Comparison of different methods of acid-base analysis in canine parvoviral enteritis

by

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Declaration

I Ryan Brandon Friedlein declare that the work on which this dissertation is based is original and that neither the whole work or any part of it has been, or is being, or is submitted for another degree at this or any other university, tertiary education institution or examining body.

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Summary

Acid-base disorders are common in critical care patients, and thus understanding these derangements is important in critical care medicine[1-3]. Disturbances should be expected in animals presenting with gastro-intestinal, renal, respiratory and neurologic diseases, as well as in shock[4]. Routine serum biochemistry results may be suggestive of an acid-base disturbance and abnormalities should prompt clinicians to investigate the patients' acid-base status further, through blood gas analysis[5]. Identification of acid-base disorders is valuable in the clinical appraisal of a patient and informing treatment regimens[6]. Since canine parvoviral enteritis (CPE) is a disease associated with extensive fluid, protein and electrolyte losses through vomiting, diarrhoea, sepsis and malabsorption, the associated losses contribute to acid-base imbalances, most importantly in the metabolic compartment[7-11].

Several methods have been used to assess acid-base status in dogs. These include the traditional Henderson-Hasselbalch approach (HH), and the quantitative approaches, namely the Stewart strong ion model and various adaptations of this approach. The hypothesis of this study was that the strong ion approach would identify acid-base changes that would not be appreciated by the HH model. Accordingly, blood was collected from 41 puppies with confirmed CPE. Blood was collected for venous blood gas and serum biochemistry, and all samples were collected at admission prior to any therapeutic interventions. Each patient's acid-base status was assessed according to the HH model, the base excess algorithm (BEA) and a simplified strong ion approach (SSA). Most data were not normally distributed as determined by Shapiro Wilk and as such all comparisons made use of non-parametric methods (Mann Whitney U for the comparisons between medians). The control group of dogs were compared to the CPE group for all measured and calculated variables and a p- value <0.05 was regarded as significant. The HH model detected acid-base abnormalities in 41% of dogs, the SSA demonstrated derangements in 46% of dogs and the BEA displayed abnormalities in 89% of dogs.

Acid-base disorders may be assessed utilising different methods with variations within each of the methods themselves. There are many ions, proteins and buffers that all affect the acid-base balance. The acid-base approaches discussed in this study namely, the HH, SSA and BEA, place emphasis on different components of a complex system in an attempt to understand and dissect the underlying pathogenesis. The contribution of one component may also dampen the effect of a component having an opposing effect on acid-base balance (for example the opposing effect of hypoalbuminaemia on the effect of phosphorus). The study also highlights the complexity of methods that use multiple variables to

generate a final result (particularly the SSA). The opposing or antagonising effects of these individual variables may mask and falsely diagnose acid-base disturbances.

The current study was chiefly descriptive. Despite this, the methods were compared and the level of discordance between the models was evaluated. A significant discordance was demonstrated between the cases diagnosed as normal by each method. In the assessment of the discordance between the methods, the HH demonstrated discordance of 42% with the SSA and 92% to the BEA. Similarly, the SSA demonstrated discordance of 36% between the HH and 86% between the BEA. Lastly, the BEA demonstrated a discordance of 50% between the HH method and 25% between the SSA. Based on these levels of discordance it becomes clear that the different methods of acid-base analysis cannot be used interchangeably.

In conclusion, the acid-base changes in CPE are complex and not easily understood using the HH model. The pathophysiologic mechanisms are more easily explained by the SSA and BEA approaches. However, it is unclear if using these methods of diagnosis change the way that CPE should be managed in clinical practise.

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   Would that I could be the peacemaker in your soul, that I might turn the discord and the rivalry of your elements into oneness and melody” Kahlil Gibran, The Prophet.

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List of Abbreviations

AG Anion gap

A_{TOT} Total quantity of weak acids

BE Base excess

BEA Base excess algorithm

CPE Canine parvoviral enteritis

HH Henderson-Hasselbalch

SID Strong ion difference

SIG Strong ion gap

SSA Simplified strong ion approach

SUM Sum of effects

XA Unmeasured ions

Chapter 1

Literature Review

1.1 Introduction

Acid base disorders are common in critical care patients and thus understanding these derangements is important in critical care medicine[1-3]. Disturbances should be expected in animals presenting with gastro-intestinal, renal, respiratory and neurologic diseases, as well as in shock[4]. Routine serum biochemistry results may be suggestive of an acid-base disturbance and abnormalities should prompt clinicians to further investigate the patients' acid-base status through blood gas analysis[5]. Identification of acid-base disorders is valuable in the clinical appraisal of a patient and informing treatment regimens[6]. Since CPE is a disease associated with extensive fluid, protein and electrolyte losses through vomiting, diarrhoea, sepsis and malabsorption, the associated losses contribute to acid-base imbalances, most importantly in the metabolic compartment[7-11]. Aggressive therapy directed at fluid, electrolyte and protein abnormalities should be applied in all CPE cases to correct the above derangements. Several methods have been used to assess acid-base status in dogs. These include the traditional Henderson-Hasselbalch approach (HH), the Stewart strong ion model (SIM [a quantitative method]) and then various adaptations of this approach[2, 6, 12-16].

In this study, three models were used to describe the acid-base disturbances of parvovirus infected puppies. Our hypothesis was that there would be significant similarities, overlap, as well as differences between the approaches, and that none of the approaches would be superior in the detection of an acid-base disturbance. A secondary purpose of this work was a descriptive comparison of the different acid-base imbalances seen in canine parvovirus (CPV) across the three approaches.

1.2 History and Evolution of Parvovirus

Canine parvoviruses are small, non- enveloped, single- stranded DNA viruses that replicate in actively dividing cells[17]. The first reported parvovirus of dogs in 1968 was the Minute virus of canines which later became CPV- 1[18]. This virus is distinct from CPV- 2 and was originally thought to be non- pathogenic, however, it was later found to cause diarrhoea and gastroenteritis in neonatal patients[18]. In 1978, CPV-2 first emerged with initial cases demonstrating myocarditis and mucoid-to-bloody diarrhoea, and within a

short period of time the virus had spread worldwide. This virus was serologically discovered to be closely related to Feline Panleucopaenia Virus (FPV) and other parvovirus infections of carnivores [18]. By 1980, the disease had spread rapidly and extensively worldwide, due primarily to contamination of clothing and shoes with faecal material and movement of dogs and owners by land, air and sea[18]. Initially in 1980- 1981, a decline in disease prevalence occurred due to heterologous vaccines (closely related to FPV) and natural infection resulting in herd immunity[19]. In 1981- 1982, veterinarians reported severe forms of the disease, with patients presenting in shock- like states with a rapidly progressing illness, severe haemorrhagic enteritis and acute mortalities, evident in both vaccinated and non- vaccinated animals[18]. This disease was subsequently identified to be caused by a mutation of the CPV, termed CPV- 2a, which then subsequently mutated to CPV- 2b in 1984[18, 20]. Another mutation occurred in 2000 resulting in CPV-2c, evident in Europe, South America and the United Kingdom[21-25]. This strain cannot be differentiated clinically from CPV-2a and CPV-2b; however some reports indicate that it may cause a more severe form of disease in adult dogs[26, 27]. Canine parvovirus- 2c has rapidly achieved global dissemination; this may be due to faster replication, a greater volume of virus yielded per shedding and more efficient transmission between dogs[28]. This however does not correlate with a worsened disease severity in puppies, or an increase in the likelihood of affecting vaccinated dogs[28]. CPV- 2a, CPV- 2b and recently CPV-2c, are now the predominant field strains, replacing the original CPV- 2 strain[19].

1.3 Epidemiology

Initially, the disease outbreak affected both puppies and young adult dogs with high morbidity and mortality rates. However, currently the disease is almost exclusively seen in puppies between 6 weeks and 6 months old, with a minority of cases involving adult populations due to immunity from vaccination or from natural infection[17]. Immunity is generally long lived and is potentially lifelong after natural infection[26], thus the only susceptible animals in a population are the newly born[29]. Maternal antibodies from vaccination or previous infection are transferred to the neonate to provide protection for the first 6 weeks of the puppy's life, with antibodies demonstrating a half-life of approximately 9.7 days[17]. Susceptibility increases as maternal immunity wanes at around 12- 14 weeks of age. Several risk factors have been identified that contribute towards the disease severity, these include: stress during weaning, overcrowded and unsanitary conditions, inadequate transfer of maternal immunity and intestinal parasites[29, 30]. An increased risk has been reported in particular breeds such as: Rottweilers, Doberman pinchers, American pit bulls, German shepherd dogs and Labrador retrievers; however it is unclear if this

data is biased toward or influenced by breed popularity, geographical distribution or inadequate vaccination protocols[17, 29]. The disease has been shown to be seasonal with an increased incidence in the summer months [31]. At the Onderstepoort Veterinary Academic Hospital, the case load of CPE infection is directly correlated with wind speeds and inversely to humidity[32]. No association has been found between gender or bodyweight in CPE enteritis however, an increased incidence in sexually intact males compared to females in dogs older than 6 months of age has been reported[31]. Both CPV and FPV are extremely stable in the environment which makes indirect transmission important and environmental decontamination difficult. Care should be taken in veterinary clinics where unvaccinated or inadequately vaccinated puppies are likely to come into contact with contaminated surfaces. Dogs admitted to hospital for treatment should be kept isolated from the rest of the hospital population. It has recently been shown that flies also play an important role in the spread of the infection amongst dogs[33]. Prevention of disease is conveyed passively through maternal antibodies from the dam to the pups that have a residual effect of affording protection in the first few weeks of life. Active prevention requires the usage of effective vaccination protocols to stimulate immunity to infection. However, a significant interference by maternally derived antibody (MDA) to vaccination is evident, which may cause vaccination failures[34]. Approximately 90% of MDA are passed through colostrum[34]. The gold standard for quantification of CPV antibodies involves measuring haemagglutination inhibition (HI) antibodies with HI titres below 1:64 to 1:80 allowing susceptibility to infection[17, 34]. High- titre, low- passage, canine- origin live attenuated CPE vaccines are the current vaccination of choice, and should be administered at six, nine weeks and 12 weeks of age[35, 36].

1.4 Pathophysiology and Clinical Signs

Viral transmission occurs via direct (faecal-oral route virus exposure) or indirect routes (exposure to fomites on contaminated surfaces) [26]. Diseased dogs can shed the virus at very high titres (up to $10^9 TCID_{50}$ per gram faeces) in their stools[37]. Primary viral replication occurs in the pharyngeal lymphoid tissue, this is then followed by dissemination to the mesenteric lymph nodes, thymus and intestinal crypt cells[26, 38]. The parvovirus infects tissues rich in rapidly dividing cells (such as the intestinal crypts), resulting in epithelial destruction and villous collapse[39]. This villous collapse results in an impaired ability of the intestinal tract to absorb and assimilate nutrients, leading to progressive malnutrition and diarrhoea in the course of disease[29]. The extensive damage to the intestinal tract also leads to translocation of gram negative bacteria into the blood stream with a resultant coliform septicaemia[17].

Escherichia coli has been the predominant bacterium, and has been cultured from the liver and lungs in affected patients indicating the systemic dissemination[40, 41] . The necrosis and damage of the epithelial and gut- associated lymphoid tissues in CPE may result in a favourable environment for the overgrowth of additional bacteria such as *Clostridium perfringens*, contributing to the haemorrhagic gastroenteritis seen[41].

1.5 Biochemistry and Haematological Abnormalities

Serum biochemistry changes are generally non-specific and worsen with disease progression. The combination of anorexia, dehydration, vomiting and diarrhoea contribute to serum hypokalaemia and potentially hypochloraemia and hyponatraemia[39]. Hypoalbuminaemia is a common finding, resulting from protein loss in the gastrointestinal tract, intestinal haemorrhage, sepsis- induced SIRS, reduced production, and dilution from fluid therapy[11]. In addition to the hypoalbuminaemia, serum electrophoresis has shown relative and absolute hypogammaglobulinaemia and hyperalpha- 2-globulinaemia, likely attributable to acute-phase protein production[11].

In a study of lipid profiles in CPE, serum total cholesterol, high density lipoproteins (HDL)-C and low density lipoproteins (LDL)-C levels were significantly lower but the mean serum triglyceride level was higher in CPE puppies than in controls[42]. This study demonstrated important clinical aspects relating to lipoprotein levels and endotoxaemia. Experimentally, increased lipoprotein levels have shown to reduce the stimulatory effects of lipopolysaccharide (LPS) and increased host survival during gram- negative bacteraemia and endotoxaemia[42].

Due to the destruction of haematopoietic leukocyte progenitor cells in various organs, primarily the bone marrow but also the spleen and thymus, the WBC count is affected negatively resulting in leucopaenia[43]. Consequently a severe leucopaenia may contribute towards septicaemia and an increased mortality in infected puppies[43]. In a study evaluating the prognostic usefulness of blood leukocyte changes in canine parvovirus, it was reported that a WBC count $\geq 4.5 \times 10^3$/mL, a lymphocyte count $\geq 1.0 \times 10^3$/mL, a monocyte count $\geq 0.15 \times 10^3$/mL and an eosinophil count $\geq 0.10 \times 10^3$/mL, as early as 24 hours after admission are accurate predictors of a better outcome in CPE enteritis[43]. Of notable importance, the WBC and particularly the lymphocyte count had a 100% positive predictive value for survival 24 hours post hospital admission[43].

C- reactive protein (CRP) is an important acute phase protein synthesised within the liver following stimulation by inflammatory cytokines, and has been shown to increase in dogs with disease conditions such as pancreatitis, sepsis, neoplasia, ehrlichiosis and leptospirosis[44-47]. The results of a study evaluating CRP in CPE puppies indicated that higher CRP levels at 12- and 24 hours post admission were associated with reduced survival times and longer hospitalization periods in survivors compared to non-survivors[48]. However, in this study, utilizing CRP as a discriminative tool in predicting outcome was only moderately accurate[48].

Endocrine markers such as serum cortisol and thyroxine concentrations have been utilized as prognostic tools in critical care in humans and dogs[49]. In a study evaluating serum cortisol and thyroxine in CPE, high serum cortisol and low serum thyroxine concentrations at 24- and 48 hours post admission were associated with mortality in puppies[49]. The study indicated that dogs with a serum cortisol that failed to normalize as well as those dogs with lower mean basal serum thyroxine concentrations had a poorer prognosis[49]. The connection behind these findings is related to the strong association between cortisol and the severity of inflammation as seen in CPE, and highlights the significance of the inflammatory reaction in these animals[39, 49].

Puppies suffering from CPE exhibit laboratory evidence of hypercoagulability. A study incorporating thromboelastography (TEG) and other coagulation parameters demonstrated a significantly lower antithrombin activity, significant increases in fibrinogen concentrations, increased TEG mean amplitude levels, moderately prolonged aPTT times, reduced fibrin degradation products and a higher prevalence for thrombosis or phlebitis[50]. These findings were consistent with a state of hypercoagulability in CPE puppies.

