Focus on acute haemorrhagic diarrhoea syndrome – Part 1

In this the first part of a two-part series *Bryn Jones*, from Willows Veterinary Centre and Referral Service, focuses on the causes, Clostridia and consequences of AHDS.

Acute haemorrhagic diarrhoea syndrome (AHDS) is encountered frequently in both first opinion and referral practice, particularly in the emergency setting. The specific syndrome was fully characterized in 2015, although has been encountered for decades, often termed or understood as 'haemorrhagic gastroenteritis' (HGE).[1] There has been much research in recent years attempting to determine the potential cause/causes of AHDS, as well as how best to treat it. This two-part article explores the aetiology and pathophysiology of AHDS, as well as its diagnosis, recognition, and ultimately appropriate treatment options.

Clinical presentation

As the name suggests, AHDS is characterized by a sudden onset of haemorrhagic diarrhoea – often a deep red or brown colour with liquid consistency (as opposed to the tarry nature of melaena). Median duration of clinical signs prior to initial presentation is 0.5–2 days, with most studies showing an average of less than a day (to be expected given the syndrome is defined by its acute nature).[1–5] 80–100% of dogs have concurrent vomiting, with 31–65% displaying haematemesis (Figure 1).[1–3,5] When present, vomiting typically precedes diarrhoea, by as much as 52 hours.[1] Tachycardia is frequently noted on presentation, compensatory for hypovolaemia. Abdominal pain is uncommon (approximately 20% of dogs).[6] Affected dogs typically trend towards hypothermia rather than hyperthermia – the latter should prompt concern for alternative differentials or the development of sepsis. The 'AHDS index' was created to report and categorize the severity of signs (Table 1).[1] Most dogs (81.5–90%) seem to be presented in the most severe category of disease.[1,5,7]

Average age has been reported as 4–7 years old.[1,2,4,5,7–17] Affected dogs seem to be smaller than average, but not all studies confirm this, and any breed and size can be affected.[1,11,18] Predisposed breeds include the Yorkshire Terrier, Miniature Pinscher, Miniature Schnauzer, Chihuahua and Maltese Terrier.[1,5,9,12] Multiple cases can be associated with geographic outbreaks, although this has been challenged.[13,19] Anecdotally, chronic inflammatory enteropathy may predispose to AHDS (and vice versa).[19,20]

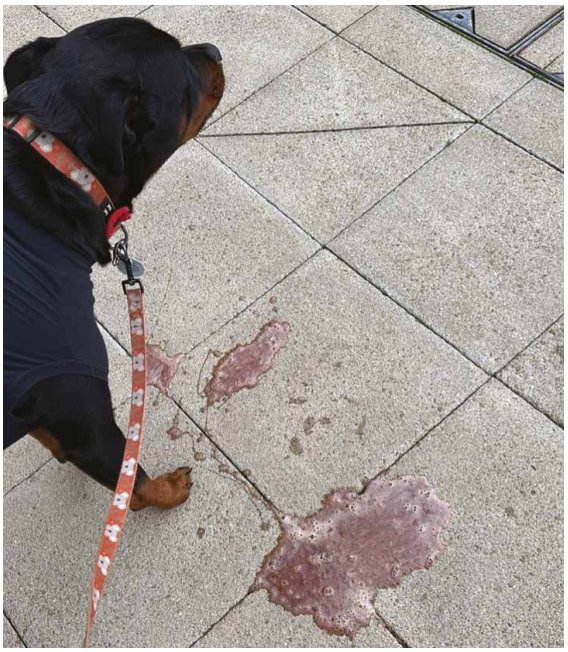

FIGURE 1: Haematemesis associated with AHDS. Credit: Ellie Farr

Score	0	1	2	3
Activity	Normal	Mildly reduced	Moderately reduced	Severely reduced
Appetite	Normal	Mildly reduced	Moderately reduced	Severely reduced
Vomiting (times/day)	0	1	2–3	>3
Faecal consistency	Normal	Slightly soft	Very soft	Watery
Defecation (times/day)	1	2–3	4–5	>5
Dehydration (%)	0	<5	5–10	>10
Total score	**0–3**	**4–5**	**6–8**	**9 or more**
Clinical significance	Insignificant	Mild	Moderate	Severe

