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Treatment of Aseptic Dogs with Hemorrhagic Gastroenteritis with Amoxicillin/Clavulanic Acid: A Prospective Blinded Study

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Background: Antibiotics generally are recommended to treat hemorrhagic gastroenteritis (HGE). Inappropriate use of antibiotics may promote risk of antimicrobial resistance and unnecessary adverse drug reactions. The necessity of antimicrobial therapy in dogs with HGE has not been demonstrated.

Objective: The purpose of this prospective, placebo-controlled, blinded study was to evaluate whether treatment with amoxicillin/clavulanic acid improves the clinical course and outcome of HGE in dogs that show no signs of sepsis.

Animals: The study included 60 dogs diagnosed with HGE between 2007 and 2009 at the Clinic of Small Animal Medicine, LMU University of Munich, Germany. The inclusion criterion was the presence of acute hemorrhagic diarrhea (<3 days). Dogs pretreated with antibiotics, with signs of sepsis, or diagnosed with any disease known to cause bloody diarrhea were excluded from the study.

Methods: Patients were randomly divided into treatment (amoxicillin/clavulanic acid for 7 days) and placebo groups. To evaluate treatment efficacy, severity of clinical signs (based on a newly developed HGE index), duration of hospitalization, and mortality rate were compared between the 2 groups.

Results: Fifty-three of 60 dogs completed the study. No significant difference between treatment groups concerning mortality rate, dropout rate, duration of hospitalization, or severity of clinical signs, either on any individual day or over the course of disease, was observed.

Conclusions and Clinical Importance: In some dogs with HGE that show no signs of sepsis, antibiotics may not change the case outcome or time to recovery.

Key words: AHD; Bloody diarrhea; Gastrointestinal; HGE.

Acute hemorrhagic diarrhea (AHD) in dogs has numerous potential causes,[1–10] including idiopathic hemorrhagic gastroenteritis (HGE).[11,12] This syndrome of unknown etiology has been defined by acute onset of bloody diarrhea and vomiting accompanied by marked hemoconcentration. A predilection for small breed dogs has been reported.[11–13] Because of the rapid onset of clinical signs, a type 1 hypersensitivity reaction to food components or bacterial endotoxin has been proposed as an inciting cause.[14] In addition, it has been suggested that *Clostridium perfringens* enterotoxin (CPE) and *Clostridium difficile* toxins A&B may be involved in the pathogenesis of this syndrome.[15] Hemorrhagic diarrhea may reflect destruction of the protective intestinal epithelial barrier, resulting in mucosal translocation of resident bacteria, which belong to the normal flora within the lumen of the intestinal tract. Decreased splanchnic blood flow, which is likely present in hypovolemic HGE patients, is an additional factor predisposing to bacterial translocation.[16] Because of the potential bacterial etiology and the risk of sepsis, antibiotics generally are recommended to treat hemorrhagic diarrhea in dogs.

Abbreviations:

AHD	acute hemorrhagic diarrhea
CPE	*Clostridium perfringens* enterotoxin
HGE	hemorrhagic gastroenteritis
TG	treatment group
PG	placebo group

Frequently, amoxicillin/clavulanic acid is used as a first antibiotic choice in these cases. This potentiated aminopenicillin is a broad-spectrum, time-dependent antibiotic drug that is effective against most Gram-positive, some Gram-negative, and anaerobic bacteria, including potentially enteropathogenic organisms (eg, some *Clostridia* spp., *Escherichia coli*, and *Salmonella* spp.).

However, in dogs, the frequency and clinical relevance of bacterial translocation and the role of bacteria as inciting agents of HGE, and thus, the necessity of with antibiotics, are not known. Moreover, inappropriate usage of antibiotics can cause disruption of the protective intestinal flora,[17,18]postantibiotic salmonellosis, *C. difficile*-associated diarrhea, and antibiotic resistance.[16,19–22] Metronidazole, a drug commonly used in patients with diarrhea, may even increase the translocation of indigenous strains of intestinal bacteria.[23] Thus, the aim of this prospective, placebo-controlled, and blinded study was to evaluate the benefit and efficacy of amoxicillin/clavulanic acid in the treatment of dogs with aseptic HGE.

Materials and Methods

Patients

This study was a prospective, randomized, blinded treatment trial. It was conducted according to the German animal welfare law.

