The Role of SID and A_{TOT} in the Metabolic Acid-Base Changes of Canine Parvoviral Enteritis

Dr R.K. Burchell (B.Sc (Hons) B.V.Sc)

Department of Companion Animal Clinical Studies

Faculty of Veterinary Science

University of Pretoria

Supervisor:

Prof. Andrew Leisewitz

Department of Companion Animal Clinical Studies

Faculty of Veterinary Science

University of Pretoria

Co-Supervisor:

Prof. Johan Schoeman

Department of Companion Animal Clinical Studies

Faculty of Veterinary Science

University of Pretoria

The Role of SID and A_{TOT} in the Metabolic Acid-Base Changes of Canine Parvovirus Infection

Richard Kenneth Burchell

Submitted in partial fulfillment of the requirements of a degree of Master of Veterinary Medicine (Small Animal Medicine)

Supervisor: Prof. Andrew Leisewitz

Professor Small Animal Internal Medicine

Department of Companion Animal Clinical Studies

Faculty of Veterinary Science

University of Pretoria

Co-Supervisor: Prof. Johan Schoeman

Professor Small Animal Internal Medicine

Department of Companion Animal Clinical Studies

Faculty of Veterinary Science

University of Pretoria

Declaration

I Richard Burchell hereby 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 to be submitted for another degree at this or any other University, Tertiary Education Institution or Examining Body.

Background

When I undertook to research this topic I was completely unprepared for the reality that would confront me, when I realized that even the hallowed basis of fundamental science such as physics and chemistry are so incompletely understood. At the very heart of the acid-base debate previously dubbed the great transatlantic debate, is the quest for supremacy in the explanation of the physicochemical reactions that drive acid-base changes. These notions are far too lofty for the minds of clinicians and as such I have attempted to be a faithful advocate for both the Henderson-Hasselbach and Stewarts Strong Ion model, by highlighting the strengths and weaknesses of the Strong Ion Model that was investigated in this dissertation. Even as this dissertation is submitted, the battle for the soul of acid-base rages on and I am reminded of the saying:

Nihil sapientiae odiosius acumine nimio

(There is nothing more hateful to wisdom than excessive cleverness)

Table of Contents

Summary

The acid-base disturbances in Canine Parvoviral Enteritis (CPV) are not well described. In addition the mechanisms causing these perturbations have not been fully elucidated. The purpose of this study was to assess acid-base changes in puppies suffering from Canine Parvoviral Enteritis (CPV) using a Modified Strong Ion Model (SIM). The hypothesis of this study was that severe acid-base disturbances would be present and that the SIM would provide patho-mechanistic insights that would not be fully appreciated by the Henderson-Hasselbalch model. The study retrospectively analysed data obtained from 42 puppies with confirmed CPV and 12 healthy controls. The CPV group had been allocated a clinical score to allow classification of the data according to clinical severity. The effects of changes in free water, chloride, lactate, albumin and phosphate were calculated using a modification of the base excess algorithm. The data for each of these variables was compared to the control group. When the data were summated for each patient and correlated to each individual component, the most important contributor to the metabolic acid-base changes according to SIM was chloride ($P < 0.001$). Severely affected animals tended to have a hypochloraemic alkalosis, whereas mildly effected puppies had a hyperchloraemic acidosis ($P = 0.0023$). In conclusion the acid base disturbances in CPV are multifactorial and complex and the SIM provides more information regarding the origin of these changes.

Acknowledgements

I would like to thank the following people for their invaluable support and assistance:

1.) Professor Andrew Leisewitz, for his guidance, wisdom and enthusiastic support, without which this project would have been impossible.

2.) Professor Johan Schoeman for sharing of his experience and wealth of knowledge and for urging me to learn to master the statistical analyses in this project.

3.) Professor Peter Thompson, for ensuring that I committed no statistical heresy, and for so effectively explaining difficult statistical concepts to me.

4.) To my wonderful wife, for her enduring love, encouragement and support, and the belief in my abilities that way transcends that of my own!

5.) Lastly to the One who makes all human endeavours' worthwhile. The First and the Last the Beginning and the End.

For it is written: "I will destroy the wisdom of the wise, I will bring to nothing the understanding of the prudent" 1 Corinthians 1: 19

List of Figures

Figure 4.6 Inter-rater agreement plot comparing the diagnosis of the acid-base changes according to the strong ion model (according to the Hopper and Haskins technique) and a simplified SID technique without using the corrected chloride

Figure 4.7 Inter-rater agreement plot comparing the diagnosis of the acid-base changes according to the strong ion model (according to the Hopper and Haskins technique) and a simplified SID technique using the corrected chloride value

Figure 4.8 Box plot comparing the SID_4 obtained from Constable's strong ion model and the base excess algorithm. The data are not significantly different, and the distributions and means are highly similar.

Figure 4.9.1 Comparison of the A_{TOT} derived from the BE algorithm and the simplified strong ion model in CPV. The data are significantly different, with the strong ion model indicating a bigger shift in the A_{TOT} value compared to the BE algorithm ($p < 0.001$).

Figure 4.9.2 Comparison of the A_{TOT} derived from the BE algorithm and the simplified strong ion model in CPV. The data are not significantly different ($p = 0.73$)

List of Abbreviations

BE Base excess

CPV Canine Parvoviral enteritis

HH The Henderson-Hasselbalch model of acid-base assessment

SID The strong ion difference.

SIG The strong ion gap

SIM The strong ion model

Chapter 1 Literature Review

1.1 Introduction

The assessment of acid-base disturbances, the mechanisms underlying these perturbations and the compensatory mechanisms that attempt to correct them has long been a subject of clinical interest[1-22]. Acid-base disorders are common in critically ill patients[17-19]. Quantitative and qualitative appraisal of acid-base disturbances in the medical setting is clinically challenging[7]. Ongoing research has improved our understanding of acid-base processes over the past few decades, and has also stimulated much debate regarding these processes and how best to assess them[23, 24]. Metabolic processes yield 50 to 100 mEq of H^+ ions each day from macromolecules such as proteins and carbohydrates, and 10 - 15 000 mmol of Carbon Dioxide (CO_2) from the metabolism of carbohydrate and fat[1]. CO_2 is rapidly converted to carbonic acid by the ubiquitous enzyme carbonic anhydrase, which readily dissociates into HCO_3^- and H^+[25]. The buffering mechanisms that maintain blood pH between a constant range are complex and include the inherent buffering of blood as a solution, electrolyte and HCO_3^- handling by the kidney and CO_2 exchange in the lungs[17, 26].

A plethora of disease processes can result in profound changes in pH homeostasis. Common causes of pH perturbations in small animals include: shock/dehydration/hypovolaemia, diarrhoea, vomiting, diabetic ketoacidosis, gastric-dilatation-volvulus, canine hypoadrenocorticism, renal failure, canine babesiosis, and lung diseases[17, 26, 27]. In spite of intense research in the area of acid-base physiology over the last century, the processes underlying acid-base disturbances are incompletely understood, and remain a subject of contention among clinicians[22, 24, 28, 29]. At present there are two paradigms, which have been put forward to explain acid-base disturbances, namely the Henderson-Hasselbalch approach and the Stewart Strong Ion model[7, 22]. The clinical utility of these two models has been and remains an area debate. The contention surrounding these models relates to the ability of each model to quantify an acid-base disturbance and to provide evidence as to the underlying aetiology[22].

The traditional understanding of pH homeostasis in mammals relates to the behavior of acids and bases in plasma, where acids are proton donors and bases are proton

acceptors. In this model, a pH disturbance will arise if acids or bases are added to or removed from plasma by biological or pathophysiological processes. The Henderson-Hasselbach approach is modelled on this rationale[1].

In contrast to the Henderson-Hasselbalch model, the Stewart Strong Ion model defines acids and bases as substances which are able to bring about a change in the hydrogen ion activity[7, 13, 15, 17, 18, 29-33]. In this model strong ions such as electrolytes and other charged molecules such as proteins are able to alter the pH through a physiochemical effect on water dissociation[18]. As such, electrolyte and protein pathologies are able to alter pH, a fact not considered by Henderson-Hasselbalch. Because of the complexity of acid-base disturbances a thorough understanding of acid-base physiology is essential in the interpretation blood gas and electrolyte data in the veterinary patient.

1.2 Concept of pH

The pH of any solution is determined by the activity of hydrogen ions as expressed by the following equation:

$$pH = -\log_{10}[H^+]$$

The pH of a solution is thus inversely proportional to the concentration of hydrogen ions[1]. Because protons are reactive and are able to alter the ionization status of proteins and thus tertiary structure, their concentration is kept within a narrow range in biological systems. The generation of H^+ in a solution can be simplified by the following equation:

$$HA \leftrightarrow H^+ + A^- \quad (1)$$

The extent of the reaction will proceed in a direction proportional to the concentrations of the reactants. In simple terms, a high concentration of HA will favour H^+ and A^- formation and *vice versa*. This is known as the law of mass action. The extent of the reaction is also affected by the ionization or dissociation constant (Ka) for an acid. Acids with high Ka values readily dissociate and are thus known as strong acids for example HCl which is virtually completely dissociated at physiological pH. Conversely, acids with lower Ka's are only partially dissociated at physiological pH[1].

Integrating equation (1) and the dissociation constant for any given acid, the following equation is derived, which expresses the pH as a function of the concentration of the reactants and the dissociation constant for the reaction:

$$pH = pKa + \log [base]/ [acid]$$

In this model the pH of the solution is determined by the ability of a compound to donate or to scavenge protons or H^+ ions[1]. This is known as the Bronsted-Lowry theory of acid-base. Acids are therefore known as proton donors, whereas bases are proton acceptors. Most physiological and biochemical texts present the concept of pH in terms of the Arrhenius concept of pH which relates to hydrogen ion activity, and thus pH is determined by the flux of hydrogen ions between various hydrogen ion "carriers."

1.3 The Henderson-Hasselbalch Equation

The Henderson-Hasselbalch or traditional approach has dominated clinical acid-base evaluation over the past 90 years. This model satisfies traditional suppositions and dogma regarding acid-base physiology[22].

In mammals, the blood pH is expressed in terms of the equation as it pertains to the bicarbonate buffer system. This is known as the Henderson-Hasselbalch equation[1, 12]. The Bicarbonate buffer system is by far the most important buffer system in mammals. In short, CO_2 is rapidly converted to carbonic acid (H_2CO_3) *in vivo* by the enzyme carbonic anhydrase[1]. There are approximately 340 molecules of dissolved CO_2 for every one molecule of H_2CO_3. Carbonic acid readily dissociates at physiological pH to HCO_3^- and the number of molecules of carbonic acid is thus negligible and therefore CO_2 is expressed as the acid and HCO_3^- the salt (normally $NaHCO_3^-$). As such, the Henderson-Hasselbalch equation can be derived as follows:

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

And therefore pH defined as follows:

$$pH = pKá + Log\ [HCO_3^-]/[H_2CO_2]$$

Summarised for simplicity as follows

$$pH = pKa + Log\ [HCO_3^-]/Sx[CO_2]$$

This approach summarizes acid-base disturbances in terms of fluctuations in CO_2 and HCO_3^- and classifies them as respiratory or metabolic[1]. An increase in CO_2 is classified as a respiratory acidosis, whereas a decrease is classified as a respiratory alkalosis. An increase in HCO_3^- is classified as a metabolic alkalosis, whereas a decrease is classified as a metabolic acidosis. When acid-base disturbances occur, a compensatory response is observed in order to rectify the change in pH[1, 28]. Compensation is defined as physiological responses that serve to normalize blood pH. In the case of metabolic acid-base disturbances pH can be corrected by increasing or reducing the amount of CO_2 through ventilation. According to Henderson-Hasselbalch the metabolic component can be altered to compensate for respiratory acid-base disease through the regulation of bicarbonate, particularly in the kidney[1].

With this in mind, the clinician interprets acid-base disturbances by evaluating CO_2 and HCO_3^- levels, and determining if alterations have occurred. The clinician then considers the reciprocal values for the given disturbance in order to determine if compensation has occurred. Table 1 lists the primary disturbances and the expected responses for acid-base disturbances according to Henderson-Hasselbalch. Table 1 demonstrates the principle underlying the interpretation of the Henderson-Hasselbalch approach.

Table 1.1 Observed and expected responses seen in simple acid base disturbances

Disorder	pH	[$H^{activity}$]	Primary disturbance	Compensatory Response
METABOLIC ACIDOSIS	↓	↑	↓HCO_3^-	↓pCO_2
METABOLIC ALKALOSIS	↑	↓	↑HCO_3^-	↑pCO_2
RESPIRATORY ACIDOSIS	↓	↑	↑pCO_2	↑ HCO_3^-
RESPIRATORY ALKALOSIS	↑	↓	↓pCO_2	↓HCO_3^-

[1]

Compensatory responses have been measured in dogs and cats and allow clinicians to estimate whether changes in pCO_2 or HCO_3^- are appropriate. These data quantify the expected changes in pCO_2 and HCO_3^- in response to simple acid base disturbances. These data assist clinicians in deciding if the compensation observed is appropriate. For example in dogs, a 1mEq increase in HCO_3^- is expected to induce a 0.7 mmHg increment in pCO_2, whereas a 1mEq decrease in HCO_3^- will induce a 0.7mm Hg decrement in pCO_2. The compensatory responses in dogs and cats are shown in table 1.2.[1].