TABLE 1: Criteria for categorization of 'AHDS index'. Reproduced from Mortier *et al*., 2015.[1]

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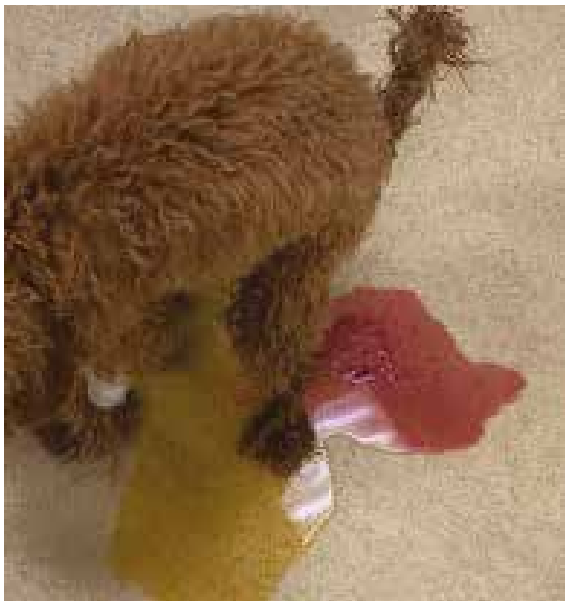

FIGURE 2: Dog passing haematochezia in hospital (and urinating at the same time), note the bright red colour and high water content. Credit: Dave Beeston

Structural pathology

Although haematochezia is usually suggestive of colonic/large intestinal haemorrhage, with melaena suggestive of small intestinal lesions, this is not universal – stool colour is more indicative of length of time in the bowel rather than necessarily the origin (Figure 2). Haemorrhagic diarrhoea, regardless of the cause, suggests loss of mucosal integrity.[21] Early histopathological assessment of AHDS primarily showed necrosis of the intestinal mucosa, hence some aversion to the term 'gastroenteritis' which implies both inflammatory lesions and involvement of the stomach.[22–25] Assessments since have observed neutrophilic enteritis too, but mostly still do not report significant gastric lesions, and the degree of inflammation has been considered disproportionate to the epithelial destruction.[3,26] Duodenum, ileum and colon all appear to be afflicted on endoscopic (typically erosive lesions) and histological assessment.[3,24] Thus, it is considered likely that both vomiting and haemorrhage originate primarily from intestinal lesions rather than any gastric involvement. More recent endoscopic and histopathological antemortem study of dogs with AHDS did find gastric lesions in 9/10 dogs.[18] As gastritis often appeared chronic (fibrotic, lymphoplasmacytic), these were considered unlikely to be AHDS-related. Duodenal lesions were acute and more typical of past investigations (neutrophilic, necrotizing enteritis). Although histological studies have not always shown inflammation, faecal inflammatory markers are increased in AHDS and quickly normalize afterwards.[8] Additionally, serum C-reactive protein concentration correlates with clinical severity and the plasma proteome signature is abnormal, both indicating some degree of systemic inflammation.[5,27]

Pathophysiology and clinical signs

In the emergency setting, AHDS is often characterized by the severity and acuity of dehydration and/or hypovolaemia. Despite this, skin tenting may not be present due to the delay in interstitial fluid shift, although 73% of affected dogs have detectable dehydration (>5%) on admission.[5,19] Affected dogs often have haemoconcentration despite blood loss due to the degree of dehydration/hypovolaemia.[1] In some instances, AHDS has been defined by a packed cell volume ≥55 or 60%, although the median has been reported as 57% with only a third of cases >60%.[1,6] Haemorrhagic diarrhoea may be a misleading term, as plasma water with minimal erythrocytes is principally lost into the lumen, as opposed to whole blood. Intestinal barrier dysfunction is supported by iohexol absorption studies, with severity of dysfunction correlating with clinical signs.[17] With loss of mucosal integrity, protein loss has been demonstrated.[8] Over half (52%) of dogs are hypoalbuminaemic following rehydration.[17]