1.6 General Therapeutics in CPE

CPV has been associated with mortality rates up to 48%, with survival rates of up to 96% if treated intensively at tertiary care veterinary institutions [17, 48, 51]. There are no specific therapeutics available and the mainstay of therapy is supportive care [26]. Generally, outpatient therapy is not recommended as these dogs rapidly deteriorate to a state of shock and collapse without appropriate care [17, 42]. Therapy requires aggressive fluid, electrolyte and colloidal support, antiemetics, antimicrobials, analgesics, enteral nutrition, anthelmintics and correction of hypoglycaemia and protein disturbances [29]. Focused correction of the protein and electrolyte disorders will have an impact on and should correct acid-base

derangements if they are present. An extensive review of the therapeutics of CPE is not within the scope of the current study.

1.7 Gastrointestinal Tract Disturbances

The gastro intestinal tract (GIT) has a critical role in the homeostasis of acid base balance[52]. Disruption of the normal function of the GIT may result from infection (viral, bacterial, fungal and protozoal), post-operative or autoimmune conditions. Depending on the electrolyte compositions of the fluids that are lost, an alkalosis, acidosis or a mixed acid-base disorder may result[52].

Gastric fluid is rich in various ions including sodium, potassium and hydrogen, with the latter increasing substantially with gastric acid production[52]. Vomiting results in the loss of large quantities of chloride as well as hydrogen[52]. 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[52]. The result of these changes if severe enough may lead to a metabolic alkalosis acid-base disorder.

The acid base disorder that develops with diarrhoea is dependent on the ions that are lost, as well as the volume of fluid loss. Sufficiently large volumes of diarrhoea need to be lost in order to negate the renal compensatory mechanisms. In human literature, it has been estimated that acid-base disturbances occur in up 70% of patients with diarrhoea[53]. Whether an acid-base disorder will occur or not is dependent on the site of diarrhoea within the intestine, the type of diarrhoea (secretory or inflammatory) and the chronicity[53]. In human literature, cases with secretory diarrhoea can lose up to 20 litres of diarrhoea per day, containing up to 140mmol/L of sodium, 105mmol/L of chloride and up to 75mmol/L of bicarbonate[52]. In cases of inflammatory diarrhoea, although the daily volumes are comparatively less (up to 3litres per day), the concentrations of sodium, chloride and potassium remain similar, however bicarbonate losses are reduced (up to 20mmol/l) [52]. Losing large volumes of stool can result in reduced extra-cellular fluid (ECF) volume, a reduction in glomerular filtrate rate, hypotension and acute renal failure[52]. Canine parvoviral enteritis is similar in the large quantities of fluids lost (through vomitus and diarrhoea) as well as in respect of hypotension, dehydration and shock[11].

In human medicine, gastrointestinal infections with *Escherichia coli* have been known to cause diarrhoea as well as a metabolic acidosis[52]. The combination of increased secretion of chloride by intestinal cells as well as inflammation downregulating both the intestinal Cl^-/HCO_3^- exchanger and Na^+/K^+-ATPase result

in diarrhoea states[52]. These findings are potentially similar in CPE where the necrosis of intestinal mural and lymphoid tissue predisposes to the colonisation of secondary bacteria such as *Escherichia coli* [40, 54].

Gastrointestinal diseases such as foreign bodies with resultant vomiting and anorexia may also cause acid-base derangements. In a retrospective study evaluating records from 138 dogs with foreign bodies in a variety of locations and of varying durations, electrolyte and blood gas results revealed a metabolic alkalosis as the most common acid-base derangement[55]. In addition, the dominant biochemical abnormalities were hypochloraemia in 51.2%, hyperlactatemia in 40.5%, and hyponatremia in 20.5% of animals[55]. Furthermore, the mean anion gap (AG) was 15.3 (range 1-29) with 58% of animals having an AG within the normal reference ranges[55]. When evaluating the pH, the mean value was 7.43 with 74.2% of animals having values within the reference range[54]. This illustrates the variety of electrolyte and acid-base disturbances that are possible in gastrointestinal disease as well as the large variation in normal and abnormal findings.

1.8 Acid-base Homeostasis

Hydrogen ions are a crucial component in numerous body systems and cellular function, and their concentrations require tight regulation[5, 56]. Alterations in hydrogen ion concentration can have drastic physiologic effects such as: diminished cardiac contractility, arrhythmias, hypotension, reduced vascular responsiveness to catecholamines, insulin resistance, disruption of enzyme systems and electrolyte imbalances[5]. A thorough understanding of the derangements in the concentrations of ions (including hydrogen) is crucial for both diagnostic and therapeutic interventions[56].

Acids are produced in one of two forms: namely volatile acids or non-volatile acids. Aerobic metabolism produces carbon dioxide (CO_2) which combines with water in a reaction catalysed by the enzyme carbonic anhydrase to produce carbonic acid (H_2CO_3) which then dissociates into H^+ and bicarbonate (HCO_3^-)[57, 58]. In contrast, protein and phospholipid catabolism results in non-volatile acids as an end-product, such as sulfuric acid and phosphoric acid[5]. Particular disease states such as diabetic ketoacidosis (DKA) and uraemia can result in excessive production of non-volatile acids, including acetoacetate, β-hydroxybutyrate and lactate[57]. The initial compensatory response to protect against acute changes in pH is the effect of multiple buffers contained in body fluids, such as plasma HCO_3^-, proteins and phosphates[57] . A buffer is a mixture of a weak acid and its conjugate base, or a weak base

and its conjugate acid, and its effects on acid-base homeostasis can be profound[57] . Consequently, when alterations in the concentration of H^+ exceed buffering capabilities, an acid-base disturbance develops[57].

Systems that are involved in acid-base homeostasis include the respiratory system, the renal system, the gastrointestinal tract (GIT) and red blood cells (RBC's) [56, 58]. Regarding the respiratory system, the principal waste product of cellular metabolism is CO_2, which is expired by the lungs[56]. Furthermore, the medulla of the brainstem and carotid and aortic bodies contain chemoreceptors that respond to alterations in H^+ concentrations and PCO_2 (partial pressure of carbon dioxide) and alter respiratory rate and effort in order to maintain equilibrium[56]. In addition, the kidneys play a crucial role in HCO_3^- regulation and are able to reabsorb and regenerate HCO_3^-, as well as excrete excessive amounts of acid or base as required to maintain pH homeostasis[5, 56, 58].

1.9 Henderson-Hasselbalch Approach

The Henderson-Hasselbalch equation was formulated in 1916 and this contributed to the development of the base excess (BE) and anion gap concepts in the 1960's[59-63]. The HH equation has assisted the diagnosis and management of electrolyte and acid-base disorders in both humans and animals for numerous years[64]. This approach is both widely recognized and relatively simple to implement and understand [65] . The method has been criticized because of its descriptive rather than mechanistic nature and it ignores important players in acid-base physiology (such as certain electrolytes and proteins). This results in poor explanations for acid-base pathophysiology, especially regarding the metabolic compartment[5, 56, 66].

The traditional approach focuses on how plasma pH is estimated by the interaction between PCO_2, HCO_3^-, the negative logarithm of the apparent dissociation constant (pK^1) for H_2CO_3 and the solubility coefficient for CO_2 in plasma[6, 64, 67]. This is represented by the following equation:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

The pH is utilized as an overall measure of acid-base status, i.e. whether blood sampled is acidaemic or alkalaemic, PCO_2 as an independent measure of the respiratory component of the acid-base balance, and plasma HCO_3^- and BE as a measure of the metabolic component[64]. The extracellular fluid BE or standardized base excess (SBE) indicates the direction and magnitude of an acid-base disturbance and

thus quantifies metabolic changes, but does not offer an understanding into the pathophysiology for the disturbance[64, 67, 68]. An accurate calculation of the SBE requires normal serum protein concentrations, which means it is often not fully assessed in critically-ill animals[64]. The HH approach further characterizes the metabolic disturbances by the anion gap calculation to detect an increase in the concentration of unmeasured anions, by using the concept of electroneutrality[6, 69].The AG is conceptually comprised of the major cations (sodium, potassium, calcium and magnesium) and the major anions (chloride, bicarbonate, sulphates, phosphates, plasma proteins and organic anions, such as lactate and citrate)[57, 61, 70] . For ease of calculation the AG is arithmetically comprised by the calculation: $(Na^+ + K^+) – (Cl^- + HCO_3^-)$. The AG does not take albumin directly into account but rather it is viewed as one of the unmeasured ions[71] . One of the limitations of the AG occurs when hypoalbuminaemia is present with concurrent increased acids such as lactate, resulting in a normal AG (due to hypoalbuminaemia) which then results in underdiagnoses of the underlying causes of a metabolic acidosis[71]. A high AG acidosis results from a gain in acid with its associated anion, while normal AG acidosis occurs from retention of protons or loss of HCO_3^-, with associated increases in plasma chloride concentration[6]. Bicarbonate has been regarded as the principal compound in the control of the metabolic component of acid-base balance[67]

The HH equation defines 4 primary acid-base disturbances:

- Respiratory acidosis (increased PCO_2)
- Respiratory alkalosis (decreased PCO_2)
- Metabolic acidosis (decreased HCO_3^- or BE)
- Metabolic alkalosis (increased HCO_3^- or BE)

Acid-base derangements are described as “simple” if there is a primary disturbance with the expected compensatory response, or “mixed” or “triple” if multiple acid-base disturbances are present concurrently (i.e. not the primary disturbance with its expected compensatory response) [57] . Development of a stepwise approach to acid-base evaluation is crucial to understanding the disturbance, creating a differential list and subsequent diagnostic and treatment plans[57]. The approach involves evaluating the pH, determining the primary disturbance, establishing whether adequate compensation for the primary disturbance exists and identification of the underlying aetiology of the acid-base disorder[57].

1.9.1 Metabolic acidosis

Metabolic acidosis is a common acid-base disturbance identified in small animal practice[5, 71] and has important diagnostic, therapeutic and prognostic implications[3, 6, 71] . This acid-base disorder has been reported to occur in up to 36% of dogs undergoing blood-gas analysis for any reason at a veterinary teaching hospital[71] . Metabolic acidosis is characterized by a reduced pH due to an accumulation of non-volatile acids or a loss of HCO_3^-, which then exceeds the buffering capabilities of the body [72]. The resultant compensatory mechanism is to reduce PCO_2 through the respiratory system (hyperventilation) [72] . The term acidaemia implies a low blood pH without the processes leading to this state. These terms have often been used interchangeably and may be misleading and confusing. Metabolic acidosis can occur either as a primary acid-base disorder, a compensatory mechanism for a primary respiratory alkalosis or in combination with a respiratory acid-base disorder, resulting in a mixed disorder[71] . This imbalance can further be characterized by an increased AG (normochloraemic and gain of an acid) or a normal AG (hyperchloraemic and loss of bicarbonate) [72] . Serum bicarbonate concentration or base excess have been utilized as markers for metabolic acidosis, however, the ideal parameter remains controversial[71] . An aspect of the controversy lies in the fact that bicarbonate is not independent of changes in the PCO_2 whereas base excess is standardized to a PCO_2 of 40mmHg[71] . In a retrospective study by Hopper *et al,* the acid-base status of 1805 dogs and cats were evaluated[71] . The most common disorder demonstrated was a metabolic acidosis occurring in 49% of animals (both dogs and cats)[71]. Furthermore the most common underlying disorder resulting in the metabolic acidosis in dogs was neoplasia and surgery, and in cats was renal disease[71] . The effects of severe acidosis (pH <7.2) are most profound on the cardiovascular system affecting cardiac output, arterial blood pressure, hepatic and renal blood flow and perfusion and well as reducing the threshold at which arrhythmias can occur[73]. In brief the treatment of a metabolic acidosis that results from an acid gain (DKA, uraemia, lactic acidosis and ethylene glycol toxicity) relies on addressing the underlying cause as well as appropriate IV fluid therapy[74]. The aim of corrective fluid therapy is to reverse the underlying derangements of hypotension, hypovolaemia and poor tissue perfusion. Treatment of cases with a hyperchloraemic metabolic acidosis requires institution of IV fluids with a lower chloride concentration and strong ion difference (SID) than the plasma[74]. In addition cases with a metabolic acidosis resulting from bicarbonate loss, the primary underlying disorder should be addressed first before the consideration of the usage of bicarbonate therapy. In cases of severe metabolic acidosis (pH<7.2) as well as if the compensatory respiratory alkalosis is detrimental to the animal, alkalinizing agents such as sodium bicarbonate ($NaHCO_3$) have been recommended by some to increase the pH[74]. This recommendation is however controversial. The

resultant negative implications of excessive $NaHCO_3$ administration include hyperosmolality with subsequent fluid overload, hypokalaemia, hypernatraemia, ionized hypocalcaemia, overshoot alkalaemia, hypercapnia and paradoxical central nervous system acidosis [72] .

1.9.2 Metabolic alkalosis

Metabolic alkalosis is characterized by an increased pH, an increased HCO_3^-, and an increased BE[72]. In order to increase the PCO_2, ventilation by the respiratory system is reduced as a compensatory mechanism[72] . Metabolic alkalosis can result from loss of non-volatile acid via the kidneys and gastrointestinal tract, administration of alkaline-containing solutions and extracellular fluid volume contraction[72]. Two types of metabolic alkalosis are described in animals: a chloride-responsive (e.g. vomiting of stomach contents, diuretic therapy and post-hypercapnia) or chloride-nonresponsive (e.g. excess quantities of mineralocorticoids, as well as adrenal-dependent hyperadrenocorticism) metabolic alkalosis[72]. The kidneys have an excellent capacity to excrete large concentrations of bicarbonate and thus metabolic alkalosis should be readily corrected [74].The kidneys ability to excrete bicarbonate may be impaired by conditions such as hyperaldosteronism, hypokalaemia, hypovolaemia and reduced renal perfusion[74]. Thus a key feature of correcting metabolic alkalosis should focus on addressing and reversing the underlying cause, normalising electrolyte derangements and restoring perfusion and circulating volume[74].

1.9.3 Respiratory acidosis

Respiratory acidosis is characterized by a reduced pH and increased PCO_2 with a compensatory increase in HCO_3^-[75] . The degree of metabolic compensation will be influenced by the chronicity of the abnormality[75]. Respiratory acidosis results from any pathologic process that affects neuromuscular control of ventilation or alveolar gas exchange[75]. Examples include: large airway obstruction, respiratory centre depression, increased CO_2 production with impaired alveolar ventilation, restrictive extra-pulmonary diseases (pleural diseases), parenchymal and small airway diseases, improper mechanical ventilation and severe obesity[75] . Treatment should once again be directed towards the underlying cause, and therapies with $NaHCO_3$ or other alkalinizing agents are contraindicated due to ineffective ventilation and removal of the CO_2 by-product from H_2CO_3 dissociation[75]. In cases where despite appropriate therapy hypoventilation still ensures, the animals should be mechanically ventilated[74].

1.9.4 Respiratory alkalosis

Respiratory alkalosis is characterized by an increased pH, decreased PCO_2 and compensatory decrease in HCO_3^- [75]. Respiratory alkalosis occurs when ventilation is excessive in relation to the expiratory requirements of CO_2 produced through normal metabolic processes[75]. Causes include: hypoxaemia, pulmonary parenchymal diseases independent of hypoxaemia, centrally mediated hyperventilation, excessive mechanical ventilation or situations that induce pain, fear or anxiety[75] . A primary respiratory alkalosis should result in a decrease in HCO_3^- concentration, the severity of which will be determined by the chronicity and severity of the alkalosis[71]. The treatment is directed towards the underlying mechanism and includes sedation, pain medications and diuretics[75]. Specific therapy directed towards the respiratory alkalosis is rarely performed[74].