Table 1.2 Theoretical Compensatory Response in Simple-Acid Base disturbances in Dogs and Cats

Disturbance	Primary Change	Dogs	Cats
Metabolic Acidosis	Each 1mEq ↓HCO_3^-	pCO_2 ↓0.7 mmHg	pCO_2 does not change
Metabolic Alkalosis	Each 1mEq ↑HCO_3^-	pCO_2 ↑0.7 mmHg	pCO_2 ↑0.7 mmHg
Respiratory Acidosis:			
Acute	Each 1mmHg ↑ pCO_2	0.15 mEq ↑HCO_3^-	0.15 mEq ↑HCO_3^-
Chronic	Each 1mmHg ↑ pCO_2	0.35 mEq ↑HCO_3^-	Unknown
Long –standing	Each 1mmHg ↑ pCO_2	0.55 mEq ↑HCO_3^-	Unknown
Respiratory Alkalosis			
Acute	Each 1mmHg ↓pCO_2	HCO_3^- ↓ 0.25mEq	HCO_3^- ↓ 0.25mEq
Chronic	Each 1mmHg ↓pCO_2	HCO_3^- ↓ 0.55mEq	Similar to dogs

[1]

Contrary to dogs and humans, cats do not appear to adequately respond to metabolic acidosis by reducing pCO_2. In one study cats were fed a diet containing NH_4Cl which induced significant changes in HCO_3^- and pH, but no changes in pCO_2 were observed. It is not entirely clear why cats fail to compensate in the face of metabolic acidosis and until this phenomenon is further elucidated the finding of acidosis with a normal pCO_2 should not be interpreted as a mixed acid-base disturbance[1]. An important concept regarding compensation is the notion that physiological responses to acid-base disturbances do not over compensate. When changes in CO_2 and HCO_3^- occur that are not appropriate for the given primary change as summarized by table 1.2, the disorder is classified as a mixed disorder. Mixed acid base disorders commonly occur in the clinical setting. A number of scenarios are possible that will give rise to a mixed disorder characterized by marked changes in blood gas data. Depending on the combination observed, little or no change to the pH or additive effects may be seen. These mixed disorders are shown in table 1.3

Table 1.3 Mixed Acid-Base Disorders

Mixed Disorder	Effect on Blood pH
Respiratory Acidosis and Metabolic Alkalosis	Neutralizing
Respiratory Alkalosis and Metabolic Acidosis	Neutralizing
Metabolic acidosis and Metabolic Alkalosis	Neutralizing
Respiratory Acidosis and Metabolic acidosis	Decrease
Respiratory Alkalosis and Metabolic Alkalosis	Increase
Metabolic Acidosis, Alkalosis and Respiratory acidosis	Normal or decreased
Metabolic Acidosis, Alkalosis and Respiratory Alkalosis	Normal or increased.

[34]

The Henderson-Hasselbalch approach to interpreting blood gas data has a number of advantages. Firstly it is simple to understand and is interpreted by evaluating the pH, pCO_2 and HCO_3^- of a patient. Each variable is assessed and if the compensation is appropriated the acid-base disturbance is classified as simple. If a normal pH is observed in the face of abnormal pCO_2 and HCO_3^- the disturbance is classified as a mixed acid base disturbance (except in cats where a metabolic acidosis without pCO_2 compensation is observed). In addition, this approach is considered to be accurate in terms of quantitative assessment of acid-base disturbances. Algebraically complex models such as the Strong Ion Model appear to offer no advantage over Henderson-Hasselbalch in quantifying the magnitude of a pH disturbance[7, 22, 35-37].

1.3.1 The base excess principle

From the table above it is evident that a number of complex acid-base clinical scenarios are possible, which cannot be adequately understood in terms of Henderson-Hasselbach alone. To this end the concept of base excess (BE) was developed in 1960[38,39].The BE is defined as the amount of strong acid or base required to titrate 1 L

of fully oxygenated blood to a pH of 7.4 at 37°C while pCO_2 is held at 40 mmHg. Because pCO_2 is kept constant BE is only changed by non-volatile or fixed acids and thus changes in BE are considered to reflect metabolic acid base disturbances. The BE principle is frequently used in conjunction with Henderson-Hasselbalch and calculated by many blood gas analysers[7].

The weakness of the BE concept is based on the fact that an *in vitro* titration cannot necessarily be extrapolated to an *in vivo* situation. BE is a reflection of the buffering capacity of blood isolated from the rest of the body and therefore cannot assess the contribution of intracellular buffers and organ systems to the acid-base status as a patient[7]. For example, a decrease in HCO_3^- may be due to the kidney compensating for a respiratory acidosis and not a metabolic acidosis *per se*[7]. The other weakness of Henderson-Hasselbalch and the BE concept is that it gives little information as to the underlying pathogenesis of the metabolic acid-base component[7, 18, 36, 37]. Furthermore, when Siggard-Anderson developed the BE model, it was determined at a constant protein concentration. BE, therefore fails to consider protein and intracellular phosphate buffers, which may cause clinically significant alterations in acid-base balance[37]. The BE of a patient is a calculated value that is derived from a normogram developed by Siggard-Andersen, which was developed in human blood and is calculated from the pH, haemoglobin concentration and actual HCO_3^-. In the normal patient the arbitrarily assigned value is 0 mEq/L. In domestic animals normal BE values violate the original BE concept, for example in dogs where the normal BE has been calculated as -6.6 mEq/L[7]. Notwithstanding these difficulties, the BE is considered to provide a fairly accurate quantification of the metabolic component and appears to correlate well with the strong ion model (discussed later)[37], however the BE is a quantitative estimation and poorly mechanistic in its assessment of metabolic acid-base disturbances[7, 36]. Clinicians desire to avail themselves of pathophysiological data, which may assist them in correcting pH disorders by addressing the underlying problem. For example, in hyperchloraemic acidosis the Hendersen-Hasselbalch equation and the BE concept will indicate an acidosis, but the actual cause of the disturbance is not evident using this method.

In summary, the weaknesses of the BE concept are: 1) it is not altered by changes in plasma proteins, 2) it assumes buffering of blood can be extrapolated to the whole body and 3) it doesn't provide much insight into the mechanisms of acid base disturbances, especially mixed disorders[7].

1.3.2 The anion gap and the law of electroneutrality

The anion gap (AG) arose from the need for clinicians to obtain more information as to the mechanisms underlying the metabolic component of acid-base disorders. In particular, it represented an attempt to quantify unmeasured organic acids such as lactate[39]. It is calculated by subtracting the major circulating cations from the major circulating anions. The anion gap is thus expressed as follows:

$$AG = (Na + K + UC) – (Cl + HCO_3^- + UA) = 0$$

Note that there is in fact no AG in reality, since the sum of all cations and anions in a solution is always equal to zero, to satisfy the law of electroneutrality. The law of electroneutrality states that the net charge of an ionic solution is always equal to zero[17, 22, 40].

The advantage of the AG is that in normochloraemic, normoproteinaemic patients, changes in the AG roughly reflect alterations in unmeasured anions (UA)[37, 40]. For example in a patient with diabetic ketoacidosis with severe dehydration a markedly increased anion gap roughly approximates the production of ketones such as aceto-acetate and beta-hydroxybutarate, due to lipolysis and ketogenesis and organic acids such as lactate, due to hypoperfusion. These data can be used to track success of treatment in correcting these imbalances[40]. The normal anion gap in dogs is 12-24 mmol[40].

The main disadvantage of the anion gap, that hinders its clinical usefulness, is that its estimation of unmeasured anions is only valid in patients with normal protein and chloride concentrations. Consider the following scenario. A patient has severe diarrhoea and vomiting and is on fluid therapy (Sodium Chloride 0.9%). The patient is hypovolaemic and blood gas data reveal an acidaemia with a significant decrease in BE. The clinician suspects that tissue lactate production has been augmented due to

tissue hypoxia and an increased flux of pyruvate to lactate due to an accumulation of NADH as a result of decreased oxidative phosphorylation. An AG is calculated which is normal. The clinician deduces that the lactate levels are thus normal and the resuscitative efforts are working, but cannot explain the reduced pH. However, closer inspection reveals that the patient is hyperchloraemic and hypoproteinaemic which effectively reduces the anion gap. In this scenario severe acid-base disturbances are present but the AG is not at all clinically useful. Chloride fluctuations are seen in association with reciprocal changes in bicarbonate levels which serves to maintain the AG at a normal level. For example addition of HCl to plasma which increases plasma chloride is associated with a reciprocal decrease in HCO_3^- and the sum of chloride and HCO_3^- therefore remains the same and the anion gap doesn't change. This is classified as hyperchloraemic normal anion gap acidosis[40].

Because the net charge of plasma proteins in dogs is negative[41], they serve as unmeasured anions denoted as A^-. Decreases in A^- (mostly albumin) results in a reciprocal increase in HCO_3^- to fill the protein gap. This decreases the anion gap. Further confusion arises when one considers that alterations in pH effect the ionization of proteins. Therefore in an acidic environment, albumin will tend towards H-Albumin as apposed to $Albumin^- + H^+$ which in itself can alter the AG[40].

AG is thus for the most part clinically unhelpful in complex acid-base disturbances, especially in patients with abnormal protein or chloride levels.

Net gain or loss of cations or anions in a solution are associated with changes in the H^+ and OH^- and therefore pH changes. This observation forms the basis of the strong ion model where pH is said to change to compensate for the change in charge, by altering the dissociation constant of water[16-18, 22, 36, 37]. Therefore a patient that gains chloride ions through a sodium chloride solution will become acidotic, due to generation of H^+ to maintain the law of electroneutrality[17, 42]. Stewart's physicochemical model of water dissociation has been an area of contention among acid-base investigators[22, 24]. Subsequent to the initial publication by Stewart, the model was vehemently refuted, since it contravened traditionally accepted dogma regarding acid-base physiology. In

particular Stewart's definition of an acid or base violated the concept of acidity in terms of the Bronsted-Lowry definition.

1.4 The Stewart Strong Ion Approach

A central theme in the understanding of the Stewart model is the concept of dependant and independent variables[6, 7, 13, 17, 18, 24, 31-33, 43-45]. An independent variable changes the system without being changed itself. A dependent variable such as HCO_3^- is altered by the independent variables when they change. For example, HCO_3^- concentrations are directly altered by changes in by pCO_2 and not *visa versa*. The traditional approach to acid-base physiology attributes changes in pH to fluctuations in pCO_2 and HCO_3^-. According to Stewart, the partial pressure of carbon dioxide is determined by ventilation. Fluctuations in pH do not affect pCO_2 directly – they affect ventilation secondary to the change in $[H^+]$. Chemoreceptors in the aortic and carotid bodies and the afferent autonomic neurons that supply these receptors transmit information to the respiratory centers in the brainstem, which in turn modulates ventilation. As such pCO_2 can be described as an independent variable[7]. This concept is paramount in the patho-aetiological understanding of acid-base physiology. The Henderson-Hasselbalch equation therefore (according to Stewart) considers only one independent variable, namely pCO_2. In 1948 Singer and Hastings proposed that plasma pH was determined by the 2 independent variables, pCO_2 and net strong ion difference (SID)[46]. This concept was ultimately developed and expanded by Peter Stewart, who proposed that a third variable, plasma concentrations of weak buffers (albumin, globulin and phosphate) also exerted and independent effect on plasma pH[17]. This variable denoted A_{tot} is largely due to plasma proteins such as albumin. These weak acids exert their effect by resulting in reciprocal changes in anions such as bicarbonate, and thus a hypoalbuminaemia will result in an alkalosis. Other weak acids such as phosphate may also contribute to the A_{tot} in the clinical setting.

The fundamental basis of the Stewart approach relates to the theory of the effect of strong ions in plasma. Strong ions (e.g. Na^+, K^+, Cl^-, SO_4^{2-}) are almost completely dissociated in biological fluids. Strong ions such as electrolytes exert their effect by serving as a point of collective charge[30, 40]. In humans and animals the strong ion

difference SID which is calculated by subtracting the major anions (such as Cl^-, lactate, keto-anions and SO_4^{2-}) from the major cations (Na^+, K^+, Mg^2 and Ca^{2+}) is normally slightly positive[30, 40]. Because the law of electroneutrality has to maintained, a solution cannot in fact have a net charge, and the 'balance' of charge is made up mostly by plasma proteins. The basis of the SID and pH changes is elucidated in terms of Stewart's theory of the physicochemical effect of SID on water dissociation. According to Stewart the SID exerts a powerful effect on water dissociation thus altering the H^+ concentration in order to maintain a neutral charge in the solution[17]. The relationship between strong ions and pH according to Stewart is illustrated by the following reaction:

$$NaCl + H_2O \rightarrow Na^+ + Cl^- + H^+ + OH^-$$

Which can be summarized as follows:

$$SID + [H^+] - [OH^-] = 0$$

Note that electroneutrality is maintained in this instance. H^+ and OH^- are the dependant variables in this system and are affected by changes in the SID, whereas sodium and chloride are the independent variables and are affected by changes outside of the system, which in turn have effects within the system[8]. The change in $[H^+]$ and therefore pH comes about as a result of the generation of H^+ from the dissociation of water, and it is in this manner that strong ions are able to change pH. It is through this mechanism that dilution acidosis and concentrating alkalosis occur, since gain or loss of pure water effects the ratio of electrolytes due their varying concentrations thereby changing the SID. Water dissociation therefore has to correct the ion difference thereby changing the pH[7, 13, 15, 17, 18, 24, 30, 31, 36, 37, 47].