Dysbiosis

Studies suggest intestinal dysbiosis in AHDS – it is unknown if it is causative or a consequence.[8,28] Dogs with AHDS have significantly lower microbiome diversity than healthy controls.[13,26] Changes variously reported include increases in *C. perfringens*-like sequences, *Sutterella* and *Fusobacteria*; and decreases in *Firmicutes*, *Lactobacillus*., *Streptococcus*, and SCFA-producing bacteria. [8,13,29] However, the exact character of the dysbiosis differs from study to study, highlighting the heterogeneity of the syndrome.[26] The intestinal microbiome appears to return to normal within 21 days.[10]

SIRS/sepsis

The risk and prevalence of sepsis in AHDS is debatable, and controversial given its impact on treatment decisions. Bacterial translocation, bacteraemia and therefore sepsis are all deemed possible sequelae to the loss of the intestinal mucosal barrier, dysbiosis and splanchnic/intestinal ischaemia. However, it seems that the oft-implicated *Clostridium perfringens* is not particularly invasive and translocation through compromised mucosa can usually be compensated for.[19] In one investigation, 11% of dogs with AHDS had positive blood cultures, but this was not significantly different to a control group.[30] The canine AHDS activity index is not affected by blood culture.[4] As discussed, studies have demonstrated systemic inflammation as a component of AHDS. The systemic inflammatory response syndrome (SIRS) can therefore be seen in some. SIRS can have an infectious (i.e. sepsis) or non-infectious cause. Diagnosis of SIRS in dogs is based on fulfilment of at least two criteria (clinical, or

expanded to include organ dysfunction – Table 2), and 12–70% of dogs with AHDS are reported to fit this, apparently dependent on when the evaluation is performed in relation to treatment.[5,7,30] Without blood cultures, sepsis is not provable, therefore is generally a diagnosis of clinical judgment – waiting for blood culture results before initiating antibiosis is neither practical nor recommended when there is strong clinical suspicion. However, SIRS criteria as a proxy for sepsis is neither sensitive nor specific.[31–33] As AHDS may or may not have primary bacterial cause/component (see later discussion), determination of sepsis versus non-infectious SIRS in affected dogs is challenging. Furthermore, many dogs with AHDS have hypovolaemia, which can influence the SIRS criteria too and improve rapidly following rehydration.[5] It seems reasonable, as some recommend, to only assess for SIRS/septic criteria after some degree of re-hydration therapy, as the number of truly septic AHDS dogs is considered low. One study used as its definition the clinical SIRS criteria after shock therapy, as well as less volume-dependent parameters like neutrophil count, glucose concentration, or total bilirubin concentration.[7] However, some of these variables too can change in AHDS without sepsis (especially neutrophil count). Therefore, classification of patients as septic or non-septic may still be inaccurate. 19/27 dogs (70%) were classified as septic using these expanded criteria, but only 2/17 of them (12%) had positive blood culture.[7] Another study showed 16% of dogs with AHDS had positive blood cultures, and that interestingly the prevalence of positive blood culture was actually higher in dogs that did not fulfil SIRS criteria.[30]

Clinical criteria	Temperature
Hypothermia	<37.5°C
Hyperthermia	>39.3°C for large dogs >39.4°C for small dogs
Tachycardia	>140 bpm
Tachypnoea	>40 bpm

Laboratory criteria	Parameter
Leucocytosis	>25 x10^9/l
Leucopenia	< 6 x10^9/l
Band neutrophilia	>3%
Hypoglycaemia	<3.9 mmol/l