From the Clinic of Small Animal Medicine (Unterer, Strohmeyer, Kruse, Hartmann) and the Clinic for Ruminants, LMU University of Munich, Munich, Germany (Sauter-Louis). This study was performed at the Clinic of Small Animal Medicine, LMU University of Munich, Veterinaerstrasse 13, 80539 Munich, Germany. *Some of the results have been presented at the ECVIM Congress, Toulouse, France, September 9–11, 2010.*

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Owners were informed about the purposes of the study, and all owners signed a written consent form.

Between August 2007 and September 2009, 60 dogs were diagnosed with HGE at the Clinic of Small Animal Medicine, LMU University of Munich, Germany. The inclusion criterion was an acute onset of bloody diarrhea (<3 days). Patients pretreated with antibiotics, those with potential signs of sepsis (rectal temperature >39.5°C, white blood cell (WBC) count <4 or > 25×10^9/L, band neutrophil count > 1.5×10^9/L) and those with hemorrhagic diarrhea because of a disease etiology unrelated to HGE (eg, drug adverse effects, gastrointestinal parasites, renal failure) were excluded from this study. Fecal culture was performed, but the presence of bacteria considered endogenous to the canine intestinal tract (eg, *E. coli*, *Clostridia* spp.) or of organisms or bacterial toxins, the roles of which in HGE have not been clarified did not result in exclusion from this study. However, if bacteria were detected that are considered potentially primary enteropathogenic bacteria (eg, *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp.), the dogs were excluded from the study.

Diagnostic evaluation of patients with AHD included a standardized history and physical examination (n = 60); abdominal ultrasound examination (n = 60); CBC (n = 60); serum biochemistry profile (n = 60); serum bile acid concentrations (n = 54); clotting profile (n = 55); fecal examination for nematode and protozoan parasites (29.5% natrium nitrate flotation solution,[a] Giardia antigen ELISA[b]) (n = 60), for parvovirosis (n = 60) (antigen ELISA,[c] electron microscopy), and for CPE (n = 50) (ELISA[d]); and, on the same day or overnight a fecal culture[e] from fresh feces (<1 hour) (n = 55) was shipped to the reference laboratory to detect various bacteria (*Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., *E. coli*). Specific methods and selective culture media were used.[e] To culture *Salmonella* spp., a selenite-bouillon was used for enrichment. After 18 hours, the culture was transferred onto a new *Salmonella* agar (Salmonella IDENT agar), which was aerobically incubated for 16–36 hours at 37°C. To culture *Campylobacter* spp., selective agars[24] were incubated in a microaerophilic environment for 48 hours at 42°C. To culture *Yersinia enterocolitica*, CIN agar was incubated for 16–36 hours at 30°C with a bouillon, which was stored at low temperature (2–8°C) for 4 days.

Additionally, from 54/60 patients, 2 separate blood cultures of 5–8 mL were taken at a 30-minute interval from the jugular vein after sterile preparation of the skin. Blood samples were fed into a blood culture system[f] and submitted for aerobic and anaerobic bacterial cultures.

Excluded from the study were dogs that had received any drug known to cause mucosal irritation (eg, NSAIDS, corticosteroids, doxycycline) 1 week before presentation or that had an underlying disease potentially responsible for the hemorrhagic diarrhea. An underlying disease was suspected if one of the following criteria was met: bleeding disorders with a prothrombin time >46 seconds, activated partial thromboplastin time (aPTT) >26 seconds, or platelet count <100 000/μL; renal failure with BUN>17.3 mmol/L or creatinine >146.0 μmol/L in conjunction with a urine specific gravity <1.030; and liver disease with more than 2 parameters that reflected abnormal liver function (eg, serum bile acids, albumin, bilirubin, BUN, cholesterol, glucose). Patients with parvovirosis; patients with a fecal culture positive for *Salmonella*, *Yersinia*, or *Campylobacter* spp.; and patients with endoparasite infestation (including *Giardia* spp.) also were excluded. To screen for focal intestinal (eg, neoplasia, intussusception, foreign body) or other visceral diseases, abdominal ultrasound examination was performed in every dog. Special attention was paid to the pancreatic region to rule out pancreatic changes. In addition to the ultrasound examination, radiographs were performed at the discretion of the clinician managing the case to rule out foreign bodies (17 dogs). Pancreatitis (which also led to exclusion from the study) was suspected if typical ultrasonographic changes, substantial abdominal pain, or both were present and if clinical improvement was not consistent with the expected clinical course of HGE.