The physicochemical Stewart model therefore implies that $[H^+]$ and therefore pH cannot be altered by changing the concentration of H^+ or OH^- but only by changing the independent variables, since water is able to yield a large amount of H^+ and OH^- by changing its dissociation constant[36]. Resultantly, in strong ion acidosis, the gain of chloride ions rather than hydrogen ions leads to an acidosis. Avid proponents of the Stewart model have surmised that low pH in the stomach is achieved by chloride ion pumping and not by hydrogen ion movement *per se*[36]. The clinical relevance of this

principle is that clinicians are not always aware of the clinical consequences of electrolyte imbalances in terms of acid-base disturbances.

Using the strong ion model there are six classifications for pH disturbances namely: 1) Respiratory acidosis, 2) Respiratory alkalosis, 3) increased SID alkalosis, 4) Decreased SID acidosis, 5) increased A_{tot} acidosis and 6) decreased A_{tot} alkalosis[34].

Although the Stewart Approach is conceptually attractive, the accurate determination of the strong ion difference (SID) is mathematically complex, utilizing 5 constants, and impractical in a clinical setting[7]. The other disadvantage of the model is that acid-base status is expressed as hydrogen ion concentration as opposed to pH. The simplified strong ion model developed by Constable (2000) is mathematically simpler, but still more cumbersome than the Henderson-Hasselbalch equation in the clinical setting. Although the consensus among many acid-base investigators is that SID exerts a powerful effect on plasma pH[7, 16-18, 36, 37, 48] accurately determining its value is difficult since it is almost impossible to measure all strong ions in plasma. Notwithstanding these shortcomings, clinically practical estimates of SID can be obtained from subtracting at least four major plasma cations and anions[8]. Various researchers have measured SID in different ways varying in the number of ions used to arrive at a value for SID[8]. Regardless of the method used to calculate the SID the purported utility of the information gained from comparing the SID in health and disease, is to allow the clinician to determine whether strong ions are contributing to acid-base disturbances[7].

SID can also be assessed according to the base excess algorithm[34]. Because buffer base is, for the most part synonymous with SID[26], and base excess reflects the deviation from buffer base the base excess algorithm calculates changes in the base excess due to changes in phosphate, albumin, chloride and free water. The base excess algorithm can be calculated as shown in box 1. Differences between the calculated SID and the BE are presumed to reflect acid-base changes that result from the presence of unmeasured anions/cations.

The SID should be differentiated from the strong ion gap. The strong ion gap (SIG) utilizes the anion gap and plasma albumin concentrations to estimate the strong ion

difference. In essence the SIG corrects the AG for changing plasma protein levels and thus improves the accuracy in determining the contribution of unmeasured anions. Consequently SIG quantifies unmeasured anions. The SIG is also known as the "charge gap" is the same as the apparent SID (SID_a) in the absence of unmeasured anions[17]. Furthermore the SIG is not affected by changing plasma pH whilst the AG is[41]. The SIG equation is species specific due to interspecies variations in the net charge of plasma proteins. The SIG in dogs was calculated by Constable and Stämpfli (2005)[41] and can be expressed as follows:

$$SIG = [UC_{strong}] - [UA_{strong}]$$

Because the anion gap considers the predominate strong ions in plasma the SIG can be expressed as follows:

$$SIG = [A^-] – AG$$

Based on this relationship the SIG has been simplified in dogs based on a normal plasma pH of 7.4

$$SIG = [alb] \text{ X } 4.9 – AG$$

When SIG is less than -5mEq/L and increase in strong anions is suspected, which will lead to a strong ion acidosis [41]. Furthermore, the AG can be corrected in the presence of hyperphosphataemia (another weak plasma buffer) as follows:

$$AG + 2.52 – 0.58 \text{ X } [phosphate]$$

In summary, the clinical utility of the SIG is to estimate the contribution of unmeasured anions such as lactate and butyrate, in acid-base disturbances. When the acidosis is not attributable to an increase in unmeasured anions, then the SIG is equal to the SID_a[17].

The strong ion model integrates a number of physiological processes in its assessment of acid-base disturbances. The strength of the model is that it is more accurate in predicting the mechanisms underlying metabolic acid-base disturbances. For example, the Stewart Model is able to explain the effect of electrolyte disturbances (common

changes in the clinical setting) on blood pH. This is of particular relevance in the case of gastro-intestinal diseases such as secretory diarrhoea which is characterized by sodium loss. This is particularly evident in children with cholera, where the cholera toxin augments the activity of alpha-GTP. The net effect is luminal sodium flux in the intestine, which is subsequently lost due to osmotic diarrhea. Traditionally acid-base disturbances in the case of diarrhoea have been attributed to bicarbonate loss, however, Constable *et al.* (2005)[54] demonstrated the importance of electrolyte changes such sodium and changes in protein concentration in calves with diarrhoea, which challenges the dogma regarding calf scours and its treatment. In this study a consistent finding among the sick calves was hyponatraemia, and severe decreased SID acidosis [41]. Treatment directed at correcting the SID restores the acid base disturbance. In this instance, the authors contend, the Henderson-Hasselbalch equation has been successful in directing the treatment of calves with diarrhea, since bicarbonate changes are normally equivalent to changes in the SID and is thus a “measure” of SID in the presence of normal blood proteins[54]. The apparent success of bicarbonate therapy in this scenario is attributed to correction of the SID and not to correction of bicarbonate deficit *per se*[54]

Box 1: The base excess algorithm (taken from Hopper and Haskins, 2008)

Changes in BE Caused By Changes in $[A_{tot}]$ in mEq/L

Albumin Contribution:

Δ Albumin = 3.7 $([alb]_{normal} - [alb]_{patient})$

Phosphate Contribution:

Δ Phosphate = 1.8 $[phosphate]_{patient}$

Changes in BE Caused by Changes in SID in mEq/L

Contribution from free water:

Δ free water = 0.25 $([Na]_{patient} - [Na]_{normal})$

Contribution from Chloride:

Δ Chloride = $([Cl^-]_{normal} - [Cl^-]_{corrected})$

Contribution from Unidentified Strong Anions (Δ XA in mEq/L)

Δ XA = [BE]patient – (Δ albumin + Δ phosphate + Δ free water + Δ chloride)

1.4.1 Criticism of the strong ion model

The Stewart Strong Ion Model was the target of intense criticism following the publication of Peter Stewart's Book (How to Understand Acid-Base, 1980)[24]. Stewart's assertions that SID was a powerful determiner of H^+ were contrary to the accepted model of acid-base physiology. The ensuing debate led to the nomenclature "traditional

approach" and "modern approach" with reference to acid-base physiology, where Henderson-Hasselbalch is the traditional approach and Stewart's model is the modern approach. A fact worth mentioning at this juncture is that most of our understanding of acid-base physiology is derived from mathematical models. This is particularly true of the Stewart Model[22]. The difficulty in elucidating the precise mechanisms of acid-base physiology stems from the tremendously complex interplay of blood and cell-based buffers as well as organ systems like the lungs and kidneys. In addition, the effect of biological variables such as SID is difficult to assess *in vitro.* This is evident in the case of the strong ion model[22]. When Stewart developed his model, he was unable to use *in vitro* or *in vivo* evaluations to corroborate his hypotheses. Resultantly, the Stewart Strong Ion Model was determined mathematically and is entirely conjectural[24]. Whilst the model was initially not adopted due to its mathematical complexity, researchers such as Peter Constable have simplified the model for the clinical setting and thus facilitated its clinical utility. The growing consensus among many acid-base investigators is that the Strong Ion Model is mechanistically superior to the Henderson-Hasselbalch equation in the assessment of acid-base disturbances[7, 16-18, 37]. This assertion is largely based on the fact that the Stewart model considers factors "ignored" by the Henderson-Hasselbalch approach. For example, the Henderson-Hasselbalch equation summarizes all metabolic acid-base disturbances in terms of bicarbonate levels. As such, the clinician is limited in their assessment of the metabolic component in terms of attempting to explain the pathophysiological mechanism that caused it. Contention as to the manner in which blood pH is maintained has been a subject of debate. Ardent supporters of the HH approach have argued that the kidneys and lungs maintain blood pH by regulating pCO_2 and HCO_3^- and that SID equilibrates with bicarbonate and not *vice versa*[22]. They argue that SID is *not* regulated by physiological processes and amounts to an elaborate mathematical way of quantifying bicarbonate changes[22]. Conversely, Stewart's supporters maintain that SID is the most powerful determiner of pH and that bicarbonate titrates with SID and does not determine pH *per se* but rather is reflective of how the SID is changing[17].

The purported superiority of Stewart's model over the Henderson-Hasselbalch equation is that the Stewart approach is mechanistic.

1.5 The Role of the Lungs and kidneys in the Regulation of Acid-Base Homeostasis

Body pH is in part regulated by the inherent buffering abilities of blood. There are a number of organ systems, however, which are pivotal in the homeostasis of acid-base regulation particularly in the long term. As mentioned previously the pCO_2 of a patient is an independent variable. The pCO_2 of a patient is determined by ventilation and alveolar exchange. Hyperventilation will serve to reduce the pCO_2 by increasing the exchange of CO_2 with atmospheric air. Because CO_2 is readily diffusible, equilibrium is quickly reached within the alveolar capillaries. Increasing ventilation will therefore lower the alveolar pCO_2 and, in turn, the pCO_2 of the alveolar capillaries. The exchange of CO_2 is thus dependant on the respiratory rate and the integrity of the blood-air barrier. Conditions that augment alveolar or interstitial secretions/fluid, will reduce CO_2 exchange by increasing the distance over which CO_2 has to diffuse[4025].

The kidneys also play an important role in the maintenance of blood pH in a number of ways. The first is through the generation of HCO_3^- in the distal tubules. Bicarbonate combines with H^+ to form carbonic acid, which is converted to CO_2 and H_2O by luminal carbonic anhydrase, both of which are absorbed. In the tubular cell, CO_2 and H_2O are converted to carbonic acid again by carbonic anhydrase, which rapidly dissociates. The H^+ is pumped back into the tubular lumen in exchange for Na^+ whilst HCO_3^- is pumped into the blood in exchange for chloride. The other way in which the kidney maintains acid base is by controlling the concentrations of electrolytes that contribute to SID[40].

1.6 Consequences of Acid-Base Disturbances

As mentioned previously pH affects the ionization status of proteins. The function of a protein is determined by its tertiary structure which is a function of amino-acid sequence, temperature and ionization. The isoelectric point is the pH at which a protein has no net electrical charge, and this pH differs for each protein. Consequently, changing $[H^+]$ changes the ionization status of proteins and thus is able to alter their ability to function[1]. This notion is largely academic to the clinician, since its effects are not tangibly quantifiable – and somewhat abstract. However, the effects of acid base disturbances in biological systems are clinically significant. Extremes of pH will

profoundly disrupt metabolic processes, which are regulated by enzymatic reactions. Furthermore alterations in mentation may also be observed due to effects of pH on brain proteins.

In addition, other more far-reaching consequences of pH disturbances are being elucidated. Of interest is the growing body of evidence as to the potential inflammatory effects of pH disturbances. In particular, cytokine 'fingerprints' related to pH challenge are being investigated in experimental models. In a survey conducted in the US, where 4525 healthy adults where compared in terms of anion gap, bicarbonate levels and C-reactive protein. Their findings showed that, a higher serum anion gap and lower bicarbonate level were associated with higher levels of inflammatory biomarkers in a healthy sample of the general population[49]. Although no meaningful conclusions can be drawn from this study at this juncture, its does provide future impetus for investigations into the effects or consequences of pH disturbances where inflammation and immune response are concerned.

The potential effect of acid-base disturbances on the immune system is of particular significance in the case of canine parvoviral enteritis, since this disease often results in profound leukopaenia, which in the presence of increased gastro-intestinal permeability, results in susceptibility in these patients to bacterial sepsis.

1.6 Acid-Base Disturbances in Canine Parvoviral Enteritis

A paucity of literature is available on the acid-base disturbances in canine parvoviral enteritis. In 1986 Heald *et al.*[50] measured arterial blood gas and venous blood electrolyte data in 17 puppies admitted for parvoviral enteritis. Diagnostic criteria for admission to the study were: 1) Clinical signs of vomiting, haemorrhagic diarrhoea and dehydration 2) Haematological changes consistent with parvoviral enteritis: leukopaenia, neutropaenia 3) No previous vaccination and 4) significant titres (≥1:40) on serum HI test. The sample size was small, however, they documented fairly consistent changes in this group. Blood pH was within the normal range in 59% of cases. Of the remaining 7 patients, 6 of them were alkalaemic and 1 was acidaemic. The most consistent electrolyte change was hypochloraemia, which was present in 5 cases and all of these patients were alkalaemic. Hyponatraemia was present in 3 cases[50].

Interestingly these findings are different from those observed by Rai and Nauriyal (1992)[51] where a significant acidaemia was demonstrated in 21 cases of parvoviral enteritis, compared to their healthy controls. Furthermore a decrease in actual and standard bicarbonate and base excess were observed. Rai and Nauriyal compared venous blood gas samples, whereas Heald *et al.,* measured arterial samples. It bears mention however that Heald *et al.,* did not record any data obtained from healthy controls.