1.9.5 Mixed disturbances

Mixed disturbances result when two or more acid-base disturbances occur simultaneously and should be suspected when: PCO_2 and HCO_3^- change in opposing directions, when a normal pH is present regardless of an abnormal PCO_2 and/ or HCO_3^-, when the pH change is greater than what is expected for a known primary disorder or not usually associated with the underlying primary disease, or when there is failure to produce the predicted compensatory response[76] . In order to investigate these changes mechanistically, additional acid-base models such as the Stewart strong ion approach should be utilized[2, 16]. As mentioned previously in a retrospective study by Hopper *et al*, primary metabolic acidosis was the most common abnormality in both dogs and cats, however mixed acid base disorders were more common in dogs representing 58% of the canine population[71]. The high proportion of disorders diagnosed by traditional means does not provide insight to the underlying pathophysiology, creating a requirement for more advanced quantitative acid-base analysis, such as the Stewart strong ion model[71].

1.9.6 Base excess

The BE represents the amount of acid or base that must be added to a litre of fully oxygenated blood exposed *in vitro* to a PCO_2 of 40mmHg at 38^0C to achieve a normal pH of 7.40[58]. The BE has also been used as a measure of the metabolic (non-respiratory) component of acid-base balance.[58]. However, it can never be truly independent of PCO_2 since HCO_3^- is dependent on PCO_2. Therefore this reflects a false assumption made for convenience. Base excess can be calculated from the results of automated blood gas analysis and pH analysis using the van Slyke equation with haemoglobin concentration (Hgb) and HCO_3^- in mmol/L [64]. Furthermore, the BE takes into account changes in free water, chloride, protein and phosphate concentrations and is affected by the presence of unmeasured strong ions[14]. The BE thus

provides insight into the direction and magnitude of an acid-base disturbance but not the underlying pathomechanism[68]. The normal range for standard BE assumes normal concentrations of A_{TOT}, with a negative BE being consistent with a metabolic acidosis and potentially an increased concentration of unmeasured anions [14, 57, 57].

1.10 Stewart Strong Ion Approach

Stewart emphasized a quantitative approach, evaluating the different contributions of strong ions that are completely dissociated in solutions and weak acids that are partially dissociated[67]. Thus the three nominated principal independent factors presented by Stewart are as follows:

1) The strong ion difference - the difference between the concentrations of strong cations (sodium, potassium, calcium and magnesium) and anions (chloride, lactate, sulphates and ketoacids), in which the strong cations and anions are fully dissociated at physiological pH[6, 67].

2) A_{TOT} - the total plasma concentration of non-volatile buffers (albumin, globulin and inorganic phosphate) where albumin accounts for most of the acid-base effects of plasma proteins, with globulins playing a minor role[56].

3) The partial pressure of CO_2[64, 64, 67, 67].

The quantitative approach makes a clear distinction between the PCO_2, SID and the non-volatile weak acids (A_{TOT}) who are independent, in that they are not affected by changes in other variables, and the dependent variables (H^+ and HCO_3^-), which are regarded as dependent variables[65, 77].

Alterations in the PCO_2, the SID or concentrations of non-volatile plasma buffers result in clinically important acid-base abnormalities[64]. The strong ion approach identifies 6 primary acid-base disorders (respiratory, strong ion or non-volatile buffer ion acidosis and alkalosis) [64]. Acidaemia results from an increase in PCO_2 and non-volatile buffer concentrations or from a decrease in SID[6, 64]. In contrast, alkalaemia results from a decrease in PCO_2 and non-volatile buffer concentration or from an increase in SID[64]. The strong ion model offers a mechanistic understanding into the mixed acid-base abnormalities[64]. An approximation of the SID can be obtained in the clinical setting with the difference in Na^+ and Cl^-, since Na^+ and Cl^- are the major contributory strong ions in plasma[6]. The simplified strong ion

equation provides a clear mechanism for understanding the contribution that plasma protein concentration has on pH, whereby changes in plasma protein concentrations directly affect A_{TOT}[6]. This subsequently leads to a direct and predictable change in plasma pH, such that decreasing plasma albumin (decreasing A_{TOT}) increases the plasma pH[64].

The Stewart approach has gained increased popularity among anaesthetists and critical care clinicians as there have been advances in quantifying metabolic acid-base changes, in particular defining the role of unmeasured ions[67]. In general, the principal approach to determining the presence of unmeasured ions has been the HH model assessing the AG[67]. The term SID has been used to describe the difference between the concentrations of strong cations and strong anions in a fluid compartment (Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^-)[14, 56]. Due to these ions being easily measured, the term apparent SID ($SID_{apparent}$) is used[56]. Another expression for the SID in plasma that does not make any assumption about which strong ion constitutes the SID is the effective SID ($SID_{effective}$) [56]. The $SID_{effective}$ incorporates the concentrations of HCO_3^-, inorganic phosphate and albumin[14, 56]. A more precise estimate of the unmeasured anions can be obtained by comparing the apparent SID ($SID_{apparent}$= $[Na^+] + [K^+] + [Mg^{2+}] + [Ca^{2+}] - [Cl^-]$ - [lactate anions]) with the SID calculated from the opposing effects of CO_2, albumin and phosphate, the effective SID. When the $SID_{apparent} > SID_{effective}$ unmeasured anions must be present, the difference has been termed the strong ion gap (SIG) [67]. This is represented by the following equation:

$SIG = SID_{apparent} - SID_{effective}$

1.10.1 The classic example of hyperchloraemic acidosis assessed by the Stewart approach

Hyperchloraemic acidosis has been characterized by hyperchloraemia and decreased plasma HCO_3^- [64, 78]. This results from an infusion of 0.9% sodium chloride (NaCl) that produces a predictable, volume-dependent acidaemia and subsequent metabolic acidosis[64, 78]. In the HH model administration of large volumes of 0.9% NaCl solution reduces the plasma HCO_3^- and consequently results in a metabolic acidosis, by a pathomechanism of *dilutional* acidosis [64]. Hence when utilising the HH equation for evaluation, it indicates the clinical condition should be treated with bicarbonate in order to restore bicarbonate concentration and normalize acid-base status[78]. However this explanation has been regarded as flawed because dilution of plasma will result in a reduction of all plasma acids and bases without a preferential reduction in HCO_3^- [64]. The strong ion theory explains that a *dilutional acidosis* is due to the effect of fluid administration on plasma SID and not on HCO_3^- only. The weaknesses of the HH approach in elucidating the pathophysiology are obvious in this example [64]. The Stewart approach

indicates that administration of large volumes of 0.9% NaCl solution alters two of the three independent variables of plasma pH, the SID and A_{TOT}. The effect on A_{TOT} is predictable and dependent on the volume infused where the greater the volume infused, the greater the effect [64]. The utilization of crystalloid solutions is the mainstay of critical care medicine with the infusion of saline, dextrose or sterile water producing an acidifying effect [67]. The extent of acidification following the infusion of a saline solution is dependent on multiple factors including: the amount and rate of saline administered, renal handling of sodium and chloride, and the transmembrane movement of strong ions[67]. Administration of a fluid with a SID <25mEq/L results in an acidifying effect, in contrast to a solution with an SID > 25mEq/L which has an alkalinising effect [64]. The intravenous fluids 0.9% NaCl and 5% dextrose are acidifying as their SID is less than that of plasma [64]. In order to correct the hyperchloraemic acidosis acid-base derangement according to the SID concept, treatment should involve administration of a solution (for example: Ringers lactate [SID of 28mEq/L] or 1.3% $NaHCO_3$ [SID of 155mEq/L]) where the strong cation concentration exceeds the strong anion concentration effectively increasing the plasma SID[64, 78]. The traditional HH equation in contrast to the strong ion approach does not provide a sound explanation for the mechanism by which treatment of hyperchloraemic acidosis corrects the acidosis [64, 78].

1.10.2 The importance of chloride

The importance of chloride should not be underestimated as chloride is the most abundant anion in multiple bodily fluids including the ECF, gastric, small and large intestinal juices as well as in glomerular ultrafiltrate, and changes in chloride will affect the animal's SID and thus the acid-base status[4]. Since chloride ion changes can occur for reasons other than changes in water balance, the chloride concentration should be corrected for changes in sodium concentration[4]. Therefore, an equation by Fencl *et al.* has been adapted for the usage in dogs and cats. The animals' chloride is 'corrected' for the changes in sodium concentration[4]. Thus corrected hypochloraemia may result from conditions whereby excessive chloride is lost in relative excess to sodium (e.g. vomiting of stomach contents, chronic respiratory acidosis, hypoadrenocorticism) or due to the administration of fluids containing high sodium concentrations relative to chloride (sodium bicarbonate and sodium penicillin)[4]. In addition, corrected hyperchloraemia may also result from excessive loss of sodium relative to chloride (e.g. diarrhoea), excessive gain of chloride in relation to sodium (e.g. ammonium and potassium chloride therapy and hypertonic fluid and saline solutions) or chloride retention (e.g. renal tubular acidosis, diabetes mellitus, chronic respiratory alkalosis, hypoadrenocorticism)[4]. Since chloride is the most abundant anion in ECF, decreasing the chloride concentration will increase the SID resulting in a hypochloraemic alkalosis[4].

Conversely increasing the chloride will decrease the SID resulting in a hyperchloraemic acidosis assuming the sodium concentration remains constant[4].

1.10.3 Quantifying the unmeasured anion concentration traditionally and quantitatively

The AG concept was derived from the theory of electroneutrality where the AG represents the difference between the unmeasured anions (UA) and unmeasured cations (UC) in serum[64]. This is represented in the following equation:

$Na^+ + K^- + UC = Cl + HCO_3^- + UC$

This can be rearranged to:

$AG = UA – UC = (Na^+ + K^+) – (Cl^- + HCO_3^-)$

An increased AG thus represents an increase in UA or a decrease in UC[64]. Approximately 66% of the AG originates from the net negative charge of serum protein, predominantly albumin, and thus the AG does not reflect an accurate method of strong ion analysis when the plasma protein concentrations are abnormal[14, 64]. Since hypoalbuminaemia may disqualify the usage of AG, an adjustment for albumin should be implemented i.e. the $AG_{corrected}$ [79].The $AG_{corrected}$ incorporates the contribution of albumin compared to the standard AG. Even though the contribution of phosphate is small, a hyperphosphataemia can increase the AG and SIG [14].

Based on the law of electroneutrality, SIG should be equal to zero because there should be no excess in cations or anions. However the SIG is usually positive due to an excess of unmeasured anions compared to unmeasured cations[14]. Anions that are not routinely measured include L-lactate, D-lactate, β-hydroxybutyrate, acetoacetate and sulphate[14, 64]. Therefore, the two most popular methods with which the unmeasured anions can be measured include the AG and SIG[64].

The SIG has been reported to be superior to the AG in its ability to identify the presence of abnormal unmeasured ions because its calculation incorporates the contribution of albumin, phosphate and strong ions not included in the AG equation[14, 15]. The AG thus lacks sensitivity and specificity since it only takes into account four known ions: Na^+, K^+, Cl^-, HCO_3^-, and its value is affected by albumin concentration[14]. The SIG incorporates the anionic charge on serum proteins and thus provides a more accurate method for quantifying the unmeasured strong ion compared to the AG[64].

1.10.4 The role of weak acids: Albumin and phosphate

In plasma, albumin is the principal weak acid with a smaller effect from phosphate [67]. Albumin is considered a major buffer of the extravascular compartment and consequently plays a vital role in the metabolic compartment of acid-base homeostasis[6]. In the intensive care unit (ICU) setting, many critically-ill human patients have decreased albumin concentrations [67]. Consequently, a reduction in plasma albumin can result in a reduced total-weak-acid concentration thus causing an alkalosis[67]. Although the loss of weak acid from the plasma results in alkalosis, there is no evidence that the body regulates albumin to maintain acid-base balance, nor is there any evidence that clinicians should treat hypoalbuminaemia as an acid base disorder[67]. Both albumin and phosphate have important roles in the Stewart and semiquantitative approaches. Components affected include: A_{TOT} (which is composed of the albumin and phosphorus contribution) and the $SID_{effective}$ (which is composed of the sum of the albumin contribution, the phosphorus contribution and the bicarbonate concentration) [15, 64, 80]. The semiquantitative method utilizes the albumin effect and phosphorus effect, amongst others, as contributors to the BE[15, 80]. Therefore albumin and phosphate play critical roles in the underlying pathomechanism of acid-base disorders when assessed by quantitative methods. Albumin also plays a role in the AG (as an unmeasured ion) however, it is not accounted for in the HH approach. Consequently the AG may be falsely within normal range in cases where there is a gain in acid with concurrent hypoalbuminaemia[71]. In order to accommodate for this deficiency, the corrected AG for albumin ($AG_{albumin}$) can be utilised as a modification of the traditional approach[6].

1.10.5 Clinical approach using the strong ion difference

When a clinician utilises the SID and A_{TOT} values, the simplified strong ion equation provides a better understanding of metabolic acid-base imbalances when compared to the HH equation. This has the potential to allow for a better evaluation of an acid-base derangement and subsequent treatment plan[64]. This is illustrated in a study where the two different models were applied to the interpretation of the analyte concentrations in sick calves (mostly with diarrhoea). The HH equation allocated the acidaemia as a result of a reduction in plasma HCO_3^- [64]. In comparison the simplified strong ion approach attributed the acidaemia to a decreased SID, secondary to hyponatraemia and increased plasma concentrations of lactate and uraemic anions, all of which contributed to decreasing the SID[64]. This allows a more precise and targeted treatment approach to these cases. For example the SID theory then dictates the administration of a strongly alkalinising intravenous solution (sodium bicarbonate [SID of 155mEq/L]). The solution however provides correcting concentrations of sodium and not bicarbonate. In

contrast the HH approach dictates the administration of bicarbonate to correct the acidaemia. It is important to note is that both approaches required the same treatment interventions albeit from different explanations.

In order to fully evaluate acid-base disorders using the SID approach, the combination of serum biochemistry (sodium, potassium, chloride, phosphorus, albumin, total protein and lactate) as well as blood gas analysis (pH, PCO_2, HCO_3^- and BE) are required[16]. An approximation of the SID ($SID_{apparent}$) can be utilised if the blood gas values are not available to gain insight into the acid-base derangement[16]. When evaluating non-respiratory acid-base abnormalities, the strong ion approach can be used to assess the contribution of the different ions to the metabolic component (the sum of effects, i.e. the total contributing effect of chloride, albumin, phosphate, free water and lactate) [16]. This sum of effects has also been termed the semiquantitative approach or BEA where these individual effects contribute to the BE[15, 16].

Mallat *et al.* utilized the Stewart approach to define metabolic acidosis in human patients with septic shock[81]. Thirty patients with septic shock were included in the study. The three main contributors to the metabolic acidosis ascribed were hyperchloraemia, hyperlactaemia and increased levels of UA[81]. The increased UA was present in 70% of patients, hyperlactaemia in 60% and hyperchloraemia in 70% of patients[81]. Hyperchloraemic acidosis is a common finding in the ICU setting and may be attributed to infusion of large volumes of isotonic saline as well as a shift of chloride from the intracellular and interstitial compartments to the intravascular compartment that occurs during endotoxaemia[81]. In a clinical setting, a metabolic acidosis with an increased SIG or high $AG_{corrected}$ despite a normal BE may indicate poor tissue oxygenation and hypoperfusion leading to hyperlactaemia and thus increasing the UA[81]. It is therefore important to understand that high UA may be present with normal BE and HCO_3^- values [81]. In addition, the Stewart approach has demonstrated important diagnostic abilities to the underlying metabolic acid-base disorders[81].

