Describing blood acid-base response in dogs with acute haemorrhagic diarrhoea syndrome using three different methods

by

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Declaration of Originality

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List of Abbreviations

ACTH: adrenocorticotropic hormone

AHDS: acute haemorrhagic diarrhoea syndrome

AG: anion gap

A_{TOT}: total quantity of weak acids

$BE_{(B)}$: base excess of blood

$BE_{(ECF)}$: base excess of extracellular fluid

HCO_3^-(act): actual bicarbonate ion concentration

HCO_3^-(std): standard bicarbonate ion concentration

HHb: deoxyhaemoglobin

K^+: potassium

Na^+: sodium

$PvCO_2$: partial pressure of carbon dioxide

PvO_2: venous partial pressure of oxygen

$SHCO_3^-$: standard bicarbonate

SID: strong ion difference

SID_{app}: strong ion difference apparent

SID_{eff}: strong ion difference effective

SIG: strong ion gap

TCO_2: total cardon dioxide

UA: unmeasured anions

XA: semiquantitative measure of unmeasured anions

UC: unmeasured cations

Abstract

Objective: To describe blood acid-base response in dogs with acute haemorrhagic diarrhoea syndrome (AHDS) using three different methods of analysis.

Hypothesis: Dogs with AHDS have increased unmeasured strong anions compared to healthy dogs.

Design: Prospective, observational study.

Setting: Private referral hospital.

Animals: A total of 20 dogs used in two groups as follows: 10 healthy age-, weight- and breed-matched dogs and 10 dogs with AHDS.

Interventions: Blood that was collected from healthy dogs were used to establish an expected normal range (minimum and maximum limits of the ranges were calculated as mean ± 2 standard deviations). Jugular venous blood, AHDS index scores (0 – 3: insignificant disease; 4 – 5: mild AHDS; 6 – 8: moderate AHDS; 9 – 18: severe AHDS) and intravenous fluid infusion volumes (mL/kg) were collected at presentation (0H) and then at set hour-intervals post presentation (4H, 8H, 12H, 16H, 20H, 24H, 36H, 48H and 60H). Blood was analysed to measure or calculate acid-base variables used in three different methods of analysis: 1) traditional, 2) Stewart theory, and 3) semi-quantitative theory approaches. Longitudinal data were compared using a general lineal mixed model with post-*hoc* comparisons using Dunnett's method (control variable: values at 0H) and significance was $P < 0.05$ and data were reported as median (minimum – maximum).

Measurements and main results: The pH, at 0H, was 7.31 (7.22 – 7.49) and classified as acidaemic with a wide anion gap of 24.6 (13.1 – 27.6) mmol/L because of a raised venous carbon dioxide tension [48 (26 – 51) mmHg], negative base excess of extracellular fluid [-5.4

((-8.0) – (-2.4)) mmol/L] and acidaemic lactate effect [-3.5 ((-5.4) – (-1.2)) mmol/L]. The pH normalised by 4H ($P < 0.0001$) in response to fluid administration where 37 (29 – 63) mL/kg was given over the 4-hour period. Whereas the AHDS clinical index score was classified as 'insignificant disease' by 48H. The pH remained within normal reference intervals until 60H and fluid rates were 3 mL/kg/hour from 8H onwards. The bicarbonate and haemoglobin buffer systems played a role in blood acid-base homeostasis.

Conclusions: The acidaemia at presentation in dogs with AHDS was related to hypovolaemia and all derangements were corrected by fluid resuscitation. All three methods of analysis were useful in interpreting the complex interplay between acidifying and alkalinising effects and blood buffers.

Keywords: acute haemorrhagic diarrhoea syndrome, acid-base analysis, strong ion approach, traditional approach

1. Introduction

Acute haemorrhagic diarrhoea syndrome (AHDS) is a common syndrome in dogs that is responsible for their hospitalisation. The aetiology of the syndrome is complex and not completely understood (Unterer et al. 2011; Mortier et al. 2015) but certain disease processes such as parvovirus infection, verminosis, coccidiosis, intestinal obstruction, pancreatitis, hypoadrenocorticism or even carbamate and cannabis exposure could confuse the diagnosis. Possible aetiologies such as diet indiscretion, dysbiosis or clostridial overgrowth can be responsible for AHDS. Dogs with AHDS present with a history of acute onset inappetence, vomiting and foetid haemorrhagic diarrhoea (Mortier et al. 2015). Furthermore, dogs can present with different degrees of disease severity, from mild vomiting and diarrhoea to collapse and severe compromise. These different degrees of illness has warranted the development of a AHDS index score which stratifies dogs into four different categories. Based on the clinical significance of disease, these categories range from 'clinically insignificant' with a total score between 0 and 3, to 'severe AHDS' with a total score between 9 and 18 (Mortier et al. 2015). Investigations into the aetiology and treatment of AHDS has been ongoing with an overall notion that a symptomatic therapy plan should be used where the initial goal is to restore intravascular volume (Unterer et al. 2011; Mortier et al. 2015). However, blood acid-base status in dogs with AHDS is scantly reported on. Mortier et al. (2015) have reported that venous blood pH has a median (minimum to maximum) of 7.34 (7.04 to 7.43), a bicarbonate ion concentration of 21.7 (8.7 to 29.3) mmol/L, and a L-lactate concentration of 2.1 (0.8 to 10.3) mmol/L. Further investigation into acid-base analysis at presentation and over time in dogs with AHDS is warranted. Both the disease process and its symptomatic treatment using intravenous fluids can alter acid-base status in these dogs (Unterer et al. 2011).

Acid-base disturbances and electrolyte disorders have been frequently reported in dog populations with a wide range of diseases including those with gastrointestinal-related illnesses (Burchell et al. 2014), especially those admitted to an intensive care unit ward (Torrente et al. 1967; Boag et al. 2005; Hopper et al. 2014a, b; Zager et al. 2018; Emejuo et al. 2020; Hasuda et al. 2020). The understanding of blood pH and acid-base status in dogs with critical illness is important, because enzyme function is most efficient at blood pH levels between 7.35 and 7.45 in most mammals which include dogs (Ilkiw et al. 1991). Moreover, enzyme function is critical to maintain whole body homeostasis, optimal organ function and metabolism (Mitchell et al. 1972; Crimi et al. 2012; Zeiler et al. 2022).

In dogs with gastrointestinal tract disease, vomiting and diarrhoea are expected with a reluctance to consume adequate volumes of food and water. In addition, the intravascular volume will be affected by the ongoing losses, all of which will influence acid-base and electrolyte balance (Boag et al. 2005). The gastrointestinal tract plays a pivotal role in acid-base homeostasis (Gennari 2006). Consequently, when there is gastrointestinal tract disease, acid-base homeostasis depends on the location (proximal versus distal gastrointestinal tract) and nature of fluid being lost (hypotonic versus hypertonic). Generally, metabolic alkalosis is more commonly diagnosed with proximal gastrointestinal tract dysfunction (vomiting and gastric ileus), whereas metabolic acidosis is more commonly diagnosed with distal gastrointestinal tract dysfunction (diarrhoea) (Emejuo et al. 2020; Hasuda et al. 2020).

Three different methods of blood acid-base analysis have been used in dogs, namely, the 1) traditional approach, 2) Stewart theory approach, and 3) semi-quantitative theory approach (Torrente et al. 1967; Hopper et al. 2014; Zager et al. 2018; Hasuda et al. 2020). Briefly, the traditional approach is focused on the blood pH as a result of the variables of the

bicarbonate buffer system in the blood. The two major influencers of pH are the dissolved carbon dioxide tension and the bicarbonate ion concentration (Henderson 1908; Hasselbalch 1917). The carbon dioxide tension is maintained by respiratory system function and is considered the *respiratory component* of acid-base homeostasis. In contrast, the bicarbonate ion concentration is considered the *metabolic component* of acid-base homeostasis. The Stewart theory approach is centred around three independent variables that influence the ionization of water. These are the 1) carbon dioxide tension, 2) strong ion difference, and 3) total amount of weak acids (Stewart 1983). This method of analysis is useful where there are derangements in electrolyte and albumin concentrations (Hopper et al. 2014a; Zager et al. 2018). The semi-quantitative theory approach is useful when there are derangements detected in base excess, because it examines the effects of five key variables that have both acidifying and alkalising properties in blood and can thus influence base excess. These five variables are sodium (free water effect), albumin, chloride, L-lactate, and phosphate (Fencl et al. 2000).

2. Literature Review

2.1 Acute haemorrhagic diarrhoea syndrome

Acute haemorrhagic diarrhoea syndrome (AHDS) was previously called haemorrhagic gastroenteritis (Unterer et al. 2011). The syndrome AHDS is common and is responsible for a significant amount of dogs presenting to a veterinary care facility.

This disease does not have a known aetiology and it has been found to be most prevalent in dogs (Burrows 1977; Unterer et al. 2011). This syndrome is mostly identified by an acute onset haemorrhagic diarrhoea with or without haematemesis. Diarrhoea episodes are usually severe, malodorous and frequent (Triolo and Lappin 2003; Unterer and Hartmann 2009). Upon presentation to the hospital, dogs are oftentimes lethargic and anorexic.

A few different aetiologies have been proposed because they have been associated with AHDS. These aetiologies include a 1) type-1 hypersensitivity reactions to food, 2) bacterial overgrowth causing endotoxin release and, 3) clostridial proliferation (Cave et al. 2002; Triolo and Lappin 2003; Unterer et al. 2011; Unterer et al. 2014).

Food allergens are low molecular weight glycoproteins, ranging between 10 – 70 kDa, that are often resistant to degradation by heating, adding acids or proteas enzymes. There is no breed, sex or age predilection, although some breeds such as Yorkshire Terriers, Miniature Schnauzers and Maltese Poodles are commonly affected (Verlinden et al. 2006). The prevalence of adverse food reactions in dogs and cats is largely unknown but seems to range between 1% and 2% of dogs presented to a primary care facility; and less than 1% of cats presented to an academic hospital has been reported in a global review of this subject (Olivry and Mueller 2017). Food intolerance and dietary indiscretion (ingestion of inappropriate materials) are probably more common in dogs than true dietary hypersensitivity (Day et al. 2005).

The upper gastrointestinal tract is usually sterile; however, bacterial overgrowth can occur in the setting of decreased gastric and intestinal peristalsis (Du Moulin et al. 1982). Changes in the intestinal flora, along with impairment of the normal barrier function of the gut, allow for the gastrointestinal system to serve as a reservoir for pathogens to enter the systemic circulation. It is postulated that barrier dysfunction and dysbiosis during the acute phase of disease are factors responsible for sensitization of the immune system (Chu et al. 2023). This postulation could ring true for AHDS, because diseased dogs undergo acute gastrointestinal damage, which results in intestinal barrier dysfunction and dysbiosis. Destruction of intestinal villi and epithelial necrosis are hallmark lesions seen in post-mortem histopathology (Unterer et al. 2014).

Understanding the pathology found at necropsy can help explain the clinical signs of AHDS seen on presentation. The principal intestinal lesions were described as superficial mucosal haemorrhagic necrosis (Prescott et al. 1978; Sasaki et al. 1999). Histopathology identified large gram-positive bacilli, namely *Clostridium perfringens* that adhered to necrotic mucosal surfaces (Badcoe 1992; Sasaki et al. 1999). This bacterium is a spore-bearing bacterium, making it extremely challenging to use gram-staining to confirm its presence. Culturing *C. perfringens* is not easy when using usual an aerobic culture techniques (Kenzaka and Ueshimo 2016). A simpler way of ruling in the possibility of *C. perfringens* as a causative agent is to perform and gram-stain a faecal smear, usually manifesting as a monopopulation of rod-shaped bacteria. Finding *C. perfringens* at post-mortem in the gastrointestinal tract implies that there is likely an association with AHDS, but it does not imply causality. Furthermore, normal post-mortem changes like autolysis are expected to occur as early as 90 minutes after death. Autolysis presents very similar to necrosis of mucosal surfaces which complicates post-mortem interpretations (Thorpe and Thomlinson 1967). An additional

conundrum with proposing *C. perfringens* as an aetiology of AHDS is that it is a normal commensal of the intestinal biome in dogs, and thus, positive cultures yield no diagnostic value (Unterer et al. 2015). Currently, no studies on macroscopic appearance or on histologic changes performed *intra vitam* in dogs with AHDS exist (Unterer et al. 2014). *Clostridium perfringens* type A strains are associated with release of *netF* toxins (which is a beta-pore-forming toxin) with a few instances, in concert with other toxins such as *netE*, *netG* and *C. perfringens* enterotoxin gene (CPE) which are known to be the main role-players seen in necrotic enterocolitis in dogs with AHDS (Sindern et al. 2019). Another interesting finding by Suchodolski et al. (2012) is that many dogs with AHDS have alterations in their microbiome. Furthermore Sindern et al. (2019) demonstrated the prevalence of *C. perfringens* encoding for *netE* and *netF* is significantly higher in dogs with AHDS compared to control dogs. Faecal samples analysed by gene sequencing revealed an increased proportion of *C. perfringens*-like sequences as well as decreases in proportions for *Blautia* spp. and *Turicibacter* spp. (Suchodolski et al. 2012).

Jejunal haemorrhagic syndrome in dairy cattle shares in a pathogenesis of protein overfeeding causing clostridial overgrowth (C. perfringens), leading to production of toxins which target cell damage and inflammation. The major finding of this disease process is acute localized necrotizing haemorrhagic enteritis in the small intestine, causing obstructive blood clots which lead to ischemic complications and devitalization of portions of the affected gut (Mamak and Börkü 2019). The fatality rate ranges from 85%-100% with most acid-base analysis showing a metabolic alkalosis with a hypochloraemia due to the clotted blood causing outflow obstruction of the abomasum (Abutarbush et al. 2005). Although this disease process

harbours similar characteristics of AHDS in dogs, it has a far more grave prognosis with severe obstructive haemorrhagic patterns identified on post mortem.