In a later study by Nappert *et al.* (2002) [52] arterial blood gas and D/L – lactate levels from puppies with parvoviral enteritis were compared to 22 healthy controls. In this study plasma pH was significantly increased in the parvoviral group but HCO_3^- was significantly decreased. The anion gap was also increased in the parvoviral group. The authors of this study attributed the pH increase to compensation, in the presence of decreased bicarbonate levels to compensation. Scrutiny of the pCO_2 levels compared to the HCO_3^- levels in the parvoviral group suggests that the decline in pCO_2 in the parvoviral group did not conform to the established compensatory values reported in the literature. A decrement of between 1 and 2 mmHg in pCO_2 would be expected in this study based on the average HCO_3^- levels, however in the study this decrement was closer to 10mmHg. The authors of this study did not consider the role of albumin and electrolytes in the acid-base balance of these patients. Of particular interest in this study is the fact that criteria for a profound metabolic acidosis were present (Decreased HCO_3^-, and increased lactate production) but the blood pH was in fact alkalaemic.

Intuitive appraisal of the metabolic derangements in parvoviral enteritis suggests that the acid-base disturbances that occur in this condition are complex and integrate all of the factors discussed so far (ie (pCO_2 ,SID, and A_{tot}) that can affect acid-base balance. Parvoviral enteritis in puppies is characterized by vomiting and diarrhoea with concomitant free water loss and dehydration (non-volatile buffer ion acidosis and lactic acidosis) electrolyte disturbances (SID changes) hypoalbuminaemia (A_{tot} alkalosis) and often anaemia due to blood loss, which will effect blood buffering.

1.7 Conclusion

Not much literature is available regarding the acid-base disturbances in CVP. In addition the pathomechanisms of pH disturbances have not, to the authors' knowledge, been fully elucidated in CPV. In the case of CPV profound metabolic acid-base disturbances would be expected with minimal changes in the respiratory compartment. Previous studies have consistently shown mild decreases in HCO_3^- levels indicative of a mild metabolic acidosis. Within this mild deviation of HCO_3^- it was postulated that the SIM would unmask profound mixed acid base disturbances which would be unapparent according to the HH model. Thus the purpose of this study was to utilize the SIM to dissect the metabolic compartment in patients with CPV to provide further insights into the pathogenesis of the acid-base disturbances in CPV. In many of the studies utilizing SIM, the data are analysed in conjunction with a cursory HH appraisal, and indeed in most cases the diagnosis of the disorder is based on the initial blood gas data. In this study blood gas data were not available and thus the authors were unable to directly compare the findings according to HH in this group of puppies. Therefore, this study was specifically directed at the non-respiratory (metabolic) compartment, to interrogate the data according to the SIM and to compare these findings to previous studies where HH was employed. This approach is valid as an initial exploration of the data because respiratory function has not been demonstrated to be altered in CVP, and consequently any changes in the respiratory contribution to acid-base balance are an attempt to compensate for a primary metabolic acid-base disturbance. A further study is being designed to compare the two models directly in a prospective trial. In addition a further advantage of assessing the utility of this model is the fact that blood gases are labile and the results of pH and HCO_3^- are affected by the pCO_2 levels, whereas electrolytes, albumin and phosphate are robust and can be assessed on stored serum. A major weakness of the study was the inability to directly compare the two models, and a major assumption of the study was that a mild metabolic acidosis would be present according to the HH which would be indicative of a mixed disorder.

2. Hypotheses and Rationale

2.1 Null and Alternate Hypotheses

To this end the following broad based hypotheses were constructed:

The null hypothesis was

- The Strong Ion Model will be similar to previous studies using the Henderson-Hasselbalch Model, where no consistent pattern of metabolic acid-base changes was evident. Expressed differently, there will be no apparent advantage in the application of the Strong Ion Model in the assessment of the metabolic acid-base changes in CPV.

The alternate hypothesis was:

- The Strong Ion Model will be superior in terms of assessing the underlying metabolic mechanisms of the acid-base disturbances in CPV.

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

In order to test these hypotheses a number of research questions were addressed in an attempt to unpack the complex acid base changes expected in CPV enteritis.

2.2 Research Questions

1. What was the nature of the metabolic acid-base derangement typical for puppies with clinically severe parvoviral enteritis, as per the Stewart method of evaluation?

2. Did the strong ion model provide additional pathomechanistic insights that would alter the way in which a clinician would manage a CPV case?

The author will scrutinize the data to assess the veracity of the following assumptions which have been made following a thorough review of the literature:

1. The Stewart approach will elucidate a complex mixed metabolic acid-base disturbance characterized by SID alkalosis and hypoalbuminaemic alkalosis, resulting in varying degrees of metabolic acidosis.
2. The sum of these effects will correlate with the severity of the disease score.
3. The Stewart approach will be more useful in assisting treatment decisions and interventions by the clinician at bedside.

2.3 Benefits Arising from the Study

Clarification of these assumptions will be of immeasurable clinical benefit because, given the cumbersome nature of the strong ion model, its utility in CPV will only be of value if it provides insights that are clinically valuable.

3. Materials and Methods

The study was retrospective and utilized data collected by Prof. JP Schoeman (JPS) from a previous study (with permission). 42 patients had sufficient data sets to be included in the study. Parvoviral enteritis was confirmed in all patients by electron microscopy which is considered the gold standard for diagnosis of canine parvovirus. Faecal analyses excluded the presence of giardia and coccidia as confounding factors in all patients. Coronavirus was excluded by EM. The age, breed and vaccination status of each patient was recorded. All puppies in the CPV group were unvaccinated dogs under 6 months of age. The control group consisted of 10 healthy, fully vaccinated puppies under 6 months of age. The control group data was collected at the time of the original study by JPS. Patients in the CPV group were categorized according to clinical severity using a clinical score previously described. The score assessed the severity of clinical disease according to vomiting, diarrhoea and appetite. The purpose of the score was to facilitate comparison of variables according to clinical severity to determine whether any of the SIM parameters were significantly different with varying levels of clinical severity.

Haematology, serum biochemistry and electrolyte data were generated in all the cases used for this study. All clinical pathology was performed in the laboratory of the Onderstepoort Veterinary Academic Hospital (OVAH) (Dept. of Clinical Pathology) which is an ISO-accredited reference laboratory. Serum samples were stored at -80ºC for subsequent analyses for both the CPV and the control group. For the purposes of this study two additional parameters were required, which were not measured in the original study, namely serum chloride and serum inorganic phosphate. Stored serum samples from the CPV and control groups were assayed for chloride and serum inorganic phosphate.

All statistics were generated using a commercial medical statistics software package (MedCalc® version 12.1.4). For all parameters tests for normality were performed, and non-parametric statistics were used for non-normal data (Mann-Whitney U test, Spearman's Rank Order Correlation, Linear Regression Analysis). Normal datasets were assessed using parametric statistics (Students T-test, Pearson's Correlation). All

statistics data were evaluated by an academic experienced in the area of epidemiology and biostatistics. (Prof. Peter Thompson, Department of Production Animals, Faculty Veterinary Science, Onderstepoort, University of Pretoria, South Africa).

3.2 Data Assessment According to the Strong Ion Model

3.2.1 Diagnosis of the acid-base disorder

To assess the acid-base data according to the strong ion model, a simplified strong ion approach was used which was derived from the approach suggested by Hopper and Haskins[53], which has been adapted from the original work of Leith and Fencl. In this model the strong ion difference is calculated by determining the contribution of the major circulating anions and cations to the acid-base load of the metabolic component. The parameters included are: free water (using the change in sodium), chloride change, phosphate change, albumin change and lactate. The formula is similar to that used in the base excess algorithm. The sum of these effects was determined for each individual as shown below:

Free water effect = $(Na_{patient} - Na_{normal}) \times 0.25$ in mEq/L

Chloride effect = $Cl_{patient} - (Cl_{patient} \times (Na_{patient} - Na_{normal}))$ in mEq/L

Lactate effect = $-1 \times lactate_{patient}$

Albumin effect = $(3 - albumin_{patient}$ in mg/dL) x 4 in mEq/L

Phosphate effect = $(phosphate_{patient} - phosphate_{normal}) \times 1.8$ in mEq/L

The sum of all of the above effects was then calculated for each individual and the diagnosis of the metabolic component was described as metabolic acidosis if the sum was less than -2.0 mEq/L or metabolic alkalosis if the sum was greater than 2.0 mEq/L. If the value of the sum was within the range of -2.0 – 2.0 mEq/L and there were significant changes in some or all of the effects which were neutralizing, the diagnosis of the metabolic component was described as neutralizing These cut off points were derived empirically and have not been validated in previous studies. Given the

contribution of many variables a tolerance range was needed to account for neutralizing effects.

Because the SIM evaluates 2 variables in the appraisal of the metabolic compartment, namely the SID and the A_{TOT} these were estimated from the above equation to yield a diagnosis of SID acidosis/alkalosis and A_{tot} acidosis/alkalosis. The SID was estimated by adding the chloride, lactate and free water effect. A negative value was deemed indicative of a SID acidosis, whereas a positive number indicated an SID alkalosis. To estimate the contribution of A_{TOT} the albumin and phosphate effects were added and negative values were considered an A_{TOT} acidosis and positive values an A_{TOT} alkalosis. A final diagnosis for the metabolic component was then reported as a metabolic acidosis, alkalosis, or neutralizing.

Given the relative complexity of the BE algorithm a simplified technique was evaluated where the SID and A_{TOT} where estimated using electrolyte data and albumin alone. These data are routinely collected as part of a minimum data base in the OVAH. The SID was first estimated using sodium, potassium and chloride. The value was designated SID_3.

$$(Na^+ + K^+) - Cl^-$$

This calculation was performed to assess its utility in CPV given the ease of calculation as opposed to the more complex technique described above. Using this method an increased SID would be consistent with a strong ion alkalosis and a decrease with a strong ion acidosis. This variable was then used in conjunction with the albumin effect alone which was used to assess the A_{TOT} component. Using these 2 variables a diagnosis of SID acidosis/alkalosis and A_{TOT} acidosis/alkalosis was reached. The diagnosis according to this method was compared to the Hopper and Haskins method using an inter-rater agreement plot with the following diagnostic groups for each method: SID acidosis A_{TOT} acidosis (A) SID acidosis A_{TOT} alkalosis (B) SID alkalosis A_{TOT} acidosis (C) and SID alkalosis A_{TOT} alkalosis (D). An additional simplified calculation was used which was exactly the same as described above, except for the fact that the corrected chloride value was used. This method requires an additional

calculation (to correct the chloride) and therefore is slightly more cumbersome than the above in a clinical setting.

Each of the effects contributing to the sum in the CPV group was compared to the control group. Furthermore, the mean value of the sum was compared to that of the control group.

Lastly, a simplified approach suggested by Constable[41] was assessed using SID and A_{TOT} values validated in canine plasma. The simplified constable method is far less cumbersome than the base excess algorithm. To estimate the SID, the SID_4 was calculated $(Na^+ + K^+) - (Cl^- +$ lactate). This value was then subtracted from the experimentally normal value previously determined (40 mEq/L). The A_{TOT} was estimated from albumin using by using previously described conversion factor:

$$A_{TOT} = \text{albumin} \times 0.425$$

The value obtained from this equation was then subtracted from the normal value determined for dogs which is 16 mEq/L[41]. This value represented the change from the normal value for $A_{TOT.}$ SID and A_{TOT} data obtained from this simplified method, were compared to the SID and A_{TOT} data obtained from the BE algorithm by means of a Students T test. Because the A_{TOT} calculated by Constable[41] was based on an albumin of 37g/L which did not necessarily represent the mid-reference range of the laboratory used in this study, the normal albumin value was estimated at 30g/L (based on control group's mean albumin concentration) representing an estimated A_{TOT} of 12.75 mEq/L. This value was substituted for the 16mEq/L value previously used and the A_{TOT} difference was calculated and compared to the data representing the A_{TOT} obtained from the BE algorithm.

3.2.2 Severity of the acid-base disorder

The variables from data groups stratified according to clinical score where compared for statistically significant differences using a Student's T test. This was performed in order to determine if a different pattern of metabolic disturbances were present at varying degrees of disease severity.

3.2.3 Pathogenesis of the acid-base disorder

The pathogenesis of the acid-base disorder was determined according to the change in each compartment that contributed to the sum. Significant changes in free water and chloride were deemed to be due to losses of fluid and electrolytes from the gastrointestinal tract (GIT). Elevated lactate was considered indicative of tissue hypoxia attributable to shock, due to anaerobic metabolism as a result of decreased perfusion of tissue. According to the SIM the pathogenesis of the metabolic disorder could be fully explained in terms of the contribution of strong ions (SID) and weak organic acids such as albumin and phosphate.

In order to elucidate the most important changes associated with acid base changes in the metabolic compartment Pearson's Correlations and linear regression analyses were performed to demonstrate the relationship between the sum and the chloride, free water, lactate, phosphate and albumin effect. The assumption of these tests was that significant contributors to the acid base changes according to SIM would be expected to be strongly correlated with the sum of effects.

4 Results

4.1 Strong-Ion Model

4.1.1 Diagnosis of the acid-base disorder

According to the SIM, 20/42 patients in the CPV group had a metabolic acidosis, 10/42 had a metabolic alkalosis and in 12/42 patients the overall effect was neutralizing. The derivation of the sum is shown in appendix 1. The diagnoses were then further classified according to whether it was characterized by strong ion acidosis/alkalosis and A_{tot} acidosis/alkalosis. Of the 20 patients with metabolic acidosis all 20 had a SID acidosis and within this group 19 had a concurrent A_{tot} alkalosis and 1 had a mild concurrent A_{tot} acidosis due to mild hyperphosphataemia. Of the 10 individuals with metabolic alkalosis (according to the sum) 9 had a SID alkalosis and 1 had a SID acidosis. All 9 of the individuals with SID alkalosis had a concurrent A_{tot} alkalosis. The 1 individual with the SID acidosis had a concurrent A_{tot} alkalosis (see appendix 2).