In a study of 312 human ICU patients, Moviat *et al.* explored the Stewart parameters in patients with an apparently normal acid-base status according to traditional HH analysis[82]. The study also determined the influence of several factors such as renal function, fluid management and presence of sepsis in the metabolic compartment in acid-base status[82]. Of the 312 patients with a normal pH, 137 were classified as having a normal acid-base state, 75 were classified as demonstrating metabolic acidosis, 74 as exhibiting metabolic alkalosis and 26 patients displayed other acid-base disorders. The study revealed

that approximately 50% of patients with a normal pH actually had underlying mixed acid-base derangements that were illuminated utilizing the Stewart approach[82]. The metabolic derangements resulted from a combination of hyperchloraemia and hypoalbuminaemia, producing acidifying and alkalinizing effects respectively, as well as a high SIG[82].

Clinically, a reduction in the SID producing a metabolic acidosis can be attributable to multiple factors. Examples include: the loss of Na^+ relative to Cl^- such as in diarrhoea; an increase in Cl^- relative to Na^+ such as with the administration of a solution with a low SID (0.9% saline); production of increased amounts of other strong ions such as with lactic acidosis and DKA and reduced hepatic function (unable to metabolize lactate) and renal dysfunction resulting in accumulation of uraemic products[58, 77]. In opposition, an increase in the SID resulting in metabolic alkalosis may be attributable to loss of Cl^- such as with vomiting and loss in urine from diuretic therapy; chloride loss from hyperaldosteronism and hypoadrenocorticism; an increase in Na^+ from excessive sodium bicarbonate administration in metabolic acidosis correction and a reduction in A_{TOT} as a result of hypoalbuminaemia (hepatic dysfunction, nephrotic syndrome, protein losing enteropathy, malnutrition and critical illness)[58, 77].

1.11 The Base Excess Algorithm/ Semiquantitative Approach

As highlighted previously, the BEA or semiquantitative approach combines concepts from the quantitative Stewart approach as well as the traditional HH approach[80]. This approach determines the influence of individual contributors to the base excess including changes in free water, chloride, phosphorus, lactate, albumin as well as the sum of these effects and the unmeasured anion effect[15]. This approach has been reported to detect acid-base abnormalities in dogs with apparently normal acid-base balance as judged by the traditional HH approach[80, 81, 83].

1.12 Acid-base Abnormalities of Canine Parvovirus

Canine parvovirus is a disease associated with extensive losses of fluids, as well as proteins and electrolytes through anorexia, vomiting, diarrhoea and malabsorption [11]. Therefore there is an expectation for the development of metabolic compartment acid-base disorders in these animals.

Within the available literature there is limited data regarding the acid-base status of parvovirus puppies. In a study by Heald *et al.* serum biochemistry, electrolyte and arterial blood gas was evaluated in 17 parvovirus puppies[7] . The majority of the puppies (59%) demonstrated normal arterial pH while 35% exhibited a metabolic alkalosis and 6% a metabolic acidosis[7]. The most common acid-base abnormality evident was a metabolic alkalosis, suspected to be attributable to vomiting of acidic stomach contents, predominantly hydrogen and chloride with smaller contributions of sodium and potassium loss as well[7]. In a study by Nappert *et al.* a documentation of the metabolic status of puppies with parvovirus as well as the serum concentrations of organic acids was studied. It was hypothesized that a metabolic acidosis developed secondarily to the production of D-lactate by the bacteria in the large colon, a phenomenon evident in ruminants[54]. The study evidenced that the blood pH of parvovirus puppies was significantly higher compared to the controls however, still within reference limits[54]. At admission the dominant abnormalities were: hyponatraemia, hypokalaemia and hypochloraemia with a decrease in HCO_3^- concentration [54]. In most puppies correction of metabolic acidosis was achieved, which the author attributed to respiratory compensation although this seems inaccurate as complete compensation is not possible[54]. The study showed that D-lactate did not increase in the serum of dogs with acute CPE infection and that the effect of fermentation by bacteria in the large bowel was not relevant in dogs with CPE.

In order to appreciate the pathophysiological complexities of acid-base alterations in canine parvovirus, a study by Burchell *et al.* evaluated retrospective biochemical data from 42 parvovirus infected puppies [12]. A modified strong ion model was employed and the effects of changes in free water, chloride, L-lactate, albumin and phosphate were calculated using a modification of the base excess algorithm. Twenty of the 42 puppies exhibited a metabolic acidosis, all of which comprised a reduced SID acidosis with 19 puppies having a concurrent A_{TOT} alkalosis. Ten out of 42 puppies demonstrated a metabolic alkalosis and the remaining 12 puppies had an overall normalisation of their acid-base status. The study demonstrated that once the variables utilised in the quantitative assessment were correlated to the sum of all the effects, the most significant contributor in the acid-base changes was chloride[12]. The study highlighted the significance of chloride changes in the pathogenesis of acid-base derangements in parvovirus[12]. The SIM provided a mechanistic interpretation and understanding that could not have been appreciated utilising the HH model.

The unique aspect of the study described hereunder (which distinguishes it from the Burchell *et al.* study) is the prospective nature which allowed us to more completely evaluate the HH approach (including the role of the respiratory compartment).

1.13 Comparison between the Different Acid-base Approaches

Mathematical simulations have shown that the SID and BE behave similarly in acid-base disturbances[79]. Consequently, there would be no theoretical advantage of SID over BE[79, 84]. Dubin *et al.* also demonstrated that SIG and $AG_{corrected}$ showed excellent agreement in a human study and inferred interchangeability and concordance between the two methods. Thus a good agreement between SID and HCO_3^-; SID and BE; and SIG and $AG_{corrected}$ suggest that these approaches are similar in diagnostic interpretation. In the study by Dubin *et al*. an observation was made that all metabolic acid-base variables had a poor ability for predicting mortality in human patients[79]. In a study by Hopper *et al.* a comparison between AG, SIG and semiquantitative analysis (developed by Fencl and colleagues) [80] was compared in dogs and cats that presented at an emergency facility[15, 69]. In this study, the number of animals identified with unmeasured ions varied depending on the method used for evaluation (AG via the HH approach, SIG via the Stewart approach and XA via the semiquantitative approach). Although a reasonably strong correlation was evident, the SIG and semi-quantitative methods diagnosed more abnormalities compared with the AG in the traditional HH approach[69].

In a similar study to the one above Mallat *et al.* showed an excellent agreement between $AG_{corrected}$ and SIG in a human study of metabolic acidosis in septic shock[81]. In another study that compared the traditional and the quantitative approaches for the assessment of acid-base status in 105 hypoalbuminaemic dogs, discernible differences were detected in the metabolic compartment of acid-base status[6]. The hypoalbuminaemic dogs showed a lower pH combined with a lower HCO_3^- and a higher AG compared to control dogs when the traditional approach was used, but lower A_{TOT} and SIG when the quantitative method was used[6]. . Decreased pH has been reported in many critically-ill animals, and this was consistent with this study which showed that the most common disturbance was a simple primary metabolic acidosis associated with a high AG[6] . Advantageously, the quantitative approach allows for the evaluation and interpretation of acid-base equilibrium in critically-ill dogs when electrolytes, albumin or phosphorus concentrations are altered[6]. As discussed above, the SID can change mainly due to variations in the free water content of plasma, changes in chloride concentration and increases in the

concentration of other strong anions[6]. The influence of SID on pH thus offers a pathomechanistic understanding of conditions such as: dilutional acidosis, contraction alkalosis, hyperchloraemic acidosis, hypochloraemic alkalosis and acidosis from unmeasured anions[6]. Utilising the quantitative approach, the A_{TOT} values in hypoalbuminaemic dogs were significantly different to the controls and statistically correlated to the severity of hypoalbuminaemia[6]. This statistical correlation is expected as albumin is integral in the calculation of A_{TOT} In addition, A_{TOT} determination allows for the identification of metabolic alkalotic processes that would otherwise not be identified with the traditional approach (33.3% vs 5.7% in the 105 hypoalbuminaemic dogs evaluated) [6]. Furthermore, strong correlations were displayed between SIG and $AG_{albumin}$ as well as SIG and $AG_{albumin\text{-}phosphate}$, but not between SIG and AG [6]. If the adjusted AG was incorporated, then the traditional approach performed equally as well as the quantitative approach[6].

Hopper *et al.* evaluated and compared 3 disparate methods of acid-base analysis in dogs and cats that presented to an emergency room[15]. These included the traditional HH approach, the Stewart strong ion approach and the semiquantitative approach. The semiquantitative approach calculated the individual contributors to the BE; including changes in free water, chloride, albumin, phosphorus and lactate concentrations[15]. This method has demonstrated the detection of acid-base abnormalities in human patients considered to have normal acid-base status by the traditional methods of evaluation [15]. The study portrayed that the Stewart approach identified more metabolic acid-base abnormalities than the traditional approach, while the semiquantitative approach identified abnormalities in all animals evaluated[15]. Thus the proposed advantages of the semiquantitative and Stewart approaches are their greater ability to detect metabolic acid-base disturbances as well as provide a clearer understanding of the underlying mechanisms of the disorders; whereas the advantage of the traditional approach is its simplicity and capacity to comment on the compensatory responses[69]. Since there is still a lack of a gold standard for comparison of the methods, it remains undetermined if the increased costs and complexity of the quantitative approaches are of any clinical benefit[15, 69]. The study displayed only a moderate correlation between the SID and BE values. The likely explanation for this is due to abnormalities in A_{TOT} and the degree of hypoalbuminaemia seen in critically-ill animals [15].

The quantitative accuracy of the simplified strong ion equation to predict serum pH in dogs has been evaluated in a recent study [13]. The study evaluated the strong ion approach to ascertain if it could be utilised to calculate the pH of serum from dogs, using published values for the non-volatile weak acids. The results indicated that no significant statistical correlation existed between the calculated and

measured pH for any dog[13]. Potential reasons for the inadequacies included inaccuracies of the constants used in the equation or in the measurements of one or more of the analytes; presence of unmeasured strong ions; intrinsic error in the calculations of pH or inaccuracies in the underlying theory, the SID equation, or both [13]. The study highlights the significance of contrasting and correlating the qualitative and quantitative approaches in various clinical settings in dogs.

Chapter 2

Rationale and Hypotheses

Since there is a deficiency of available literature regarding the acid-base disturbances in CPE, the purpose of this study was to descriptively evaluate the traditional Henderson-Hasselbalch, the simplified strong ion approach and the base excess algorithm acid-base diagnosis of puppies suffering from CPE Post admission the cases were not followed through to completion and subsequently an in depth evaluation of treatment adjustments and changes according to the acid-base diagnosis was not performed.

2.1 Null and Alternate Hypotheses

The null hypothesis:

- There was no difference between the Henderson-Hasselbalch approach, the Stewart strong ion approach and the base excess algorithm in the quantification and assessment of acid-base disturbances in CPE.

The alternate hypothesis:

- The Strong ion approach and base excess algorithm quantified a higher number of acid-base disturbances in CPE as well as provided a clearer understanding to the underlying pathophysiology of the disturbance.

The alternate hypothesis has been derived from the consensus in the literature that there is little difference between the models on a quantitative scale but that the Stewart model and base excess algorithm are superior in terms of the qualitative understanding of acid-base disturbances.

2.2 Benefits Arising from this Study

This study has contributed to the understanding and description of acid-base disturbances in CPE. As mentioned previously, disparity exists in the literature regarding acid-base disturbances.

This study and the data produced may be used in future studies comparing treatment modalities or for prognostic purposes. This however was not within the scope or purpose of the current manuscript.

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2.3 Objectives

The objectives of this study were as follows:

1) To evaluate the acid-base disturbances of puppies with CPE according to reference ranges generated from age and weight matched control cohort of puppies.

2) To diagnose the acid-base disturbances according to the Henderson-Hasselbalch model, the simplified strong ion approach and the base excess algorithm in puppies with CPE.

3) To provide an overview of the discordance between the models.

Chapter 3

Materials and Methods

3.1 Experimental Design

The study was an observational prospective cohort case-control study that included client-owned dogs naturally infected with parvovirus. Two aliquots of venous blood were collected from the jugular vein from CPE positive dogs on admission. A single venous blood sample was collected anaerobically into a commercially available heparinized syringe[a] from 41 dogs diagnosed with acute CPE on admission to the isolation ward at the Onderstepoort Veterinary Academic Hospital (OVAH). The diagnosis of parvovirus was based on a rapid bedside ELISA SNAP test[b] in dogs with appropriate clinical signs. Values for acid-base analysis and biochemistry were also generated from a control population of 16 puppies for comparison. The control group of puppies were clinically healthy based on a history from the owner, a complete physical examination, a peripheral thin stained blood smear and a faecal examination. The heparinized samples were used to generate blood gas and electrolyte data using an automated analyser[c] (including pH, HCO_3^-, PCO_2, Na^+, K^+, Cl^-, Ca^{2+}). The analyser utilised direct potentiometry. The second venous sample was collected and utilised for biochemistry (albumin, phosphorus and lactate) and results were generated using an automated analyser[d] as well as a bedside lactate measuring device[e]. All samples were collected prior to any treatment and the blood gas and electrolytes were determined within 30 minutes of collection. In addition between half and one millilitre of blood was stored in an EDTA tube and archived at -80°C in a biobank initiative for future studies.

Inclusion criteria:

- Puppies between the age of 6 and 36 weeks of any breed and either sex, weighing between 2 kg and 30 kg.
- Only puppies demonstrating severe clinical signs of CPE that warrant hospitalisation and therapy in the opinion of the attending clinician [i.e. depression/ collapse, vomiting (more than 4 times in 24 hours), watery or bloody diarrhoea or anorexia for more than 8 hours].
- The puppies must have been diagnosed as CPE positive on a commercial bedside rapid ELISA SNAP test.

- Puppies that had been vaccinated were included within the study as long as vaccination was provided no less than 14 days previously.
- Puppies diagnosed with verminosis, giardiasis or coccidiosis with concurrent CPE were included in the study and treated accordingly.
- Informed client consent (Appendix 2) was required with a client information sheet (Appendix 3) provided to the owner for further information.

Exclusion criteria:

- Any dogs diagnosed with unrelated co-morbidities such as babesiosis or *Ehrlichia* (by peripheral blood smear) or canine distemper virus (CDV) (clinical muco-purulent ocular-nasal discharge, neurological signs, naso-digital hyperkeratosis, enamel hypoplasia, positive diagnostic tests for CDV- for example rapid CDV antigen test kits) were excluded.

All dogs then received standard therapy for CPE (according to the OVAH guidelines). For the purposes of this study, the continued management and treatment of these CPE positive dogs was not evaluated or interfered with.

3.2 Statistical Methods

A commercial statistical software package was used for all statistical analysis[f]. Most data were not normally distributed as determined by Shapiro Wilk and as such all comparisons made use of non-parametric methods (Mann Whitney U for the comparisons between medians). The control group of dogs were compared to the CPE group for all measured and calculated variables and a p- value <0.05 was regarded as significant.