TABLE 2: SIRS criteria for dogs. Reproduced/adapted from multiple sources [5,28,30,33]

Cause of AHDS

As the GI tract is the canine shock organ, any critical illness can result in haemorrhagic diarrhoea; however, AHDS is defined by disease originating in the GI tract itself, without an apparent underlying trigger. Many structural diseases can also cause GI haemorrhage, including neoplasia, ulceration (e.g. secondary to NSAID administration) and rarely torsion/volvulus or angiodysplasia/vascular ectasia. Coagulopathies, particularly platelet disorders, can also result in acute GI haemorrhage. Exogenous toxin ingestion, hypoadrenocorticism or severe pancreatitis can occasionally result in haemorrhagic diarrhoea.[34–37] The peracute nature of AHDS has led some to postulate a type 1 hypersensitivity reaction to food components, but there is currently no evidence to support this. Food engorgement without torsion has led to clinical and histological signs consistent with fatal AHDS.[38] Again however, AHDS is notable for the fact that an identifiable cause is not immediately obvious.

Many studies into AHDS have focussed on identifying causative infectious agents.[13,39] A multitude of agents have been reported in conjunction with the syndrome. What is less clear is the clinical relevance of these findings – the presence of an organism does not always indicate that it is the underlying cause.

Viral causes

Canine Parvovirus (CPV) is a well-known infection that can also cause haematemesis, haematochezia, haemoconcentration, shock and leucopenia (the latter more commonly than in AHDS). Parvoviral enteritis and AHDS should be considered separate entities. Though there are many overlapping features, some key differences include the age of dogs affected, the average severity of presentation/findings and the lack of an immediately identifiable cause in AHDS. Although puppies are most frequently affected by CPV, older dogs can be affected too, especially if vaccination compliance is sub-optimal.

Dogs with AHDS shed more viruses than healthy controls.[40] Paramyxovirus shedding has been identified more frequently in AHDS than healthy controls.[40] Canine Coronavirus was found to be shed more in healthy dogs than AHDS, suggesting that this is not a significant cause.[40] SARS-CoV-2 (the causative agent of Covid-19), including the concerning Spike protein mutation, was isolated from one dog with haemorrhagic diarrhoea, but the clinical significance is questionable as it was far from proven that this was the causative organism.[41] A hypervirulent strain of Canine Vesivirus was associated with a small fatal outbreak in Virginia, USA, but all other known strains appear to cause no or mild disease.[42]

Circovirus (CV) has been reported in association with fatal haemorrhagic diarrhoea in puppies, and a dog affected by haemorrhagic diarrhoea alongside vasculitis and granulomatous lymphadenitis, so could theoretically be an important cause in some dogs.[43–45]

On further faecal investigation, CV was detected in only 12/175 (6.8%) dogs with diarrhoea, including only 2/55 (3.6%) AHDS dogs, suggesting this is not a major causative agent.[9]

Bacteria

Bacterial infections have also been frequently investigated. Acute haemorrhagic enteritis in people caused by *E. coli* enterotoxins has a similar clinical presentation to AHDS. A multi-drug-resistant *E.coli* with Shiga-toxin-producing gene was implicated as a potential cause of fatal septic haemorrhagic diarrhoea that developed in a dog 6 hours post-operatively following cutaneous mass excision.[46] *Salmonella* spp. or *Campylobacter* spp. have not been isolated in great numbers from dogs with AHDS.[24] A few reports described the presence of *Providencia alcalifaciens* in dogs with diarrhoea potentially associated with one geographical outbreak.[13,47] However, it has been argued that the seemingly contagious nature and high mortality rate mean that this was not representative of 'true' AHDS.[19]