Within the neutralizing group 8 of the 12 had a SID acidosis and all 8 of these had an A_{tot} alkalosis. The remaining 4 individuals had a SID alkalosis and within this group 2 had an A_{tot} alkalosis and the remaining 2 had an A_{tot} acidosis. From these data it was concluded that the dominant metabolic acid base change was an acidosis characterized by a SID acidosis, which was partially offset by a concurrent A_{tot} alkalosis. Notwithstanding the number of significant changes in the metabolic compartment of the puppies with CPV the sum of effects was not significantly different from the control group.

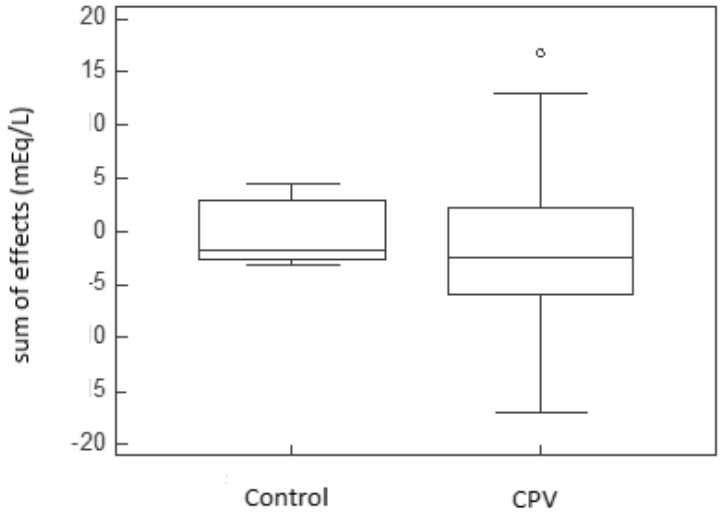

Figure 3.1 Comparison of sum of effects of CPV patients with healthy controls. In all graphs the boxes indicate interquartile range (IQR) the solid horizontal lines represent the median, and the whiskers 1.5 ± IQR. The data were not significantly different (p < 0.9).

Significant differences where noted between CPV and control group in the sodium levels and serum chloride and albumin (hyponatraemia and hypoalbuminaemia in CPV group) but not serum phosphate or lactate where there was considerable overlap between the CPV and control group.

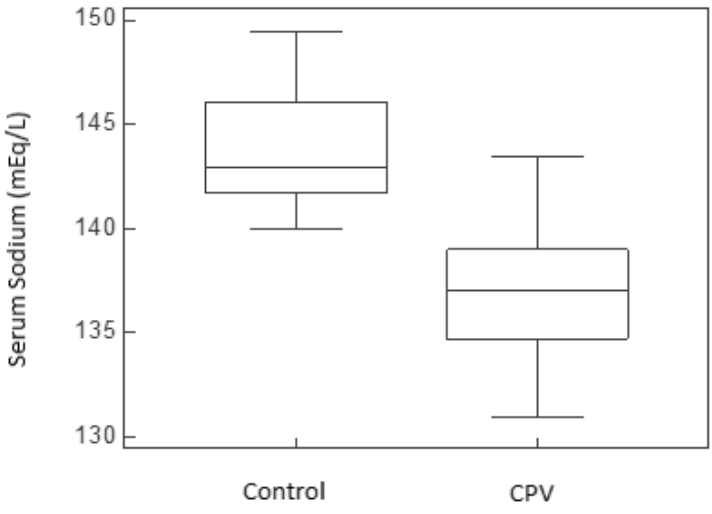

Figure 3.2 Comparison of sodium levels of CPV patients with healthy controls. The data are significantly different (p < 0.001, t-test)

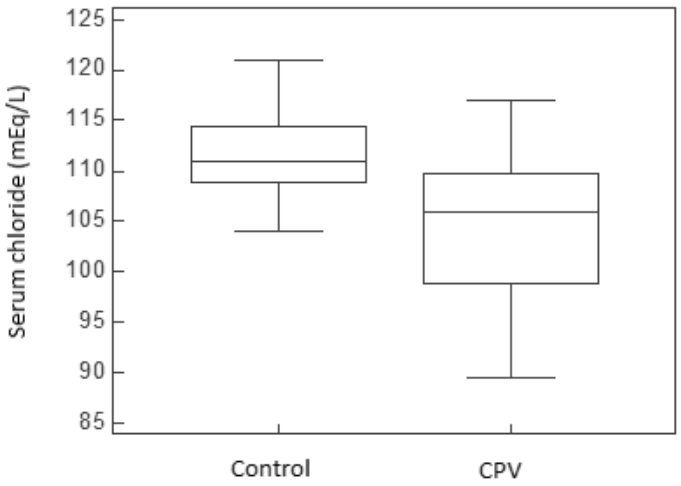

Figure 3.3 Comparison of chloride levels of CPV patients with healthy controls. The data are significantly different ($p < 0.001$).

Within the constituents of the sum, significant free water excess and albumin changes were noted when the data were compared to the controls, these data graphically represented below.

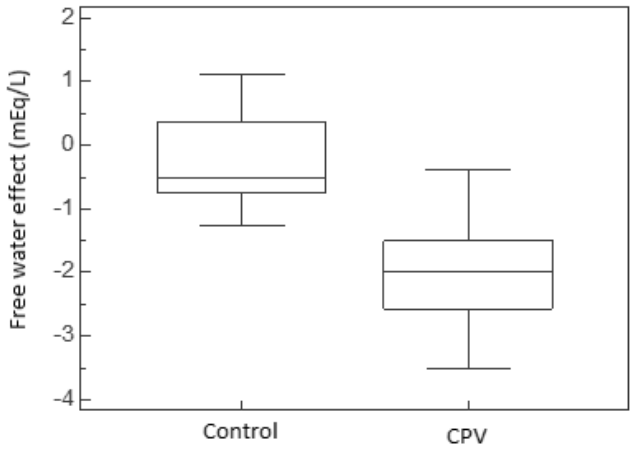

Figure 3.4 Comparison of the free water effect of CPV patients with healthy controls. The data are significantly different ($p < 0.001$, t-test)

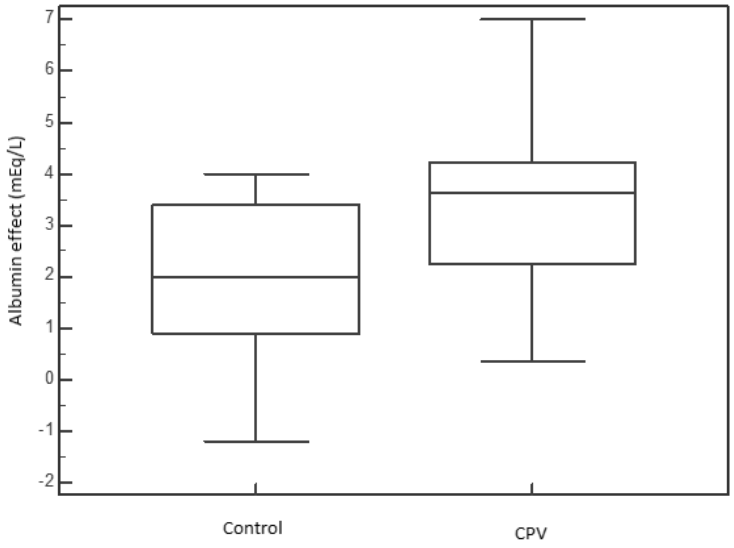

Figure 3.5 Comparison of the albumin effect of CPV patients with healthy controls. The data are significantly different (p = 0.004, t-test)

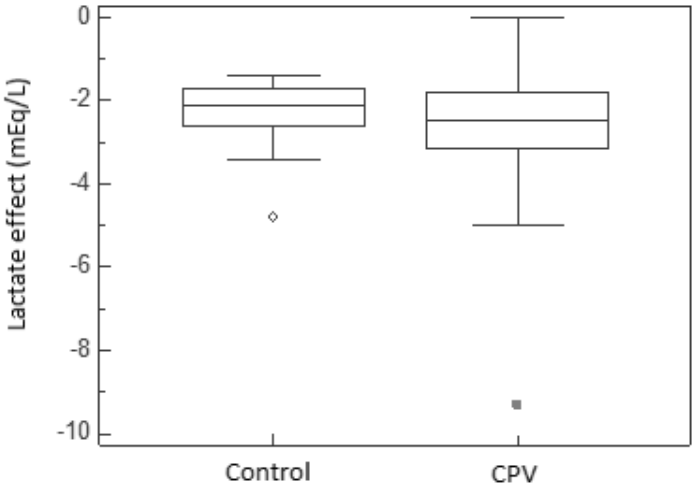

Figure 3.6 Comparison of the lactate effect of CPV patients with healthy controls. The data are not significantly different (p = 0,241)

When chloride levels were corrected for free water changes yielding the chloride gap or the true contribution of chloride, there was no statistical difference between the CPV and the control group as graphically represented below.

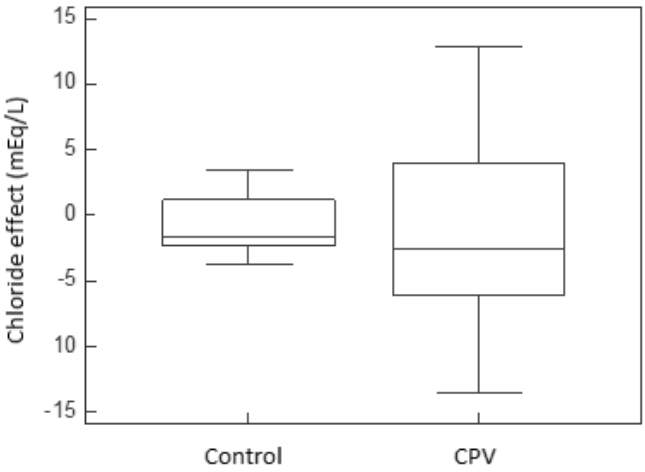

Figure 3.7 Comparison of the chloride effect of CPV patients with healthy controls. The data are not significantly different (p = 0.99, t-test)

4.1.2 Comparison of severity and clinical score

When the SIM data were compared according to clinical severity the only statistically significant difference was observed within the chloride effect. Individuals within the severe group tended to have a corrected hypochloraemic alkalosis, whereas those in the mild group tended to have a hyperchloraemic acidosis (see below).

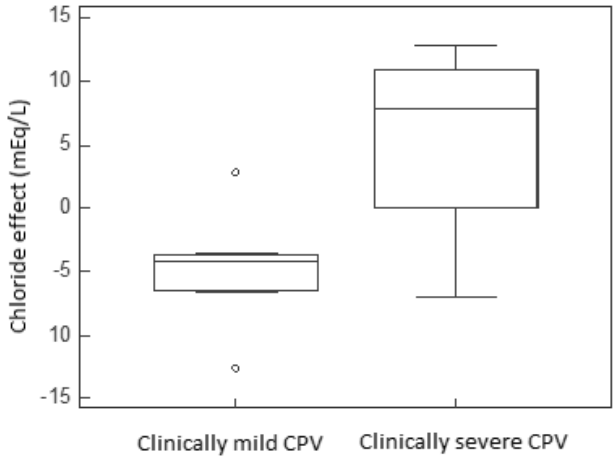

Figure 3.7 Comparison of the chloride effect of CPV patients with clinically mild and clinically severe CPV enteritis. The data are significantly different (p = 0.0077, t-test)

4.2 Pathogenesis of the disorder

4.2.1 Description of pathogenesis according to the SIM

According to the SIM model the pathogenesis affecting the changes in the metabolic compartment were significant electrolyte imbalances and protein loss. Chloride was the most important electrolyte. Significant changes in chloride were noted in most cases of CPV. Hypochloraemia (corrected) generally occurs secondary to loss of hypertonic fluid. Vomiting is a common cause of hypochloraemia and occurs as a result of chloride loss which is highly concentrated in gastric juice. Corrected hyperchloraemia may be due to chloride retention, or loss of chloride poor fluid. In the case of CPV the hyperchloraemia was judged to be due to the diarrhoea and excessive loss of sodium relative to chloride.

Significant changes in free water were also noted in many cases, and were due to a relative free water excess (FWE). A relative FWE may arise due to excessive administration of high water concentration fluids, or loss of fluids with a lower water concentration. In the case of CPV, all of the data were measured on the day of admission prior to the institution of aggressive fluid administration, and changes in free water were therefore due to the loss of water poor or solute rich fluids.

Another important contributor to the pathogenesis was albumin loss. In many cases where a strong ion acidosis was present the concurrent presence of hypoalbuminaemia was neutralizing. The application of the SIM facilitated a comprehensive description of the underlying pathogenesis of the metabolic component in CPV on a case by case basis (appendix 2).

4.2.2 Statistical assessment of the pathogenesis

Linear regression analyses of each of the abovementioned components and the sum indicated that chloride was the most significantly related with changes in the sum. Free water changes on the other hand correlated poorly with the sum, which was opposite to what would have been expected based on inspection of the significant differences between free water effect in the CPV and control group. The regression analyses of the remaining components and their respective p values are shown below.