The aetiology of AHDS is not completely understood. Consequently, a diagnosis of this syndrome is often based on a diagnosis-by-exclusion. It is vital to take a descriptive history, noting non-steroidal anti-inflammatory drug administration as well as possible intoxications such as ingestion of insecticides containing pyrethroids or carbamates, or chocolate containing theobromine (milk and dark chocolates made from cocoa), or poisonous plants such as cycads (*Zamia loridana, Cycas revoluta, Macrozamia riedlei*) and syringa berries (*Melia azedarach*). Abdominal pain elicited on cranial abdominal palpation could indicate a co-morbidity with pancreatitis, a common finding in dogs with AHDS. Dogs presenting with this co-morbidity present with more severe clinical signs of vomiting and haemorrhagic diarrhoea. Evaluating specific electrolytes such as sodium (Na^+) and potassium (K^+) will assist with identifying typical hypoadrenocorticism (Addison's disease). Faecal analysis (to exclude giardiasis, coccidiosis and verminosis), complete blood count, point-of-care ELISA test (to rule out parvovirus infection) and abdominal ultrasound examination (to rule out gastrointestinal obstruction or pancreatitis) should be performed before a diagnosis of AHDS is made.

It has been proposed that AHDS could possibly result in a chronic enteropathy later on in the dog's life (Skotnitzki et al. 2022). To assess the long-term impact of AHDS, longitudinal studies are warranted (Skotnitzki et al. 2022). A loss of tolerance to food components and altered intestinal microbiota after severe intestinal injury are potential mechanisms and risk factors for the development of chronic enteropathy (Killian et al. 2018). Another risk factor is instability of the microbiome in early life, due to the immune system

and intestinal microbiome still being immature (Nylund et al. 2014; Johnson and Depaulo 2017).

2.2 Acid-base analysis

The blood pH is maintained within a normal reference range of between 7.35 and 7.45 despite the body continuously producing acids and bases as metabolic products and byproducts (Adams and Polzin 1989). The pH is defined (and calculated) as the negative logarithmic of hydrogen activity in solution ($pH = -\log[H^+]$) (Sorenson 1968). It is imperative for the body to maintain a normal pH, because this is where the body functions optimally through its cellular enzyme systems and maintaining the correct concentrations of ionized forms of electrolytes, especially calcium and magnesium (Cameron 1989). These two electrolytes play a pivotal part as co-factors in many enzyme-controlled metabolic reactions and trans-membrane transportation systems (Rosol and Capen 1997; Pasternak et al. 2010).

Acid-base derangements are a frequent occurrence in animals with gastrointestinal-, renal-, and pulmonary dysfunction and intravascular and total body water volume status disruptions, and general anaesthesia, for example (Perez et al. 1987; Bailey and Pablo 1998; Boag et al. 2005; DiBortola 2011; Unterer et al. 2011; Mortier et al. 2015). Understanding an acid-base derangement in animals with a disease process could help guide therapy and allow for disease risk stratification and prognostication.

Acids and bases are constantly fluctuating between a state of acidosis or alkalosis depending on the disease process that is affecting their homeostasis. There are buffer systems in the extracellular and intracellular compartments what maintain the pH within its narrowly regulated range. The extracellular buffering systems include the bicarbonate buffer system, plasma proteins, and phosphate. Whereas intracellular buffer systems include

cytosolic proteins that are present in high concentrations, making them the most important intracellular buffer system, along with phosphate. An efficient buffering system is made up of either a weak acid or a weak base with an ionising dissociation constant (pKa) close to plasma pH. For the most part, most extracellular buffers are weak acids with a pKa of approximately 6.1. These buffers can relinquish their hydrogen ion if necessary, and the conjugate base which can accept hydrogen ions minimises changes in free hydrogen ion concentrations. Non-buffer related mechanism to maintain blood pH are transcellular movement of ions, such as potassium ions being exchanged for hydrogen ions (Adler and Fraley 1977). This transcellular exchange occurs along concentration gradients while maintaining electrical neutrality between extracellular and intracellular fluids (see later: 2.2.2 Stewart theory approach).

Blood gas analysis is a vital diagnostic tool used in intensive care unit wards, because it makes it possible for us to analyse the oxygenation, ventilation, acid-base balance and electrolytes of dogs and other animals. Blood gas analysers are available as handheld point-of-care devices or as benchtop analysers. Ideally, lithium heparinised syringes are used for blood collection, with analysis done promptly to minimise pre-analytical errors (Rieser 2013). Common sites of collection for arterial blood sampling include dorso-pedal-, metatarsal-, auricular- and femoral arteries. Common venous blood collection sites include peripheral (least ideal), jugular vein (most practical), central venous and mixed venous (most preferred) (Shiroshita et al. 2023). The blood sample should be collected over two respiratory cycles and any air aspirated into the syringe should be expelled immediately, and the sample sealed anaerobically to prevent exposure to room air (Davis 2020). The sample should be analysed promptly after collection. Blood gas analysers directly measure pH (pH probe), partial pressure of oxygen (Clark electrode) and the partial pressure of carbon dioxide (PCO_2; Severinghaus electrode), electrolytes and analytes (various ion selective electrodes) and

saturations of various forms of haemoglobin (using co-oximetry). These measured values are then used to calculate other variables printed out on a typical blood gas strip, these include actual and standard bicarbonate ion [HCO3-(act) and HCO3-(std), respectively] concentrations (using the Henderson-Hasselbach equation; see 2.2.1 Traditional theory approach), and blood and extracellular fluid base excess [$BE_{(B)}$ and $BE_{(ECF)}$, respectively] based on variations of the Van Slyke equation (Rieser 2013).

When deciding whether to choose venous or arterial sampling of blood, clinical status of the patient is important to take into consideration as well as factors that affect blood circulation such as sampling site, blood flow rate and puncture site temperature (Brandenburg and Dire 1998; Ak et al. 2006; Byrne et al. 2014). Arterial blood gas is the gold standard in respiratory conditions, because arterial samples give good indications of events occurring at the pulmonary level (Ak et al. 2006). Blood from any venous site will accurately reflect the acid-base status of the patient, provided the patient has a normal circulatory status (Sutton et al. 1967; Phillips and Peretz 1969; Ilkiw et al. 1991). A study performed by Ralston (1985) suggested that venous blood gas analyses are sensitive to changes occurring in the systemic capillary bed and may be a more accurate indicator of the intracellular metabolic status (Ralston et al. 1985). Kelly (2010) found that in human patients who are not in a state of shock have sufficient agreement between venous pH, HCO_3^-(act) and $BE_{(ECF)}$ with the arterial values and thus venous blood gas can be used clinically and are interchangeable. This gives us opportunity to run venous blood gases routinely as a patient-side diagnostic tool, provided patients are not in a shocked state. Weil et al. (1986), argued that mixed venous pH plays a pivotal role in interpretation of the balance between whole body oxygen delivery and oxygen consumption. This finding in mixed venous pH was seen to be of more relevance than when comparing to the arterial pH during his study on acid-base status in cardiopulmonary

resuscitation. Respiratory acidosis was an important component of tissue acidaemia during cardiopulmonary resuscitation, where arterial blood gas failed to reflect these abnormalities.

In addition, our study required multiple blood samplings for blood gas analysis which made arterial collection a non-feasible option, due to prolonged arterial catheterisation being associated with rare, but serious complications (Frezza and Mezghebe 1998; Scheer et al. 2002; Valentine et al. 2005; Lorente et al. 2006). Furthermore, arterial blood sampling using repeated needle sticking has limitations due to the collection procedure being painful, not easily accessible in awake patients and has the potential to cause arterial injury and thrombosis (Saberian et al. 2023). Therefore, we elected to place a catheter into the jugular vein for repeated sampling of venous blood.

Table 1 was taken from Ilkiw et al. (1991) which tabulates the mean acid-base values from arterial, mixed venous, jugular and cephalic blood samples from conscious dogs .

Table 1 Demonstrates the mean variables for pH, PCO_2 ,PO_2 HCO_3^- TCO_2 BE and $SHCO_3^-$ of arterial blood, mixed venous, jugular and cephalic blood samples in conscious dogs

Value	Arterial Blood	Mixed venous blood	Jugular blood	Cephalic venous blood
pH (U)	7.395	7.361	7.352	7.360
PCO_2 (mmHg)	36.8	43.1	42.1	43.0
PO_2 (mmHg)	102.1	53.1	55.0	58.4
HCO_3^- (mEq/L)	21.4	23.0	22.1	23.0
TCO_2 (mEq/L)	22.4	24.1	23.2	24.1
BE (mEq/L)	-1.8	-1.1	-2.1	-1.2
$SHCO_3^-$ (mEq/L)	22.8	23.0	22.2	23.2

PO_2 : partial pressure of oxygen, TCO_2: total cardon dioxide, BE: Base excess, $SHCO_3^-$: standard bicarbonate.

2.2.1 Traditional theory approach

Background to this theory

The traditional theory is based on the Henderson-Hasselbalch equation, as follows:

$$pH = pK_a\,(H_2CO_3) + Log_{10}\left(\frac{HCO3-}{H2CO3}\right)$$

where pK_a represents the acid dissociation constant of carbonic acid (H_2CO_3) and is 6.1 (Henderson 1908; Hasselbalch 1917). The HCO_3^- represents the actual bicarbonate ion concentration, which is the conjugate base of the acid, carbonic acid. In addition, carbonic acid is an effective buffer because it can dissociate into either HCO_3^- and a hydrogen ion, or dissociate into dissolved carbon dioxide and water (H_2O), depending on concentration gradients, as follows:

$$H_2O + \text{dissolved } CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

This chemical formula for the production and dissociation of H_2CO_3 is the main biochemical mechanism of the bicarbonate buffer system of plasma.

Henry's Law states that when a gas is in contact with a fluid, the amount of gas that dissolves into fluid is directly proportional to the partial pressure of that gas. Taking Henry's Law into effect, the H_2CO_3, can be rewritten as the dissolved CO_2 in plasma by multiplying the PCO_2 by Henry's constant (0.03 mole/L x atmospheric pressure), because it is a blood-gas-

partitioning coefficient (Nargis et al. 2013). Then the revised Henderson-Hasselbalch equation becomes:

$$pH = 6.1 + Log_{10}\left(\frac{HCO3-}{0.03\ x\ PCO2}\right)$$

The blood pH is directly proportional to HCO_3^- therefore as HCO_3^- increases, so does the pH increase and become more basic. Whereas PCO_2 is inversely proportional to blood pH, so as the PCO_2 increases, the pH will simultaneously decrease, becoming more acidic.

The [HCO_3^-] is controlled by the renal system and thus termed the metabolic-component of acid-base balance (DiBartola 2006). Whereas the PCO_2 is controlled by ventilation and thus termed the respiratory-component of acid-base balance. The ideal ratio of [HCO_3^-] to [0.03 x PCO_2] is 20:1 and maintaining this ratio became the premise to the concept of respiratory or metabolic compensation.

Interpretation of acid-base status using this theory

Interpretation of acid-base using the traditional approach uses PCO_2 in the determination of the respiratory component of acid-base balance and HCO_3^- as the metabolic component. An increased PCO_2, in arterial blood traditionally, is classified as a respiratory acidosis. Metabolic acidosis is interpreted with a decreased HCO_3^- and metabolic alkalosis with an increased HCO_3^- (Scott and Klutts 2006).

Metabolic derangements often occur at the cellular level and in the post-capillary blood, after blood-tissue exchanges of nutrients and byproducts of metabolism have occurred and is best analysed using venous blood (Brandenburg and Dire 1998). The renal system plays

a crucial part in acid-base regulation and compensates through adjusting the amount of HCO_3^- that is being filtered. Reabsorption of HCO_3^- occurs predominantly in the proximal tubule and collecting duct. The H_2O within the distal tubular cell dissociates into a hydrogen ion (H^+) and hydroxide ion (OH^-); and in the presence of carbonic anhydrase, the OH^- combines with CO_2 to form HCO_3^-, which is transported back into the peritubular capillary, while H^+ is secreted into the tubular lumen and joins with freely filtered HCO_3^- to form CO_2 and H_2O, which are also reabsorbed (Imenez and Mohebbi 2022). Thus, the distally reabsorbed HCO_3^- ions are newly generated and not the same as those that were filtered. The renal system is a lasting compensatory mechanism which begins within 12 hours of detecting an acid-base derangement and can continue compensation for up to 5 days (Clayton-Smith and Sharma 2021).

Respiration (rather ventilation) plays a vital role in the control of pH variations because CO_2 is finely regulated by changes in tidal volume and respiratory rate (the product is minute ventilation). A decrease in pH is sensed by central chemoreceptors and leads to increases in minute ventilation (by increasing tidal volume or respiratory rate, or both); Carbon dioxide is then exhaled and blood pH increases as a consequence. In contrast to chemical buffering, which is immediate, pulmonary regulation and compensation occurs over minutes to hours (Carlson 1997).

In dogs, calculations to determine the adequacy of metabolic or respiratory compensation have been validated (de Morais and DiBartola 1991). The compensatory responses to primary acid-base disorders are as follows: for metabolic acidosis, respiratory compensation is when PCO_2 decreases by 0.7 – 1.2 mmHg per 1.0 mmol/L decrease in HCO_3^- ; for metabolic alkalosis, PCO_2 will increase by 0.7 mmHg per 1.0 mmol/L increase in HCO_3^-;

for acute and chronic respiratory acidosis, HCO_3^- will increase by 0.15 mmol/L and 0.35 mmol/L, respectively for each 1.0 mmHg increase in PCO_2. For acute and chronic respiratory alkalosis, HCO_3^- will decrease by 0.25 mmol/L and 0.55 mmol/L, respectively for each decrease in 1.0 mmHg of PCO_2 (de Morais and DiBartola 1991; de Morais and Leisewitz 2006; DiBartola 2006).

Mixed acid-base derangements are present when there is the presence of more than one primary disturbance (i.e., two metabolic disturbances, one respiratory and one metabolic disturbance). Various scenarios prompt the suspicion for a mixed acid-base disturbance such as a normal pH which is accompanied by an abnormal HCO_3^- and/or PCO_2, a change in pH that cannot be attributed to one disorder alone, the change in HCO_3^- that is not proportional to the change in the anion gap, and PCO_2 and HCO_3^- change in opposite directions as well as when the compensatory response is unexpected, with either too much or too little response.

