# Short and long-term impact of antibiotic exposure and acute epithelial injury on gut microbiome and gut health

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Summary

Acute enteritis is a well-recognized trigger of chronic diseases in humans. Mechanisms triggering chronicity include environmental changes, barrier dysfunction and dysbiosis. Dogs with acute hemorrhagic diarrhea syndrome (AHDS) represent a perfect model for acute intestinal damage of the mucosa/intestinal barrier dysfunction and changes of the intestinal microbiome/metabolome. We already showed that dogs with acute, severe intestinal damage and dysbiosis have a high risk for developing long-term intestinal diseases. Further goals of our research is to establish non -invasive markers for intestinal barrier integrity/function, to prove the hypothesis: „development of food allergen sensitization during the phase of acute enteritis“, to evaluate short and long-term impact of dysbiosis and antibiotic exposure on intestinal health, to evaluate the usefulness of antibiotic treatment in dogs with acute diarrhea, to identify specific risk factors inducing chronic intestinal and extraintestinal disorders and to establish new treatment strategies in preventing chronicity, such as modulation of the intestinal microbiota (e. g. fecal microbiota transplantation, probiotics) and therapeutic agents improving intestinal integrity.

Research Objectives

Acute hemorrhagic diarrhea syndrome (AHDS) in dogs is defined as sudden onset of severe bloody diarrhea frequently associated with vomiting as first clinical sign observed by the owner and a significant loss of fluid into the intestinal lumen. In dogs, different aetiologies have been discussed, including intestinal type 1 hypersensitivity reaction to food components or bacterial endotoxin, and enterotoxigenic clostridial strains.

A major focus of our research was to describe the pathology of AHDS using endoscopic gastrointestinal biopsies, and to potentially identify bacteria in these biopsy samples. These investigations should provide new insight into the pathogenesis and role of bacteria in this syndrome. We showed that mucosal lesions were characterized by necrotizing and neutrophilic inflammation and were restricted to the large and small intestines. Therefore, the term “HGE,” which is often used in the current literature and implies the involvement of the stomach, is misleading. Thus, we renamed the syndrome into “acute hemorrhagic diarrhea syndrome.”

In addition, a prominent feature of the small intestinal lesions was the adherence of large rod-shaped bacteria to the necrotic mucosal surfaces. The histologically detected bacteria were identified as C. perfringens Type A by immunohistochemical staining, bacterial culture and genotyping. Based on their pathomechanisms, both Clostridium perfringens enterotoxin (CPE) and Clostridium difficile toxin A/B (CDT A/B) could potentially cause the extensive fluid loss and

intestinal necrotic lesions characteristically observed in dogs with AHDS. Consequently, the objectives of the next study were to determine the prevalence of the toxins CPE and CDT A/B, as well as the prevalence of the genes that encode for these toxins in the faeces of dogs with AHDS. CPE was detected significantly more often in the feces of dogs with AHDS than in the feces of dogs in the control group. However, the prevalence of CPE in the feces of dogs with AHDS was only 24%, and there was no difference in severity of clinical signs, duration of hospitalisation, or outcome between CPE-positive and -negative dogs with AHDS. The results of this study suggested that CPE does not play a significant role in dogs with AHDS. Recently, novel pore-forming toxin genes designated netE and netF were identified in a Clostridium perfringens type A strain isolated from a dog with acute hemorrhagic diarrhea. Thus, we aimed to determine the prevalence of C. perfringens genes encoding for netE and netF in the feces of dogs with AHDS and to evaluate any association between selected clinical variables and the presence of these toxin genes. We included 174 dogs in this study and showed that the prevalence of C. perfringens encoding for netE and netF is significantly higher in dogs with AHDS compared to control dogs, but in dogs with AHDS, no significant difference was detected in any clinical and laboratory variables evaluated between netE- positive and netF-positive and netE-negative and netF-negative dogs.

Summarizing all current information, it can be assumed that NetF is likely a major virulence factor responsible for AHDS. However, C. perfringens produces numerous extracellular enzymes and minor toxins, such as collagenase (κ-toxin), neuraminidase, caseinase (λ-toxin), deoxyribonuclease (η-toxin), hyaluronidase (μ-toxin), and urease. Individually, they potentially play only a minor role in the pathogenesis of C. perfringens associated diseases, but their cumulative effect is likely profound. Further studies are needed to define the exact role of pore forming toxins and CPE in the pathogenesis of AHDS and to discover novel toxins responsible for virulence.