Addison's disease also led to exclusion. None of the included dogs had hyponatremia, hyperkalemia, or both and lack of a stress leukogram. To further rule out Addison's disease, each of the dogs was followed for at least 12 months after discharge. Follow-up information was obtained by telephone communication with the owner or referring veterinarian. Two dogs had a recurrence of diarrhea. In those 2 dogs, baseline cortisol concentration was measured to rule out Addison's disease, but these results were within the reference range.

To confirm an unbiased distribution, data with regard to history, signalment, physical examination, laboratory parameters and fecal examination were compared between the treatment (TG) and placebo groups (PG) on the day of study inclusion.

Treatment

All dogs were randomly divided into a TG and PG (n = 30/group) by means of a computer-generated schedule. Patients in the TG received amoxicillin/clavulanic acid for 7 days. During hospitalization (for at least 3 days), the drug was administered as injections (7 mg/kg SC q24h[g]), followed by oral administration (12.5 mg/kg PO q12h[h]) given by the owners at home. Patients in the PG were not treated with any antimicrobial agent during hospitalization and received a placebo tablet[i] at home. Additional therapy was standardized and equal for both groups: fluid therapy (crystalloids; fluid amount depended on dehydration, maintenance demands, and ongoing losses), antiemetics (maropitant[j] 1 mg/kg SC q24h, on days 1 and 2), gastric antacids (ranitidine[k] 2 mg/kg IV q8h, on days 1 and 2), and DIC prophylaxis (dalteparin natrium[l]: q12h SC, day 1: 75 IU/kg; day 2: 38 IU/kg; day 3: 19 IU/kg).

Evaluation of Treatment Efficacy

The day of presentation and study inclusion was defined as day 1. Every day, clinical signs were assessed and quantified by a clinician blinded to the treatment by a simple, newly developed scoring system, the "canine HGE activity index" (Table 1). This index represents a modified form of the canine inflammatory bowel disease index[25] and includes the parameters attitude, appetite, vomiting, stool consistency, stool frequency, and dehydration. Each parameter was scored (0 = normal, 1 = mild, 2 = moderate, 3 = severe), and the sum of scores yielded a total cumulative score. On days with no bowel movement, the stool consistency and frequency were scored as 0. After discharge of the patient, the same clinical parameters, with the exception of dehydration, were evaluated and documented by the owner.

After 3 days of obligate hospitalization, dogs fulfilling the following criteria were discharged from the hospital: normal activity, no dehydration, no vomiting, and no watery diarrhea (only dogs with grades 1 and 2 diarrhea were allowed to leave the hospital).

To evaluate treatment efficacy, the canine HGE activity index (on any individual day and over the whole course of disease), duration of hospitalization, and dropout and mortality rates were compared between the TG and PG.

Statistical Analyses

Statistical analyses were conducted with Microsoft Office 2007 Excel, SAS (version 9.2; http://www.sas.com), SPSS (version 17.0; http://www.spss.com), and Statcalc (http://www.n.cdc.gov/epiinfo). Mean, median, and range were determined for the parameters age, weight, duration of disease, duration of bloody diarrhea, and blood test results. Data were investigated for normality by visual plots (Boxplots and Q-Q-Plots). Mann-Whitney *U*-tests were conducted

Table 1. Criteria for assessment of the canine HGE activity index.