The traditional approach has incurred criticism because it is descriptive in nature and qualifying or quantifying complex acid-base derangements is a major short coming, especially in clinical decision making in critically ill patients, yet it is commended for its simplicity and application to the clinical setting (Balasubramanyan et al. 1999; Fencl et al. 2000; Sirker et al. 2002; Boniatti et al. 2009; Fidkowski and Helstrom 2009).

Additional assessments used in the traditional theory approach

There are two additional variables that have been incorporated into the traditional theory approach of acid-base analysis. These are base excess (BE) and anion gap (AG).

Base excess

Base excess was established through a quest for the discovery of a stand-alone marker defining metabolic acidosis and alkalosis, which was independent of respiratory derangements and able to quantify disease severity (Severinghaus and Astrup 1985). The concept of a buffer-base was introduced to consider all non-carbonic buffers (CO_2 independent) (Singer 1948), but this buffer-base model experienced wide variability between non-carbonic buffer concentrations (Astrup et al. 1960). To address this limitation of wide variability, the concept of base excess was developed. Base excess is defined as the amount of base (in mmol) that would be required to be added or removed in order to restore 1 litre of blood to a pH of 7.4 (assuming PCO_2 is normal), at 37°C. If there is a deficit of base, there is a metabolic acidosis and base would need to be added to correct the pH. In contrast, if there is a base excess, there is a metabolic alkalosis and base would need to be removed to normalise the pH. Most equations used for calculating the base excess are human-derived and stem from the fundamental equation derived by Siggaard-Andersen, called the Van Slyke equation, which is:

$$BE = Z \times [[cHCO_3^- \ P) - {}_{C7.4}HCO_3^- \ P)] + beta \times (pH - 7.4)]$$

where Z is a constant which depends solely on total haemoglobin concentration in blood (Siggaard-Anderson 1977), ${}_{C7.4}HCO_3^-$ is plasma bicarbonate at reference pH and beta is the slope of the PCO_2 buffer line (mmol/L) for whole blood.

The BE can be evaluated for whole blood, where the interstitial component is not considered, or for the extracellular fluid, $BE_{(ECF)}$, where haemoglobin (Hb) is not included in the equation. The two BE values yield similar results in clinical practice and guide clinicians

similarly in therapeutic interventions, provided the Hb is within normal reference intervals. However, $BE_{(ECF)}$ is considered the more reliable variable of the two and should be focused on in clinical practice (Kofstad 2001; Morgan 2011).

A BE value outside of the normal reference range indicates an acidotic (less than lower limit) or alkalotic (greater than upper limit) process occurring. A limitation of interpreting BE is that it does not provide insight into the underlying mechanism(s) of the acid-base derangement. Variables such as chloride, L-lactate, albumin and ketoacids can explain derangements in BE (Constable 2002; Hopper et al, 2014a,b; Morgan et al. 2011). These variables could behave in opposite directions, cancelling each other's effect and leading to difficulty in interpretation of a normal BE in the face of a sick patient (Langer et al. 2022). The BE measured from either arterial or venous blood have been shown to be indicators of shock, intra-abdominal injury and to be predictive of mortality after injury in human trauma patients (Davis et al. 1991; Blow et al. 1999; Meregalli et al. 2004; Callaway et al. 2009). While BE measured in arterial and venous blood are highly correlated in stable, well-perfused patients, this agreement deteriorates with worsening shock, particularly in patients with haemorrhage and hypotension (Stillion and Fletcher 2012).

The equations used to calculate the various effects are validated for humans, but not yet for dog's despite being applied in many studies. There is a predisposition for the data to risk over interpretation because of the equations used and because reference intervals have not yet been established for the different species.

Anion gap

The AG is defined as the difference in the serum (plasma) concentrations of the measured cations (Na^+, K^+) and anions (Cl^-, HCO_3^-) and is traditionally calculated using the following equation:

$$\text{Anion Gap} = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$$

The AG was centered around the concept of electroneutrality, recognising that total cations must equal total anions:

$$[Na^+] + [K^+] + [UC] = [Cl^-] + [HCO_3^-] + [UA]$$

where [UC] is the concentration of unmeasured cations and [UA] is the concentration unmeasured anions (Emmet et al. 1977).

Unmeasured anions such as L-lactate and phosphate can cause widening on the anion gap. Most plasma proteins are negatively charged at physiologic pH and, thus, hyperproteinaemic states may be associated with an increased anion gap. Normal AG values are in the range of 12 to 24 mmol/L in dogs (de Morais and Constable 2012; DiBartola 2012). Changes in albumin tend to complicate the identification of increased unmeasured anions because it changes the AG proportionally. The anion gap can be corrected for change in the albumin with the following equation for dogs (Constable and Staempfli 2005):

$$AG_{adjusted} = AG + 4.2 \times (3.77 - [\text{albumin in g/dl}])$$

For human blood, the equation is slightly different where (Emmett 1977):

$AG_{adjusted}$= AG + 2.3 x (4.0 - [albumin in g/dl])

Occasionally the presence of unidentified anions can be detected by examining the anion gap (Shull 1978). This may be associated with either normal or increased anion gap, the determination of which may allow more rapid identification of the metabolic derangements resulting in acidosis. An increase in anion gap is seen in particular disease processes that are responsible for consuming HCO_3^- which decreases its amount, and subsequently widens the anion gap. A widening of the anion gap will cause an increase in the accumulation of acids, which will result in a metabolic acidosis.

2.2.2 Stewart theory approach

The Stewart approach is a physicochemical method based on principles of laws of mass action, conservation of mass and electroneutrality of many overlapping biochemical processes occurring in the body. Overall, three independent variables impact blood acid-base balance, namely total weak acids (A_{TOT}), PCO_2 and strong ion difference (SID) (Stewart 1978; Stewart 1981; Stewart 1983). The change in acid-base balance is related to the dissociation of water of body fluids, as follows:

$$[H_2O(acid)] + [H_2O(Base)] \leftrightarrow [H_3O^+] + [OH^-]$$

where water can act as either an acid or as a base. Ions found in plasma can be categorise into non-buffer ions (strong ions) and buffer ions (Constable 1997). The reason strong ions are non-buffer ions is because at a physiological pH, they are fully dissociated in

solution and do not easily revert to a unionized form and thus have no buffering effect. Although strong ions do not have a buffering effect, they do have an effect on the ionization of water through the physiochemical principles relevant to this theory (Stewart 1978; Stewart 1981; Stewart 1983). Buffer ions do not fully dissociate at a physiological pH and are made up of plasma weak acids and bases (Constable 2000).

Weak acids do not fully dissociate in biological solutions. Proteins are the main weak acids in plasma and their concentration is largely controlled by the liver, with changes occurring over days. Phosphorus also contribute to [A_{TOT}] and its contribution increases during hypoalbuminaemia (Siegling-Vlitakis et al 2007). The A_{TOT} is calculated as follows:

A_{TOT} = Albumin contribution + Phosphorus contribution

with an increase in A_{TOT} resulting in a metabolic acidosis and a decrease resulting in metabolic alkalosis. A weak acid ionizes slightly in an aqueous solution and as with strong acids, ionization yields a hydrogen ion and its conjugate base (Schwartz 1963). There has been no direct method for measuring A_{TOT} through any method with Stewart, nevertheless, Constable and colleagues developed a method to experimentally determine A_{TOT} values for plasma from a number of species (Constable 1997) which found that A_{TOT} differed between species. Studies in the dog suggest that A_{TOT} is approximately 15 – 18 mmol/L (Zweens et al. 1977; Anderson and Jennings 1988; Constable and Stämpfil 2005).

The PCO_2 can contribute to a change in pH. As described previously (see 2.2.1 Traditional theory approach), the PCO_2 is part of the bicarbonate buffer system of plasma. The amount of PCO_2 influences the production of HCO_3^- and hydrogen ions when combining with water. Ideally the PCO_2 should be measured using an arterial blood sample, because

PCO_2 changes according to pulmonary function. The PCO_2 measured in arterial blood is different to that measured in venous blood, venous blood is often higher because carbon dioxide is a metabolic byproduct of cellular respiration (Kelly 2010).

The strong ion approach characterizes different acid-base disturbances including respiratory, strong ion, and non-volatile buffer ion components. The unmeasured strong anion concentration is quantified by calculating the strong ion gap, which attributes a value to the net anionic charge on serum proteins and phosphate and more accurately quantifies the unmeasured strong anion charge in animals with abnormal serum protein concentrations than does the anion gap (Constable and Stampfli 2005). The strong ion approach requires species-specific values for the total plasma concentration of non-volatile weak acids (A_{TOT}) and the effective dissociation constant (K_a) for plasma non-volatile buffers (Constable 1997).A reliable reference range for canines was developed by Constable and Stampfli (2005).

SIG = [albumin] x 0.49 – AG (Kellum 1995)

SIG = [total protein] x 0.29 – AG (Constable 2002)

The SID is calculated in two ways, the apparent SID (SID_{app}) and the effective SID (SID_{eff}) (Figge et al. 1992):

SID_{eff} =[HCO3-] + Albumin contribution + Phosphorus contribution

SID_{app}= [total strong cations] – [total strong anions]

In theory, an increased SID implies the presence of unmeasured anions (i.e., alkalosis). If the SID is decreased, this indicates the presence of unmeasured cations (i.e., acidosis). In all biological fluids, both SIDs are almost always positive (Chawla and Drummond 2008). However, it is not practical to measure all known plasma strong ions in every patient. Therefore, a simplified equations have been proposed, and in dogs, as with many other mammals, an accurate approximation of SID_{app} equation is (Constable 1997):

$SID_{app} = ([Na^+] + [K^+]) – ([Cl^-] + [L\text{-lactate}])$

Diarrhoea, vomiting and fluid therapy can influence the strong ion concentrations, as well as HCO_3^- and albumin, which has the potential to affect both SID calculations (Benjamin 2016).

2.2.3 Semi-quantitative method

Fencl et al. (2000) criticised the fact that the BE and AG do not consider the influence of non-bicarbonate buffers, namely albumin and phosphate on metabolic acid-base derangements, and defined the semi-quantitative method. The semi-quantitative method provides great insight into the underlying mechanism of metabolic acid-base disturbances. Free water, albumin, chloride, L-lactate, and phosphate are all examined individually, and have an effect on the base excess. All of these variables have acidifying and alkalinizing effects. Using this method, unmeasured anions (XA) are quantified by the difference between the BE and sum total of the individual effects (Hopper et al. 2014a). Insight into these processes allows for an

intricate and detailed analysis of what could be responsible for the metabolic derangements. In a study by Osborne et al. (2021) the semi-quantitative theory approach to acid-base analysis diagnosed coexisting metabolic acidotic and alkalotic processes in many dogs. Although many of the abnormalities identified with the semi-quantitative approach were minor, recognition of these individual processes may enhance understanding of acid–base pathophysiology and help guide treatment (Osborne et al. 2021).

Post-analytical calculation required for this approach are cumbersome and can be prone to unintentional mathematical errors. Without detailed computer spreadsheets, these approaches become impossible to apply in a quick, emergency patient-side setting (Hopper et al. 2014a). Bearing in mind all these challenges that a clinician faces in interpreting acid-base, these intricate approaches have been recommended in critically ill patients but have not been shown to improve management of all cases (Hopper et al. 2014a, b; Zeiler et al. 2022).

2.3 Gastrointestinal disease and acid-base balance

The gastrointestinal tract plays an integral part in body acid-base homeostasis, ensuring that the physiological processes of the body are still capable of proceeding amidst a disease process. The stomach's parietal cells secrete Cl^- and H^+ into the gastric lumen. This H^+ is derived, via carbonic anhydrase, from carbon dioxide produced within the cell binding to water which forms carbonic acid which dissociates into H^+ and HCO_3^-. The HCO_3^- moves into the circulating volume of blood. The presence of HCO_3^- causes an alkaline effect (Sirker et al. 2002). Also, HCO_3^- is found in pancreas secretions where a net movement of HCO_3^- from plasma into pancreatic cells and thereafter into the duodenal lumen, neutralises the H^+ being secreted by stomach parietal cells (Sirker et al. 2002).

A prevalent clinical finding of patients that are suffering a gastrointestinal disease process is often the act of vomiting. Hydrogen and chloride are lost in large quantities when an increased frequency of vomiting is noted (Gennari 2006). The compensatory response to this loss is a reduction of bicarbonate loss (reduced pancreatic secretion) via the duodenum and an increase in the serum bicarbonate concentrations. Burchell et al. (2020) noted an increase in bicarbonate because of a chloride loss, as seen in puppies with parvovirus enteritis who were frequently vomiting. The result of these changes, if severe enough may lead to a metabolic alkalosis.

Another clinical finding associated with gastrointestinal disorders is diarrhoea, which can cause a vast degree of acid-base derangements. Profound hyponatraemia and hypochloraemia has been documented in critically ill calves with experimentally-induced and naturally acquired coccidiosis (Stockdale et al 1981) and horses with Equine neorickettsiosis. Eimeriosis causes severe hyponatraemia and hypochloraemia in calves, with a mechanism of action leading to decreased absorption of sodium and chloride (Urgibl-Bauer et al, 2025) by the proximal large intestine, allowing for plasma to move into the large intestine's lumen, due to the damage of the mucosal lining and not active hypersecretion (Daugschies et al 1997). Worsening of a clinical picture will occur if the patient is already hypovolaemic, more losses through diarrhoea could push a clinical case into decompensated shock.

2.4 Gaps in the literature

Acid-base derangements have not been specifically studied in longitudinal detail in dogs with AHDS. As a consequence, there is paucity in the literature regarding what acid-base derangement occur in AHDS and whether convalescence and symptomatic treatment impact these derangements. The latest literature on AHDS treatment is symptomatic care, including fluid resuscitation which can also impact acid-base status (Mortier et al. 2015).

2.5 Aims, objectives and hypotheses

2.5.1 Aims and objectives

The aim of this study was to describe blood acid-base status at presentation and over time in dogs diagnosed with AHDS using three different methods. The objectives were to collect venous blood from a cohort of healthy dogs and dogs with AHDS. Various acid-base variables used in three different methods of analysis were measured or calculated from the blood. The healthy dog cohort was used to establish an expected normal range and to determine if the dogs with AHDS had acid-base derangements.