Clostridial disease

Clostridial disease has been investigated in relation to AHDS for decades. Clostridia are anaerobic, gram-positive, spore-forming bacilli. *Clostridium perfringens* and *Clostridioides difficile* have been explored particularly, due to their endotoxin release, which can cause extensive fluid loss and intestinal necrotic lesions explaining the findings in AHDS.[48] Other clostridial organisms have been rarely associated with AHDS too.[49]

In one small study of intestinal biopsy samples rather than faecal testing, all dogs with AHDS had Clostridia isolated, with 6/9 identified as *C. perfringens* (in a control group only 1/11 dogs had *C. perfringens*).[3] Through another study utilizing immunohistochemistry, all 10 dogs with AHDS had clostridial antigen detected.[18] *C. perfringens* is widely dispersed and inhabits most terrestrial and aquatic environments, as well as the mammalian gut even in health. Some propose that stress, or alterations in local immunity or motility induce dysbiosis and predispose to overgrowth of *C. perfringens*, but there does not appear to be evidence for this in dogs.[3,50]

C. perfringens isolates have traditionally been classified by their phenotype/toxinotype (types A-E, with further addition of types F and G), which is determined by the combination of (often similarly-named) major toxins it can produce – including alpha (CPA), beta, epsilon, iota, enterotoxin (CPE), and now NetB (Table 3). [51,52] A variety of newer toxins have also been described (up to 20 in total). Studies into toxin production variously study detection of the toxin itself (e.g. CPE), and/or of the gene encoding for that toxin (e.g. *cpe*). Type A isolates are most associated with disease in humans, ruminants and chickens. In pre-2018 studies CPE was associated mainly with type A strains. However, the toxinotyping groups have now been re-classified so that type F are now the predominant CPE-producing strains (Table 3). In people, CPE is implicated in food poisoning, nosocomial diarrhoea and antibiotic-associated diarrhoea.[53–56]

C. perfringens (previously *C. welchii*), mostly type A (prior to 2018), has been isolated from dogs with AHDS, often with large numbers of bacilli adherent to enteral mucosal surfaces and sometimes with the *cpe* gene.[3,23,25,38,59] Both CPA and CPE isolation appear more common in dogs with diarrhoea, but crucially all have also been isolated from dogs without diarrhoea.[60–63] Regarding AHDS specifically, some studies have shown increased prevalence of *C. perfringens* compared to controls, and others showed increased isolation of CPE.[13,24,29,48]

C. difficile is another organism strongly associated with toxin release and enteral disease in humans, particularly nosocomial or antibiotic-induced disease. *C. difficile* toxins 'A' and 'B' have been the most extensively studied. *C. difficile* has been isolated only in low numbers (25%) from dogs with AHDS.[24,28]

Against this evidence in favour of clostridial pathogens as a cause of diarrhoea/AHDS is the simple fact that *C. perfringens* and *C. difficile* are also commonly found in the faeces of healthy dogs.[29,63–65] Isolation rates of *C. perfringens* as

Toxinotype	Alpha (CPA)	Beta	Epsilon	Iota	Enterotoxin (CPE)	NetB
A	+					
B	+	+	+			
C	+	+			+/-	
D	+		+		+/-	
E	+			+	+/-	
F	+				+	
G	+					+

TABLE 3: Updated toxinotype classification of *C. perfringens*. Reproduced/adapted from multiple sources [51,52]

