could not be analyzed from every dog on every day (Supplementary Table S2). The analysis was possible in the following numbers of dogs: day 1, 8 of 12 (ST group), 8 of 12 (FMTT group), and 8 of 8 (AT group); day 7, 10 of 12 (ST group), 10 of 12 (FMTT group), and 7 of 8 (AT group); day 21, 11 of 12 (ST group), 10 of 12 (FMTT group), and 7 of 8 (AT group); and day 42, 11 of 12 (ST group), 10 of 12 (FMTT group), and 8 of 8 (AT group).

For analysis, fecal samples were sent on dry ice to the Gastrointestinal Laboratory at Texas A&M University. Samples were assessed for the Di, the C. perfringens 16S rRNA gene, enterotoxin- and NetF-encoding C. perfringens, and C. difficile. The Di is based on qPCR results of the abundance of 7 bacterial taxa (namely, Faecalibacterium, Ruminococcus, Streptococcus, Escherichia coli, Blautia, Fusobacterium, and P. hiranonis) and total bacterial abundance. For assessment of the severity of dysbiosis, a classification of the Di, which has been shown to correlate with metagenomic analysis, was used.22,23 Di ≥ 2 was considered significant dysbiosis, and Di between 0 and 2 was considered mild-moderate dysbiosis. Samples that had a Di < 0 but some bacteria outside the reference interval were characterized as minor changes.

For the C. perfringens 16S rRNA, validated primers were used.29-31 The PCR was performed in a single 30-cycle step by use of the HotStarTaq Plus Master Mix Kit (Qiagen NV). This step was followed by incubation at 94 °C for 3 minutes and then 28 cycles at 94 °C for 30 seconds, at 53 °C for 40 seconds, at 72 °C for 1 minute, and finally an elongation step at 72 °C for 5 minutes.32

The C. perfringens enterotoxin gene and NetF gene were analyzed for each sample with qPCR. Total fecal DNA was extracted and isolated by use of a validated protocol based on a bead-beating procedure with a PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc).28 Validated primers were used for quantification of NetF coding and enterotoxin genes. The PCR was performed with specific conditions: (1) 20 seconds at 95 °C, (2) 5 seconds of 40 cycles at 95 °C, (3) 20 seconds at 72 °C, (4) 10 seconds at 72 °C.
and (3) 10 seconds at the optimized annealing temperature. The master mix of the assays consisted of 10 μL of TaqMan reaction mix. This mixture contains 5 μL TaqMan Fast Universal PCR Master Mix no AmpErase UNG (2X) (Applied Biosystems), 1 μL water, 1 μL of 1% bovine serum albumin, 0.4 μL of each primer, 0.2 μL of probe, and 2 μL of DNA (1:10 or 1:100 dilution). The PCR protocol for SYBR-based assays was performed at 95 °C for 2 minutes, 40 cycles at 95 °C for 5 seconds, and 10 seconds at the optimized annealing temperature. The PCR was performed by use of 10 μL of SYBR-based reaction mix, which contains 1.6 μL of water, 5 μL of SsoFast EvaGreen Supermix (Bio-Rad Laboratories Inc), 2 μL of DNA (1:10 or 1:100 dilution), 0.4 μL of each primer (final concentration, 400 nM), and 1 μL of 1% bovine serum albumin (final concentration, 0.1%).

Clostridioides difficile was also measured with a qPCR assay. A master mix consisting of 2 μL of DNA, 5 μL of SsoFast Probes Supermix, 2.35 μL of water, 0.15 μL of probe, and 0.25 μL of each primer was used. Cycling conditions included incubation for 2 minutes at 95 °C, 40 cycles for 5 seconds at 95 °C, and 10 seconds at the optimized annealing temperature. A commercial qPCR thermal cycler (CFX96 Real-Time PCR Detection System; Bio-Rad Laboratories Inc) was used for the reactions.