2.5.2 Hypotheses

Primary descriptive hypotheses

1) Dogs with AHDS have a metabolic acidaemia due to increased unmeasured strong anions compared to healthy dogs.
2) Dogs with AHDS have a low actual bicarbonate ion concentration which increases by more than 2 mmol/hour after onset of diarrhoea.

3. Material and Methods

3.1 Study design

The data presented in this prospective, observational case-controlled study was approved by the ethics committee of the University of Pretoria (REC 119-22) and the ARRIVE 2.0 guidelines were followed for reporting. The study took place at Valley Farm Animal Hospital, Pretoria, Gauteng, South Africa (-25.780916, 28.35078) which is a 24-hour referral hospital. A simple opportunistic sampling technique was used to include a cohort of AHDS diseased dogs.

3.2 Sample size

A group of 10 dogs were required based on a comparison of means sample size calculation using previously published blood pH values (alpha: 0.05; beta: 0.20; difference of means: 0.04; standard deviation in both groups: 0.03; with equal sample size ratio) (Mortier et al. 2015). Furthermore, for the diseased dog cohort, serial data were collected and analysed over time and a second sample size calculation was used, based on the assumption that the actual bicarbonate ion (HCO_3^-) will change by 2 mmol/L over (Mortier et al. 2015) 3 days (power curve for 1-way ANOVA where alpha: 0.05, beta: 0.2; standard deviation: 1.0 mmol/L) and confirmed that 10 diseased dogs were adequate for longitudinal analyses.

3.3 Animal housing

Written and signed informed owner consent was obtained prior to including any dog in this study. The inclusion criteria for the diseased dogs were as follows: a body mass of 5.0 kg or more, presence of vomiting and/or haemorrhagic diarrhoea and a haematocrit of above 0.57 L/L (Busch and Unterer 2022). Due to AHDS being a diagnosis of exclusion (Mortier et al.

2015); faecal analysis (to exclude giardiasis, coccidiosis and verminosis), complete blood count, point-of-care ELISA test to rule out parvovirus infection and abdominal ultrasound examination (to rule out gastrointestinal obstruction or pancreatitis) were performed before a diagnosis of AHDS was made. Exclusion criteria for the diseased dogs were any dog with co-morbidities such as cardiac, liver or renal conditions.

Diseased dogs were admitted to hospital and housed in the intensive care unit and cared for by experienced veterinarians and veterinary nurses for the duration of treatment and data collection. Dogs were offered a commercial gastrointestinal diet and had free access to water during their convalescence.

3.4 Experimental procedures

All dogs underwent data collection for the present acid-base study as well as unrelated longitudinal haematology, biochemistry and endocrine investigations. For the endocrine study, standard adrenocorticotropic hormone (ACTH) stimulation tests were performed at set timepoints by sampling venous blood and storing serum before and 1 hour after an injection of a synthetic ACTH (5 mcg/kg, IV; Synacthen).

For the diseased dogs, hair on the ventral neck, in the region of the jugular vein was shaved and aseptically prepared. A bleb of local anaesthetic (lidocaine 2%) was injected into the subcutaneous region around the intended catheter insertion site. A catheter (22 gauge, 50 mm, Arrow arterial catheterization set; Arrow International) was inserted percutaneously using an over-the-wire insertion technique. The catheter was sutured in place using 4/0 nylon and a light bandage was wrapped around the neck to protect the catheter. This catheter was used exclusively for serial blood sampling. The push-pull method for blood collection was used to ensure good quality samples without causing hospital-acquired anaemia due to serial

sampling (Lynch et al. 2016) or haemodilution. This method was to first flush the catheter with 5 mL of 0.9% sodium chloride (Fresenius Kabi) and using the same syringe, 5 mL of blood was then aspirated (pulled) into the syringe and then reinfused (pushed) back into the catheter (Barr et al. 2017). This push-pull process was repeated 3 times. A new 5 mL syringe was then attached to the catheter and a sample of blood was drawn and then the catheter was flushed and fluid-locked with 5 mL of 0.9% NaCl.

A second catheter (22 gauge, 25 mm, Smiths Medical jelco) was inserted into one of the cephalic veins for fluid therapy and intravenous treatments as per the attending veterinarian's discretion but using a standardized treatment protocol.

Fluid therapy using a balanced isotonic crystalloid (lactated Ringer's solution: LRS) (lactated Ringer's solution; Fresenius Kabi) was initially bolused at 20 mL/kg over 1 hour followed by an infusion at twice the calculated daily maintenance rate (hourly fluid infusion rate for maintenance (mL/hour) = [[1.2 x [weight in kg]$^{0.75}$] x 70]/ 24 hours))(Matthews 1996). for the first 24 hours. Additional fluid boluses were given if the attending veterinarian deemed the dog to be hypovolaemic (decreased mentation, poor peripheral pulses, rapid heart rate, prolonged capillary refill time) or if the original presenting haematocrit did not decrease by 5% within 4 hours. The fluid rate and volume of fluids administered per hour were recorded on monitoring sheets. Maropitant citrate (1 mg/kg subcutaneously) was injected every 24 hours in dogs that were vomiting. Dogs that were painful on abdominal palpation were given a proprietary mixture of dipyrone-hyoscine at 0.2 mg/kg of hyoscine subcutaneously every 12 hours. The use of intravenous amoxicillin-clavulanic acid antibiotic (12.5 mg/kg Co-amoxyclav, Sandoz) was restricted unless warranted by a white cell count value outside of the normal reference interval (5.7 to 14.2 x 10^9/L) (Cornell reference). Food intake was monitored and if dogs were not demonstrating any commitment to eating within 12 hours of admission, they

were syringe fed a commercial gastrointestinal diet at 50% to 75% of their calculated resting energy requirements (RER) where RER = (30 x Body Weight [kg]) + 70 = kcal per 24 hours (Chan 2015).

An AHDS scoring system, proposed by Mortier et al. (Table 2), was used to score the dogs on their activity, appetite, frequency of vomiting, faecal consistency and frequency as well as dehydration (%) (Mortier et al. 2015).

Mortier et al. (2015) did not divulge into the details pertaining to estimating dehydration status, where subjective measurements based on mucus membrane moistness, capillary refill time, haematocrit measurements, degree of sunken eyes within the orbit and skin elasticity was used. Serial measurements of body weight provide the best indication of hydration status (DiBortola 1992). The percentage decrease in body weight is assumed to be equal to the degree of dehydration in acute fluid loss, depicting the decrease in extracellular fluid volume (Constable et al. 1998).

Table 2 Canine Acute Haemorrhagic Diarrhoea Syndrome (AHDS) index score using six domains. The AHDS index is calculated by adding up the score (0 to 3) assigned per domain. The score can range from a minimum of 0 (clinically insignificant) to a maximum of 18 (severe AHDS).

Parameter	0	1	2	3
Activity	Not reduced	Mildly reduced	Moderately reduced	Severely reduced
Appetite	Not reduced	Mildly reduced	Moderately reduced	Severely reduced
Vomiting	0	1	2 – 3	> 3
Faecal consistency	Normal	Slightly soft	Very soft	Watery diarrhoea
Defecation (times/day)	1	2 – 3	4 – 5	> 5
Dehydration (%)	0	< 5	5 – 10	> 10

AHDS index: 0 – 3 Clinically insignificant; 4 – 5 Mild AHDS; 6 – 8 Moderate AHDS; > 9 Severe AHDS

3.4 Data collection

Each 5 mL blood sample was immediately aliquoted into storage tubes, in the following order: serum activator tube (3 mL; Vacucare), lithium-heparin tube (0.5 mL; Idexx), and ethylenediaminetetraacetic acid (EDTA) tube (1.5 mL; Vacucare). The EDTA tubes were submitted daily in batches to an offsite veterinary clinical pathology reference laboratory (OVAH Clinical Pathology Laboratory) for complete blood count analysis using a benchtop, analyser (Advia 2120, Siemens). All EDTA blood samples were analysed within 12 hours of collection (Schapkaitz and Pillay 2015). Complete blood counts were compared to a blood smear made from the same blood sample. Blood placed in lithium-heparin tubes was analysed within 5 minutes of collection using an onsite daily calibrated benchtop blood gas analyser

(Rapidlab 500 System; Siemens) to measure or calculate acid-base variables, haematocrit, haemoglobin content, saturation of oxyhaemoglobin and deoxyhaemoglobin using co-oximetry, electrolytes and L-lactate. Blood stored in the serum activator tube were allowed to rest for 1 hour before being centrifuged at 2000 times gravity for 10 minutes before being aliquoted into “date and timepoint” and “dog” marked cryovials and stored in an onsite -80°C freezer until all study samples were collected. The completed sample collection was sent to the same offsite laboratory for batch analysis. For the present study, serum albumin and phosphorus were measured using a daily calibrated and quality controlled (inspecting Levy-Jennings plots) reference analyser (Cobas Integra 400 plus; Roche diagnostics) by an experienced laboratory technician.

In diseased dogs, blood was collected at presentation (0H), then at 4-hour intervals (4H, 8H, 12H, 16H, 20H) until 24 hours (24H), then collected at 12-hour intervals (36H, 48H) until the final collection at 60 hours (60H). Not relevant to this study but blood (2 mL) was collected 1 hour after ACTH stimulation at 0H, 24H and 48H. AHDS scores were obtained at presentation and then at all timepoints before blood was sampled.

3.5 Data Analysis

The volume of fluid administered was calculated for each dog as the volume (mL) given between each timepoint divided by the body weight (kg). Then the rate of fluid administration was calculated as the volume of fluid per kg given between each timepoint divided by the number of hours lapsed between two consecutive timepoints. Acid-base status was assessed using three different methods of analysis 1) a traditional approach, 2) Stewart theory approach and 3) semi-quantitative theory approach. Table 3 contains all equations that were used to calculate variables of interest within each method of analysis.

Table 3 Equations used to calculate variables used for acid-base analysis using a traditional approach, Stewart theory approach and a semi-quantitative theory approach. Published ranges were used to help describe the variable values over time in a dog population with acute haemorrhagic diarrhoeal syndrome.

Variable	Formula	Healthy dog range	
		Range	Unit
Traditional approach			
Anion gap	$([Na^+] + [K^+]) - ([HCO_3^-] + [Cl^-])$	17 – 24	mmol/L
Stewart theory approach			
Albumin contribution	Measured albumin x ((0.123 x pH) – 0.631) x 10	-	
Phosphate contribution	Measured phosphorus x 0.323 x ((0.309 x pH) – 0.469)	-	
A_{TOT}	Albumin contribution + phosphorus contribution	11 – 16	mmol/L
SID_{app}	$([Na^+] + [K^+] + [Ca^{2+}]) - [Cl^-]$	39 – 47	mmol/L
SID_{eff}	$[HCO_3^-]$ + albumin contribution + phosphorus contribution	29 – 39	mmol/L
Strong ion gap	SID apparent – SID effective	5 – 11	mmol/L
Semi-quantitative theory approach			
Corrected chloride	Measured $[Cl^-]$ x (mid-normal $[Na^+]$/measured $[Na^+]$	-	
Free water effect	0.25($[Na^+]$ – mid-normal $[Na^+]$)	(-1.0) – 0.8	mmol/L
Chloride effect	Mid-normal$[Cl^-]$ – corrected $[Cl^-]$	(-5.3) – 3.8	mmol/L
Phosphate effect	0.58(mid-normal phosphorus – measured phosphorus)	(-1.8) – 2.6	mmol/L
Albumin effect	3.7(mid-normal albumin – measured albumin)	(-3.0) – 3.0	mmol/L
L-Lactate effect	-1 x L-lactate	(-3.2) – (-0.7)	mmol/L
Sum of effects	Free water effect + chloride effect + phosphorus effect + albumin effect + L-lactate effect	(-7.5) – 1.7	mmol/L
Unmeasured ion effect	Base excess – sum of effects	(-1.3) – 3.1	mmol/L

For the traditional approach, the measured pH, $PvCO_2$ and calculated HCO_3^- (act) and HCO_3^- (std), $BE_{(B)}$ and $BE_{(ECF)}$ by the blood gas analyser were used. Furthermore, the anion gap (AG) was calculated and haematocrit, haemoglobin concentration, venous partial pressure of oxygen, venous oxygen saturation, oxyhaemoglobin and deoxyhaemoglobin saturation, corrected chloride and potassium concentrations, and total carbon dioxide content were investigated.

For the Stewart theory approach, the pH, $PvCO_2$ were assessed , and $SID_{(app)}$, $SID_{(eff)}$, A_{TOT} and strong ion gap were calculated. For the semi-quantitative theory approach, first the range of the electrolytes in the healthy cohort were determined to identify the mid-normal range value (Addendum 1). These mid-normal range values were used to calculate free water effect, chloride effect, phosphate effect and albumin effect. In addition, the L- lactate effect, sum of effects and total unmeasured ions effect were calculated.

These published normal ranges from previously reported values (Hopper et al. 2014a, Zaldivar-Lopez et al. 2011) were used to determine if the diseased dog variables were within expected normal ranges.

For diseased dogs, data were assessed for normality by inspecting histograms, evaluating descriptive statistics and using Shapiro-Wilk test for normality and it was concluded that the data was nonparametric. All longitudinal data were analysed using a general linear mixed model (fixed factors: time; random factors: dog). Model fits were determined by expecting linearity and randomisation of the residuals. Significant observations were compared using Dunnett's method of post-*hoc* multiple comparisons where values of variables at 0H was used as the control variable. The shift of each variable over time were analysed using the Jonckheere-Terpstra trend test. Data reported in text and tables were median and range (minimum and maximum) for each timepoint. Data reported

in figures were in the form of composite boxplots where median values with the 95% confidence interval of the median, 1st quartile and 3rd quartile, minimum and maximum values for each timepoint. In figures, red (acid-base) or green (co-oximetry and haemoglobin concentration) reference lines depicted previously published reference ranges. Data were analysed using commercially available statistical software and significance was interpreted as a $P < 0.05$.