Activity	0: Normal	1: Mild	2: Moderate	3: Severely decreased
Appetite	0: Normal	1: Mild	2: Moderate	3: Severely decreased
Vomiting	0: No	1: 1×/day	2: 2–3×/day	3: > 3×/day
Stool consistency	0: Normal	1: Slightly soft	2: Very soft	3: Watery diarrhea
Stool frequency	0: Normal	1: 2–3×/day	2: 4–5×/day	3: >5×/day
Dehydration	0: No	1: < 5%	2: 5–10%	3: > 10%

HGE, hemorrhagic gastroenteritis.

to identify statistical differences in values between the PG and the TG, as well as between dogs excluded postinclusion and those completing the study, for laboratory values as well as for HGE index values per day. Frequency data (number of dogs with CPE, positive blood culture, or diarrhea on any given day and the number of dogs excluded postinclusion) were analyzed by a chi-square test. The time course of the HGE index in the 2 groups was compared by a mixed model (ProcMIXED in SAS), whereby the animal was used as a random effect and the model corrected for repeated measurements within an animal. This analysis was conducted twice, including and excluding the animals that left the study during the study course, in order to estimate the effect of these animals on the results of the analysis (according to the study protocol and according to intention-to-treat analysis). For all statistical tests, the significance level was set to $\alpha = 0.05$.

Retrospectively, a power analysis was conducted by PASS 2008 (http://www.ncss.com/pass.html) to calculate the power of detecting a difference in the dropout proportion between the TG and PG.

Results

Study Population

The median age and weight of all dogs with HGE were 5.4 years (range, 0.6–15.4) and 9.7 kg (range, 1.6–41.6), respectively. The population comprised 46.7% males (10/28 neutered) and 53.3% females (22/32 spayed). The median duration of disease and, in particular, of hemorrhagic diarrhea until presentation was 18 (range, 2–96) and 12 hours (range, 1–72), respectively. The baseline characteristics of HGE patients shown in Table 2 confirm a balanced distribution between TG and PG.

There was no significant difference in any laboratory parameter between both groups, with the exception of aPTT ($P = .030$) and albumin ($P = .009$) on day 1 (Table 2). Of all dogs, 18.5% (10/54) had a positive blood culture: 3/10 were in the PG (*Staphylococcus intermedius*, unclassified coryneform rod, *Corynebacterium accolens*), and 7/10 were in the TG (*S. intermedius*, *Staphylococcus warneri*, *Staphylococcus xylosus*, *Acinetobacter lwoffii*, *Corynebacterium* spp., *Staphylococcus epidermidis*, *Arthrobacter* spp.). In 30.0% of the dogs (15/50), CPE could be detected on fecal examination; 25.9% (7/27) of dogs in the PG had CPE-positive fecal samples, whereas 34.8% (8/23) of dogs in the TG had CPE-positive fecal samples. There was no significant difference regarding positive blood culture ($P = .298$) or positive fecal CPE results ($P = .710$) between both groups.

Treatment Efficacy

There was no significant difference concerning the canine HGE activity index on any individual day of the study (Table 3) or over the whole course of disease between the PG and TG (mixed model; $P = .487$). In addition, there was no difference in fecal consistency between groups. A rapid improvement of clinical signs could be observed during the first 48 hours in the PG (mean HGE score day 1, 11.6; day 3, 3.0) as well as in the TG (median HGE score day 1, 12.4; day 3, 1.5). However, on day 5, 50.0% (12/24) of the dogs in the PG and 27.6% (8/29) in the TG ($P = .080$) still had soft stool consistency (grade 1 in the HGE index).

The duration of hospitalization was not different between the 2 treatment groups (PG: median, 3.0; range, 3.0–7.0; TG: median, 3.0; range, 3.0–4.0; $P = .740$).

Focusing on patients with positive blood culture, there was no statistically significant difference between both treatment groups in outcome, including duration of hospitalization and canine HGE activity index on any day of the study period. Comparing only the cases with CPE-positive results, no significant difference between both treatment groups concerning the canine HGE activity index on any day other than day 5 ($P = .030$) could be detected. On day 5, the TG (median, 1.25; range, 0–3) had a significantly lower HGE score compared with the PG (median, 4; range, 1–4). Complications because of antibiotic therapy were not observed.

Postinclusion Dropouts

Of 60 dogs, 53 completed the study. Of the 7 excluded dogs, 6 dogs were in the PG and 1 was in the TG. The number of dropouts was not significantly different between both groups ($P = .103$). A retrospective power analysis calculated the power to detect this difference (6 versus 1 dropout per group) as 58%. Dogs were removed from the study because of inadequate clinical improvement (cases 1, 2, and 6), fever (case 3), leukopenia (case 3), and left shift (band neutrophils $> 1.5 \times 10^9$/L; cases 1 and 2).