Statistical analysis
Excel version 16.71 (Microsoft Corp) and Prism version 9.5.1 (GraphPad Software Inc) were used for the statistical analyses. Normal distribution was tested with the Shapiro-Wilk normality test. For data over time, a mixed-effects model adjusted for multiple comparisons with the Geisser-Greenhouse correction was used. Correlations were performed with the Spearman rank correlation test or Pearson correlation coefficient depending on the distribution. A P value < .05 was considered significant. In order to see a difference of 1 to 3 points between the 2 groups (the group with FMT and the group without FMT), 15 patients per group were required, with σ = 1.5, power > 80%, and P < .05. The power analysis was performed to compare the ST and FMTT groups. Due to the decision of the treating veterinarian, who classified the dogs as septic (all showed at least one of the criteria listed in Table 1), the AT group was formed retrospectively. Within the AT group, no distinction was made between FMT and AT plus ST plus AT, as it was assumed that the antibiotics had the most significant influence on the microbiome. Therefore, all dogs that received antibiotics were combined as 1 group, regardless of the underlying randomized therapy.

Results

Study population
Thirty-two dogs were included in the study. Initially, 16 dogs were randomized to the ST group and 16 dogs to the FMTT group. After enrollment and during hospitalization, 8 of the 32 dogs received antibiotics. One dog from the FMTT group received 12.5 mg/kg of amoxicillin–clavulanic acid every 8 hours on day 3 because of a nephrolith, and a urinary infection was found incidentally. The other 7 dogs received antibiotics based on the decision of the treating veterinarian, who classified the dogs as septic (all showed at least one of the criteria listed in Table 1). Of the 7 dogs in which sepsis was suspected, 4 were in the ST group and the other 3 were in the FMTT group. Of these 7 dogs, 6 received amoxicillin–clavulanic acid at a dosage of 20 mg/kg, IV, every 8 hours, and 1 dog received marbofloxacin at a dosage of 4 mg/kg, IV, for 1 day, and then 2 mg/kg, IV, every 24 hours. This dog was given marbofloxacin because of previous allergic signs to amoxicillin–clavulanic acid. Dogs that were in the FMTT group and received antibiotics still received FMT. Antibiotic therapy was started on day 2 in 6 of 7 dogs and on day 3 in 1 of 7 dogs.

Signalment
The signalment of the different treatment groups is shown in Table 2. The groups did not show a significant difference in age or weight (P > .05).

Clinical severity
Overall, dogs were hospitalized for a mean of 3 days (range, 1 to 5 days). No significant differences in clinical severity based on the AHDS index between the treatment groups at any time point except day 2 were detected. On day 2, the AT group showed a significantly higher (P = .046) AHDS index (mean, 9; range, 4 to 11) compared to the FMTT group, in which the dogs showed a mean AHDS index of 5.7 (range, 2 to 10), as shown in Figure 1.

Table 2—Signalment of the different treatment groups for dogs with acute hemorrhagic diarrhea syndrome.

<table>
<thead>
<tr>
<th></th>
<th>ST group</th>
<th>FMTT group</th>
<th>AT group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>8.1 (1.2-14.1)</td>
<td>5.5 (1.3-10.9)</td>
<td>7.2 (1.5-12.5)</td>
<td>P = .14**</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>11.3 (1.8-19.2)</td>
<td>15.8 (4.5-46)</td>
<td>12.1 (4.4-32)</td>
<td>P = .65**</td>
</tr>
<tr>
<td>Sex</td>
<td>8 females (3 spayed)</td>
<td>5 females (1 spayed)</td>
<td>5 females (3 spayed)</td>
<td>P = .34**</td>
</tr>
<tr>
<td></td>
<td>4 males (2 neutered)</td>
<td>7 males (2 neutered)</td>
<td>3 neutered males</td>
<td>P = .79**</td>
</tr>
</tbody>
</table>