4. Results

All dogs with AHDS survived and were discharged as dogs with an AHDS index score of "insignificant disease" after the 60H timepoint. The median (minimum to maximum) body mass and age of dogs with AHDS were 7.4 (5.8 to 18.2) kg and 4.8 (1.0 to 12.0) years old, respectively. The breeds of dog were Jack Russell Terrier (*n* = 3), Miniature Schnauzer (*n* = 2), Dachshund (*n* = 2), Yorkshire Terrier (*n* = 1), Staffordshire Bull Terrier (*n* = 1) and French Bulldog (*n* = 1). Dogs with AHDS had an index score of 13 (8 to 16) at 0H that improved significantly ($P < 0.0001$) in a decreasing trend ($P < 0.0001$) over time to a score of 1 (0 to 4) at 60H (Figure 1 and Table 4). The intravenous infusion rate of lactated Ringer's solution was 10 (7 to 16) mL/kg/hour for the first 4 hours and was decreased significantly ($P < 0.0001$) over time to a rate of 3 (3 to 3) mL/kg/hour from 16H until 60H when it was discontinued. One (1/10) case had antibiotics (amoxicillin clavulanic acid 12.5mg/kg IV every 8 hours instituted due to the white cell count well above the reference range.

Table 4 Volume and rate (mL/hour) of lactated Ringer's solution administered to 10 dogs with acute haemorrhagic diarrhoea syndrome (AHDS) from presentation (0H) until 60 hours (60H). The AHDS index score was assigned over time where the score can range from a minimum of 0 (clinically insignificant) to a maximum of 18 (severe AHDS).

Variable	Unit	0H	4H	8H	12H	16H	20H	24H	36H	48H	60H
Volume	mL/kg	0	37	14	14	13	13	13	39	39	39
		0	29-63	11-46	11-30	11-14	11-14	11-14	34-41	24-41	34-41
Rate	mL/kg/hr	0	10	3	3	3	3	3	3	3	3
		0	7-16	3-8	3-3	3-3	3-3	3-3	3-3	3-3	3-3
AHDS	-	13	12	10	10	10	9	5	4	2	1
score		8-16	7-16	6-15	4-15	4-15	4-14	3-12	0-10	0-5	0-4

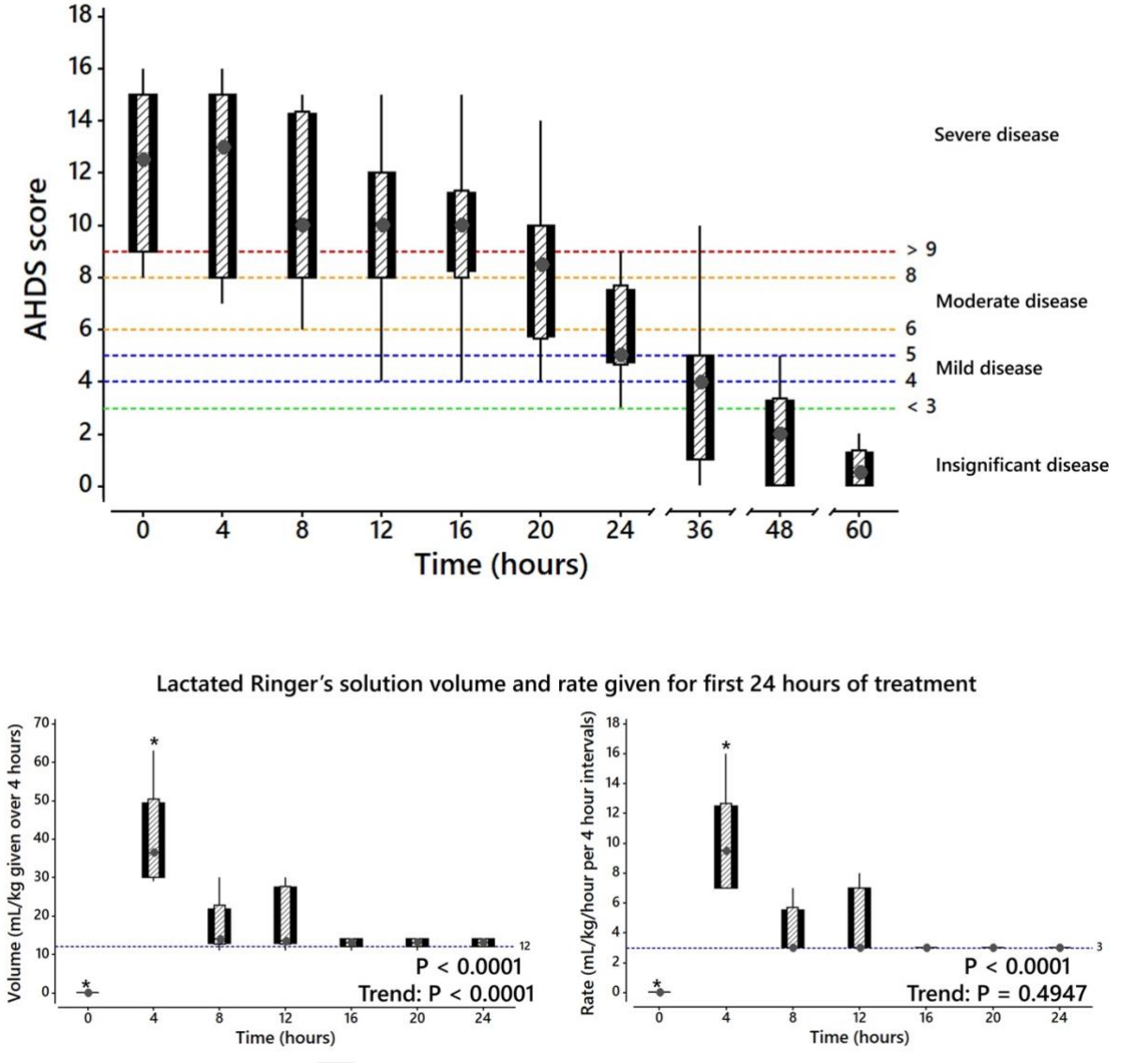

Figure 1 Composite boxplot and whiskers of an acute hameorragic diarrhea syndrome (AHDS) index score assiged to 10 dogs with AHDS from presentation (0) until discharge (60). These dogs were given lactated Ringer's solution as part of their treatment and the volume and hourly rate of fluid administered for the first 24 hours are plotted. The composite boxplot is made up of the median (black dot), the 95% confidence interval of the median (inner white box), the interquartile range (outer black box) and the range (whiskers). Where *P* is significance from a general linear mixed model and Trend: *P* is significance from the Jonckheere-Terpstra trend test.

The blood pH at 0H was 7.31 (7.22 to 7.49) and acidaemic compared to published normal reference ranges (Figure 2 and Addendum 2). The pH significantly (P < 0.0001) normalised from 4H in an increasing trend (P < 0.0001) that stabilised with narrow interquartile ranges from 16H until 60H. Over time, from 4H onwards the pH was within the calculated range. However, from 16H onwards, the pH was above the upper reference limit for published range, which indicated an alkalemia.

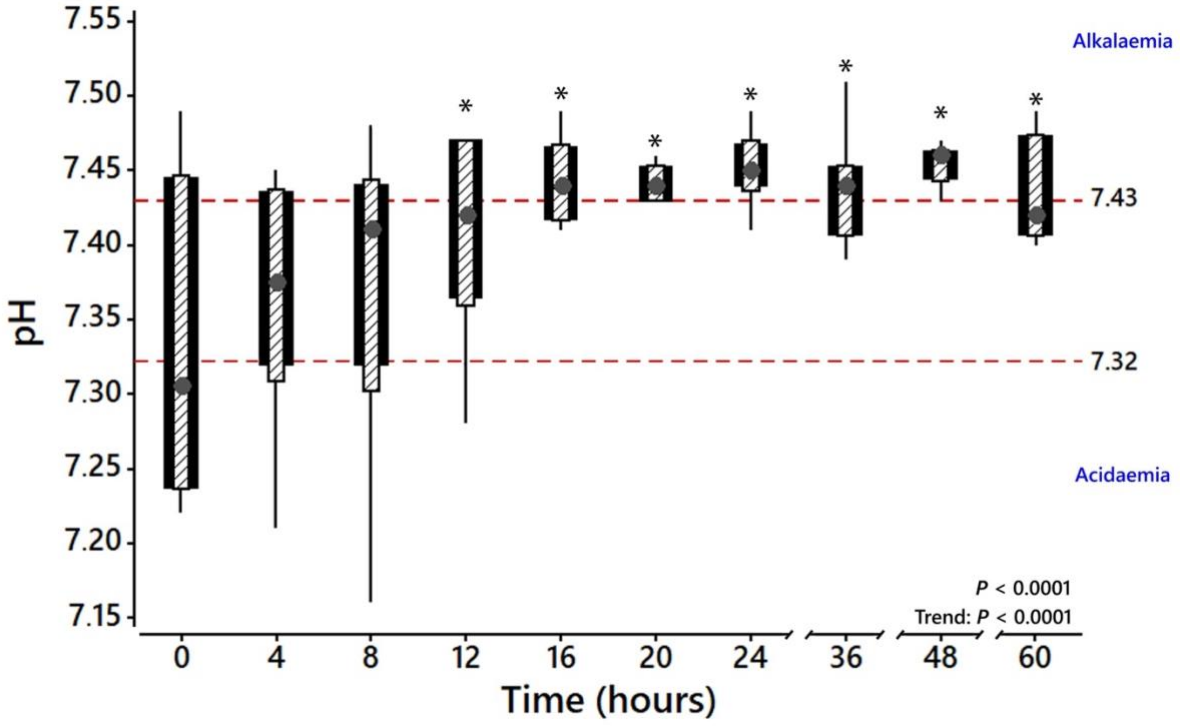

Figure 2 Composite boxplot and whiskers of venous blood pH at presentation (0) and over time during treatment and convalescent (until 60 hours) in 10 dogs with acute haemorrhagic diarrhoea syndrome (AHDS). The composite boxplot is made up of the median (black dot), the 95% confidence interval of the median (inner white box), the interquartile range (outer black box) and the range (whiskers). Red reference lines indicate expected normal ranges that have been published. Where P is significance from a general linear mixed model and Trend: P is significance from the Jonckheere-Terpstra trend test.

With respect to the normal expected ranges using the traditional approach, the calculated range was wider compared to the published range for most variables with the exception of AG (Figure 3). The AG was contrasting between the normal range. The acidaemia with increased AG at 0H manifested from an increased $PvCO_2$ and more negative $BE_{(ECF)}$ when using the published ranges. Whereas, from 16H onwards, the alkalemia arose from a low $PvCO_2$ and positive $BE_{(B)}$ and $BE_{(ECF)}$ when applying the published ranges.

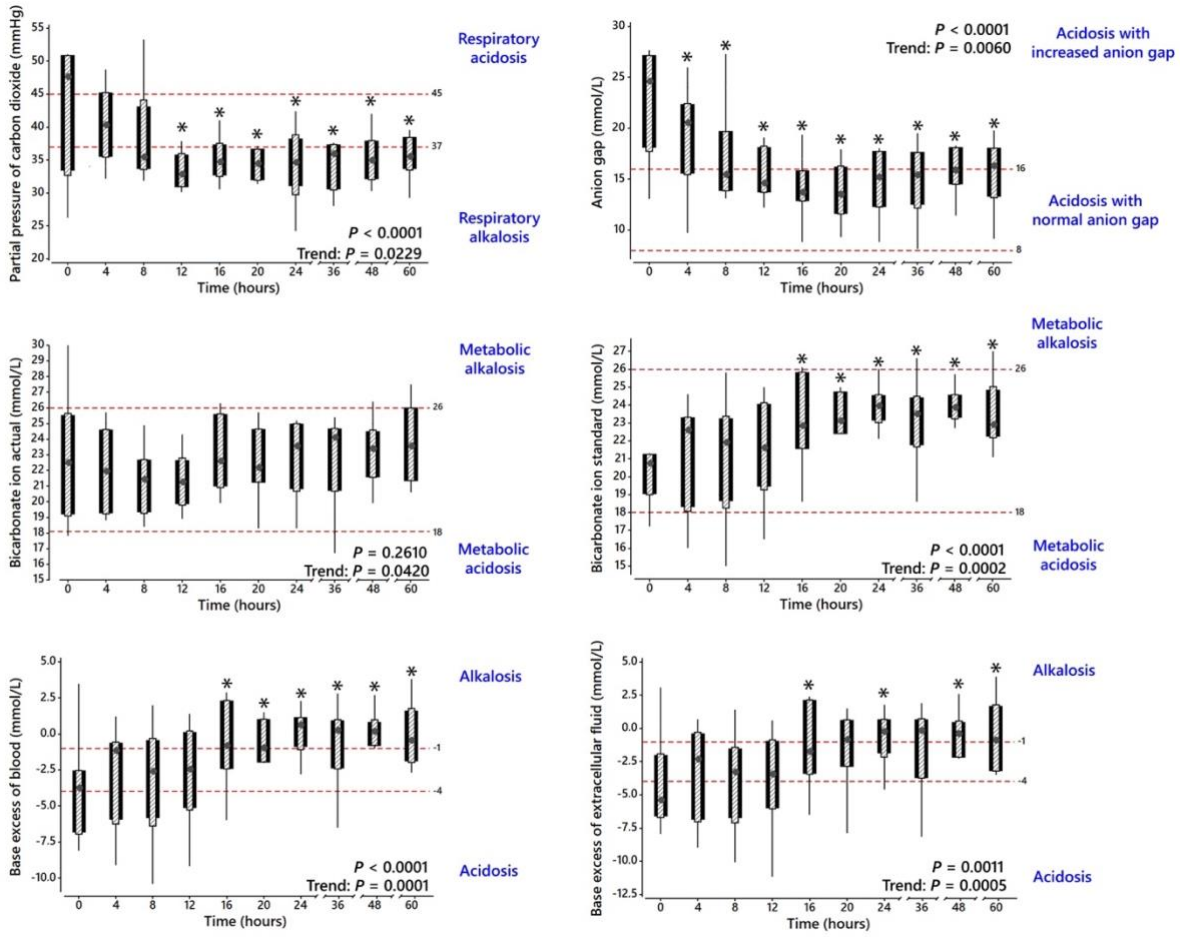

Figure 3 Composite boxplot and whiskers of venous blood derived variables used in the traditional acid-base status analysis collected at presentation (0) and over time during treatment and convalescent (until 60 hours) in 10 dogs with acute haemorrhagic diarrhoea syndrome (AHDS). The composite boxplot is made up of the median (black dot), the 95% confidence interval of the median (inner white box), the interquartile range (outer black box) and the range (whiskers). Red reference lines indicate expected normal ranges that have been published. Where *P* is significance from a general linear mixed model and Trend: *P* is significance from the Jonckheere-Terpstra trend test.