Two dogs died acutely and unexpectedly: case 4, assigned to the PG, was judged as clinically stable at presentation and had a WBC count of 15.0×10^9/L and 1.4×10^9/L band neutrophils on initial blood work. This dog died 8 hours after presentation. Necropsy showed necrotizing enteritis possibly associated with *C. perfringens*. Case 5 was assigned to the TG, showed a good clinical improvement over the first hours and died suddenly on day 2. Initial bloodwork revealed a WBC count of 18.7×10^9/L and band neutrophil count of 1.3×10^9/L. Necropsy findings were consistent with enteritis. Because of autolysis, the exact histopathologic changes could not be determined. One dog from the PG was euthanized

Table 2. Comparison of baseline characteristics and CBC, coagulation panel, and chemistry results from the day of presentation of patients with HGE randomly divided into a placebo (n = 30) and an amoxicillin/clavulanic acid treatment group (n = 30).

	Placebo Group		Treatment Group		
Parameters	Proportion		Proportion		***P*-Value**
Sex male/female (n)	14/16		14/16		1.000
Positive blood culture (n)	3/26		7/28		.298
Positive fecal CPE (n)	7/27		8/23		.710
	Median	Range	Median	Range	*P*-value
Age (years)	5.9	1.1–14.6	5.4	0.6–15.4	.324
Weight (kg)	11.2	2.1–41.6	8.5	1.6–40.0	.147
HGE index score[a]	12.5	3.0–16.0	13.0	6.0–17.0	.376
Disease duration (h)[b]	16	1–72	9	0–48	.256
RBC ($\times 10^{12}$/L)	8.5	6.7–10.8	8.9	5.9–11.6	.154
HGB (mmol/L)	12.6	9.7–16.1	13.1	8.9–16.5	.088
PCV (%)	56	0.35–0.76	59	39–75	.082
WBC ($\times 10^9$/L)	12.8	4.9–20.2	12.5	5.7–23.4	.610
Monocytes ($\times 10^9$/L)	0.61	0.00–1.91	0.34	0.00–5.01	.075
Lymphocytes ($\times 10^9$/L)	1.24	0.00–4.65	1.06	0.15–3.23	.185
Bands ($\times 10^9$/L)	0.51	0.00–1.50	0.28	0.00–1.10	.294
Neutrophils ($\times 10^9$/L)	11.1	2.7–15.5	10.6	3.1–19.9	.817
Eosinophils ($\times 10^9$/L)	0.00	0.00–0.53	0.00	0.00–1.10	.378
Basophils ($\times 10^9$/L)	0.00	0.00–1.00	0.00	0.00–0.15	.313
Platelets ($\times 10^9$/L)	327	177–752	433	161–898	.121
PT (seconds)	19.0	10.4–36.5	18.4	11.7–23.6	.396
aPTT (seconds)	12.2	9.7–18.2	10.9	7.7–13.9	**.030**
ALT (U/L)	55.5	18.0–1651.0	62.0	17.0–174.0	.994
ALP (U/L)	52.5	21.0–199.0	38.5	16.0–202.0	.249
Total bilirubin (μmol/L)	2.37	0.39–11.43	2.38	0.50–5.42	.819
Bile acids (μmol/L)	3.7	0.1–33.5	7.6	0.1–67.2	.111
α-Amylase (U/L)	588	197–6741	705	300–2049	.388
Cholesterol (mmol/L)	4.70	1.93–9.08	5.51	2.50–9.57	.300
Triclycerides (mmol/L)	0.41	0.10–2.00	0.51	0.30–1.00	.054
Total protein (g/L)	59.7	34.2–80.3	64.0	34.9–83.3	.135
Albumin (g/L)	35.0	21.7–47.9	37.8	20.3–46.9	**.009**
Urea (mmol/L)	6.43	2.65–11.87	6.95	2.80–17.03	.098
Creatinine (μmol/L)	67.5	34.0–146.0	65.0	27.0–138.0	.830
Glucose (mmol/L)	5.9	4.0–9.6	6.4	3.5–12.3	.808
Sodium (mmol/L)	142	131–147	143	134–151	.164
Potassium (mmol/L)	3.8	3.4–5.3	3.8	3.3–6.7	.518
Ionized calcium (mmol/L)	1.2	1.0–1.4	1.2	1.0–1.3	.632
Phosphorus (mmol/L)	1.3	1.0–2.1	1.3	0.67–4.4	.539
Chloride (mmol/L)	110	104–117	111	96–119	.417

Values of age, weight, HE score, disease duration, and blood examination are stated as median and range. Bolded *p* values are significant.