Age and weight for each treatment group are presented as the median and range. A P value < .05 is considered significant. AT = Antibiotic treatment. FMTT = Fecal microbiota transplantation. ST = Symptomatic treatment. The superscript letters indicate the corresponding treatment groups. *AT group. **FMTT group. #ST group.
It has to be highlighted that all dogs in the antibiotic group except the one with the nephrolith and the urinary infection were classified as clinically severe and potentially septic on day 1 (AHDS index ≥ 9). Moreover, the mean hospitalization time for dogs in the AT group was 4 days (range, 3 to 5 days), which was significantly longer compared to the dogs in the ST group (mean, 2 days; range, 1 to 4 days; \( P < .01 \)) and the dogs in the FMTT group (mean, 3 days; range, 1 to 5 days; \( P = .04 \)).

**Assessment of the microbiome and clostridial organisms**

Some dogs independent of the treatment groups showed an increased DI (DI > 0) on day 1 of the study. Significant dysbiosis (DI > 2) was found in 5/24 dogs (3/8 from the ST group and 2/8 from the AT group). The increase in DI was transient in the FMT and ST groups as the majority of dogs had a DI < 0 at day 42. In the AT group, in contrast to the ST and FMT groups, several dogs had a persistently increased DI until day 42, with as many as 3 of 8 dogs showing significant dysbiosis on day 42. No significant difference between the different therapy groups in terms of DI was found except on day 2: on this day, the AHDS index was significantly higher in the AT group than in the FMT group (\( P = .046 \)).

![Figure 1](image1.png)

**Figure 1**—Comparison of the acute hemorrhagic diarrhea syndrome (AHDS) index between the different treatment groups. The AHDS index of each dog is shown as an individual dot. The horizontal lines show the mean values. The gray dots represent the symptomatic treatment (ST) group. The black dots represent the fecal microbiota transplantation treatment (FMTT) group. The red dots represent the antibiotic treatment (AT) group. No significant differences between the treatment groups at any time point were found except on day 2: on this day, the AHDS index was significantly higher in the AT group than in the FMTT group (\( P = .046 \)).

In 75% (5/8 from the ST group, 7/8 from the FMTT group, and 6/8 from the AT group) of the dogs, *C. perfringens* as assessed by the 16S rRNA gene, indicating total *C. perfringens* organisms, increased above the reference interval on day 1. In all but 2 dogs (1 each from the ST and AT groups), *C. perfringens* abundance returned to within the reference interval. Regarding the different treatment groups, there was only a significant difference on day 7 between the ST and AT groups (\( P < .01 \)). On this day, the dogs in the AT group showed a lower *C. perfringens* log DNA than the ST group. Otherwise, no significant differences were detected between the different treatment groups at any other time point.

A total of 67% (5/8 from the ST group, 6/8 from the FMTT group, and 5/8 from the AT group) of the dogs showed *C. perfringens* encoding for enterotoxin above the reference interval on day 1. In all groups, independent of therapy, the increase was transient, and in all but 3 dogs (1 from each treatment group), the abundance returned to within the reference interval on day 42. No significant correlation was observed between clinical severity (AHDS index) and the presence of enterotoxin-encoding *C. perfringens* at any day. No significant difference in the abundance of the *C. perfringens* enterotoxin gene could be determined between the different treatments with the exception of day 7, in which dogs in the ST group had a significantly higher abundance of *C. perfringens* encoding for enterotoxin compared with the AT group (\( P = .01 \)).

On day 1, 67% of all dogs were positive for NetF-toxin–producing *C. perfringens*. A rapid decrease in the prevalence of these organisms in all groups independent of treatment then occurred. By day 7, 22% of all dogs (and by day 21, only 1/28 of the dogs) from the ST group were still positive (Figure 3). No significant differences in NetF-encoding *C. perfringens* between the different groups on any day were found.