The haematocrit ($P < 0.0001$) and haemoglobin concentration ($P < 0.0001$) both significantly trended in a decreasing direction (both $P < 0.0001$) and were within published normal ranges by 8H onwards (Figure 4). The venous partial pressure of oxygen ($P = 0.1911$), venous saturation of oxygen ($P = 0.3751$), oxyhaemoglobin ($P = 0.3703$) and deoxyhaemoglobin ($P = 0.5952$) saturations, respectively, were not different over time. The corrected chloride was 108 (103 to 112) mmol/L at presentation which was at the lower end of the normal reference interval and it increased significantly by 8H to 114 (109 to 117) mmol/L and plateaued until 60H ($P < 0.0001$). The potassium concentration in plasma was 3.9 (2.9 to 5.6) mmol/L and did not change over time ($P = 0.5000$). The total carbon dioxide content was 23.6 (16.0 to 28.7) mmol/L and did not change over time ($P = 0.1556$).

The published ranges were contrasting for the variables used in the Stewart theory approach. In particular, the SID apparent and strong ion gap, where the published range did not really overlap which made interpretation a challenge (Figure 5). For SID apparent, there was an alkalotic effect from 0H, 4H, 48H and 60H, respectively, when comparing the values to the published range, noting a significant decrease ($P < 0.0001$) in SID apparent from 0H onwards. The SID effective, strong ion gap and A_{TOT} were mostly unremarkable over time. The A_{TOT} was mostly within expected normal ranges for the study duration.

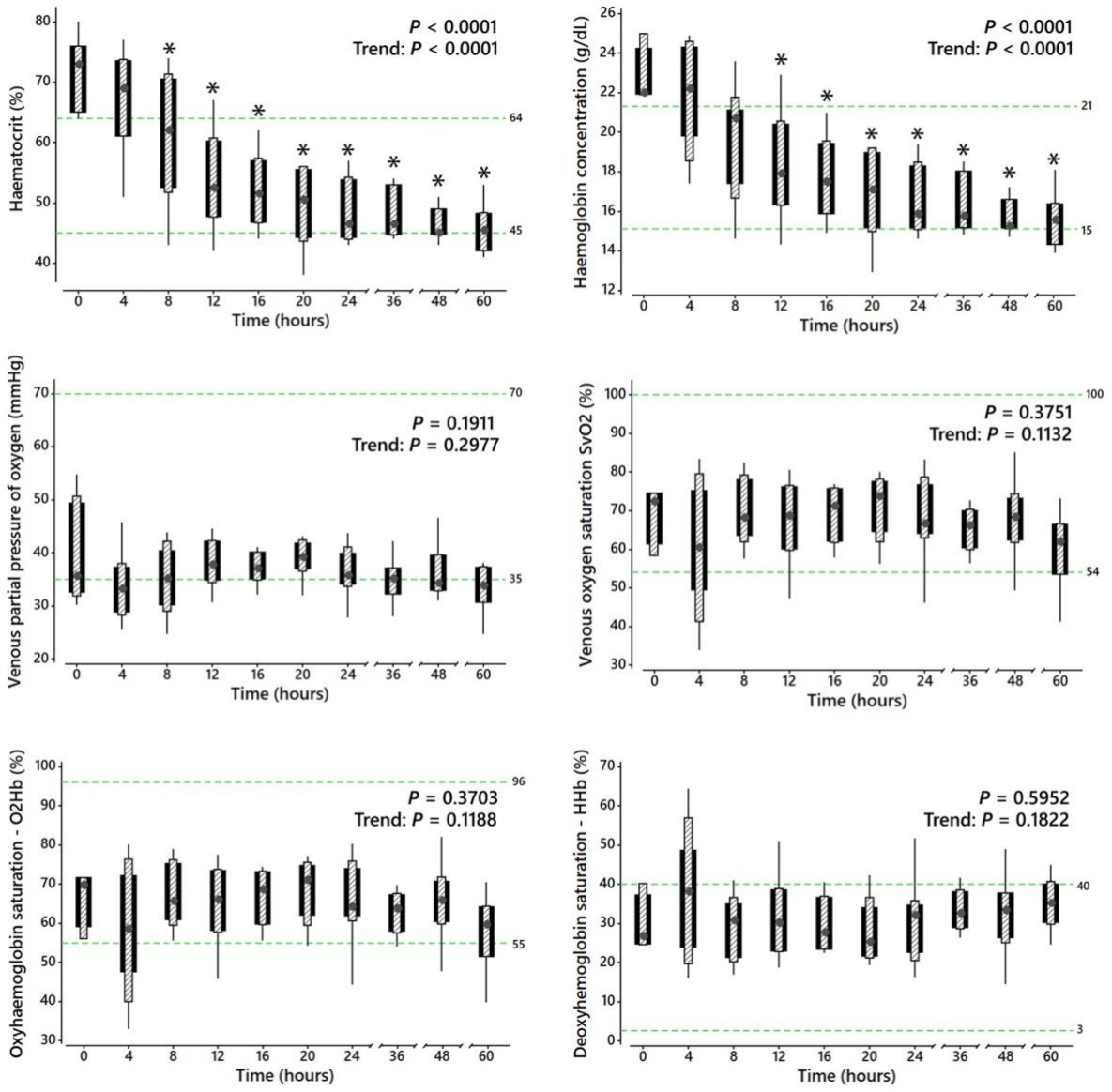

Figure 4 Composite boxplot and whiskers of venous blood haematocrit, haemoglobin concentration, venous partial pressure and saturation of oxygen, oxyhaemoglobin and deoxyhaemoglobin saturations collected at presentation (0) and over time during treatment and convalescent (until 60 hours) in 10 dogs with acute haemorrhagic diarrhoea syndrome (AHDS). The composite boxplot is made up of the median (black dot), the 95% confidence interval of the median (inner white box), the interquartile range (outer black box) and the range (whiskers). Green reference lines indicate expected normal ranges that have been published. Where *P* is significance from a general linear mixed model and Trend: *P* is significance from the Jonckheere-Terpstra trend test.

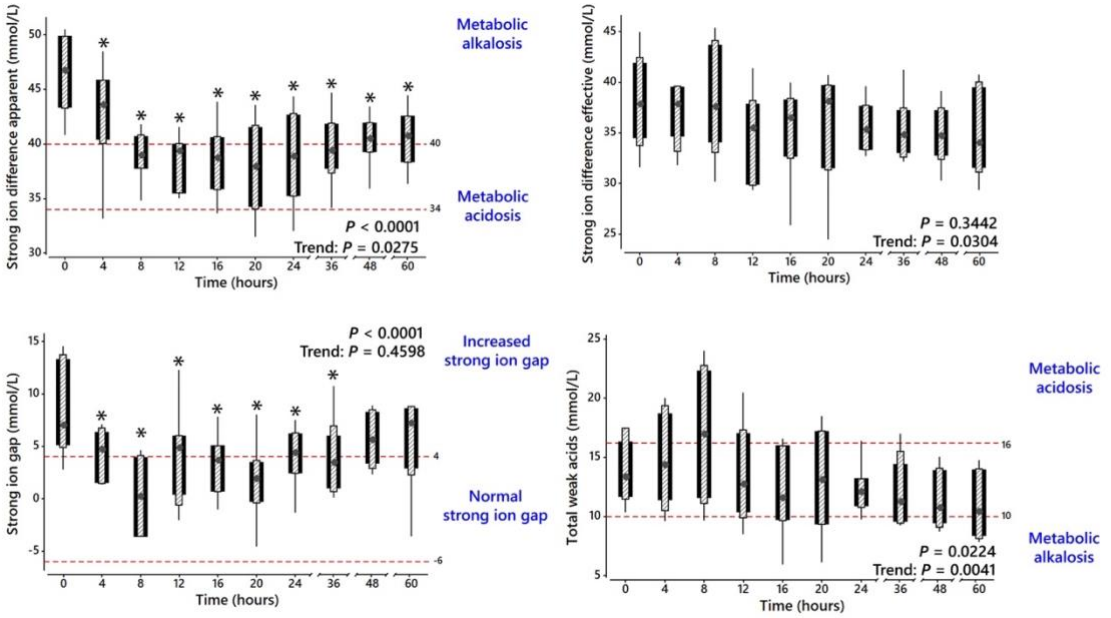

Figure 5 Composite boxplot and whiskers of venous blood derived variables used in the Stewart theory approach to acid-base status analysis collected at presentation (0) and over time during treatment and convalescent (until 60 hours) in 10 dogs with acute haemorrhagic diarrhoea syndrome (AHDS). The composite boxplot is made up of the median (black dot), the 95% confidence interval of the median (inner white box), the interquartile range (outer black box) and the range (whiskers). Red reference lines indicate expected normal ranges that have been published. Where *P* is significance from a general linear mixed model and Trend: *P* is significance from the Jonckheere-Terpstra trend test.

The reference ranges were narrowly matched for all the variables used in the semi-quantitative theory approach (Figure 6). There were concurrent acidifying (free water effect, phosphate effect, lactate effect) and alkalizing effects (albumin effect, unmeasured anion effect) over time that could explain the resultant pH at most timepoints. The acidaemia at presentation (0H) was due to an acidifying effect of L-lactate and a number of other unmeasured acids. Whereas the alkalemia from 16H onwards was attributed mostly to an alkalizing effect of albumin and a correction of the free water effect that was resolved by 20H.

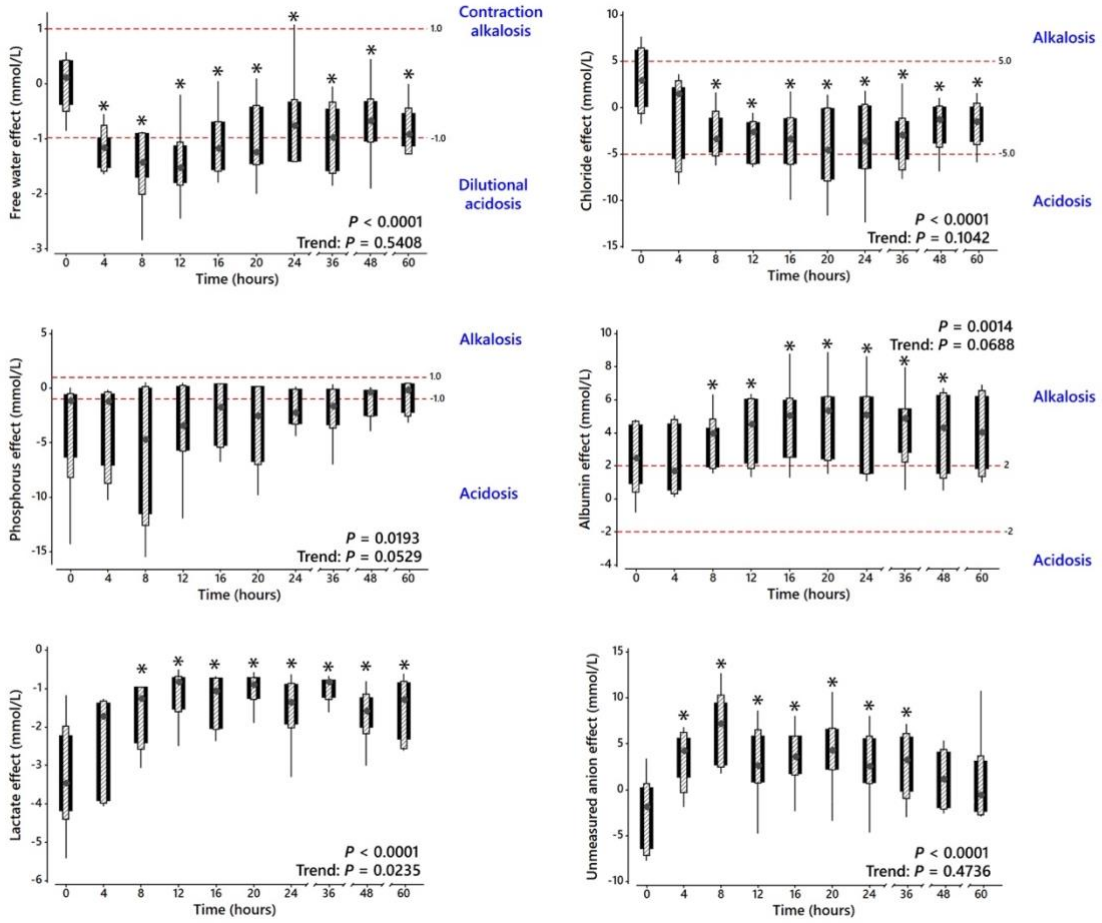

Figure 6 Composite boxplot and whiskers of venous blood derived variables used in the semi-quantitative theory approach to acid-base status analysis collected at presentation (0) and over time during treatment and convalescent (until 60 hours) in 10 dogs with acute haemorrhagic diarrhoea syndrome (AHDS). The composite boxplot is made up of the median (black dot), the 95% confidence interval of the median (inner white box), the interquartile range (outer black box) and the range (whiskers). Red reference lines indicate expected normal ranges that have been published. Where *P* is significance from a general linear mixed model and Trend: *P* is significance from the Jonckheere-Terpstra trend test.

5. Discussion

Dogs with AHDS were acidaemic at presentation because of a raised venous partial pressure of carbon dioxide and L-lactate effect. The blood pH normalised within hours during resuscitation of the intravascular compartment with a balanced isotonic crystalloid solution. Each approach to acid-base interpretation and analysis provided a unique outlook on aspects of blood physiology in relation to the time course of AHDS and its concomitant symptomatic treatment, mainly using intravascular fluids.