CPE, *Clostridium perfringens* enterotoxin; h, hours; kg, kilogram; n, number; RBC, red blood cells; HGB, hemoglobin; PCV, packed cell volume; WBC, white blood cells; PT, prothrombin time; PTT, partial thromboplastin time; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HGE, hemorrhagic gastroenteritis.

[a]Disease activity index at presentation.

[b]Duration of hemorrhagic diarrhea until presentation.

24 hours after presentation. This dog did not show any clinical improvement and developed substantial leukopenia (WBC 1.1 $\times$ 10^9/L). Thoracic radiographs revealed intrathoracic masses. No final diagnosis was achieved because of financial constraints.

Discussion

There is evidence that resistance to antimicrobials is increasing among bacteria isolated from pets.[26] Fewer than half of *E. coli* isolates from pet and farm animals are susceptible to first-generation cephalosporins and aminopenicillins.[27,28] Inappropriate usage of prophylactic antibiotics, especially β-lactam antibiotics, can lead to multiresistant bacteria, including methicillin-resistant *Staphylococcus aureus*, which affects mortality in humans.[29] Thus, unnecessary antibiotic treatment should be avoided. In addition, antimicrobial therapy can disrupt the normal microbial flora, which is important for defense against pathogens.[30,18] In humans, *C. difficile* infection is a toxin-mediated intestinal disease that develops in patients after antibiotic treatment. *C. difficile* can only colonize the gut if the normal intestinal microbiota is disturbed or absent. Although this association between

Table 3. Comparison of the HGE activity indexes from any individual treatment day between placebo and amoxicillin/clavulanic acid treatment group.

	Placebo Group		Treatment Group		
Day	Median	Range	Median	Range	*P*-Value
HGE index day 1	12.5	3.0–16.0	13.0	6.0–17.0	.376
HGE index day 2	3.0	0.0–12.0	3.0	0.0–11.0	.861
HGE index day 3	2.0	0.0–13.0	1.0	0.0–8.0	.072
HGE index day 4	2.0	0.0–11.0	2.0	0.0–6.0	.529
HGE index day 5	2.0	0.0–7.0	2.0	0.0–6.0	.715
HGE index day 6	1.0	0.0–5.0	1.5	0.0–5.0	.286
HGE index day 7	1.0	0.0–5.0	1.0	0.0–4.0	.910

HGE, hemorrhagic gastroenteritis.

antimicrobial therapy and *C. difficile* infection is infrequently observed in dogs, non-hospital-associated reservoirs are emerging, and *C. difficile* is capable of spreading in animal hosts.[31]

In acute intestinal diseases, antimicrobials are indicated only in animals with confirmed bacterial infection (eg, positive blood culture, fecal culture, or both of a potentially enteropathogenic organism in association with signs of sepsis), a predisposition for bacterial translocation (eg, disruption of intestinal epithelial barrier) and increased risk of sepsis (eg, immunosuppression, porto-systemic shunting). In HGE, the presence of blood in the feces might reflect a breach of the intestinal integrity. This is most likely the reason why several authors recommend a routine prophylactic antibiotic treatment in these patients. However, there is no documented evidence that patients with HGE truly have an increased risk for bacterial translocation or sepsis, and the efficacy of antibiotic therapy in the treatment of HGE has not been demonstrated in controlled studies.

There is evidence that CPE and *C. difficile* toxin A might be associated with AHD in dogs.[15] However, toxin production can be stimulated by antibiotic treatment, and therefore gastrointestinal infections in humans with Shiga toxin-producing *E. coli* are not treated with antimicrobials.[32] The benefit of antimicrobial treatment of gastrointestinal infections with toxigenic bacteria in dogs has not been evaluated.

Therefore, the goal of this study was to examine whether treatment with amoxicillin/clavulanic acid improves disease outcome in dogs with HGE. The choice of amoxicillin/clavulanic acid was based on its broad spectrum, which covers some potentially enteropathogenic organisms, the rare occurrence of associated adverse effects, and its high margin of safety.[33] Additionally, amoxicillin/clavulanic acid is available in most veterinary practices, is considered a first-line drug, and is licensed for dogs.