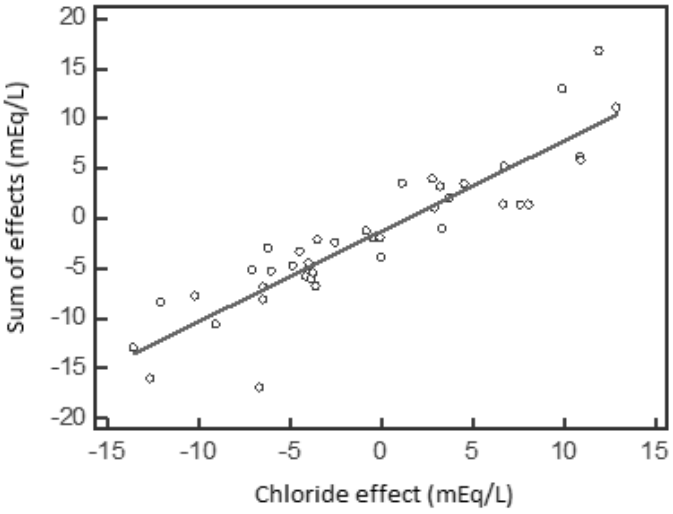

Figure 4.1 Linear regression analysis with chloride gap (or effect) as the independent variable and the sum as the dependent variable showed significant dependence of the sum on chloride changes ($p < 0.001$).

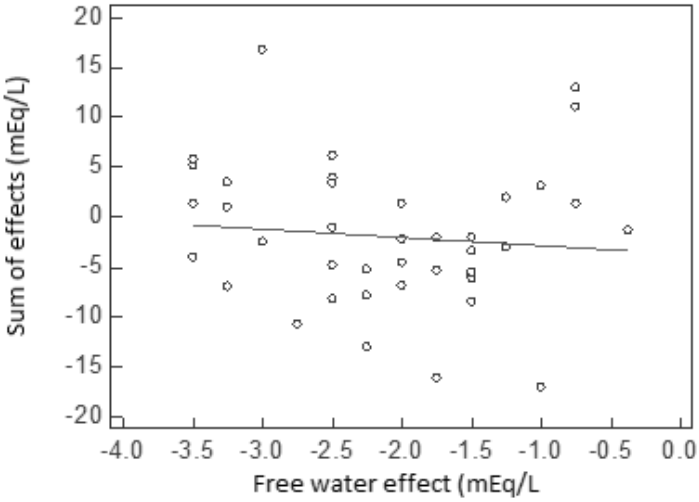

Figure 4.2 linear regression analysis of free water and the sum of effects. The relationship was not significant ($p = 0.106$)

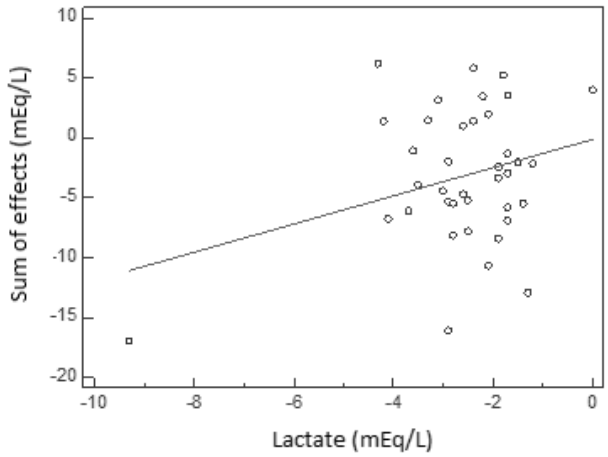

Figure 4.3 Linear regression analysis of lactate and the sum of effects. The relationship was not significant (p =0.06)

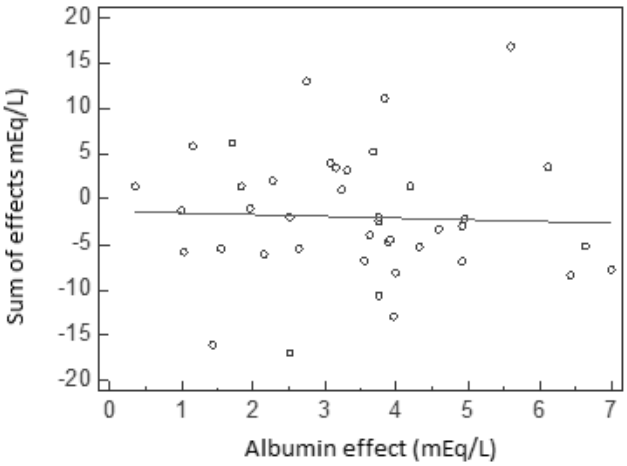

Figure 4.4 Linear regression analysis of the albumin effect and the sum. The relationship is not significant (p = 0.5)

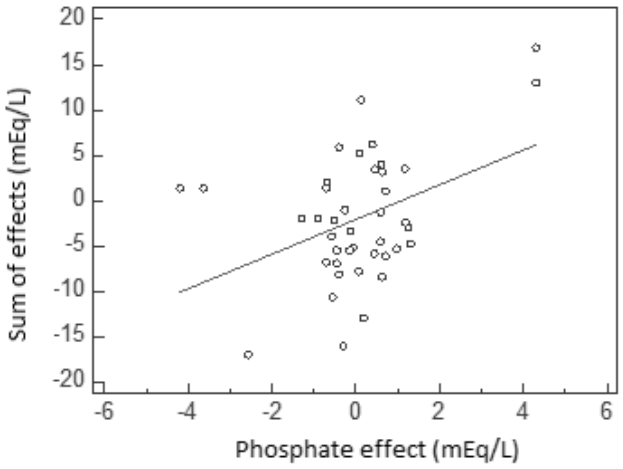

Figure 4.5 Linear regression analysis of the phosphate effect and the sum. Although the p – value suggests a significant value (p = 0.049) inspection of the curve indicates that the shape of the slope is due to a cluster of outliers. Removal of the outliers renders the relationship non-significant.

Therefore according to the SIM chloride was the most important determiner of the metabolic compartment. Expressed differently, chloride changes in terms of magnitude and direction of change, most consistently predicted changes in the sum and therefore the metabolic acid base compartment in CPV.

4.2.3 Diagnostic agreement between models

When the diagnoses reached with the SIM approach described by Hopper and Haskins was compared to the simplified approach utilizing electrolytes (without corrected chloride) and albumin as described above were compared a kappa plot was obtained: Briefly the inter-rater (kappa) agreement plot was obtained as follows. The mEq/L value obtained by calculating the sum of effects was assumed to represent the measure of acidity or alkalinity within the metabolic compartment (without the effects of unmeasured anions or cations). A negative number indicated an acidosis and a positive an alkalosis. Using the Hopper and Haskins method, the effects of free water, chloride and lactate were added and if this number was negative a strong ion acidosis was diagnosed (or alkalosis if positive). The same was then done for the A_{TOT} using the albumin and phosphate effect. In so doing the following diagnosis could be reached which were assigned letters A-D: strong ion acidosis A_{TOT} acidosis (A), strong ion acidosis A_{TOT}

alkalosis (B), strong ion alkalosis A_{TOT} acidosis (C) strong ion alkalosis A_{TOT} alkalosis (D). To arrive at the same diagnoses using the simplified method (SID_3) and albumin, the value of SID as estimated by the values of sodium and chloride (not corrected) and the albumin effect alone (denoting the A_{TOT}) were used. Both of these calculations are quick and easy to perform in the clinical setting. Using this method the diagnoses were assigned to the same categories (A-D) and were compared using the inter-rater agreement plot below. The x-axis indicates the categories according to the Hopper-Haskins method (designated SIM dx) and the y axis indicates the number of individuals in each category. The legend indicates the colours of the blocks and respective diagnoses (A-D) when the simplified technique was used (designated SID Dx). For example in the SIM dx category B (strong ion acidosis A_{TOT} alkalosis) 10 of those patients had the same diagnosis according to SID dx, and 16 were diagnosed as strong ion alkalosis A_{TOT} alkalosis (D). The variations in the diagnoses as indicated by the different colour blocks within the major diagnostic groups indicates that there is not good agreement between the two methods and that the simplified model may tend to under-estimate the presence of a strong ion acidosis.

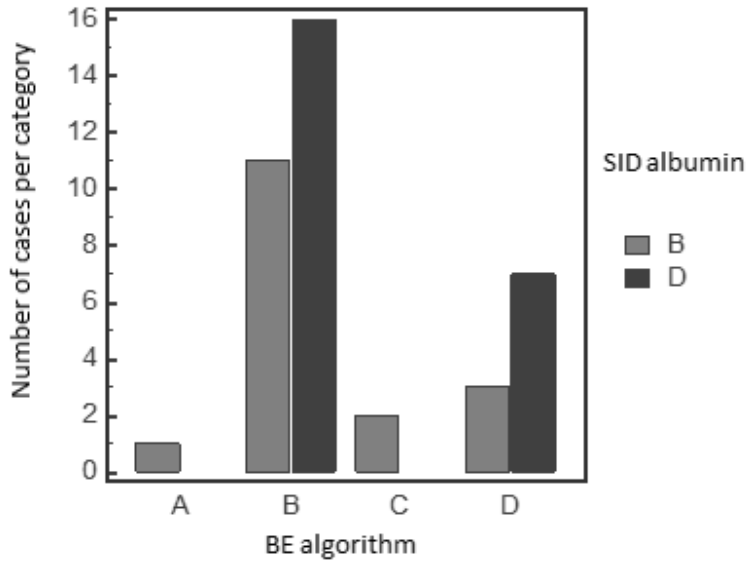

Figure 4.6 Inter-rater agreement plot assessing the diagnostic agreement between the BE algorithm and a simplified SID model, without corrected chloride. There was not good diagnostic agreement between these two models. The disagreement in the B column is significant, as many of the B individuals according to SIM were D's (SID alkalosis A_{tot} alkalosis) when the simplified model was applied. (Kappa = 0.062)

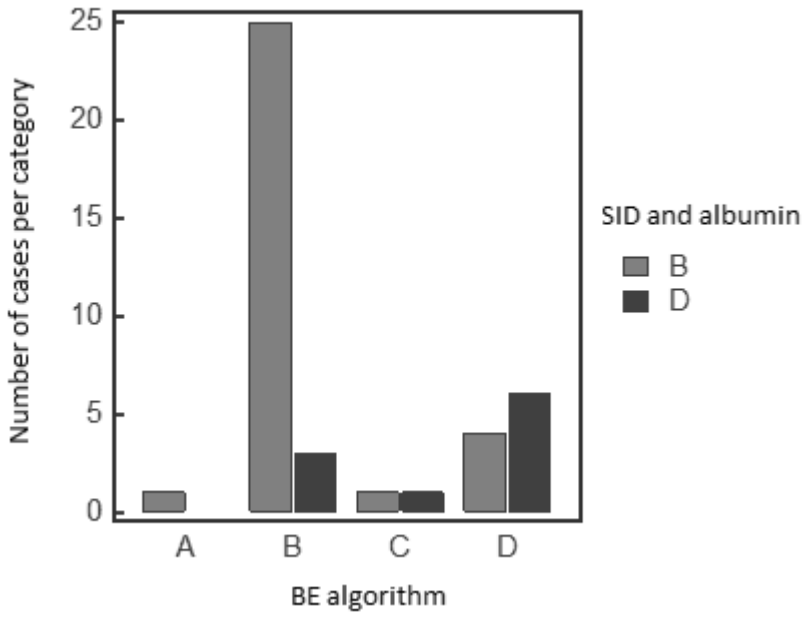

Figure 4.7 Inter-rate agreement plot assessing the diagnostic agreement between the BE algorithm model and the simplified SID model calculated using corrected chloride. Although the kappa number indicated moderate agreement (kappa = 0.47) the overall agreement was good. Both B and D indicate an A_{tot} alkalosis and differ in the SID diagnosis. Therefore, the simplified model tended to more frequently diagnose SID alkalosis compared to the SIM model.

When the kappa plot was repeated with a slight modification, which was the use of the corrected chloride value, and the above procedure was repeated (figure 4.7), the inter-rater agreement plot indicated good agreement between the two models. It was therefore concluded that by using sodium and corrected chloride alone, the value of the strong ion difference could be reliably estimated in CPV and that albumin alone would, for the most part predict the contribution of $A_{TOT.}$

The SID_4 of the CPV group was 35 ± 2.98mEq/L, compared to 39.9mEq/L which has been experimentally determined and 39.2 ± 6 mEq/l for the control group. The difference was statistically significant (p =0.01). Finally the data obtained from the BE algorithm were compared to the data for the same variables obtained using Constables' simplified strong ion model. The change in SID_4 ($SID_{patient}$ – 40mEq/L) obtained using this model was statistically compared to the SID estimated from summating the SID variables from the BE algorithm (sodium, chloride and lactate effect). The mean changes in SID were -6.022 ± 6.33 and -5.43 ± 6.91 mEq/L for the SID_4 and BE algorithm derived SID respectively.