In our study, 29% of all dogs showed a reduced abundance of *P. hiranonis* on day 1. This decrease was transient in the ST and FMT groups, as *P. hiranonis* normalized in all dogs except in the AT group.
In the AT group, 4 of 8 dogs still showed reduced *P hiranonis* on day 42. No significant differences between the treatment groups were detected except on day 7 (Figure 4). On this day, the AT group (median, 3.3; minimum to maximum, 0.1 to 6.0) showed a significantly (*P = .03*) lower *P hiranonis* log DNA compared to the FMTT group (median, 6.4; minimum to maximum, 5.3 to 7.1).

Clostridioides difficile was detected in a total of 8 of 32 dogs at different time points (3/24 on day 1, 4/28 on day 7, 3/28 on day 21, and 4/29 on day 42). Five of the 8 dogs also showed a reduction in *P hiranonis*. No significant differences in the abundance of *C difficile* between the different treatment groups at any time point were found. To better visualize the development of *C difficile* between the different treatment groups, a figure was included in the supplementary material (Supplementary Figure S1).

**Discussion**

In this study, symptomatic therapy was compared with the use of FMT in dogs with AHDS regarding the clinical course, changes in potentially enteropathogenic bacterial strains, and DI. From both groups, a third group was established during the clinical trial that consisted of the dogs that received antibiotics in addition to their respective therapy. The study showed that neither FMT nor antibiotic therapy was associated with a faster clinical improvement. In addition, no AT regime was superior to standard treatment with or without FMT alone in reducing the number of *C perfringens* strains.

Fecal microbiota transplantation aims to modulate the recipient’s microbiome to become more similar to that of the donor.17,21,25 Fecal microbiota transplantation is used more frequently in clinical practice in an attempt to modulate intestinal dysbiosis and improve gastrointestinal signs. In a study30 of dogs with lymphoplasmacytic enteritis, a significant improvement in clinical signs at 2 weeks after FMT was detected and a significant increase in *Fusobacterium* spp was also noted. In a case series37 of 16 dogs with chronic diarrhea, the DI normalized 1 week after FMT and the concentrations of *Faecalibacterium* spp and *P hiranonis* increased. In a recently published study,25 FMT was found to be useful as an adjunct to standard treatment for dogs with chronic enteropathy. In that study, 75% of the dogs showed less diarrhea and were more active. In addition, FMT has resulted in faster improvement of diarrhea and shorter hospitalization times in dogs with parvovirosis.20 Based on the similar clinical appearance and histopathologic findings,5 we hypothesized that the use of FMT in dogs with AHDS would be associated with faster clinical improvement and normalization of the microbiome. In our study, all dogs showed a very rapid clinical improvement and none of the dogs died during the observation time due to AHDS, independent of the treatment modality. Dogs in the AT group, which had been retrospectively allocated into this group due to the suspicion of sepsis, presented a tendency toward a higher AHDS index on the first 2 days compared to the other treatment groups. This finding could be explained by the bias of administering antibiotics to more severely affected dogs. Overall, no significant differences in the short-term recovery and clinical course between treatment groups were found. These findings agree with the results from other studies on FMT in dogs with AHDS. In the study by Jugan et al,38 FMT was compared to probiotics and no significant difference in terms of clinical improvement could be determined between the 2 treatment modalities. Similarly, in a study by Gal et al,39 no faster clinical improvement in clinical signs could be obtained with the use of FMT. The modality of FMT could possibly affect the clinical outcome. In this study, FMT was administered to the dogs through a rectal enema. Given that dogs with AHDS exhibit increased fecal defecation frequency and potentially tenesmus, some dogs expelled the FMT shortly after administration. This fact was a limitation of the study, and it is possible that employing an alternative FMT
method, such as delivering feces into the duodenum via gastroduodenoscopy or using lyophilized capsules for oral therapy, would have yielded different results. However, in general, demonstrating any positive treatment impact is challenging due to the self-limiting nature of AHDS, both in terms of clinical signs and inherent intestinal dysbiosis. Subtle variances in clinical improvement and the restoration of the intestinal microbiota might become apparent with a larger number of dogs per study group. Another limitation of the study was that dogs in which an FMT was not performed did not receive a placebo procedure. A placebo FMT could have significantly influenced the fecal consistency and could have provided different results. However, there was no significant difference in fecal consistency between the groups, which is why this was also not considered a primary limitation.