No significant differences between treatment groups regarding mortality rate, duration of hospitalization, or severity of clinical signs were observed in this study. There are some possible explanations for this finding: (1) HGE is not caused by a primary bacterial infection, (2) involved bacteria are not susceptible to amoxicillin/clavulanic acid, or (3) bacterial infection/toxin production is self-limiting, and the clinical course is not influenced by antimicrobials. In the initial publications about HGE, an anaphylactic response to bacterial endotoxin was discussed as an inciting cause.[11,14] Cytotoxic drug therapy is a predisposing factor for endotoxin release.[33] The dogs of the present study did not show any adverse effect to the bactericidal antibiotic amoxicillin/clavulanic acid, and clinical improvement was rapid in both treatment groups. Thus, endotoxemia does not seem to be a predominant feature in this disease.

Enteric *C. perfringens* infection and CPE production have been proposed to cause acute gastrointestinal disease[34–36] and hemorrhagic diarrhea.[7,15,37,38] Assuming that the fecal CPE ELISA has an adequate sensitivity, the 30% rate of CPE-positive fecal samples in the present study population indicates that CPE is not the exclusive cause of HGE. Enterotoxigenic *C. perfringens* can be part of the normal flora, and CPE can be detected in healthy dogs.[36] As suggested previously by Marks and colleagues (2002), it is more likely that changes in the intestinal environment of dogs with diarrhea promote increased proliferation and transient overgrowth of enterotoxigenic strains of *C. perfringens*, leading to detectable amounts of CPE in feces. This is supported by the fact that amoxicillin/clavulanic treatment was not associated with a better clinical response than placebo in the CPE-positive dogs. The single difference of canine HGE activity index in CPE-positive dogs between the TG and PG on day 5 was assessed as being clinically irrelevant.

Five percent of the patients in this study had positive blood culture results. Because blood culture may yield false-negative results, the true prevalence of bacteremia might have been underestimated. On the other hand, some bacteria cultured may have represented contaminants (eg, *Staphylococcus* spp.). The blood culture results in this study show that bacteremia can be a feature of dogs with HGE. However, bacterial translocation also can be observed in healthy dogs,[39] and none of the dogs with positive blood cultures had signs of sepsis or had to be unblinded and removed from the study. Additionally, no benefit of antibiotic therapy could be documented in blood culture-positive patients with HGE. These results suggest that bacteremia is infrequent, seems to be transient even without antibiotic treatment and is thus of minor clinical importance in dogs with HGE.

The etiology of HGE is still unclear, and the diagnosis is obtained after excluding other differential diagnoses. To eliminate most diseases that can cause AHD and to recognize conditions that would prompt antibiotic treatment (eg, parvovirosis, sepsis), strict inclusion, and exclusion criteria were applied to the dogs in this study. Despite these strict criteria, postinclusion removal of 7 dogs was necessary. Although the number of dropouts and the mortality rate were not significantly different between the PG and TG, the excluded dogs will be described in detail.

In the PG, cases 3 and 7 had to be removed because they developed severe neutropenia and were suspected to be septic or to have parvovirus infection. Severe inflammation because of bacterial translocation typically is characterized by a left shift. Because no band neutrophils

were evident on the repeated CBC in either dog, parvovirosis was more likely despite the initial negative fecal parvovirus antigen test. Fecal antigen tests have a low sensitivity (15.8–26.3%) compared with polymerase chain reaction testing.[40] Therefore, parvovirus infection might have been undetected in these 2 dogs.

Unblinding occurred in cases 1 and 6 of the PG because of inadequate clinical improvement. Case 1 was bright and alert but had still diarrhea on day 3. CBC was repeated, and because of the increased number of band neutrophils (1.6×10^9/L), the dog was removed from the study. Case 1 had signs of large bowel diarrhea (mucus in feces, tenesmus) for 3 additional days but was asymptomatic on day 7, when discharged. Case 6 showed good clinical improvement over the first 2 days of hospitalization and was discharged on day 3. Because of relapse of diarrhea during the first 24 hours at home, the dog was represented at the hospital and unblinded by the clinician. On the same day, case 6 was sent home with amoxicillin for 7 days and fenbendazole for 3 days and recovered uneventfully. Both dogs (cases 1 and 6) were stable over the entire course of disease. It is unknown if they would have benefited from initial antibiotic treatment.