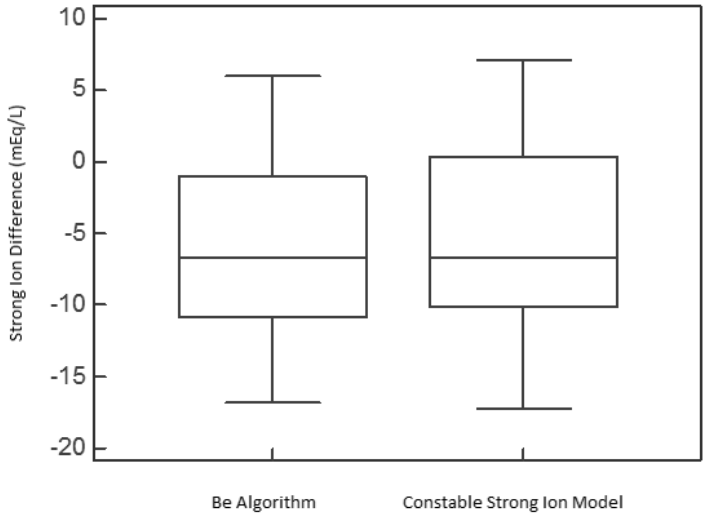

Figure 4.8 Box plot comparing the SID_4 obtained from Constable's strong ion model and the base excess algorithm. The data are not significantly different, and the distributions and means are highly similar.

A_{TOT} of the CPV group was 8.9 ± 2.9 mEq/L compared to 9.03 ± 6.1 mEq/L in the control group. The groups were not statistically different (p = 0.5) When the change in A_{TOT} calculated from the BE algorithm was compared to that obtained from the simplified strong ion model using published reference range the data sets were significantly different (figure 4.9.1). However, when the A_{TOT} was adjusted to an albumin level of 30g/L and the data were compared again, there was no statistically significant difference between the A_{TOT} values (Figure 4.9.2).

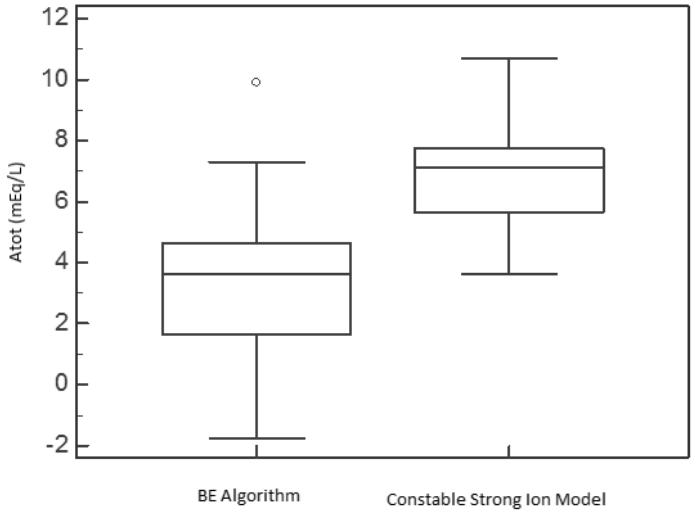

Figure 4.9.1 Comparison of the A_{TOT} derived from the BE algorithm and the simplified strong ion model in CPV. The data are significantly different, with the strong ion model indicating a bigger shift in the A_{TOT} value compared to the BE algorithm (p < 0.001).

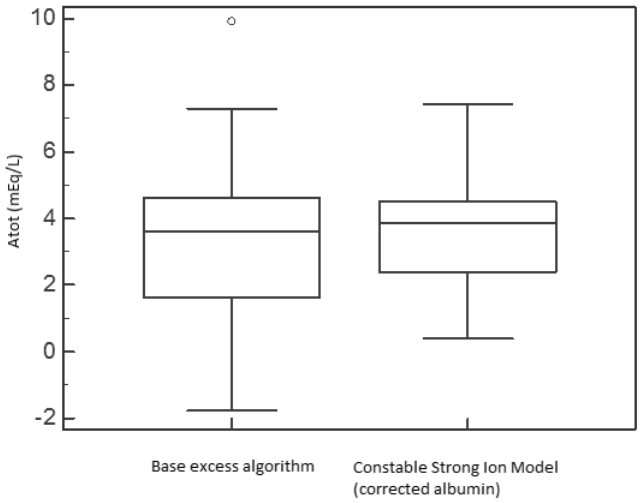

Figure 4.9.2 Comparison of the A_{TOT} derived from the BE algorithm and the simplified strong ion model in CPV. The data are not significantly different (p = 0.73)

5) Discussion

According to SIM significant strong ion changes were present in most cases and metabolic acidosis was the predominate change. The contribution of electrolyte changes as exemplified by the significant changes in free water and chloride were not surprising findings given the severity of fluid losses in CPV. Both vomitus and diarrhoea are electrolyte rich fluids and therefore these were expected to be abnormal in many cases as was demonstrated. Sodium was consistently low in the CPV group resulting in a relative free water excess. According to SIM principles a free water excess will invoke an acidosis due to a decrease in the strong ion difference (SID). Conversely, a free water deficit (such as in diabetes insipidus) would result in an increased SID alkalosis. Chloride was also significantly different in the CPV group and tended to be low compared to the control group. However, when the chloride was corrected for free water changes and used to calculate the chloride gap, there was no significant difference in the CPV and control group. This is due to the fact that many of the CPV patients corrected into the normal or hyperchloraemic range. In addition, an A_{tot} alkalosis was also common due to significant albumin losses. These findings gave insight into the complexity and variability of the acid base changes in CPV and explained why minor changes in bicarbonate concentrations have been observed in previous studies in the face of significant metabolic disturbances.

In this study lactate levels were not significantly different between the study and control group. This may be partly due to the narrow range of lactate and due to the fact that there was a large overlap between the groups. Stratification of the data according to clinical severity did not yield a significant difference between the groups in terms of lactate levels. This could be due to the fact that the groups were relatively small and that the small changes in lactate are clinically significant. Visual inspection of the data indicated a more consistently elevated lactate in the more severely affected group. This raises interesting questions as to the role of lactate in the pathogenesis of the acid-base disturbances in CPV. Further studies with larger numbers are needed to clarify this issue.

When the sum was correlated with its constituents the most significant relationship was with chloride. This was an interesting finding, since it would have been expected that a significant relationship would have been observed between the sum and free water changes, however this was not the case. Interestingly the two most consistently changed variables, namely sodium and albumin showed the least significant relationship with the sum. This finding appears to emphasise the significance of chloride changes in the pathogenesis of acid-base changes in CPV (and in acid base balance generally). Therefore, regardless of a consistent hyponatraemic acidosis and hypoalbuminaemic alkalosis, the direction and magnitude of the changes in chloride will determine the outcome of the sum in most cases.

The importance of chloride changes was further highlighted in this study by the fact that it was the only variable in which differences were observed according to clinical severity. The more severely effected puppies according to clinical score tended to have a hypochloraemic alkalosis, whereas more mildly affected individuals tended to have a hyperchloraemic acidosis. These changes may reflect differences in the frequency and severity of vomiting, as vomitus tends to be a chloride rich fluid. Further studies are needed to determine if chloride changes correlate with outcome. This could not be undertaken in this study due to the low number of individuals that succumbed to CPV in this data set. Given the fact that many CPV patients had low sodium and chloride levels and were alkalotic, perhaps 0.9% saline as the mainstay of intravenous fluid support deserves further attention given the fact that it is an acidifying solution and has a higher concentration of sodium and chloride.

The preponderance of metabolic acidosis is consistent with a previous study utilising the HH model to assess acid-base changes in CPV[51]. When these data were compared to previous studies using the HH technique the data appeared to be consistent in some respects with the previous findings[51, 52]. Most of the deviations in the sum were only a few mEq/L outside of the -2.0 to +2.0 mEq/L tolerance range. In the previous bicarbonate-centred approaches, similar mEq/L deviations were noted in bicarbonate[51, 52].

The significant electrolyte and albumin changes, which are not appreciated by the HH model led to the adoption of the hypothesis that the models would be different in terms of the qualitative analysis. If significant electrolyte changes and albumin changes were not present this hypothesis would have been rejected. The strong correlation of chloride and the sum was the main motivation for accepting this hypothesis. The bicarbonate-centred approach does not consider the role of chloride in acid-base disturbances. Therefore in acid-base diseases where no significant chloride changes are present such as diabetic ketoacidosis, the HH model (and anion gap) may not be significantly different from the SIM in the description of the acid base changes as both would attribute the changes in pH to unmeasured anions. Therefore the finding of severe and significant chloride changes automatically resulted in significant differences in the description of the pathogenesis of the acid-base changes in CPV according to the two models.

The assumption of this study was is that in the absence of unmeasured anions or cations (UA/C) that the sum will be roughly equal to the base excess (the base excess algorithm). In this study neither base excess nor bicarbonate levels were available and thus the data could not be directly compared to those derived from a standard HH evaluation. Therefore one of the benefits of the BE algorithm, namely the detection of UA/C was not possible. In addition blood pH was not available and therefore only metabolic acidosis/alkalosis could be determined according to SIM principles. When the sum was assumed to be equivalent to the BE a metabolic acidosis was the most common finding.

Application of Constable's strong ion model using the SID_4 yielded very similar results to the SID data obtained using the BE algorithm. A particular advantage of this technique was the fact that it is far easier to calculate. Furthermore, using this technique, the expected changes to plasma pH evoked by SID can be estimated[41]. When data experimentally obtained for A_{TOT} from the same study[41] were compared to the CPV group (16mEq/L) based on albumin concentrations, the CPV group was markedly lower, however, the control group was also markedly lower and not significantly different from the CPV group. Many of the control patients had low normal albumin values, possibly

related to age and undiagnosed worm burdens, and thus it was speculated that albumin may underestimate A_{TOT} in this setting, where it is significantly lower than the experimentally normal values for adult dogs. This highlights the importance of the establishment of normal range values for a particular test population. Notwithstanding this limitation, it was concluded that the Constable technique compares favourably with the more cumbersome BE algorithm. A central calculation in the Constable equation, ie the calculation of the SIG or effective SID could not be determined due to the fact that no anion gap data were available. This calculation would be of great interest in the CPV setting in a prospective study. Finally this study was able to demonstrate the utility of a simplified SIM technique making use of standard electrolyte and albumin data. When SID was calculated without corrected chloride there was significant diagnostic disagreement with the Hopper and Haskins technique. When SID was calculated with corrected chloride there was very good diagnostic agreement between the two models. This method, therefore, compared well with the more cumbersome Hopper and Haskins approach in reaching a diagnosis of strong ion and A_{tot} changes. This approach could be used at bedside and the data are obtainable from numerous chemistry machines which do not have the stringent sample handling requirements of standard blood gas analysis. A prospective study is needed to determine the relationship between bicarbonate and the strong ion difference in the appraisal of the metabolic compartment in CPV. This study was also able to demonstrate how acid-base analysis in a veterinary clinical setting can be approached in a different way and provide additional insights not apparent in a standard HH evaluation. Thus it was evident that understanding the SIM principles the SID could be calculated in a number of ways yielding similar results.

With reference to the research questions posed in this study it was determined that the acid base disturbances typical for CPV was a SID acidosis with a concomitant A_{tot} alkalosis. Changes in chloride were determined to be the most important in the acid-base disturbances in CPV. Of interest was the lack of correlation between the clinical score and the overall severity of the acid-base disturbance. This may be due to the complexity of the changes which often neutralize the metabolic compartment in severe mixed cases. Notwithstanding the lack of correlation in the sum, the chloride effect was significantly different between the mild and the severe group. Although this study was

not intended to investigate treatment recommendations, it did raise some questions as to the fluid selection in the treatment of CPV, given the consistently low sodium levels. urther studies are needed to further address this question.

It is difficult to determine whether the SIM would alter clinical decisions in the retrospective setting, however, this study enumerated panoply of different patho-mechanistic changes not appreciated in previous studies, and a prospective study is needed to further assess this aspect of the research questions.

6. Conclusions

The SIM findings were similar to those of previous HH studies demonstrating mixed metabolic disorders, but provided a wealth of additional patho-mechanistic information. It was concluded that the SIM model is conceptually superior in the evaluation of the acid-base disturbances in CPV given its broader and more in depth dissection of the metabolic compartment in CPV. Future direct comparisons may serve to fine tune some of the research questions which could not be conclusively answered.

7. References

1. Di Bartola SP. Chapter 9 - Introduction to Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Fourth Edition). Saint Louis: W.B. Saunders; 2012: 231-252.

2. Adrogue HJ, Gennari FJ, Galla JH, Madias NE. Assessing Acid-Base Disorders. Kidney International 2009;76: 1239-1247.

3. Boyle M, Baldwin I. Introduction to an Alternate View of acid/base Balance: The Strong Ion Difference Or Stewart Approach. Australian Critical Care 2002;15:14-20.

4. Cohen Y, Nimord A, Ascher-Landsberg J, et al. Reference Values for Strong Ion difference—A Novel Tool for Fetal Metabolic Assessment. European Journal of Obstetrics & Gynecology and Reproductive Biology 2009; 145: 145-148.

5. Constable PD. Strong Ion Difference: New Applications in the Study of Cattle Diseases: SIVAR National Congress - Cremona 7-8 may 2010. 2010; 16: 135-138.

6. Constable PD. Calculation of Variables Describing Plasma Nonvolatile Weak Acids for use in the Strong Ion Approach to Acid-Base Balance in Cattle. American Journal of Veterinary Research 2002; 63: 482-490.

7. Constable PD. Clinical Assessment of Acid-Base Status: Comparison of the Henderson-Hasselbalch and Strong Ion Approaches. Veterinary Clinical Pathology 2000; 29: 115-128.

8. Constable P. A Simplified Strong Ion Model for Acid-Base Equilibria: Application to Horse Plasma. Journal of Applied Physiology 1997; 83: 297-311.