The AT group was included retrospectively, as some dogs required antibiotic administration. This was also a limitation, as this group lacked standardization. Nevertheless, we considered it useful to include the antibiotic group and analyze the comparisons between the 3 groups, as antibiotics have the strongest effect on the microbiome and, in contrast, FMT has a normalizing effect on the microbiome. Furthermore, our results are consistent with other studies\(^1\) that have shown that, in terms of clinical improvement in dogs with AHDS, in most cases (uncomplicated forms of AHDS), antibiotics are not beneficial/necessary and can cause major disruptions in the microbiome that can be long-lasting.

The DI allows a reliable evaluation of global shifts in the intestinal microbiome.\(^2\) In general, dogs with acute diarrhea have mild-moderate changes in the microbiome, which quickly return to normal; significant and persistent increases in the DI, especially with a decrease in *P. hirano*{\textit{n}}is, are usually seen only after antibiotic therapy or in chronic enteropathies.\(^12\) In our study, some dogs showed an increased DI at baseline. This increase was transient in the ST and FMTT groups. Compared to the ST and FMTT groups, the AT group continued to show an increased DI on day 42. A long-term effect of antibiotic therapy on the microbiome could conceivably be the reason for the consistently significant intestinal dysbiosis in those dogs. Thus, it can be concluded that the use of FMT did not produce a significantly more rapid improvement in the changes in the microbiome but that antibiotics have a negative influence on the intestinal microbiome.

A single dog in the ST group must be highlighted, as this dog showed significant dysbiosis from days 1 to 42. During the examinations, a diaphragmatic hernia was found incidentally. As mentioned previously, significant dysbiosis usually results from antibiotic therapy or chronic enteropathy.\(^12\) Since this dog showed no evidence of chronic enteropathy and had not received any recent AT, the diaphragmatic hernia and its potential influence on the intestinal motility could have been a possible cause of the significant dysbiosis.

In our study, 29% showed a reduction in the abundance of *P. hirano*{\textit{n}}is on day 1. This decrease was transient except in the AT group. Within the AT group, 4 out of the 8 dogs exhibited a decrease in *P. hirano*{\textit{n}}is levels, which extended through day 42. Notably, *C. difficile* was identified in all 4 of these dogs. The correlation between these 2 bacterial species has been established in various studies.\(^12\) Similar to the occurrence of significant dysbiosis, a reduction in *P. hirano*{\textit{n}}is is usually found only in dogs with chronic enteropathies or after the use of antibiotics.\(^12\) Thus, in the 4 dogs, the reason for the decreased *P. hirano*{\textit{n}}is on day 42 is most likely a consequence of the antibiotic therapy. However, these dogs showed a reduction in *P. hirano*{\textit{n}}is already on day 1, so it is also conceivable that the dogs had subclinical persistent dysbiosis and were more susceptible to antibiotic-induced dysbiosis.

In our study, *C. difficile* was detected in 3 dogs on the day of presentation. The limited occurrence implies that *C. difficile* is unlikely the causative agent of AHDS. This finding is consistent with the results of the study by Busch et al.\(^12\) in which no significant association between clinical severity and the presence of the *C. difficile* toxin A/B in dogs with AHDS could be seen. On day 42, 4 dogs were positive for *C. difficile*, whereas the log DNA level was significantly lower than at baseline. All dogs were asymptomatic. This finding suggests that *C. difficile* is detectable in some dogs, but its presence has no clinical relevance and, accordingly, does not require specific therapy. What should be mentioned, however, is that 3 of the 4 dogs (all from the AT group) that were positive for *C. difficile* on day 42 showed a reduction in the presence of *P. hirano*{\textit{n}}is on both days 1 and 42. A reduction in the abundance of *P. hirano*{\textit{n}}is (and therefore, abnormal bile acid conversion) is associated with the presence of *C. difficile*, as a normal bile acid metabolism suppresses the proliferation of *C. difficile*.\(^12\) Thus, it is possible that the dogs had subclinical dysbiosis that favored the presence of *C. difficile*.