[a] BD A-Line, arterial blood collection syringe, Becton, Dickinson and Company, UK

[b] SNAP® Parvo, © 2015 IDEXX Laboratories, Inc, One IDEXX Drive, Westbrook, Maine, USA

[c] Rapidpoint 405 – Blood gas**,** Siemens, PO Box 198 Isando 1600, South Africa

[d] Cobas Integra 400 Plus- chemistry, Roche, PO Box 1927, Randburg 2125, South Africa

[e] Lactate Pro, Arkray, Tecil, Barcelona

[f] IBM® SPSS Statistics® version 24, New York 10540, USA

3.2.1 Reference ranges

Age and weight matched 'control' puppy data was used to generate reference ranges for comparison with the diseased group for all the acid-base variables. The intention of including the control group of puppies was for the purpose of accurate comparison and elimination of laboratory variables. Differences in reference ranges exist between laboratories, studies, equipment, altitude as well as animal ages. Thus the control group of puppies aimed to provide a meaningful group for comparative purposes. The reference intervals used for the HH and SSA were the median ± 2 standard deviations for each variable[15] (Textbox 1 and 2). The minimum and maximum values for the control dogs were used as reference ranges for the BEA[15] (Textbox 3).

3.3 Data Assessment According to the Henderson-Hasselbalch Model

In the definition of acid-base changes, the term acidosis and alkalosis refer to the pathophysiologic processes that cause net accumulation of acid or alkali in the body. The terms acidaemia and alkalaemia refer specifically to the pH of the ECF[57].

Four parameters were assessed in the application of this model, namely blood pH, PCO_2 and HCO_3^- and AG. Bicarbonate and BE were calculated by an automated analyser using the HH and van Slyke equations, respectively [85]. Additionally the AG was calculated by the formula $([Na^+] + [K^+]) - ([HCO_3^-] + [Cl^-])$ [15]. Blood pH was assessed to determine if the patient was acidaemic or alkalaemic, followed by assessment of the metabolic compartment (HCO_3^-) and the respiratory compartment (CO_2). The acid-base diagnosis was classified as simple or mixed. In addition the metabolic compartment was sub classified according to the AG. Diagnostic criteria for the HH were shown in textbox 1. The formulae for calculated variables are provided in table 1.

1) Simple disturbances
a. Metabolic acidosis: pH < 7.28, HCO_3^- < 19mmol/L
$PCO_2 = 44 - (\Delta HCO_3^- \times 0.7) \pm 3$
b. Metabolic alkalosis: pH > 7.42, HCO_3^- > 27mmol/L
$PCO_2 = 44 + (\Delta HCO_3^- \times 0.7) \pm 3$ ΔHCO_3^- = Mid-normal HCO_3^-[22 mmol/L]– Measured HCO_3^-
c. Respiratory acidosis: pH < 7.28, PCO_2 > 51mmHg
$HCO_3^- = 22 + (0.15\text{-}0.35 \times \Delta PCO_2) \pm 2$
d. Respiratory alkalosis: pH > 7.42, PCO_2 < 35mmHg
$HCO_3^- = 22 - (0.25–0.55 \times \Delta PCO_2) \pm 2$ ΔPCO_2 = Mid-normal PCO_2 [44mmHg] – Measured PCO_2
2) Mixed disturbances
- Response in the secondary system not within predicted range as provided above
3) Metabolic acidosis further classified by anion gap
a. Metabolic acidosis associated with increased AG: AG > 20 mmol/L
b. Metabolic acidosis not associated with increased AG: AG ≤20 mmol/L

Textbox 1: Diagnostic criteria for Henderson-Hasselbalch acid-base analysis in dogs. PCO_2,partial pressure of CO_2 in venous blood; HCO_3^-, bicarbonate; PCO_2, partial pressure of carbon dioxide; AG, anion gap.

3.4 Data Assessment According to the Simplified Strong Ion Approach

In this model, denoted the SSA, three parameters were assessed, namely the strong ion difference ($SID_{apparent}$ and $SID_{effective}$), A_{TOT} and the SIG. The $SID_{apparent}$ was calculated using the following equation: $([Na^+] + [K^+] + [Ca^{2+}]) - [Cl^-]$ (Table 1). The $SID_{effective}$ was calculated using sum of the HCO_3^-, albumin contribution and phosphorus contribution. The A_{TOT} was calculated by summating the albumin and phosphorus contribution and the SIG by means of the equation: $SIG = SID_{apparent} - SID_{effective}$. In veterinary and human literature there are many variations of the SID equation[15, 86] . The SID, A_{TOT} and SIG equation used in this study was derived from Hopper *et al*[15].Consequently, in this study, lactate was not incorporated into the $SID_{apparent}$ equation, so its influence was not within the calculated variables in SSA; however, its contribution was evaluated in the lactate effect in the BEA. The diagnostic criteria cut-off parameters were generated from a control population of puppies and the data of each case consequently assessed. Using this model, deviations from the normal range derived from the control dog data were classified as follows: increased SID alkalosis/decreased SID acidosis, increased A_{TOT} acidosis/decreased A_{TOT} alkalosis and an increased unmeasured anion acidosis (SIG). The diagnostic criteria for Stewart acid-base analysis are provided in textbox 2. The formulas for calculated variables are provided in table 1.

Parameter	Formulae
Anion gap	$([Na^+] + [K^+]) - ([HCO_3^-] + [Cl^-])$
$SID_{apparent}$	$([Na^+] + [K^+] + [Ca^{2+}]) - [Cl^-]$
Albumin contribution	Measured albumin × ((0.123 × pH) − 0.631) × 10
Phosphorus contribution	Measured phosphorus × 0.323 × ((0.309 × pH) − 0.469)
A_{TOT}	Albumin contribution + Phosphorus contribution
$SID_{effective}$	$[HCO_3^-]$ + albumin contribution + phosphorus contribution
Strong ion gap	$SID_{apparent} - SID_{effective}$
Free water effect	$0.25([Na^+] - \text{mid-normal } [Na^+])$
Corrected chloride	Measured $[Cl^-]$ × (mid-normal $[Na^+]$/measured $[Na^+]$)
Chloride effect	Mid-normal $[Cl^-]$ − corrected $[Cl^-]$
Phosphate effect	0.58 (Mid-normal [phosphorus] − measured [phosphorus])
Albumin effect	3.7 (Mid-normal albumin − measured [albumin])
Lactate effect	−1 × [lactate]
Sum of effects	Free water effect + chloride effect + phosphate effect + albumin effect + lactate effect
Unmeasured anion effect	Base excess − sum of effects

Table 1: Formulas for calculated variables for the Henderson-Hasselbalch, simplified strong ion approach and base excess algorithm. SID, strong ion difference; A_{TOT}, total quantity of weak acids; Na^+, sodium, mid-normal 144mmol/L ; K^+, potassium; HCO_3^-, bicarbonate; Ca^{2+}, calcium; Cl^-, chloride, mid-normal 109mmol/L; Phosphorus, mid-normal 2.8mg/dL; Albumin, mid-normal 29g/L[1, 15, 65, 85, 87].

1) Strong ion difference
a. Increased SID metabolic alkalosis: $SID_{apparent}$ > 44 mmol/L
b. Decreased SID metabolic acidosis: $SID_{apparent}$ < 36 mmol/L
2) Total weak acids
a. Increased A_{TOT} metabolic acidosis: A_{TOT} > 15 mmol/L
b. Decreased A_{TOT} metabolic alkalosis: A_{TOT} < 11 mmol/L
3) Unmeasured Anions:
a. Increased SIG: SIG > 7 mmol/L

Textbox 2: Diagnostic criteria for the simplified strong ion approach acid-base analysis. SID, strong ion difference; A_{TOT}, total quantity of weak acids; SIG, strong ion gap.

3.5 Data Assessment According to the Base Excess Algorithm

In the second quantitative model, denoted the BEA, seven parameters were assessed namely the free water effect, chloride effect, albumin effect, lactate effect, phosphorus effect, sum of effects and the unmeasured ion effect. This approach combines concepts from the quantitative Stewart approach as well as the traditional HH approach. The model determines the influence of individual contributors to the BE including: changes in free water, chloride, phosphorus, lactate and albumin [15]. The diagnostic criteria for the BEA are provided in textbox 3. The formulas for calculated variables are provided in table 1.

Free water effect:
· Dilutional acidosis: Free water effect < -0.8 mmol/L
· Contraction alkalosis: Free water effect > 0.5 mmol/L
Chloride effect:
· Acidosis: Chloride effect < −3.3 mmol/L
· Alkalosis: Chloride effect > 3.5 mmol/L
Albumin effect:
· Acidosis: Albumin effect < −2.4 mmol/L
· Alkalosis: Albumin effect > 2.4 mmol/L
Phosphorus effect:
· Acidosis: Phosphorus effect < −1.5 mmol/L
· Alkalosis: Phosphorus effect > 0.8 mmol/L
Lactate effect:
· Acidosis: Lactate effect > −0.1 mmol/L
Unmeasured Ions (XA):
XA =BE – (Free water effect + chloride effect + albumin effect +phosphorus effect + lactate effect)
a. Unmeasured acids: XA < −3.1 mmol/L
b. Unmeasured alkalis: XA > 6.2 mmol/L

Textbox 3: Diagnostic criteria for the base excess algorithm acid-base analysis. BE, base excess.

Chapter 4

Results

4.1 The 16 Control Puppies

Sixteen puppies were included as the control population. The population consisted of 11 males (69%) and five females (31%) with a median age of 3.5months (range 1.5 to 6months) and a median weight of 5.2kg (range 3kg to 25kg). There were 11 mixed breed (69%) and five pure breed (31%) puppies in the control population.

4.2 The 41 Canine Parvoviral Enteritis Dogs

Forty-one dogs were included in the study, 14 females (34%) and 27 males (66%), with a median age of four months (range 2 to 9 months) and a median weight of 6.6kg (range 2.4kg to 30kg). Thirty-one pure breed dogs (76%) and 10 mixed breed dogs (24%) were included. There was no statistical difference between age, weight or sex between the control and CPE groups. The data comparing the CPE and control population with respect to age and weight is provided in table 2.

Parameter	**Unit**	**N Control**	**Control Median**	**Control 95% CI Lower; upper bound**	**N CPV**	**CPV Median**	**CPV 95% CI Lower; upper bound**	**P value**
Age	Months	16	3.5	2.6-4.3	41	4	3.6-5	0.387
Weight	kg	16	5.6	4.6-11.3	41	6.6	6.5-11	0.447

Table 2: Data generated for comparison between the control group of puppies and the parvovirus infected group of puppies. P, significance level as determined by Mann Whitney U test comparing the control group with the canine parvovirus group; N control, the number of control puppies included in the analysis; N CPE, the number of parvovirus infected puppies; 95% CI, the 95% confidence interval.

4.3 Henderson-Hasselbalch Model

The data utilized to generate the HH diagnosis is provided in table 3. The diagnostic results of the HH method of analysis are provided in table 4. The diagnostic classification was divided into normal acid-base status (assessing pH, CO_2 and HCO_3^- only), simple disorders and mixed disorders. In addition, the AG was also evaluated and separated into those cases with a normal pH and elevated AG and those cases with an acidaemic pH and elevated AG. Twenty-four puppies (59%) demonstrated a normal blood acid-base status, while 11 displayed simple disorders (27%) and four showed mixed disorders (10%). One additional puppy did not fit the classification scheme exactly and demonstrated an acidaemia (yet a normal HCO_3^-) with an elevated AG. This case is further mentioned in the AG abnormalities. The most common simple disorders were respiratory acidosis in five cases, metabolic alkalosis in three cases, and metabolic acidosis in three cases (Table 4). Four cases demonstrated a mixed disorder that consisted of combinations of metabolic and respiratory acidosis and alkalosis (Table 4). In total there were nine cases with an elevated AG, of which two demonstrated metabolic acidosis with elevated AG, one case displayed acidaemia (pH < 7.28, yet a normal HCO_3^-) with a raised AG, while six cases displayed an elevated AG, yet with normal pH, PCO_2 and HCO_3^- (Table 4). There was no statistical difference between the control and CPE population with respect to any of the HH parameters (pH, CO_2, HCO_3^-, AG and BE) (Table 3).

Parameter	Unit	N Control	Control Median	Control 95% CI Lower; upper bound	N CPE	CPE Median	CPE 95% CI Lower; upper bound	p value
pH		16	7.35	7.33;7.37	41	7.33	7.3;7.35	0.359
CO_2	mmHg	16	43	42;47	41	44	43;48	0.783
HCO_3^-	mmol/L	16	23	22;25	41	22	22;25	0.88
AG	mmol/L	16	16	14;17	41	17	16;18	0.287
BE	mmol/L	16	-1.6	-2.8;-0.4	41	-2.6	-3.7;-1.1	0.334

Table 3: Data generated for comparison between the control group of puppies and the parvovirus infected group of puppies in the Henderson-Hasselbalch analysis. P, significance level as determined by Mann Whitney U test comparing the control group with the canine parvovirus group; N control, the number of control puppies included in the analysis; N CPE, the number of parvovirus infected puppies; 95% CI, the 95% confidence interval; CO_2, carbon dioxide; HCO_3^-, bicarbonate; AG, anion gap; BE, base excess.

Classification	**N**
Normal	**24**
Simple disorders	**11**
Respiratory acidosis	5
Metabolic acidosis	3
Metabolic alkalosis	3
Mixed disorders	**4**
Metabolic acidosis and respiratory acidosis	1
Metabolic acidosis and respiratory alkalosis	1
Metabolic alkalosis and respiratory acidosis	1
Metabolic alkalosis and respiratory alkalosis	1
Elevated AG	**9**
Metabolic acidosis with an elevated AG	2
Acidaemia (pH <7.28, normal HCO_3^-) with an elevated AG	1
Elevated AG (normal pH, CO_2 and HCO_3^-)	6

Table 4: The Henderson-Hasselbalch acid-base diagnosis of the 41 parvovirus infected dogs. AG, Anion gap; N, number of cases; CO_2, carbon dioxide; HCO_3, bicarbonate.

4.4 Simplified Strong Ion Approach

The data utilized to generate the SSA diagnosis is provided in table 5. The diagnostic results for the SSA approach are provided in table 6. The SSA model revealed a normal acid-base status in 22 dogs (54%), one or more acidotic processes in three dogs (7%), one or more alkalotic processes in five dogs (12%) and a combination of acidotic and alkalotic abnormalities in 11 dogs (27%). The most common individual abnormality was an SIG acidosis in 14 dogs, followed by a $SID_{apparent}$ alkalosis in 10 dogs and an A_{TOT} alkalosis in seven dogs. A significant statistical difference ($p<0.05$) between the control and CPE population was demonstrated with respect to A_{TOT} ($p=0.015$) as well as SIG ($p=0.001$) (Table 5).