9. Corley KTT, Marr CM. Pathophysiology, Assessment and Treatment of Acid-Base Disturbances in the Horse. Equine Veterinary Education 1998; 10: 255-265.

10. Coskun A, Sen I, Guzelbektes H, et al. Comparison of the Effects of Intravenous Administration of Isotonic and Hypertonic Sodium Bicarbonate Solutions on Venous Acid-Base Status in Dehydrated Calves with Strong Ion Acidosis. Journal of the American Veterinary Medical Association 2010; 236: 1098-1103.

11. Doberer D, Funk G, Kirchner K, Schneeweiss B. A Critique of Stewart's Approach: The Chemical Mechanisms of Dilutional Acidosis. Intensive care medicine 2009; 34:1528.

12. Durward A, Murdoch I. Understanding acid–base Balance. Current Paediatrics 2003; 13: 513-519.

13. Fencl V, Leith DE. Stewart's Quantitative Acid-Base Chemistry: Applications in Biology and Medicine. Respiratory Physiology 1993; 91: 1-16.

14. Gatz G. The Stewart Approach to Acid-Base Analysis: Not Disqualified Yet. Intensive care medicine 2009; 35: 212.

15. Greenbaum J, Nirmalan M. Acid–base Balance: Stewart's Physicochemical Approach. Current Anaesthesia & Critical Care 2005; 16: 133-135.

16. Gunnerson KJ, Kellum JA. Acid-Base and Electrolyte Analysis in Critically Ill Patients: Are we Ready for the New Millenium. Current Opinion in Critical Care 2003;9: 468-473.

17. Kellum JA. Disorders of Acid-Base Balance. Critical Care Medicine 2007; 35:2630-2636.

18. Kellum JA. Determinants of Plasma Acid-Base Balance. Critical Care Clinics 2005; 21: 329-346.

19. Kellum JA. Metabolic Acidosis in the Critically Ill: Lessons from Physical Chemistry. Kidney International Supplement 1998: S-81.

20. Kellum JA, Kramer DJ, Pinsky MR. Strong Ion Gap: A Methodology for Exploring Unexplained Anions. Journal of Critical Care 1995; 10: 51-55.

21. Kellum JA, Song M, Almasri E. Hyperchloremic Acidosis Increases Circulating Inflammatory Molecules in Experimental Sepsis. Chest 2006;130: 962-967.

22. Kurtz I, Kraut JA, Ornekian V, Nguyen M. Acid Base Analysis: Critique of the Stewart and Bicarbonate Centred Approaches. American Journal of Renal Physiology 2008;9: 1009-1031.

23. Derksen R, Scheffer GJ, van der Hoeven JG. Quantitative acid–base Physiology using the Stewart Model. does it Improve our Understanding of what is really Wrong? European Journal of Internal Medicine 2006; 17: 330-333.

24. Morgan TJ. The Stewart Approach - One Clinician's Perspective. Clinical Biochemistry Reviews 2009; 30: 41-54.

25. DiBartola SP. Chapter 10 - Metabolic Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Fourth Edition). Saint Louis: W.B. Saunders; 2012: 253-286.

26. de Morais HA, Leisewitz AL. Chapter 12 - Mixed Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Fourth Edition). Saint Louis: W.B. Saunders; 2012: 302-315.

27. de Morais HA, Bach JF, DiBartola SP. Metabolic Acid-Base Disorders in the Critical Care Unit. Veterinary Clinics of North America: Small Animal Practice 2008; 38: 559-574.

28. Neligan PJ, O'Donoghue R. 56 - How Should Acid-Base Disorders Be Diagnosed and Managed? In: Clifford S. Deutschman A2MS A2MD A2FCCM, Patrick J. Neligan, MA, MB,FRCARCSI, eds. Evidence-Based Practice of Critical Care. Philadelphia: W.B. Saunders; 2010: 389-396.

29. Srisawat N, Gunnerson KJ, Kellum JA. Can we really use the Stewart-Fencl Method to Analyze Acid-Base Derangement in Clinical Practice?—Author Response. Journal of Critical Care 2011;26: 97-98.

30. de Morais HA, Constable PD. Chapter 13 - Strong Ion Approach to Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Fourth Edition). Saint Louis: W.B. Saunders; 2012: 316-329.

31. Gilfix BM, Bique M, Magder S. A Physical Chemical Approach to the Analysis of Acid-Base Balance in the Clinical Setting. Journal of Critical Care 1993; 8: 187-197.

32. Kellum JA. Acid-Base Balance: Albumin and Strong Ions. In: Anaesthesia Science. Blackwell Publishing Ltd; 2006; 2008:188-197.

33. Story DA, Kellum JA. Acid-Base Balance Revisited: Stewart and Strong Ions. Seminars in Anesthesia, Perioperative Medicine and Pain 2005; 24: 9-16.

34. DiBartola SP. Chapter 10 - Metabolic Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Third Edition). Saint Louis: W.B. Saunders; 2006:251-283.

35. de Morais HA. Editorial: Has Stewart Finally Arrived in the Clinic. Journal of Veterinary Internal Medicine 2005;19: 489-490.

36. Sirker A, Rhodes A, Grounds R, Bennet E. Acid-Base Physiology; the "Traditional" and "Modern" Approaches. Anaesthesia 2002; 57: 348-356.

37. Wooten W. Science Review: Quantitative Acid-Base Physiology using the Stewart Model. Critical Care 2004; 8: 448-452.

38. SIGGAARD-ANDERSEN O, Engel K, Jorgensen K, Astrup P. Micro Method Determination of pH, Carbon Dioxide Tension, Base Excess and Standard Bicarbonate. Scandinavian Journal of Clinical Laboratory Investigation 1960; 12: 172-176.

39. Oh M, Carrol H. Current Concepts: The Anion Gap. New England Journal of Medicine 1977; 297: 814-817.

40. Engleking L. Textbook of veterinary physical chemistry, 1st ed. Oxford: Elsevier; 2004.

41. Constable PD, Stampfli H. Experimental Determination of Net Protein Charge and A_{Tot} and Ka of Non Volatile Buffers in Canine Plasma. Journal of Veterinary Internal Medicine 2005;19: 507-514.

42. Stewart PA. Independent and Dependent Variables of Acid-Base Control. Respiratory Physiology 1978;33: 9-26.

43. de Morais HA, Constable PD. Strong Ion Approach to Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Third Edition). Saint Louis: W.B. Saunders; 2006: 310-321.

44. Navarro M, Monreal L, Segura D, Armengou L, Añor S. A Comparison of Traditional and Quantitative Analysis of Acid-Base and Electrolyte Imbalances in Horses with Gastrointestinal Disorders. Journal of Veterinary Internal Medicine 2005; 19: 871-877.

45. Powner DJ, Kellum JA, Darby JM. Concepts of the Strong Ion Difference Applied to Large Volume Resuscitation. Journal of Intensive Care Medicine (Blackwell Publishing Limited) 2001;16: 169-176.

46. Singer R, Hastings A. An Improved Clinical Method for the Estimation of Disturbances of Acid-Base of Human Blood. Medicine 1948; 27: 223-242.

47. McCullough SM, Constable PD. Calculation of the Total Plasma Concentration of Nonvolatile Weak Acids and the Effective Dissociation Constant of Nonvolatile Buffers in Plasma for use in the Strong Ion Approach to Acid-Base Balance in Cats. American Journal of Veterinary Research 2003; 64: 1047-1051.

48. de Morais HA, Constable PD. Chapter 13 - Strong Ion Approach to Acid-Base Disorders. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice (Third Edition). Saint Louis: W.B. Saunders; 2006: 310-321.

49. Farwell WR, Taylor EN. Serum Anion Gap, Bicarbonate and Biomarkers of Inflammation in Healthy Individuals in a National Survey. CMAJ 2010;182:137-141.

50. Heald RD, Jones B, Schmidt D. Blood Gas and Electrolyte Concentrations in Canine Parvoviral Enteritis. Journal of the American Animal Hospital Association 1986;22:745-745-748.

51. Rai A, Nauriyal D. A Note on Acid-Base and Blood Gas Dynamics in Canine Parvoviral Enterits. Indian Journal of Veterinary Medicine 1992;12:87-87-88.

52. Nappert G, Dunphy E, Ruben D, Mann FA. Determination of Serum Organic Acids in Puppies with Naturally Acquired Parvoviral Enteritis. Can J Vet Res 2002; 66:15-18.

53. Hopper K, Haskins SC. A Case-Based Review of a Simplified Quantitative Approach to Acid-Base Analysis. Journal of Veterinary Emergency and Critical Care 2008; 18: 467-476.

54. Constable PD, Stampfli HR, Navetat H, Berchtold J, Schelder F. Use of a Quantitative Strong Ion Approach to Determine the Mechanism for Acid–Base Abnormalities in Sick Calves with or without Diarrhea. Journal of Veterinary Internal Medicine 2005; 19: 581-589

Appendix 1: Calculation of the contribution of each of the SIM components to the sum of effects

Na	Cl	Corr CL	Chloride Gap	free water effect	alb effect	PO4 effect	lactate effect	SIDapp	Sum
139.00	**109.4**	114.12	-4.12	-1.5	1.04	0.45	-1.7	-5.62	-5.83
138.00	105.10	110.43	-0.43	-1.75	2.52	-0.882	-1.5	-2.18	-2.04
135.00	108.4	116.42	-6.42	-2.5	4	-0.396	-2.8	-8.92	-8.1
141.00	113.4	116.61	-6.61	-1	2.52	-2.556	-9.3	-7.61	-16.95
136.00	112.7	120.15	-10.15	-2.25	7	0.072	-2.5	-12.40	-7.83
135.00	99.8	107.19	2.80	-2.5	3.08	0.612	0	0.30	3.99
135.00	98.2	105.47	4.52	-2.5	3.16	0.468	-2.2	2.02	3.45
134.00	110	119.02	-9.02	-2.75	3.76	-0.54	-2.1	-11.77	-10.66
139.00	117	122.05	-12.05	-1.5	6.44	0.63	-1.9	-13.55	-8.38
137.00	107.3	113.56	-3.56	-2	3.56	-0.702	-4.1	-5.56	-6.80
142.00	99.8	101.90	8.091	-0.75	1.84	-3.618	-4.2	7.34	1.36
140.00	112.2	116.20	-6.20	-1.25	4.92	1.26	-1.7	-7.45	-2.97
132.00	97.5	107.10	2.89	-3.25	3.24	0.72	-2.6	-0.35	1.00
132.00	99.1	108.85	1.140	-3.25	6.12	1.188	-1.7	-2.10	3.49
136.00	109.8	117.06	-7.06	-2.25	6.64	-0.054	-2.5	-9.31	-5.23
131.00	99.4	110.02	-0.02	-3.5	3.64	-0.558	-3.5	-3.52	-3.94
141.00	103.8	106.74	3.25	-1	3.32	0.648	-3.1	2.25	3.12
137.00	97.6	103.29	6.70	-2	4.2	-4.176	-3.3	4.70	1.4
136.00	115.9	123.56	-13.56	-2.25	3.96	0.198	-1.3	-15.81	-12.9
137.00	107.2	113.45	-3.45	-2	4.96	-0.504	-1.2	-5.45	-2.20
142.00	95.1	97.10	12.8	-0.75	3.84	0.126	-5	12.140	11.10
143.50	109.7	110.84	-0.84	-0.375	1	0.594	-1.7	-1.22	-1.32
135.00	92.3	99.13	10.86	-2.5	1.72	0.414	-4.3	8.36	6.19
131.00	93.3	103.27	6.72	-3.5	3.68	0.09	-1.8	3.22	5.19
131.00	92.5	102.38	7.61	-3.5	0.36	-0.702	-2.4	4.11	1.37
139.00	109.1	113.85	-3.80	-1.5	2.16	0.72	-3.7	-5.30	-6.12
131.00	89.5	99.06	10.93	-3.5	1.16	-0.378	-2.4	7.43	5.81
135.00	99.3	106.65	3.34	-2.5	1.96	-0.252	-3.6	0.84	-1.04
142.00	98	100.07	9.92	-0.75	2.76	4.32	-3.3	9.17	12.96
133.00	103.2	112.51	-2.51	-3	3.76	1.206	-1.9	-5.51	-2.44
139.00	109.7	114.43	-4.43	-1.5	4.6	-0.108	-1.9	-5.93	-3.34
133.00	90	98.12	11.87	-3	5.6	4.32	-2	8.87	16.8
138.00	110.4	116	-6	-1.75	4.32	0.99	-2.9	-7.75	-5.34
135.00	106.9	114.81	-4.81	-2.5	3.88	1.314	-2.6	-7.31	-4.72
137.00	107.7	113.98	-3.98	-2	3.92	0.594	-3	-5.98	-4.47
139.00	105.5	110.05	-0.053	-1.5	3.76	-1.278	-2.9	-1.55	-1.97
140.00	102.6	106.26	3.73	-1.25	2.28	-0.684	-2.1	2.48	1.98
138.00	116.7	122.61	-12.61	-1.75	1.44	-0.288	-2.9	-14.36	-16.11
132.00	106	116.43	-6.43	-3.25	4.92	-0.45	-1.7	-9.68	-6.91
139.00	109	113.70	-3.70	-1.5	2.64	-0.144	-2.8	-5.20	-5.50
139.00	109	113.76	-3.705	-1.5	1.56	-0.45	-1.4	-3.5	-5.49

Appendix 2 Summary of the diagnoses according to the strong ion model