Nevertheless, it is remarkable that across the treatment groups, all but 2 dogs still showed minor changes in the microbiome on day 42. One recent study\(^4\) showed that dogs have an increase in the risk of developing chronic gastrointestinal signs after an AHDS episode. As one highly possible cause of this increased risk, an intestinal barrier dysfunction present during the acute phase of AHDS resulting in sensitization of the immune system to feed components was discussed.\(^4\) It is also conceivable that mild changes in the microbiome could persist for an extended period of time and thus be associated with chronic gastrointestinal signs in some dogs.\(^6\) Therefore, longer follow-up periods of these veterinary patients are necessary to assess the development of chronic enteropathies and the impact of AHDS on the intestinal microbiome. It would also be interesting to see whether microbiome changes persist longer in dogs that have received antibiotics and whether that group of dogs has an increased risk of developing chronic gastrointestinal signs later in life.

Regarding NetF-encoding *C. perfringens*, nearly 70% of the dogs were positive at presentation, but this finding was transient in all the groups, and on day 42, NetF-encoding *C. perfringens* could be detected in only 1 dog. These results are in agreement with results of other studies\(^6\) in which > 50% of dogs with AHDS were positive for NetF-encoding *C. perfringens* on the day of presentation, and these specific clostridial strains disappeared very quickly even without specific therapy. In our study, no significant differences between the different
treatment groups at any time point were found. This finding supports the conclusion that NetF-toxin-producing *C. perfringens* disappears even in the absence of any specific therapy (FMT or antibiotics). Although 1 dog from the AT group was suddenly positive for NetF-encoding *C. perfringens* again on day 42, the dog had no other changes in the microbiome and was classified as normal. One dog from the AT group was suddenly positive for NetF-encoding *C. perfringens* again on day 42. The dog was asymptomatic, showed no other changes in the microbiome and was categorized as normal based on the DI. This is not completely surprising, as NetF-toxin-producing *C. perfringens* has also been detected in some healthy and asymptomatic dogs, although it has been clearly linked to the pathogenesis of AHDS. This occurrence resembles necrotizing enteritis in chickens, which is caused by NetB-gene–encoding *C. perfringens*. However, research indicates that an infection solely associated with NetB-gene–carrying *C. perfringens* in chickens does not trigger a disease outbreak; instead, a coinfection, such as with coccidia, is necessary. A comparable mechanism might also be applicable to dogs with AHDS, suggesting that additional risk factors may be required for the development of hemorrhagic diarrhea in some dogs. Consequently, the identification of NetF in this dog could be coincidental. In summary, while NetF significantly contributes to the pathogenesis of AHDS, it resolves swiftly and does not necessitate specific treatment.

*Clostridium perfringens* enterotoxin and *C. perfringens* 16S rRNA increased in a majority of AHDS dogs on the day of presentation. While NetF is likely a major virulence factor responsible for AHDS, other extracellular enzymes and minor toxins produced by overgrowing *C. perfringens* strains may play a role in the pathogenesis of this syndrome. Nevertheless, the increase in *C. perfringens* enterotoxin-encoding strains and total *C. perfringens* was transient in all 3 groups and returned in nearly every dog to the reference range regardless of the type of therapy.

In conclusion, affected dogs showed a rapid clinical improvement with symptomatic therapy, and antibiotic interventions were not beneficial in most cases. In addition, Clostridia strains suspected to be associated with AHDS rapidly disappeared irrespective of therapy. While FMT is unlikely to have a significant beneficial impact on the recovery of the intestinal microbiome and the improvement of clinical signs in the acute phase of AHDS, further research is needed to assess its potential on producing a reduction in the frequency of long-term consequences.

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**References**


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.