Parameter	Unit	N Control	Control Median	Control 95% CI Lower; upper bound	N CPE	CPE Median	CPE 95% CI Lower; upper bound	P value
Albumin	g/L	16	29	27;31	41	27	26;30	0.271
Phosphorus	mmol/L	16	2.8	2.7;3.1	41	2.3	2.3;2.7	0.003
Potassium	mmol/L	16	4.3	4.3;4.7	41	3.8	3.6;4	<0.001
Sodium	mmol/L	16	144	142;144	41	140	138;141	0.001
Chloride	mmol/L	16	109	107;110	41	104	101;105	<0.001
Lactate	mmol/L	15	1.6	1.3;2.2	35	1.4	1.2;1.9	0.464
Calcium	mmol/L	16	1.4	1.3;1.4	41	1.3	1.3;1.3	0.019
HCO_3^-	mmol/L	16	23	22;25	41	22	22;25	0.88
A_{TOT}	mmol/L	16	13	12;14	41	12	12;13	0.015
$SID_{apparent}$	mmol/L	16	40	39;42	41	41	40;42	0.338
$SID_{effective}$	mmol/L	16	37	35;38	41	35	34;37	0.263
SIG	mmol/L	16	3	3;5	41	6	5;7	0.008

Table 5: Data generated for comparison between the control group of puppies and the parvovirus infected group of puppies in the simplified strong ion approach. P, significance level as determined by Mann Whitney U test comparing the control group with the canine parvovirus group; N control, the number of control puppies included in the analysis; N CPE, the number of parvovirus infected puppies; 95% CI, the 95% confidence interval; HCO_3^-, bicarbonate; SID, strong ion difference; A_{TOT}, total quantity of weak acids; SIG, strong ion gap.

Metabolic acid-base diagnosis	**N**
Normal	22
One or more acidotic processes	3
One or more alkalotic processes	5
Both alkalotic and acidotic processes	11
Individual abnormalities	**N**
Increased SID alkalosis	10
Decreased SID acidosis	0
Increased A_{TOT} acidosis	0
Decreased A_{TOT} alkalosis	7
Increased SIG acidosis	14

Table 6: The simplified strong ion approach diagnosis of the 41 parvovirus infected dogs. SID, strong ion difference; A_{TOT}, total quantity of weak acids; SIG, strong ion gap; N, number of cases.

4.5 Base Excess Algorithm

The data utilised to generate the BEA diagnosis is provided in table 7. The diagnostic results of the BEA are provided in table 8. In the BEA evaluation 35 out of 41 dogs were included in the diagnostic results. Six dogs were excluded due to an absence of a lactate measurement which influenced the lactate effect, sum of effects and unmeasured ion effect. The BEA detected a normal acid-base status in four dogs (11%), one or more acidotic processes in seven dogs (20%), one or more alkalotic abnormalities in eight dogs (23%) and a combination of alkalotic and acidotic processes in 16 dogs (46%). The most common individual abnormalities were a free water effect acidosis in 20 dogs (57%), followed by a phosphorus effect alkalosis in 19 dogs (54%), an unmeasured anion effect in 18 dogs (51%), a chloride effect alkalosis in 14 dogs (40%), albumin effect alkalosis in three dogs (9%), albumin effect acidosis in three dogs (9%), as a well as a phosphorus effect acidosis in two dogs (6%). Statistically, a significant difference was evident between the control and CPE population in the free water effect ($p=0.001$), phosphorus effect ($p=0.003$), sum of effects ($p=0.003$) and the unmeasured ion effect ($p<0.001$), the results which are displayed in table 7.

Parameter	Unit	Control N	Control Median	Control 95% CI Lower; upper bound	CPE N	CPE Median	CPE 95% CI Lower; upper bound	P value
Free water effect	mmol/L	16	0	-0.4;0.1	41	-1	-1.5;-0.7	0.001
Chloride effect	mmol/L	16	-0.3	-1.4;0.8	41	1.9	1.1;3.7	0.11
Albumin effect	mmol/L	16	0.1	-0.9;0.8	41	0.7	-0.4;0.9	0.271
Phosphorus effect	mmol/L	16	0	-0.6;0.2	41	0.9	0.2;0.9	0.003
Lactate effect	mmol/L	16	-1.6	-2.2;-1.3	35	-1.4	-1.9;-1.2	0.27
Sum of effect	mmol/L	16	-2.5	-3.4;-1.3	35	0.5	-0.9;1.5	0.003
Unmeasured ion effect	mmol/L	16	0.7	-0.7;2.1	35	-3.1	-3.7;-1.8	<0.001

Table 7: Data generated for comparison between the control group of puppies and the parvovirus infected group of puppies in the base excess algorithm. P, significance level as determined by Mann Whitney U test comparing the control group with the canine parvovirus group; N control, the number of control puppies included in the analysis; N CPE, the number of parvovirus infected puppies; 95% CI, the 95% confidence interval;.

Metabolic acid-base diagnosis	**N**
Normal	4
One or more acidotic processes	7
One or more alkalotic processes	8
Both alkalotic and acidotic processes	16
Individual abnormalities	
Free water effect acidosis	20
Free water effect alkalosis	0
Chloride effect alkalosis	14
Chloride effect acidosis	0
Albumin effect acidosis	3
Albumin effect alkalosis	3
Phosphorus effect acidosis	2
Phosphorus effect alkalosis	19
Lactate effect acidosis	0
Increased unmeasured anions	18
Increased unmeasured cations	0

Table 8: The base excess algorithm diagnosis of the 35 parvovirus infected dogs. N, number of cases.

4.5 Discordance between the models

Within each model the CPE cases diagnosed as normal were compared to determine their discordance. This data is displayed in table 9. The HH approach diagnosed 24 CPE cases as normal. Of the HH cases classified as normal the SSA approach detected 14 cases and the BEA detected two cases that were also classified as normal. Thus the level of discordance between the HH and SSA was 42% (10/24) and between the HH and BEA was 92% (22/24).

The SSA diagnosed 22 cases as normal. Of those classified as normal by the SSA, the HH diagnosed 14 as normal and the BEA diagnosed three as normal. Therefore, the degree of discordance between the SSA and the HH was 36% (8/22) and between the SSA and BEA was 86% (19/22).

The BEA classified four cases as normal. Of these four cases the HH diagnosed two as normal and the SSA three. Thus the level of discordance between the BEA and HH was 50% (2/4) and between the BEA and SSA was 25% (1/4).

The table also displays how the normal cases from one method were classified according to the other methods. For example in the 24 cases classified as normal by the HH, the SSA detected an SID alkalosis in seven cases, an A_{TOT} alkalosis in three cases and an SIG acidosis in nine cases. The remainder of the results according to the individual changes that each model detected according to the other approaches are tabulated.

		HH	SSA	BEA
N Total		**24**	**22**	**4**
HH	**Normal**		**14**	**2**
	Mixed		3	1
	Simple respiratory acidosis		3	0
	Simple metabolic acidosis		1	0
	Simple metabolic alkalosis		1	1
SSA	**Normal**	**14**		**3**
	SID Alkalosis	7		0
	SID normal	3		4
	ATOT alkalosis	3		0
	ATOT normal	7		4
	SIG Acidosis	9		1
	SIG normal	1		3
BEA	**Normal**	**2**	**3**	
	Chloride effect alkalosis	9	4	
	Free water effect acidosis	4	1	
	Albumin effect alkalosis	1	0	
	Albumin effect acidosis	2	2	
	Phosphorus effect alkalosis	11	6	
	Phosphorus effect acidosis	1	2	
	Unmeasured anion acidosis	10	10	

Table 9: Discordance between the acid-base diagnoses for CPE cases provided by the three models where each approach diagnosed a normal acid base balance. HH, Henderson-Hasselbalch; SSA, strong ion approach; A_{TOT}, the total quantity on weak acids; BEA, base excess algorithm; N, number of normal cases.

Chapter 5

Discussion

5.1 The Importance of Reference Intervals

Reference intervals for different patient populations are critical in the evaluation of biochemical and acid-base data. Age has been reported to have a substantial influence on biochemical and acid-base variables as neonates are more susceptible to water and electrolyte abberations[88, 89]. Different reference intervals exists between puppies, adult and geriatric canine populations, as well as between arterial and venous samples[15, 89-92]. In a study by Vanova-Uhrikova *et al.* 224 venous blood samples and 119 arterial blood samples were collected from apparently healthy adult dogs and evaluated[90]. Both traditional HH and quantitative Stewart parameters were subsequently calculated and statistically analysed. There were similarities and differences between the current study and the published study with respect to the traditional and quantitative parameters evaluated. Multiple equations with respect to the SID, SIG and AG have been described[6, 13, 15, 86, 90] including the equations utilised in the current study. Comparatively, the mean PCO_2 in the current study was 7mmHg higher, the HCO_3 was 1.5mmol/L higher, the AG was 2.2mmol/L lower and the pH 0.031 lower than those values in the study by Vanova-Uhrikova *et al*[90]. These differences, excluding the pH and PCO_2, were within the standard deviation (SD) of the Vanova-Uhrikova *et al.* study values. Thus the puppies in the current study demonstrated higher PCO_2 values than healthy adult dogs[90], a possible explanation is provided later. Comparing the Stewart parameters, the $SID_{effective}$ was 3.5mmol/L higher, the $SID_{apparent}$ 1.6mmol/L lower and the SIG 5.2mmol/L lower than the values in the Vanova-Uhrikova *et al.* study[90]. Only the $SID_{apparent}$ variable was within the SD range. A possible explanation for the differences between the puppy and adult published population would be the influence of increased serum phosphorus concentrations that is present in puppies. Since the SIG is determined by the $SID_{apparent}$ – the $SID_{effective}$, a difference would also be expected.

Regarding biochemical data only, a study by Rortveit *et al.* evaluated venous blood from 101 clinically healthy puppies aged 16 to 60 days[89]. The data was stratified into three age groups. Pertaining to the biochemical variables, differences were demonstrated between the phosphorus, calcium, potassium, total protein, globulin and albumin[89]. The phosphorus, potassium and calcium were reported higher than in adult populations, while the albumin, total protein and globulin were lower than adult populations[89]. Similar values between the adults and puppies were evident with respect to sodium and

chloride. Data relating to the third group in that study (46-60days) will be highlighted here because of the overlap between this group and the control group used in the current study. In our study, the median values for the control puppies total protein was 2g/L higher, the albumin 2.8g/L higher, the phosphorus 0.3mmol/L lower, the potassium 1.4mmol/L lower, the calcium 1.4mmol/L lower, the chloride values were the same, and the sodium was 1mmol/L lower. It is important to note that the major strong ions in blood (sodium and chloride) values were almost identical between the present and the Rortveit *et al.* study. Both studies also demonstrated hyperphosphataemia in the puppy populations, explained by the increase bone metabolism and turnover in this age group[89].

An additional study that aimed to determine reference ranges for paediatric canine populations evaluated 257 venous blood samples from 68 puppies between ages four days and 84 days[93]. The data generated was also compared to venous blood samples from 30 healthy adult dogs. Statistically significant differences were evident between sodium, potassium, chloride, calcium and magnesium[93]. Bicarbonate was only significantly different from the adult population when puppies were 4 days old, which is an important and interesting finding. In human literature it has been reported that the compensatory effects of the human renal system stabilize shortly after birth, which may be similar in puppies[93]. The stratified age groups in the O'Brian *et al.* study that are most similar to the current study population are puppies aged 70-77 days and those aged 84 days. Comparing the same age groups in the O'Brian *et al.* study and the current CPE study control puppy population, the median pH of the CPE study was 0.1 units lower and the HCO_3^- was 3-4mmol/L lower [93]. It should however be noted that the median chloride, sodium and potassium values of our control group were almost identical in comparison[93]. The above studies highlight the importance of age specific reference intervals as well as the similarities and differences between the current control puppy group and age appropriate published normal ranges.

5.2 Overview of Findings

In the present study, HH detected acid-base abnormalities in 39% of dogs, the SSA demonstrated derangements in 46% of dogs and the BEA displayed abnormalities in 94% of dogs. Similar to Hopper *et al.* the HH approach and SSA both detected a similar number of acid-base abnormalities in the study whereas the semiquantitative approach (BEA) demonstrated derangements in 100% of the cases [15, 69]. In an additional study by Torrente *et al.* acid-base disturbances were demonstrated in 90% of animals using the HH model and derangements in 89% using the quantitative (simplified strong ion) model [66]. Since there is no gold standard of evaluation the above models should not be compared and contrasted directly since the HH model is disadvantaged by the fact that it considers only BE/ HCO_3^- in the evaluation of the entire metabolic compartment as opposed to the BEA and SSA which assess multiple components. In viewing the results in a descriptive fashion only, there is clearly a discrepancy between the two quantitative approaches (SSA and BEA) in the number of abnormalities demonstrated. An overview of the discordance of normal cases is discussed further on.

5.3 The Henderson-Hasselbalch Model

Using the traditional HH model CPE dogs may demonstrate an alkalosis or an acidosis depending on the predominant clinical feature of either vomiting (loss of hydrogen, potassium and chloride ions) or diarrhoea (loss of bicarbonate and albumin)[54, 94]. According to pH, there were seven acidaemic dogs of which three were due to a simple respiratory acidosis, two were classified as simple metabolic acidosis, one dog demonstrated a mixed metabolic acidosis and respiratory acidosis and one dog had an elevated AG (Table 4). There were also two alkalaemic dogs, both of which were classified with mixed acid-base disorders. The three cases that classified with a simple metabolic alkalosis were likely attributed to loss of hydrogen rich fluid via the vomitus or due to retention of bicarbonate by the kidneys[72], or a combination. The median PCO_2 in the control population was 43mmHg compared to 44mmHg in the CPE disease population and hence there was no difference in this regard between the two groups. These values are however higher when compared to healthy adult dog populations; for which a median PCO_2 value of 37mmHg is given in one study[90] or mid normal PCO_2 of 41 provided in another[15]. It is possible that this difference could be explained by the altitude at which this variable is determined. Unfortunately reference intervals for PCO_2 for puppies at various altitudes are not available. In addition, significant differences have been reported for pH and PCO_2 between venous and arterial blood[92, 95]. Therefore arterial sampling would have provided lower PCO_2 values in the CPE and control populations. However the difficulties associated with sampling of arterial blood in small puppies necessitated the use of venous blood. A simple respiratory acidosis due to hypercapnia is most commonly due to decreased ventilation and less commonly due to ventilation/perfusion mismatches or a hypermetabolic state[75]. An elevated venous CO_2 can also be the result of poor perfusion[96, 97]. Lung disease is an uncommon cause of respiratory acidosis due to the high solubility of CO_2, thus even with severe lung oedema, respiratory acidosis is uncommon. Respiratory disease is not a common feature of CPE and none of the dogs had obvious clinical signs of respiratory disease. Thus, in this study the respiratory acidosis was most likely due to a combination of muscle weakness, sepsis and poor perfusion with an elevated arteriovenous PCO_2 difference [11, 96, 97]. Statistically however there were no significant differences between the HH parameters of the control and CPE populations as a whole. Although acid-base abnormalities were detected by the HH approach, the clinical importance of these aberrations is unknown.