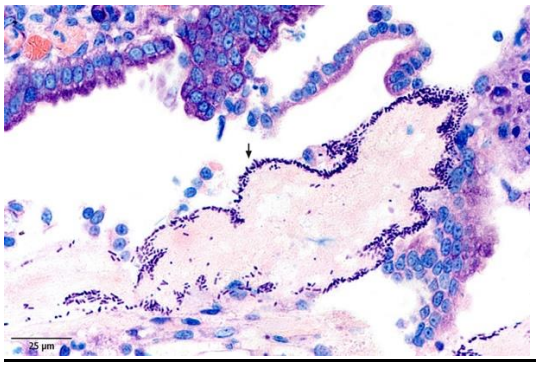
The peracute loss of intestinal mucosal integrity in AHDS results in a rapid movement of fluid and electrolytes into the gut lumen leading to significant dehydration and hypovolemic shock. Therefore, rapid volume replacement and symptomatic treatment is necessary and usually results in a short course of the disease lasting from 24 to 72 hours. Because of the potential bacterial etiology and the risk of sepsis, antibiotics generally are recommended to treat hemorrhagic diarrhea in dogs. However, our study group showed that the incidence of bacteremia in dogs with AHDS is low and not different from those of healthy control dogs and that in dogs with aseptic AHDS, antibiotics do not change outcome or time to recovery. In a further study evaluating the influence of amoxicillin-clavulanic acid on the proportion of amoxicillin-resistant E. coli in dogs with acute diarrhea, we demonstrated a significant increase of resistant E. coli isolates, which persist for as long as 3 weeks after treatment. Thus, only dogs with signs of systemic inflammation should be treated with antibiotics.

Acute enteritis is a well-recognized trigger of chronic diseases in humans. Two independent studies performed by our research group showed that also dogs with an episode of severe acute intestinal damage have an increased risk for chronic gastrointestinal disorders later in life. Forty-two per cent of dogs that survived a canine parvovirus infection and about 30% of dogs with an episode of AHDS develop chronic diarrhea later in life. There is evidence that in acute disease, barrier dysfunction as well as dysbiosis can lead to loss of oral tolerance and sensitize the immune system to food components and the intestinal microbiota.

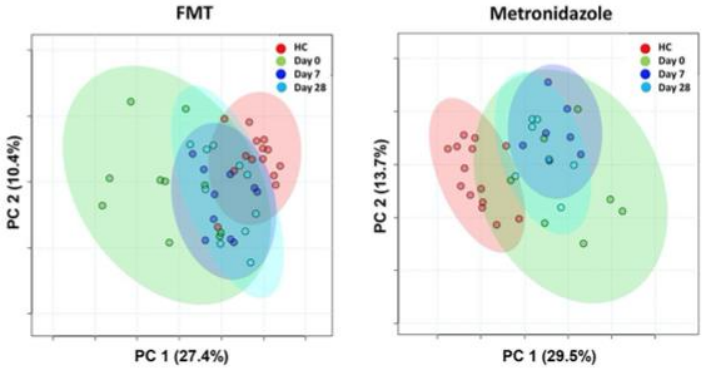
In dogs with AHDS, increased fecal markers such as calprotectin and S100A12 as well as α1-proteinase inhibitor reflect intestinal damage and associated intestinal inflammation. Fecal microbiome analysis of the 16S rRNA gene revealed profound alterations and qPCR assays significant increases in genus Clostridium perfringens and Sutterella in dogs with AHDS when compared to healthy dogs. These findings show that dogs with AHDS represent a perfect model for acute intestinal damage of the intestinal barrier and changes of the intestinal microbiome. Currently, we try to identify new treatment strategies with the aim to rapidly restore intestinal barrier function and normobiosis. We already documented that dogs receiving probiotic treatment showed an accelerated normalization of Blautia, C. hiranonis, Faecalibacterium, and Turicibacter compared to dogs that were only treated symptomatically. Additionally, the abundance of C. perfringens encoding enterotoxin was significantly lower in dogs receiving probiotics. Fecal microbiota transplantation (FMT) might also be good treatment strategy to correct dysbiosis and

potentially replace pathogenic bacterial strains. It could already be shown by our research group, that in a group of dogs with acute diarrhea reveiving FMT, the dysbiosis index decreased over time (= normalisation of the intestinal microbiota), while metronidazole treatment led to a significant increase in the dysbiosis index (= dysbiosis of the intestinal microbiota) at day 7 and 28 compared to FMT.
Further goals of our research is to establish non-invasive markers for intestinal barrier integrity/function, to prove the hypothesis: „development of food allergen sensitization during the phase of acute enteritis“, to evaluate short and long-term impact of dysbiosis and antibiotic exposure on intestinal health, to further evaluate the usefulness of antibiotic treatment in dogs with acute diarrhea, to identify specific risk factors inducing chronic intestinal and extraintestinal disorders and to establish new treatment strategies in preventing chronicity, such as modulation of the intestinal microbiota (e. g. fecal microbiota transplantation, probiotics) and therapeutic agents improving intestinal integrity.