The carbon dioxide tension is used in the traditional and Stewart theory approach to acid-base analysis and interpretation. In both methods, the respiratory component of acid-base balance is interpreted using the carbon dioxide tension, and if its tension is raised then a respiratory acidosis is diagnosed. Interpreting the arterial carbon dioxide tension has been preferred over assessing the $PvCO_2$ but in trauma and hypotensive and hypovolaemic human patients and dogs, this sentiment has been questioned (Kelly 2010; Williams et al. 2014; Chung et al. 2019; Rudkin et al. 2020; Chong et al. 2021; Kadwa et al. 2022). Conflicting outcomes between the agreement of arterial carbon dioxide tension and $PvCO_2$ have been reported. However, $PvCO_2$ can be used instead of arterial carbon dioxide tension in critical human patients and dogs who are haemodynamically stable (Chong et al. 2021; Kadwa et al. 2022). Unfortunately, we did not obtain arterial blood samples for analysis. However, at presentation, the $PvCO_2$ was raised and classified as a respiratory acidosis and we speculate that rather than a respiratory issue, the $PvCO_2$ was raised for other reasons 1) a wide venous-to-arterial carbon dioxide tension gap (Ltaief et al. 2021), and 2) a right shift in the carbon dioxide dissociation curve (Mallat et al. 2016; Ltaief et al. 2021). Furthermore, to strengthen our speculation, the dogs did not appear to hypoventilate on clinical examination and their

venous partial pressure of oxygen (PvO_2) were within normal reference intervals, albeit on the lower end of the reference interval. An important factor to consider why the PvO_2 was low-normal is that the study was performed at an altitude of 1650 meters above sea level with an average atmospheric pressure of 650 mmHg. Although the fraction of inspired oxygen is 0.21, the atmospheric pressure is lower and thus a lower oxygen tension in the alveolar to participate in gas exchange (Gallagher and Hackett 2004.). The wide venous-to-arterial carbon dioxide tension gap is plausible because the dogs were hypovolemic and hyperlactatemic and both responded to fluid resuscitation. The dogs were not hypoxaemic and thus the hyperlactataemia was more likely due to decreased liver metabolism because of the low circulating blood volume (Pang and Boysen 2007). The venous-to-arterial carbon dioxide tension gap is widened following cardiac output reduction because of a low flow-induced tissue carbon dioxide stagnation phenomenon (Mallat et al. 2016). Due to the increasing transit time, a higher than usual addition of carbon dioxide per unit of blood passing efferent microvessels leads to hypercapnia in the venous blood. As long as alveolar ventilation and gas exchange are sufficient, which we speculate was normal in the dogs with AHDS, a wider gradient will occur between $PvCO_2$ and arterial carbon dioxide tension (Benjamin et al. 1987). A right shift in the carbon dioxide dissociation curve can be identified when the total carbon dioxide content remains the same but there is an increase in the tension of carbon dioxide, which was identified in the AHDS dogs at presentation. Moreover, an increase in body temperature, increase in haemoglobin concentration and metabolic acidosis cause a right shift in this curve (Ltaief et al. 2021).

For the traditional approach, the actual and standard HCO_3^- concentrations were within normal intervals, which was an unexpected outcome. In dogs with diarrhoea, HCO_3^- is lost via the intestinal tract which contributes to a metabolic acidosis (Armstrong 2013). The

normal HCO3- concentrations could be because of 1) an interplay between the variables of the bicarbonate buffer system, and 2) the concurrent buffering by blood proteins, specifically haemoglobin. The bicarbonate buffering system is the most important buffer of blood plasma. In plasma, carbon dioxide is dissolved in water to form carbonic acid which dissociated into HCO_3^- and hydrogen ions. The pH is defined as the negative logarithm of hydrogen ion concentration and forms the premise of the traditional Henderson-Hasselbalch equation of blood pH (Henderson 1908; Hasselbalch 1917). Briefly, blood pH is equivalent to the sum of the partitioning coefficient of carbonic acid (pKa = 6.1) and logged ratio of the bicarbonate concentration to the dissolved carbon dioxide (log ($[HCO_3^-]/[0.03 \times PvCO_2]$)). The ratio $[HCO_3^-]:[0.03 \times PvCO_2]$ of [20]:[1] results in a blood pH of 7.4. Hence, if the $PvCO_2$ is raised, as in the AHDS dogs, then the HCO_3^- will also raise in concentration to restore the ratio. This is the premise of compensation, although not calculated in this study population. Haemoglobin within the erythrocytes has an important function of either oxygen transportation or as a buffer (Mallat et al. 2016). In dogs with AHDS, the haematocrit was elevated, despite normal haemoglobin concentrations, suggesting a relative haemoconcentration. This relative haemoconcentration is a classic pathomechanism of AHDS and occurs in animals that are losing more plasma compared to cellular components of blood (Unterer et al. 2014). High $PvCO_2$ pressures will drive carbon dioxide into the erythrocyte, and once intracellular it will bind with water to form carbonic acid under the catalysation of carbonic anhydrase. The carbonic acid in the cytoplasm will dissociate into HCO_3^- and a hydrogen ion (Mallat et al. 2016; Ltaief et al. 2021). The hydrogen ion will be buffered by loosely binding to haemoglobin to form reduced haemoglobin (HHb, deoxyhaemoglobin). The HCO_3^- concentration increases in the cytoplasm and with constant production, shifts from the cytoplasm into the plasma by a counter exchange with a chloride ion (Doddamani et al. 2020), called the Hamburger effect

(chloride shift or Lineas phenomenon). We speculate that this haemoglobin buffering system was occurring at a high rate because the corrected chloride concentration was on the lower limit of the normal reference interval. However, the low-normal corrected chloride could also be due to gastric stasis and emesis (Boag et al. 2005).

Another mechanism to decrease plasma hydrogen ion concentration is by intracellular exchange with potassium (Adrogue and Madias 1981; Carlson 1997). In dogs with acidaemia, the potassium concentrations can be raised (Magner et al. 1988). A metabolic acidosis with hyperkalaemia can be due to mineral (inorganic) acids such as ammonium chloride (NH_4Cl) and hydrochloric acid (HCl) (Adrogue and Madias 1981; Carlson 1997). However, the same does not occur when the acidosis is associated with organic acids (lactic acid, ketoacids) where the potassium concentration is variable but mostly within normal refence intervals (Adrogue and Madias 1981; Carlson 1997). We speculate that hydrogen-potassium ion exchange was not a major contributor to restoring the blood pH to normal over time, because the dogs with AHDS had an unchanging potassium concentration that was within the normal reference interval.

For the Stewart approach, review of other published ranges in dogs with other disease processes magnify the need to obtain normal ranges in healthy dogs (Zaldivar-Lopez et al. 2011; Vanova-Uhrikova et al. 2017). Regardless of this concern, when the published ranges were used for interpretation, there was a strong ion alkalotic effect at presentation and at 4 hours, whereas the $PvCO_2$ was raised at presentation. The overriding result was an acidotic pH at presentation. Further research is required to establish normal reference intervals for dogs before this approach to acid-base analysis can be useful.

The semi-quantitative approach was helpful to further investigate the alterations seen on the $BE_{(B)}$ and $BE_{(ECF)}$. The $BE_{(ECF)}$ emulated the change in blood pH better than the $BE_{(B)}$, perhaps

because of the elimination of the effects of haemoglobin as a buffer (Mentel et al. 2011). At presentation, the acidaemia was explained by a decrease in L-lactate effect and other unmeasured anion effect. However, the ensuing alkalosis was most likely because of the decrease in albumin concentration to within a normal reference interval over time raising the albumin effect. The authors speculate that the deeper investigation of BE using the semi-quantitative theory approach was helpful and can have benefit in the time course of treatment in dogs with AHDS. We further speculate that fluid therapy to resuscitate the intravascular compartment can be guided by investigating the free water effect, albumin effect and L-lactate effect. Lastly, in dogs with diarrhoea, it would be important to consider that sodium and albumin can be lost by the intestinal tract which could falsely present as a dilutional acidosis (free water effect) or alkalinising albumin effect, respectively (Boag et al. 2005; Torrente et al. 2014; Zager et al. 2018; Hasuda et al. 2020).

The study had some notable limitations, the first being that compensation was not calculated. Although there are validated calculations for compensation in dogs (de Morais and DiBortola 1991), the authors elected to not calculate the adequacy of compensation because of the $PvCO_2$ being raised in the absence of clinical hypoventilation. Secondly, the sample size was small, despite the sample size calculations which focused on only two variables of interest, pH and act HCO_3^-. Thus, we only had the power to reliably detect large effects in the other variables. Despite our small sample size (10 diseased dogs), there were no dramatic clinical alterations in blood acid-base except at 0H which were corrected early on in the disease process and treatment. With this information in mind, we were sceptical that collecting data from more dogs would have altered the outcome of our study. However, the observations herein will assist future researchers in estimating accurate samples sizes when using other variables of interest. Thirdly, the oscillometric blood pressure readings were not recorded for

many of the dogs with AHDS over time. Thus, we could not include this as data despite its use in providing goal orientated fluid resuscitation during the initial treatment. Lastly, we did not correct for the albumin and the widened anion gap, due to this being out of the scope of the current study.

6. Conclusions and Future Research

Dogs with acute haemorrhagic diarrhoea syndrome present with acidaemia because of hypercapnia, more negative base excess and acidifying L-lactate effect. The acidaemia was due to hypovolaemia and once the circulating volume was restored, acid-base homeostasis was restored. In dogs with AHDS, the bicarbonate and haemoglobin buffering systems are efficient at maintaining acid-base homeostasis. The traditional and semi-quantitative theory approaches to acid-base analysis were more informative compared to the Stewart theory approach only because of inconsistencies with reference ranges.

Acid-base is a very valuable tool for prognostication as well as guiding fluid therapying dogs. Fluid deficits lead to hypoperfusion, consequently affecting the body's ability to produce energy. Hypoxic tissue requires anaerobic glycolysis which will increase the manufacturing of L-lactate and hydrogen ions (Allen and Holm 2008; Gillespie et al 2017) perpetuating a change in the acid-base status and pH of the patient. Future research into acid-base analysis in dogs with AHDS, focusing on the various formulas and discerning if it would be of any benefit to correct AG with albumin. The equations used for both SIDs also had substantial variation and that would also be preferable to standardise these calculations. The current published reference intervals for acid-base parameters using less commonly used methods of analysis are too variable with very large reference intervals, making interpretation of data difficult as

abnormal values were still seen to be within the normal interval. Further research is indicated to establish normal reference intervals for these variables used in the Stewart and semi-quantitative approaches.

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8.Addendum Tables

Addendum Table 1 Selected measured and calculated acid base variables of 10 diseased dogs using the 3 approaches of acid-base analysis (Traditional, Stewart and Semi-quantitative) undergoing fluid resuscitation using lactated Ringer's solution following diagnosis with AHDS. Data collected before fluid resuscitation (0H), after receiving a fluid bolus of 20ml/kg with 4 hours lapsed since presentation (4H) and at set time points (8H,12H,16H,20H,24H,36H, 48H and 60H) until discharge form the hospital. immediately after hemorrhage but before starting fluid resuscitation (T0), 60 minutes of fluid resuscitation (T60) and at 120 minutes of fluid resuscitation (T120). Data presented as median and range (min to max).

Variables	Unit	0H	4H	8H	12H	16H	20H	24H	36H	48H	60H
pH	mmol/L	7.31	7.38	7.41	7.42	7.44	7.44	7.45	7.44	7.46	7.42
	mmol/L	(7.22 to 7.49)	(7.21 to 7.45)	(7.16 to 7.48)	(7.28 to 7.47)	(7.30 to 7.49)	(7.31 to 7.46)	(7.25 to 7.56)	(7.39 to 7.51)	(7.29 to 7.47)	(7.40 to 7.49)
Traditional approach											
$PvCO_2$	mmHg	48	40	35	33	35	35	34.6	35.9	35.0	35.5
	mmHg	26 to 51	32 to 49	32 to 53	30 to 44	31 to 41	31 to 37	24 to 42	28 to 38	30 to 42	29 to 40
HCO3- std	mmol/L	20.8	22.6	21.9	21.6	22.9	23.1	24.0	24.2	23.9	22.9
	mmol/L	17.2 to 26.7	16.0 to 24.6	16.5 to 25.8	16.5 to 25.0	18.6 to 26.1	18.0 to 25.0	17.8 to 26.0	18.6 to 26.6	19.4 to 25.7	21.1 to 27.0
HCO3- act	mmol/L	22.5	22.0	21.5	21.3	22.6	22.2	23.6	24.1	23.4	23.6
	mmol/L	17.8 to 30.2	18.8 to 25.7	18.4 to 24.9	15.1 to 24.3	19.9 to 26.3	18.3 to 25.7	18.3 to 25.2	16.7 to 25.4	19.9 to 26.4	20.6 to 27.5
BE (b)	mmol/L	-3.8	-1.1	-2.6	-2.5	-0.8	1.0	0.7	0.3	0.2	-0.5
	mmol/L	-8.1 to 3.5	-9.1 to 1.2	-10.4 to 2.0	-9.2 to 1.4	-6.0 to 2.9	-7.1 to 1.5	-8.6 to 2.3	-6.5 to 2.8	-6.4 to 2.7	-2.7 to 3.8
BE (ecf)	mmol/L	-5.4	-2.3	-3.3	-3.5	-1.8	-0.8	-0.3	-0.2	-0.4	-0.9