5.4 The Unmeasured Ions

Each of the three methods of acid-base analysis provides an estimate of unmeasured ions, namely the AG with the HH approach; the SIG with the SSA approach; and the unmeasured ions effect (XA) with the BEA approach. An elevated AG was demonstrated in nine cases using HH analysis (Table 4), an increased SIG acidosis in 14 dogs using SSA (Table 6), and an increase in XA in 18 cases utilizing the BEA (Table 8). Considering case numbers only, the three methods clearly show discordance in the measurement of unmeasured ions. This difference may be as a result of the quantitative approaches having an increased diagnostic detection of abnormalities to the traditional approach or that the quantitative models (with the multiple variables incorporated into calculation) are more prone to error and overdiagnosis[69]. Similar to the current study, the BEA also detected the highest number of cases with unmeasured ions in the Hopper *et al.* study[69]. However a direct comparison between the studies cannot be made as the control reference ranges in the Hopper *et al.* study were numerically different and from an apparently healthy adult dog population. As alluded to previously, each method has its own strengths and weaknesses therefore direct comparison between the methods may not be a useful approach. An advantage of the quantitative methods over the traditional analysis is in their ability to unmask the effects of underlying imbalances of acid-base balance. Thus these methods are more likely to uncover the effect and contributions of unmeasured ions.

The AG assists in classifying cases with a metabolic acidosis into those that have unmeasured anions versus those without[98]. The most common cause for an increased AG is the accumulation of endogenous lactic acids, ketoacids (β-hydroxybutyrate), uraemic acids, phosphates and sulphates or exogenous acids such as ethylene glycol (glycolic acid) and acetylsalicyclic acid[69, 76]. Albumin exerts an important negative charge that contributes to a normal AG. There was no statistical significance between the control and CPE populations with respect to albumin; as well as only a few changes in the SSA and BEA with respect to albumin were evident. In the current study, the AG was not normalized for albumin and therefore additional AG abnormalities may have been present as a result. Additional comparisons that incorporate the AG corrected for albumin may be warranted in the future. The diagnostic accuracy of the AG is improved with the incorporation of albumin in the identification of unmeasured anions[69]. In the current study an elevated AG was the most frequent abnormality with the HH approach (Table 4). Surprising here is the fact that by definition only two dogs had an AG metabolic acidosis (HCO_3^-<19mmol/L and pH<7.28) whilst nine cases had AG abnormalities (Table 4). Aberrations in phosphorus detected by the BEA phosphorus effect were present in 20/35 cases (Table 8) highlighting the importance of evaluating

the metabolic compartment by other methods (since phosphorus/ phosphoric acid is an unmeasured ion). There was also a significant difference in serum phosphate and the phosphate effect between the control and CPE groups (Table 7). The inability of the HH approach to assess the underlying metabolic compartment limits its usefulness. There were no abnormalities detected in the lactate effect utilising the BEA or statistical differences between blood lactate and lactate effect in the control or CPE groups (Table 7). Therefore lactate was not an important unmeasured ion contributing to the AG in this study population. Lactate has been reported as a marker of inadequate tissue perfusion, an important prognostic indicator and has been associated with survival in various diseases[54, 91, 99-101]. In a study by McMichael *et al.* reference ranges for blood lactate were determined from 247 blood samples from 68 puppies aged four days to 80 days[91]. For the purposes of comparative discussion only those puppies aged 70 and 80 days will be compared to the current study control group. The mean value of venous blood lactate from our generated control population (1.72mmol/L) was almost identical to the published mean values (70 days old, 1.64mmol/L, SD 0.69; 80 days old, 1.79mmol/L, SD 0.71)[91]. The findings also demonstrated that lactate was significantly higher in puppies within the first 28 days of life, but from 70 days onwards the values were indistinguishable from an adult dog population [91].

Strong ion gap abnormalities were detected in 14 cases via the SSA (Table 6). The SIG formula is derived from the following equation: $SID_{apparent} - SID_{effective}$. In normal animals, the SIG approaches zero and increases are as a result of unmeasured anions[69].The $SID_{apparent}$ is in turn derived from contributions of sodium, potassium, calcium and chloride. In contrast to this, the $SID_{effective}$ is derived from the sum of the bicarbonate, albumin and phosphorus contributions (Table 1). Thus multiple components are integral in the calculation of the SIG. Parvovirus infected puppies clinically display vomiting (loss of chloride, sodium, hydrogen, bicarbonate and potassium), anorexia (reduced phosphorus and albumin), dehydration (increases in sodium and chloride due to loss of free water) and diarrhoea (loss of bicarbonate, albumin and potassium) [8, 94, 102]. All these clinical signs and biochemical alterations affect the key components that determine the SIG. It is not surprising that a statistically significant SIG difference was present between the control and CPE population. Similarly in a previous study, the SIG detected more abnormalities in unmeasured ions compared to the AG[15]. In addition the SIG was strongly correlated to the AG[15]. It however can be argued that the increased complexity in calculation does not provide clinical benefit over the traditional AG. The $SID_{apparent}$ has marked similarities with the HH AG calculation with the exception of not incorporating bicarbonate yet including calcium. This can also be viewed as an estimation of unmeasured ions in the author's opinion. The current study demonstrated an SID alkalosis in 10 cases (Table 6), which was the second most common abnormality detected by the

SSA. There was however no difference between the control and CPE populations with respect to the SID (Table 5). The SID can be affected by two conditions, namely a relative concentration change in ions due to free water changes or by an absolute change in strong ion concentrations. A SID alkalosis could be a result of dehydration causing a contraction alkalosis, an increase in the strong ion sodium or a loss of the strong ion chloride (such as with vomiting) [103]. The SID alkalosis in the current study was likely due to varying degrees of dehydration in the infected puppies as well as a reduction in serum chloride, which was demonstrated in the infected puppies compared to the control puppies. Serum chloride was also statistically different between the groups, emphasising its role and importance (Table 5).

The BEA detected a substantial proportion of animals with increased unmeasured anions (51%). Similar to the calculation of the SIG, the XA incorporates six parameters (chloride, free water, albumin, phosphorus, lactate effect and base excess) into its calculation (Table 1). Evaluating multiple components increases the diagnostic sensitivity ;however, an inherent degree of error may be incorporated with each calculation leading to a potential cumulative error and over- or underdiagnoses. There was also a statistically significant difference in the sum of effects and XA between the CPE and control group (Table 7). These findings are not surprising as significant differences were detected with respect to the serum chloride, sodium, potassium, phosphate and total protein between the CPE and control populations (Table 7).

5.5 The Contribution of Proteins

In evaluating the acid-base status using the quantitative methods, the SSA incorporates albumin into A_{TOT} and the BEA evaluates the albumin effect. Hypoalbuminaemia is a common and expected finding in canine parvovirus, and may be the result of a combination of protein loss in the gastrointestinal tract, intestinal haemorrhage, sepsis- induced SIRS, reduced intake and production and dilution due to fluid therapy[11, 104]. Since albumin is a weak acid, hypoalbuminemia would result in a metabolic alkalosis, detected by SSA (A_{TOT} alkalosis) or by BEA methods (albumin effect alkalosis) [15]. An albumin effect alkalosis has been demonstrated previously as a consistent finding in CPE using the BEA[12]. In the current study, the SSA approach detected albumin-related abnormalities (via A_{TOT}) in six cases, of which all demonstrated an A_{TOT} alkalosis (Table 6). Statistically there was a significant difference between the control and diseased populations with respect to A_{TOT} (Table 5).

The BEA detected albumin effect abnormalities in six cases, of which three were an albumin effect alkalosis and three an albumin effect acidosis (Table 8). Since A_{TOT} is affected by both the phosphorus contribution as well as the albumin contribution, the presence of a concurrent hyperphosphataemia may conceal the effect of hypoalbuminaemia, resulting in an underdiagnosis by the SSA[15]. In the evaluation of a single parameter (albumin effect via the BEA) both the expected (alkalosis) and unexpected (acidosis) diagnostic changes in CPE were present, however, there was no statistical difference evaluating the albumin effect or the serum albumin between populations (Table 7). In summary, both the SSA and BEA detected differing numbers of protein-related acid-base abnormalities, which accentuated the importance of albumin and phosphate in canine parvovirus, as well as the diagnostic ability of the quantitative approaches to assess the impact of changes in protein on acid-base status.

5.6 The Phosphorus Effect

The second most frequent abnormality observed by the BEA was a phosphorus effect alkalosis. Hypo- and hyperphosphataemia have been reported in CPE[94], with hyperphosphataemia related to dehydration. A state of hyperphosphataemia can also be seen in metabolic acidosis (caused by lactic acidosis and diabetic ketoacidosis), in which the organic acids result in the breakdown of adenosine triphosphate to adenosine monophosphate and inorganic phosphate[105]. This was however not the case in this study population. Although there was a statistically significant difference between the populations with respect to serum phosphate and phosphorus effect, the median value of phosphate was within the control reference range (Table 7). This once again emphasises the importance of assessing a laboratory diagnosis in light of its clinical significance. It is also likely that the role of phosphate was overemphasized because the cases were growing puppies in which the phosphate levels are expected to be higher than in adult dogs[105]. This implies that the numerous aberrations detected were likely of limited clinical significance. Additionally it is noteworthy that the reference interval cut-off points vary considerably between studies. Our control population (with intervals generated) consisted of a small population which may not truly represent the very young population we studied. The inclusion of a larger control population may alter the number of detected abnormalities according to the phosphorus effect. In addition, narrow references ranges have the potential to inflate the number of diagnosed abnormalities (producing a larger number of false positives or abnormalities without clinical relevance). It can be argued that the phosphorus effect range in the current study is not wide enough to substantially affect pH and could be considered within the margin of error.

5.7 The Free Water and Chloride Effect

A common clinicopathologic feature present in CPE is hyponatraemia and hypochloraemia[94], which would be expected to result in a free water effect acidosis and a chloride effect alkalosis, respectively[12]. A free water acidosis was present in 20 cases, the most common BEA abnormality, while no cases demonstrated a free water effect alkalosis (Table 8). The free water effect was also statistically significant between the populations (Table 7). The degree of hyponatraemia varied between the two groups with the CPE population displaying a median value below the control range (Table 7). The extent of dehydration would depend on the severity of clinical disease and the duration of illness. A hyponatraemia with volume depletion is generally caused by gastrointestinal fluid losses (vomiting and diarrhoea), third-space losses (pleural effusion, peritonitis or pancreatitis) or renal losses (hypoadrenocorticism and diuretic therapy) [106]. The most likely explanation in the CPE population would be sodium-rich fluid loss via the gastrointestinal tract, resulting in the free water effect acidosis seen. A chloride effect alkalosis, the fourth most common BEA abnormality was evident in 14 dogs (Table 8); however, statistically there was no significant difference between the CPE and control populations (Table 5). In the statistical analysis the serum chloride demonstrated statistical significance whereas the chloride effect did not; this was due to chloride being corrected to a median value of 109mmol/L and adjusted for sodium concentrations. An additional explanation would be the small population size or the large statistical variance in the CPE population. As discussed previously hypochloraemia was evident in the CPE group with this group having a significantly lower chloride than the control population. The chloride effect has previously been reported to be the most important component in the metabolic acid-base disturbances as well as in the BEA in CPE[12]. In a study by Burchell *et al.* that utilised a clinical scoring system, more severely affected puppies demonstrated a hypochloraemic alkalosis compared to mildly affected puppies that displayed a hyperchloraemic acidosis[12]. Due to the small number of CPE puppies in our study stratifying them by a disease score would have made numbers in groups too small to be useful. The chloride effect has demonstrated importance, but should only be viewed in the context of the entire strong ion set. The chloride effect is however a construct of convenience rather than the actual value of its true effect, since it assumes that the mean of the population confidence interval value is the “normal value” for every individual, and thus it will underestimate and overestimate individuals.

5.8 The Quantitative Models: SSA and BEA

Concurrent metabolic acidosis and alkalosis were evident in 10 cases in the SSA (Table 6) and in 21 cases in the BEA analysis (Table 8). This suggests an increased diagnostic detection for the physicochemical approaches, especially the BEA, in detecting underlying metabolic disorders as well as coexisting acid-base abnormalities. Once again since no gold standard of acid-base analysis exists, a direct comparison between the models should not be performed. It may be argued that the BEA actually over-diagnosed acid-base disturbances and the usefulness of this may be questioned. This overdiagnosis may have negative clinical consequences if the acid-base disorder is the focus of treatment, as opposed to a holistic patient approach. As a result, it would be unwise to assert superiority of one model over the other because both models are predicated on the assumption of their inherent accuracy. Previous studies comparing the HH and Stewart approaches have demonstrated diagnostic similarity (in terms of magnitude and direction of change) between the models, and have suggested that the differences between the models are in the interpretation of the underlying pathology[107-111]. The findings of the aforementioned studies support sufficiency of both models in the explanation of acid-base disturbances although from a different perspective.

Therefore, some authors suggest that the application of each method is a matter of preference rather than improved performance of one model over the other [112]. The results of this study demonstrated differing findings between the HH model, the SSA and BEA both quantitatively and qualitatively. This is because, as we have shown, CPE is characterized by a complex collection of acid-base derangements.

5.9 An Overview of Discordance between the Models

An important comparison in this study is the evaluation of the discordant diagnoses between the three models. If the approaches diagnosed the same cases as normal then a conclusion regarding diagnostic equivalence could be inferred. In so far as the proportion of normal cases diagnosed between models, the HH demonstrated discordance of 42% with the SSA and 92% with the BEA. As such there was a disagreement in the classification of normal cases in 42% and 92% of cases, respectively. The SSA provided a discordant diagnosis in 36% of cases when compared to the HH method and in 86% of cases when compared to the BEA. When comparing the BEA to the HH the degree of discordance was 50% and compared to the SSA it was 25%. The number of discordant diagnoses was lowest between the BEA and SSA and was highest between the HH and BEA models. Based on these levels of discordance it is clear that the different methods of acid-base analysis cannot be used interchangeably. As mentioned throughout the study, a statement regarding the superiority of one model over the other cannot be made. If the HH is utilised as the benchmark (as is the author's preference) then the BEA vastly over-detected acid-base abnormalities. In the 24 cases diagnosed as normal by the HH approach; the BEA diagnosed a phosphorus effect alkalosis in 11, an unmeasured anion acidosis in 10, a chloride effect alkalosis in nine, a free water effect acidosis in four, an albumin effect acidosis in two and an albumin effect alkalosis and phosphorus effect acidosis in one case each (Table 9). Thus the HH approach failed to diagnose 22/35 cases (63%) with apparent acid-base anomalies according to the BEA, or seen another way, that the BEA overdiagnosed a significant proportion of cases. Likewise the HH approach failed to detect 10/41 cases (24%) that were diagnosed as abnormal according to the SSA approach (Table 9). This confirms the findings in a previous study that showed that the agreement between the HH and quantitative methods for the diagnosis of acid–base abnormalities was poor [6]. Therefore the alternate hypothesis holds true in that SSA and BEA diagnosed a larger number of acid-base disturbances in CPE at the same time as providing a clearer understanding to the underlying pathophysiology of the causes of the disturbances.