In 2 other dogs from the PG (cases 2 and 4), complications because of a primary enteropathogenic infection or a secondary bacterial translocation were likely. Case 2 was lethargic on day 3 and developed a degenerative left shift (5.6×10^9/L band neutrophils; 3.4×10^9/L mature neutrophils). Sepsis was suspected in this patient, and treatment with amoxicillin/clavulanic acid in combination with enrofloxacin was instituted. On the next day, the dog was alert, began to eat, and did not have diarrhea. Case 4 died acutely 8 hours after admission, although the patient appeared clinically stable and had no fever, clinically relevant neutrophilia, or left shift. On necropsy, a large number of *Clostridia* spp. were found adhering to the necrotic epithelial surface. In both cases, a primary enteric bacterial infection could have been missed on initial diagnostic evaluation. Only patients with positive fecal cultures for *Salmonella*, *Yersinia*, or *Campylobacter* spp. were excluded from the study. Dogs with other potentially enteropathogenic organisms (eg, *Escherichia* spp., *Clostridia* spp.) were not excluded because many of these organisms are normal constituents of the indigenous intestinal flora and virulence factors are not well characterized.[36,41,42]

Case 5 was the only dog in the TG that did not complete the study. This patient died suddenly on day 2. On necropsy, signs of enteritis were present, but because of autolysis, no definitive diagnosis could be established.

There was no different proportion of dropouts between the TG and the PG. However, in retrospect, the power for detecting the observed difference in the dropout rate was determined to be only 58%. The original power analysis was not based on the dropout rate but on the HGE index. A power, determined in a power analysis to find the appropriate sample size, usually is considered adequate if $> 80\%$. This means that the sample size of 30 dogs per group, which was used in our study, is considered rather low in terms of interpretation of the differences in the dropout rate between the groups. Therefore, these results must be viewed with caution.

There was wide variation in the severity of clinical signs, and some dogs with HGE were presented as medical emergencies. Marked clinical improvement usually was observed within the first 24–48 hours. Clinical treatment response was not improved in patients that received antibiotics. This leads to the assumption that symptomatic treatment, including aggressive IV fluid therapy, seems to be more important than antibiotics in the treatment of aseptic dogs with HGE. With adequate supportive therapy and close patient monitoring, the mortality rate is low.

The results of this study suggest that in some dogs with aseptic HGE, antibiotics may not change the case outcome or time to recovery. Therefore, future studies might focus on ways to identify which dogs benefit from antibiotic therapy and which do not. Because ruling out an enteric bacterial infection is challenging and bacterial translocation is a potentially life-threatening complication, dogs with acute bloody diarrhea of unknown cause that are not treated with antibiotics should be monitored very closely. The administration of amoxicillin/clavulanic acid was not associated with obvious adverse effects. The development of bacterial resistance after treatment with antibiotics, however, was not evaluated in this study.

Footnotes

[a] Natriumnitratflotationslösung, Janssen-Cilag, Neuss, Germany
[b] ProSpecT *Giardia* Microplate Assay, Remel Inc, Lenexa, KS
[c] Snap Parvo Test, IDEXX Laboratories Inc, Westbrook, ME
[d] *Clostridium perfringens* enterotoxin ELISA, Tech Lab Inc, Blacksburg, VA
[e] IDEXX Vet Med Labor GmbH, Division of IDEXX Laboratories Inc, Ludwigsburg, Germany
[f] Oxoid system blood culture medium, Oxoid Limited, Basingstoke, UK
[g] Synulox RTU, Pfizer Pharma GmbH, Karlsruhe, Germany
[h] Synulox tablets, Pfizer Pharma GmbH
[i] P-Dragees, Lichtenstein von Winthrop Arzneimittel GmbH, Mülheim-Kärlich, Germany
[j] Cerenia, Pfizer Pharma GmbH
[k] Sostril, GlaxoSmithKline Manufacturing S. p. A., Verona, Italy
[l] Fragmin D, Pfizer Pharma GmbH

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