## Figures




Plump, rod-shaped, gram-positive bacteria identified as *C. perfringens* on the necrotic villus tip in the duodenum of a dog with AHDS




PCA plots showing changes based on untargeted metabolomics in healthy control dogs (HC) vs. dogs with acute diarrhea treated with either FMT as a single enema, or with metronidazole. Dogs with acute diarrhea treated with fecal microbiota transplantation (FMT) clustered closer to healthy dogs on day 28. Dogs with acute diarrhea treated with metronidazole did not cluster closer to healthy dogs on day 28 than before.

## Key Findings

Over the last years we focused our research in gastroenterology on a specific acute gastrointestinal disease syndrome called “hemorrhagic gastroenteritis”. We were able to exactly describe the affected dog population, characteristic histopathologic findings of intestinal lesions, the pathogenic role of C. perfringens, the influence of different toxins, and the appropriate treatment. Our initial research had immediate impact on reducing untargeted antibiotic usage in small animal gastroenterology and led to renaming of the syndrome into “acute hemorrhagic diarrhea syndrome” (AHDS). We recognized that dogs with AHDS represent a perfect model for acute intestinal damage of the mucosa/intestinal barrier dysfunction and changes of the intestinal microbiome/metabolome. We already showed that dogs with acute, severe intestinal damage and dysbiosis have a high risk for developing long-term intestinal diseases. This is very likely due to sensitisation of the immune system during the acute phase of the intestinal disease.

## Selected publications

1. Hauck C, Silke Schmitz S, Burgener IA, Wehner A, Neiger R, Kohn B, Rieker T, Reese S, Unterer S. Prevalence and characterization of hypoadrenocorticism in dogs with signs of chronic gastrointestinal disease: A multicenter study. J Vet Intern Med Accepted 21st February 2020.

2. Werner M, Suchodolski JS, Straubinger RK, Steiner JM, Lidbury JA, Neuerer F, Katrin Hartmann K, Unterer S. Effect of amoxicillin-clavulanic acid on clinical scores, intestinal microbiome, and amoxicillin-resistant Escherichia coli in dogs with uncomplicated acute diarrhea. J Vet Intern Med 2020; 1-11.
3. Sindern N, Suchodolski JS, Leutenegger CM, Mehdizadeh Gohari I, Prescott JF, Proksch AL, Mueller RS, Busch K, Unterer S. Prevalence of Clostridium perfringens netE and netF toxin genes in the feces of dogs with acute hemorrhagic diarrhea syndrome. J Vet Intern Med. 2019; 33(1): 100-105.
4. Kilian E, Suchodolski JS, Hartmann K, Mueller RS, Wess G, Unterer S. Long-term effects of canine parvovirus infection in dogs. PLoS One 2018; 13(3): e0192198.
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7. Ziese AL, Suchodolski JS, Hartmann K, Busch K, Anderson A, Sarwar F, Sindern N, Unterer S. Effect of probiotic treatment on the clinical course, intestinal microbiome, and toxigenic Clostridium perfringens in dogs with acute hemorrhagic diarrhea. PLoS One 2018; 13(9): e0204691.
8. Mueller RS, Unterer S. Adverse food reactions: Pathogenesis, clinical signs, diagnosis and alternatives to elimination diets. Vet J 2018; 236: 89-95.
9. Anderson A, Hartmann K, Leutenegger C M, Proksch A L, Mueller R S, Unterer S. The role of canine circovirus in dogs with acute hemorrhagic diarrhoea. Vet Rec 2017; 180(22): 542.
10. Heilmann RM, Guard MM, Steiner JM, Suchodolski JS, Unterer S. Fecal markers of inflammation, protein loss, and microbial changes in dogs with the acute hemorrhagic diarrhea syndrome (AHDS). J Vet Emerg Crit Care (San Antonio) 2017; 27(5): 586-589.

## Funding


| Funder | Project title | Start date | End date |
|---|---|---|---|
| Pharmaceutical company | Enterococcus faecium versus metronidazole in dogs with acute diarrhea | 2020 | 2021 |
| Texas A&M University/Veterinary Nutrition Company | Microbiome and metabolome analyses following FMT in dogs with chronic enteropathy | 2018 | 2019 |
| Pharmaceutical company | PRE02 - Target animal safety study in healthy dogs | 2015 | 2017 |