5.10 Clinical Relevance

There is a paucity of clinically relevant literature relating acid-base changes to the outcome in CPE. It was the purpose of this study to provide a descriptive evaluation of acid-base changes between the different models and not correlate acid-base diagnosis with outcome. This aspect was however not within the scope of the current study. As mentioned earlier, in a retrospective study by Burchell *et al.* a clinical scoring system was applied to CPE puppies at admission and the strong ion model was used to analyse outcome based on this clinical stratification[12]. The most significant association between clinical score and acid base diagnosis was that the more severely-affected puppies tended to have a hypochloraemic alkalosis, whereas mildly-affected puppies tended to have a hyperchloraemic acidosis. This data was not correlated to outcome and hence prognostic inferences cannot be made. Similarly, in the current study, puppies were not followed from initial diagnosis to outcome and conclusions regarding prognosis could thus not be made. Furthermore, none of the CPE cases received specific treatment directed at correcting their acid-base diagnosis. Importantly in all acid-base diagnoses the primary underlying disorder should be addressed. The clinical signs (for example: vomiting, diarrhoea, dehydration, anorexia, pain and malnutrition) that result in the acid-base disorders should always be addressed as a primary concern. Once appropriate and intensive treatment is applied to these manifestations of disease the acid-base aberrations should normalise. Of the methods studied, the quantitative approaches (specifically the BEA) would theoretically provide the most insight into which ions require therapeutic attention. There are multiple aspects that further studies could address in the future but it seems unlikely that allowing an acid base diagnosis alone to set the treatment agenda would make significant impact on case outcome. This study provided a snap shot in time of an acid-base diagnosis. Perhaps a kinetic study conducted over time would provide more information on the effect of correcting acid-base imbalances on the outcome in CPE.

5.11 Limitations

We identified the following limitations in this study: the normal ranges and cut-off points for the various measured and calculated variables differ between equipment and studies. Consequently, the differing values will alter the interpretation of some of the results as well as influence the inclusion or exclusion of cases. Moreover, there are several variations of the Stewart SID calculation, each making use of different measured variables[6, 15, 86]. Although the various analytes have only a small impact on the final outcome, the model used does impact slightly on the final assessment. Additionally, parameters in the SSA and BEA required multiple calculations in order to arrive at the final value. Each calculation has an inherent degree of error that may potentially result in a cumulative error in the final result. Lastly, our control values were generated from a relatively small group of puppies and a larger group may have reduced the standard variation and altered the significance of the comparisons.

Chapter 6

Conclusion

In summary, the various models detected numerous acid-base disturbances in CPE. In an overview of the analysis the HH analysis detected abnormalities in 41% of cases, with the most common simple disorder consisting of a simple respiratory acidosis, and the most common abnormality an elevated AG. The SSA detected abnormalities in 46% of cases, the most common derangement consisting of an increased SIG acidosis. Lastly, the BEA detected disorders in 89% of cases with the most common aberration comprising a free water effect acidosis. In the evaluation of the discordance between normal cases within the models, the level of disagreement was lowest between the BEA and SSA, and was the highest between the HH and BEA. Acid-base disorders may be assessed utilising different approaches with variations within each of the approaches themselves. There are myriads of ions, proteins and buffers that all affect whole body acid base balance. The acid-base approaches discussed in this study namely, the HH, SSA and BEA, place emphasis on differing components in an attempt to dissect the underlying pathogenesis. As mentioned previously there is a lack of a gold standard for comparison of the methods; however, in the authors' opinion the quantitative methods are more appropriate for the assessment of changes in CPE as the contributions of individual ions and components are assessed more clearly. Regardless treatment of CPE should follow standardised protocols directed towards the clinical condition and biochemical abnormalities and not be directed by the acid-base diagnosis. The study also highlights the complexity of methods that use multiple variables to generate a final result (particularly the SSA). The opposing or antagonistic effects of these individual variables can also falsely diagnose acid-base disturbances in multiple cases. It is therefore clear that the acid-base changes in CPE are complex and that they accrue due to a number of different primary disorders illustrated more clearly in the quantitative models compared to the HH. The HH model appeared unable to account for apparent metabolic changes to acid-base, whereas the SSA and BEA suggested that metabolic changes were present due to a combination of acidotic and alkalotic processes between individual ions, electrolytes and proteins.

Chapter 7

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Appendixes

Appendix 1: Raw biochemistry and Henderson-Hasselbalch acid-base data of the 41 canine parvovirus dogs.

CPE	Age	Weight	CO_2	pH	HCO_3	Base Excess	TP	Lactate	Phosphate	Albumin	Potassium	Sodium	Chloride	Calcium
Unit	Months	Kg	mmHg		mmol/L	mmol/L	g/L	mmol/L	mmol/L	g/L	mmol/L	mmol/L	mmol/L	mmol/L
1	4	6.6	43	7.26	18	2.1	57.3	1.3	2.14	30	4.0	139.8	100	1.22
2	5	11	38	7.36	21	1.5	42	0.9	2.13	21	4.2	123	87	1.21
3	2	2.8	44	7.3	21	-3.1	50.2	1.2	2.23	28	4.1	140.7	107	1.27
4	2	5.6	53	7.37	29	-0.2	56.7	None	2.37	40	4.6	147	108	1.43
5	3	14	44	7.38	25	8.4	42.4	1.6	1.96	25	3.3	130	93	1.23
6	7	6.6	55	7.33	28	-2.9	43.8	0.1	2.49	25	2.9	139	106	1.31
7	5	5.1	53	7.34	28	-4.1	49.9	1.8	2.3	22	3.4	143	110	1.27
8	3	2.8	59	7.23	24	-5.3	41.3	1.2	2.21	31	3.6	139.4	107	1.33
9	2		41	7.34	22	-9.5	47.8	None	3.21	23	4.1	146	111	1.43
10	5	22.2	40	7.36	22	-6.8	42.1	3.3	3.14	23	4.0	146	113	1.41
11	5	30	38	7.38	21	6.3	46	None	1.41	21	2.6	122	81	1.16
12	3	3.8	44	7.39	25	-3.9	45.6	1.8	2.21	27	4.0	138.7	104	1.35
13	3	3	49	7.31	24	-4.3	51.9	None	2.48	29	4.0	130	96	1.3
14	3	8.2	39	7.36	21	-4.6	48.5	1.9	2.27	32	4.0	138	107	1.39
15	4	2.8	45	7.32	22	5.1	53.1	1.4	2.17	29	3.5	131	90	1.28
16	6	11	37	7.36	20	-2.2	53.6	3.7	2.98	28	3.5	139	99	1.28
17	9	11.2	57	7.37	32	-3.2	56.3	None	3.27	29	3.3	149	104	1.38
18	3	5.2	48	7.41	30	-7.9	47.9	2.3	4	23	2.4	142.3	104	1.22
19	4	14.2	37	7.4	22	-1.6	47.8	2.8	2.47	26	4.8	140.6	103	1.36
20	3	5	36	7.33	18	-7.1	52.1	0.1	2.78	35	4.0	143	110	1.36
21	5	4.6	51	7.31	25	-3.6	56.5	1.7	2.05	39	4.5	139	103	1.32
22	3	9.2	43	7.33	22	-1.5	50.8	1.4	2.19	24	3.5	146	109	1.35

23	3	9.2	41	7.25	17	4.4	41.5	1.1	2.26	26	3.8	133.2	96	1.34
24	5	14.2	48	7.38	28	-7.1	44.7	1.1	2.35	28	3.7	138.6	106	1.37
25	7	13.4	41	7.3	19	0.8	44.8	1.3	2.79	25	3.6	137.1	97	1.32
26	7	13.4	45	7.39	26	-1.4	37.7	None	2.77	22	4.7	141	108	1.43
27	3	6.6	54	7.21	21	-4.4	53.2	0.8	2.39	36	4.1	144.8	109	1.42
28	3	3	48	7.29	22	-3.9	52.6	0.1	2.58	27	3.8	143.2	108	1.43
29	2	7.2	52	7.29	24	-3.9	58.9	1.1	2.62	34	3.8	134.3	101	1.38
30	6		55	7.3	26	-2.3	51.6	2.2	2.53	31	4.0	141	103	1.37
31	3	3	74	7.16	26	-8	57.2	2.3	3.5	31	4.3	140	103	1.25
32	4	8.6	44	7.48	32	-1.7	46.7	0.1	2.96	31	3.7	143.3	107	1.4
33	5	9.8	40	7.49	30	-7.2	45.8	3.3	4.15	26	4.0	138	99	1.3
34	3	6.4	34	7.35	18	-7	52	2	2.14	22	3.5	141	107	1.26
35	4	7.6	45	7.31	22	-5	49.3	0.1	2.55	34	4.0	141	107	1.34
36	3	6.6	46	7.34	24	2.5	52.8	2.3	2.19	33	4.2	146	105	1.31
37	8	12	62	7.25	25	0.6	48.5	1.1	2.24	28	4.6	140.8	104	1.4
38	2	2.4	44	7.26	20	2.1	66.4	1.4	2.48	35	3.0	142.8	97	1.29
39	2	5.2	57	7.29	26	-2.9	53.8	0.1	1.54	27	3.7	138.9	107	1.24
40	5	14.2	36	7.42	23	0.2	47.9	3.2	2.2	24	3.6	137.6	100	1.33
41	4	6.7	38	7.31	19	7.2	53.2	1.9	1.59	38	3.7	140	98	1.22

Appendix 2: Informed consent form

INFORMED CONSENT FORM

We, the undersigned, hereby agree that the animal(s), as specified below, may be used by the researcher(s), as specified below, in the procedures as explained below:

1. To be completed by the researcher(s)

- **NAME OF THE RESEARCHER(S):**

Dr Ryan Brandon Friedlein, BVSc (Hons)
Prof Andrew Leisewitz, BVSc (Hons) MMedVet (Med) ECVIM-CA PhD
Dr Richard Burchell, BVSc MMedVet (Med) DECVIM-CA

- **NAME OF RESEARCH PROJECT:**

Comparison of different methods of acid-base analysis in canine parvoviral enteritis

- **PURPOSE OF RESEARCH PROJECT:**

Parvovirus puppies have multiple blood electrolyte and acid-base status abnormalities. Obtaining information regarding these abnormalities will aid future understanding of the disease and may help optimising the ideal treatment applied to these animals. Early recognition and understanding of these

abnormalities will prompt more effective and efficient treatment being applied and may achieve an improved survival or outcome in these cases.

- **DETAILED PROCEDURE(S) TO BE PERFORMED:**

Various blood tests as performed on venous blood samples collected in the normal course of treatment in CPE. Faeces and urine will be collected for archiving. The remainder of the treatment and testing costs for the routine treatment of CPE will be paid by the owner and not by the research budget.

- **RISK(S) INVOLVED IN SPECIFIED PROCEDURE:**

None beyond the normal course of patient investigation in CPE.

- **IDENTIFICATION OF ANIMAL TO BE USED:**

2. To be completed by the animal's owner or person duly authorized to sign on his/her behalf:

- **NAME OF OWNER:**

- **HAVE YOU RECEIVED DETAILED INFORMATION REGARDING THE PROPOSED STUDY?**

- **HAVE ALL THE RISKS INVOLVED IN THE PROCEDURE BEEN EXPLAINED TO YOU AND DO YOU FULLY UNDERSTAND THESE RISKS?**

- **DO YOU GRANT FULL CONSENT FOR THE PROCEDURE/ TESTS TO BE PERFORMED?**

3. The undersigned parties further agree that no compensation regarding costs for the treatment and testing in the normal course of CPE will be payable to the animal's owner or anybody else and that only research associated costs will be covered by the researchers.

4. The undersigned parties further agree that this form would serve to fully indemnify the University of Pretoria and the undersigned researchers against any future claims resulting from the specified procedure by or on behalf of the animal's owner.

5. The undersigned parties further agree that no material of any kind, including data and research findings, obtained or resulting from the procedure, would be passed on to any third party or used for any purpose other than that specified in this form, except with the written consent of the undersigned owner of the animal.

_____ _____

SIGNATURE RESEARCHER(S) **SIGNATURE OWNER**

_____

SIGNATURE WITNESS

DATE: _____

Appendix 3: Client Information sheet

Parvovirus in Dogs

What is Parvovirus?

Parvovirus infection, commonly called "parvo," is a disease of dogs that affects the intestinal tract and causes vomiting, diarrhoea, fever, and decreased ability to fight infection. It is especially severe in puppies. Doberman pinschers and Rottweilers are more susceptible and have more severe signs of parvo than other breeds, but puppies of any breed or mixed breed puppies can die from this disease. Parvo is a relatively new disease entity in dogs that was first identified in the late 1970s. The virus did not exist before that time. It is believed that this is a disease caused by a virus of the cat or other species that adapted itself to dogs. When the virus first emerged, dogs of all ages became infected. Now that the disease is in its second decade, usually only young dogs are infected. This is because the virus is so contagious and so commonly found in the environment that most older dogs have become immune through vaccination or infection early in life. Oral intake of virus-infected materials transmits the infection to susceptible dogs. Parvovirus multiplies in the intestinal tract of infected dogs, and a billion virus particles per teaspoon of stool can be passed during an infection. The virus is sturdy and persists in the environment for at least 6 months. It is impossible to eliminate the virus from contaminated soil without killing all vegetation. For inside facilities, thorough washing and rinsing followed by careful application of a chlorine bleach solution containing one ounce of bleach per quart of water is needed. Avoid skin and eye contact with the bleach solution.

What are the symptoms of Parvovirus?

Infection of puppies usually results from exposure to contaminated soil, and signs of disease are seen from 4 to 14 days after exposure. The initial signs are depression, loss of appetite, and fever. Vomiting and blood-streaked diarrhoea develop within 1 or 2 days. These signs progress quickly to dehydration and death in severely affected dogs. Puppies 6 to 8 weeks of age have a higher death rate than older dogs. The age of onset of infection depends on exposure to the virus as well as the pups' level of antibodies against parvovirus. Bitches that are immune by vaccination or previous exposure to the virus pass some of their antibodies to their puppies in milk. Depending on the amount of antibodies passed to the puppies, the antibodies protect them for a few weeks to as long as 3 months. The puppies' bodies

gradually degrade or break down the antibodies and the puppies must then produce their own immunity to be protected.

What treatment is needed?

The best approach to parvo is prevention of disease with vaccination. Puppies should be started on vaccines at 6 weeks of age and exposure to infected environments should be minimized until the vaccination series is complete. Puppies should be vaccinated every 3 to 4 weeks until 16 weeks of age. The long course of vaccination is necessary because of the maternal antibodies passed from the mother to the pups. Although these antibodies protect against infection, they also interfere with an effective response to vaccination. Low levels of maternal antibodies interfere with vaccination but may not protect puppies from infection. Advances in parvovirus vaccines have resulted in improved vaccines that provide effective protection despite some maternal antibodies. It is advised that the exposure of puppies be minimized until vaccines given at 16 weeks of age have been administered. The mainstay of treatment involves supportive care with the usage of intravenous fluids, antibiotics, stomach protectants, anti-nausea medications, pain killers and supplemental nutrition.

What is the prognosis?

The initial damage to the body in parvo occurs because the virus destroys the cells in which it reproduces. Unfortunately, no antivirus treatment exists at this time. Treatment of dogs infected with parvo depends on the severity of the infection. Dogs with mild infections can recover with nursing care, but those with severe infection become severely dehydrated. These dogs require intravenous fluid to maintain their hydration because they are unable to take in fluids and are losing large amounts of fluids because of vomiting and diarrhoea. In addition to the fluid loss, the virus destroys the lining cells of the intestinal tract, which allows bacteria from the intestine to enter the body. When this bacterial invasion occurs (septicaemia), antibiotics must be given to kill the bacteria in the bloodstream. In addition to allowing bacterial entry into the blood stream, the parvovirus damages the bone marrow, where white blood cells are produced. Neutrophils, a specific type of white blood cell necessary for destroying invading bacteria, are severely reduced in numbers. Immune dysfunction causes some dogs with parvo to die in spite of extensive treatment with fluid and antibiotics.