high as 71–100% have been reported in dogs without diarrhoea.[53,60,61,65] Many studies have shown that diarrhoeic dogs have similar isolation rates of *C. perfringens* and *C. difficile* as non-diarrhoeic dogs.[24,53,60,61] However, *C. perfringens* is rarely identified in the intestine through culture or histopathology in healthy dogs or those with other GI disease, as has been noted in AHDS.[19] Additionally, in some studies, dogs with diarrhoea had increased isolation of CPE and *C. difficile* toxins A and B, supporting the idea that it is not the bacteria themselves but the type of toxin they produce that influences pathogenicity.[24,48,53,60] However, other studies (some looking at AHDS specifically) have found conflicting results, where diarrhoeic and non-diarrhoeic dogs had similar isolation rates of *cpe*, *cpa*, *C. perfringens* toxins and *C. difficile* alpha/beta toxins.[4,8,14,48,61] In one study, there was no significant difference between CPE or *cpe* detection in disease severity, length of hospitalization or mortality, with the conclusion that CPE does not play a significant role in AHDS.[48] It attributed any increased prevalence instead to secondary dysbiosis, highlighting that all studies investigate faeces after the onset of diarrhoea rather than before. Identification of faecal sporulating Clostridia, CPE or *cpe* are no longer considered diagnostic for AHDS. Clostridia were cultured from the blood of both healthy and AHDS-affected dogs.[30] Finally, it seems true that many dogs with AHDS do not have evidence of either of these clostridial pathogens or their toxins.[24]

More recently, a pore-forming toxin (NetF) associated with the *netF* gene was identified in *C. perfringens* type A in both canine AHDS and the similar 'Fatal Foal Necrotizing Enterocolitis' in horses.[66] Histopathology showed that 5/5 of the *C. perfringens* isolates from dogs with AHDS were capable of NetF production.[18] Studies revealed that 46–70% of dogs with AHDS had *netF*, implying it is certainly not present in all cases, but significantly more than dogs that were healthy or diagnosed with CPV. [10,12,28] There was no difference between *netF*-positive and -negative dogs in relation to clinical severity or outcome.[12] These strains disappear quickly even without specific therapy – by day 42, NetF-encoding *C. perfringens* could be detected in only 1/32 dogs in one study, and in no dogs within 7 days in another.[10,28] The fact that this toxin generally disappears after resolution of clinical signs, whereas other clostridial toxins persist, is further support for their pathogenicity in AHDS. Of further interest, in CPV-infected dogs with similar pathological changes to AHDS, *netF* was not detected, stymieing any idea that this could be a secondary response.[12]

Summary of evidence

Always in investigating pathogens from the GI tract of dogs with disease, it is important to remember that correlation does not equal causation, and that a different inciting cause may lead to alterations in the bacterial populations and virulence toxins.[53] Secondary clostridial overgrowth could be a sequel of another underlying cause e.g., a yet undiscovered virus. It may be that isolation of the organism directly from the intestinal mucosa is a more accurate assessment than simply faecal isolation.

Koch's postulates (an admittedly outdated model) have not been satisfactorily fulfilled for any of the suggested causes of AHDS, consistent with a complex and presumed multifactorial aetiology. More nuanced models such as Molecular Koch's postulates trend towards closer fulfilment for the NetF toxin, but further research is still required.[66] Crucially, AHDS does not appear to be transmissible.[19] It seems likely that NetF release from *C. perfringens* type A/F strains, possibly alongside other toxins such as CPE, is a key virulence factor associated with the necrotic enterocolitis in many dogs with AHDS.[19,67] Potentially, different triggering causes are responsible for clostridial overgrowth. All evidence highlights the syndromic nature of AHDS – that is that dogs share common clinical and clinicopathological abnormalities but not necessarily share an aetiology.[26]

Reflect on your reading

1. Which usually comes first in dogs affected by AHDS – vomiting or diarrhoea?
2. Name two reasons why 'haemorrhagic gastroenteritis' may be a potentially misleading term?
3. Which *Clostridum perfringens* toxin shows the most promise for involvement in AHDS?

   a) CPE
   b) CPA
   c) NetB
   d) NetF

Answers available online in the BSAVA Library.

About the author

Bryn graduated from the University of Nottingham in 2014 before spending some years in a small animal hospital in South-West Wales. After completing an internship at the RVC and a 3-year Small Animal Medicine residency in AMVS in Hampshire, Bryn settled at Willows at the start of 2023. Bryn has a keen interest in nephrology, hepatology and particularly gastroenterology.

References and further reading are available at **www.bsavalibrary.com**.

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