	mmol/L	-8.0 to -2.4	-9.0 to 3.1	-10.1 to 1.4	-11.2 to 0.6	-6.5 to 2.4	-7.9 to 1.5	-8.9 to 1.8	-8.2 to 1.9	-6.7 to 2.6	-3.5 to 3.9
$CtCO_2$	mmol/L	23	23	23	22	24	23	25	25	25	25
	mmol/L	19 to 28	20 to 27	20 to 26	16 to 25	21 to 28	19 to 27	20 to 26	18 to 27	21 to 28	22 to 29
Anion gap	mmol/L	24.6	20.5	15.5	14.6	13.7	13.5	15.2	5.4	15.9	16.3
	mmol/L	13.1 to 27.6	9.7 to 26.0	13.1 to 27.3	12.2 to 25.5	8.8 to 19.4	9.3 to 18.0	8.8 to 18.0	8.1 to 19.5	1.4 to 18.3	9.1 to 19.8
Stewart theory approach											
SID apparent	mmol/L	46.7	43.3	39.0	39.4	38.7	37.9	38.9	39.4	40.5	40.7
	mmol/L	40.8 to 50.5	33.1 to 48.5	34.8 to 47.1	35.0 to 41.6	33.7 to 43.8	31.5 to 43.6	32.0 to 44.4	27.7 to 44.7	35.9 to 43.3	36.3 to 44.5
SID effective	mmol/L	37.8	37.8	37.6	35.5	36.5	38.1	35.3	34.8	34.7	34.0
	mmol/L	31.6 to 45.0	31.8 to 39.7	30.2 to 45.4	29.3 to 41.4	25.9 to 40.0	24.2 to 40.7	24.5 to 39.7	26.0 to 41.2	30.3 to 39.1	29.3 to 40.8
Atot	mmol/L	13.4	14.4	17.0	12.8	11.6	13.1	12.1	11.3	10.7	10.4
	mmol/L	10.3 to 23.4	9.6 to 20.1	9.7 to 24.0	8.5 to 20.5	5.9 to 16.6	6.1 to 18.5	6.1 to 16.4	9.2 to 17.0	8.7 to 15.0	7.8 to 14.8
SIG	mmol/L	7.0	4.7	0.2	4.9	3.6	1.9	4.4	3.5	5.6	7.2
	mmol/L	4.6 to 13.8	1.34 to 6.9	-3.6 to 4.6	-2.1 to 12.3	-1.0 to 7.8	-4.6 to 8.0	-1.3 to 7.5	0.1 to 10.8	2.3 to 8.9	-3.6 to 8.8
Semi-quantitative theory approach											
Free water effect	mmol/L	0.1	-1.2	-1.4	-1.5	-1.2	-1.2	-0.8	1.0	-0.7	-0.9
	mmol/L	-0.9 to 0.6	-1.7 to -0.6	-2.9 to -0.9	-2.5 to -0.2	-1.8 to 0.1	-2.0 to 0.1	-1.4 to 1.1	-1.9 to -0.1	-2.0 to 0.5	-2.3 to 0.0
Chloride effect	mmol/L	3.0	1.5	-3.4	-2.6	-3.4	-4.6	-3.6	-3.0	-1.3	-1.5
	mmol/L	-1.8 to 7.7	-8.3 to 3.6	-6.3 to 1.6	-6.4 to -0.6	-10.0 to 1.8	-11.7 to 1.4	-12.4 to 1.8	-15.8 to 2.6	-7 to 1.1	-6 to 1.6
Phosphate effect	mmol/L	-1.2	-1.2	-4.7	-3.4	-1.8	-2.6	-2.2	-1.7	-0.4	-0.2
	mmol/L	-14.4 to 0.0	-10.3 to 0.1	-15.6 to 0.5	-12.0 to 0.5	-6.8 to 0.5	-9.8 to 0.2	-4.4 to 0.2	-7.0 to 0.4	-4.0 to 0.1	-3.1 to 0.5
Albumin effect	mmol/L	2.5	1.7	4.0	4.5	5.0	5.3	5.1	4.9	4.3	4.0
	mmol/L	-0.9 to 4.8	0.1 to 5.1	1.5 to 6.3	1.3 to 6.4	1.3 to 8.8	1.5 to 8.9	1.0 to 8.6	0.5 to 8.0	0.5 to 6.7	1.0 to 6.9
Lactate effect	mmol/L	-3.5	-1.7	-1.3	-0.8	-1.1	-0.9	-1.4	-0.8	-1.6	-1.3
	mmol/L	-5.4 to -1.2	-4.1 to -1.2	-3.1 to 0.0	-2.5 to -0.5	-2.4 to -0.7	-1.9 to -0.6	-3.3 to -0.6	-2.4 to -0.7	-3.0 to -0.8	-2.6 to -0.6

Sum of effects	mmol/L	-0.8	-7.0	-9.9	-3.5	-3.3	-4.2	-3.0	-2.6	-1.8	-0.2
	mmol/L	-5.6-3.7	-8.0-0.9	-13.3-(-3.7)	-11.0-(-1.6)	-10.7-0.6	-12.0-(-0.7)	-7.5-0.7	-11-3.1	-6.4-4.0	-7.7-2.9
Unmeasured anion	mmol/L	-1.9	4.2	7.2	2.6	3.5	4.3	2.6	3.3	1.1	-0.6
effect	mmol/L	-7.9 to 3.4	-1.9 to 6.8	1.7 to 12.7	-4.8 to 8.6	-2.4 to 8.0	-3.4 to 10.6	-4.7 to 8.0	-3.0 to 7.2	-2.6 to 5.4	-3.0 to 10.8

PvCO2: venous carbon dioxide tension; HCO3- std: standard bicarbonate ion concentration; HCO3- act: actual bicarbonate ion concentration; BE (b): base excess blood; BE (ecf): base excess extracellular fluid; CtCO2: total content of carbon dioxide in blood; SID: strong ion difference; Atot: total weak acids; SIG: strong ion gap.

9. Appendices Appendix i

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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Faculty of Veterinary Science
Research Ethics Committee

14 October 2024

LETTER OF APPROVAL

Ethics Reference No	**REC119-22**
Protocol Title	**Acid-base and endocrine response in dogs with acute haemorrhagic diarrhoea syndrome (AHDS)**
Principal Investigator	**Dr AF Michaletos**
Supervisors	**Prof JP Schoeman**
	Prof GE Zeiler

Dear Dr AF Michaletos,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC119-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

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Lefapha la Disaense tša Bongakadiruiwa

Appendix iii

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	Y
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	Y
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	Y
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Y
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly.	Y
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	Y
		c. For each analysis, report the exact value of *n* in each experimental group.	Y
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	N/A
		b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	N/A
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	N/A
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Y
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Y
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Y
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Y
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Y
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Y
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	Y
		a. What was done, how it was done and what was used.	Y
		b. When and how often.	Y
		c. Where (including detail of any acclimatisation periods).	Y
		d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report:	Y
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	N
		b. If applicable, the effect size with a confidence interval.	

Appendix iv

Date: AHDS STUDY CONSENT FORM

Owner:
Patient: **Client Number:**

NB: Circle Primary Contact:
Cell
Cell 2
E-mail
Dog sitter -

Breed: **Age:** **Sex:** **Weight:** kgs **Color:**
Admitted For: **Hospitalising Vet:**

INFORMED CONSENT FOR AHDS STUDY DONE BY DR ANTHEA MICHALETOS (RESEARCHER) AT VFAH, FUNDED BY IDEXX

I, the undersigned, an adult major accept the following conditions:

1. Acute haemorrhagic diarrhoea syndrome (ADHS) is a common disease in dogs that can result in severe morbidity and possibly mortality if not timeously treated. These dogs have diarrhea and vomiting which can cause alterations in the concentrations of electrolytes, albumin and bicarbonate ions, all of which can alter the acid-base balance. However, there is a paucity in literature detailing the effect this syndrome has on acid-base balance. Furthermore, these animals are under a transient state of increased energy requirements and stress which can alter the physiology of the HPA axis and HPT axis. These endocrine responses are poorly documented in dogs with this syndrome. Together, this profiling of the acid-base balance and endocrine response could assist in interpreting a ADHS severity score.
2. Benefits of the study would be endocrine response and acid-base response will be elucidated in dogs with AHDS, acid-base and endocrine responses could be useful in gauging disease severity and the principle investigator will get an MSc.
3. Benefits to the patient would be testing of endocrine function as well as close monitoring of acid-base in ill patients, ensuring critical evaluation of patient.
4. Potential side effects of this study could include mild anaemia, catheter site inflammation and possible pheblitis from catheterization.
5. NO restitution in cases of injury.
6. NO compensation will take place.
7. Any additional tests and procedures needed for the study will be the financial responsibility of the researcher.

8. This form is in addition to the hospitalisation form. This form must be used in conjunction with the Hospitalisation Form.
9. I give permission for a simple central catheter to be placed in my dog as a part of the treatment protocol. I acknowledge that this procedure has been explained to me.
10. I give permission for regular blood sampling to take place (under physiological controls that will not endanger my dog) from my dog as a part of data collection. I acknowledge that this procedure has been explained to me.
11. I have been informed about the risks associated with regular blood collection.
12. I understand that my pet's contribution will be to the benefit of science and better understand a very common disease process in dogs and assist in improving an AHDS severity score.
13. I understand that that I will receive no financial benefit or public acknowledgement for my pet's part in the trial.
14. I understand and consent that all the information will be published with no financial gain.
15. I acknowledge that my pet is up to date with vaccination, deworming and parasite control.
16. **I acknowledge that I have read and understand these conditions.**

Date:
Full Name of Owner / Legal Agent:

…… SIGNATURE:
……

Valley Farm accepts no responsibility for lost belongings!
Items left for more than 2 months will be donated to charity.

Appendix v

Data collection sheet

Date: Breed:
Patient name: Weight:
Age: Presenting complaint:

Time	0	4	8	12	16	20	24	36	48	60
Sample										
L-Hep (1 mL)										
EDTA (1 mL)										
Serum (3 mL)										
Procedure/Analysis										
VBG										
CBC										
Blood smear										
CRP										
Alb										
Phos										
TT4										
Cortisol										
Store serum										
Store EDTA										
ACTH stim										
US scan										

Appendix vi

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

22 June 2023

Approval Certificate
New Application

AEC Reference No.: REC119-22
Title: Acid-base and endocrine response in dogs with acute haemorrhagic diarrhoea syndrome (AHDS)
Researcher: Dr AF Michaletos
Student's Supervisor: Prof GE Zeiler

Dear Dr AF Michaletos,

The **New Application** as supported by documents received between 2023-04-12 and 2023-05-29 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-05-29.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Dogs -	70
Samples	Number
bblood (Samples from live animals)	385

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-06-22.
3. Please remember to use your protocol number (REC119-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
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Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix vii

agriculture, land reform & rural development

Department
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Ms Mama Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MamaL@dalrrd.gov.za
Reference: 12/11/1/1/32 (3987 SdlR)

Responsible person: Dr Anthea Francis Michaletos
Institution: Valley farm animal hospital
829 Old farm Road
Faerie glen
Pretoria east
Email: antheamichaletos@gmail.com
Tel: 0849499192

Dear Dr Anthea Francis Michaletos

PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Title of research project / study: "Acid-base and endocrine responses in acute haemorrhagic diarrhoea syndrome dogs."

Your application, dated 09/06/2023, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) to perform the research project or study stipulated above, refers.

1. Based on the information provided in your application, the Director of Animal Health has no objection to this study. The study may continue if statement 1.1 to 1.6 hereunder (as applicable) are, and remain, accurate. **Should the scope of your research project change in any way you are required to inform the Section 20 Secretariat and may not proceed with any activities until written permission to do so have been granted by the National Director: Animal Health.**

1.1. No work will be done with controlled and notifiable animal diseases (list can be obtained / requested from this office), which includes any animal diseases which do not occur in South Africa;
1.2. No imported material of animal origin or imported animal pathogens will be utilized in the study;
1.3. No samples that originate from a biobank will be used in the study;
1.4. No clinical studies will be performed in the target species, either in a laboratory or in the field;
1.5. The areas where the samples are to be collected are not under restriction for controlled or notifiable diseases to which the species of animal, from which the samples are obtained, is susceptible;
1.6. No samples or products that have not been passed as fit for human consumption will be obtained from an abattoir.

2. In addition to the conditions mentioned in point 1, you are responsible for ensuring that your research project or study complies with all or part of the following, as applicable:

2.1. Permission to perform research under Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) does not relieve the researcher of any responsibility which may be placed on him/her by any other Act of the Republic of South Africa, including the Veterinary and Para-Veterinary Professions Act, 1982 (Act No 19 of 1982), the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1947), the Medicines and Related Substances Control Act, 1965 (Act No 101 of 1965), the Genetically Modified Organisms Act, 1997 (Act No 15 of 1997) and the National Environmental Management: Biodiversity Act, 2004 (Act No 10 of 2004);
2.2. No part of the study may begin until valid ethical approval has been obtained in writing from the relevant South African authority;
2.3. All biological or potentially infectious material must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and the National Road Traffic Act, 1996 (Act No. 93 of 1996);
2.4. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984), must be reported immediately to the responsible state veterinarian;
2.5. Samples or material may not be outsourced or used for further/other research without prior written approval from the Director of Animal Health;

2.6. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study and only a registered waste disposal company may be used for the removal of waste generated during or by the study;

2.7. Records must be kept for five years for auditing purposes.

Written permission from the Director of Animal Health must be obtained prior to any deviation from the conditions. Application must be sent in writing to MarnaL@dalrrd.gov.za.

Failure to obtain written permission as above may be considered a contravention of the Animal Diseases Act, 1984 (Act No 35 of 1984).

Expiry date of this permit: 09 June 2026

Kind regards,

Dr Mpho Maja
DIRECTOR: ANIMAL HEALTH
Date:
2023 -06- 09

SUBJECT: Permission to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984)

Appendix viii

agriculture, land reform & rural development
Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X250, Pretoria 0001
Enquiries: Ms. Mama Laing · Tel: 012 319 7442 · Fax: +27 12 319 7470 E-mail: MamaL@dalrrd.gov.za
Reference: 12/11/1/1/32 (6639 CMa)

Dr Anthea Francis Michaletos
Valley farm animal hospital
Valley Farm Animal Hospital
829 Old Farm Road
Faerie Glen
Pretoria East
E-mail: antheamichaletos@gmail.com

Dear Dr Anthea Francis Michaletos,

RE: AMENDMENT OF SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) – EXTENSION OF THE EXPIRY DATE

Title of research project / study: "Acid-base and endocrine responses in acute haemorrhagic diarrhoea syndrome dogs"

An amendment is hereby granted on the Section 20 approval that was issued for the above-mentioned study on 2023-06-09.

1. As requested, the validity of the section 20 approval is extended to August 2025.
2. All other conditions as specified in the Section 20 approval of 2023-06-09 remain in full effect. This includes the validity of laboratory approvals in terms of SANAS and DALRRD.

Kind regards,

Malke pp.

DIRECTOR: ANIMAL HEALTH

Date: 2024 -08- 22

10. Declarations

The authors declare no conflict of interest.

The authors declare that artificial intelligence was not used in this study or during the preparation of the manuscript.

The data set is available upon reasonable request.

AF Michaletos