RESEARCH PAPER

Describing acid-base balance using three different methods of analysis in a feline acute haemorrhage-resuscitation model

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Abstract

Objective To describe acid-base status using the Henderson-Hasselbalch, Stewart and semi-quantitative methods of analysis in a feline haemorrhage-resuscitation model.

Study design Randomized crossover study.

Animals A total of six domestic cats (mean age, 21 months; weight, 4.9 kg).

Methods Venous blood samples were taken before haemorrhage, after haemorrhage at 30 minute intervals during fluid resuscitation and at 24 hours. The cats were anaesthetized and underwent following treatments: no purposeful haemorrhage and resuscitation (NoPHR), purposeful haemorrhage followed by either lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Voluven) for resuscitation. LRS and Voluven were administered at 60 and 20 mL kg^{-1} $hour^{-1}$, respectively, for 120 minutes. Variables used for the analysis methods were measured or calculated from the blood samples and then compared among treatments over time using a general linear mixed model ($p < 0.05$; data reported as mean and standard deviation).

Results The total blood loss at 120 minutes was 10.2 ± 2.3, 29.3 ± 9.0 and 29.1 ± 6.3 mL kg^{-1} for NoPHR, LRS and Voluven, respectively. Total volumes of LRS and Voluven administered were 120 and 40 mL kg^{-1}, respectively. All cats became acidaemic during anaesthesia regardless of treatment. The Henderson-Hasselbalch method indicated that anaesthetized cats undergoing severe haemorrhage and resuscitation manifest a mixed acidosis. The Stewart method indicated two counter metabolic processes that contributed to the overall pH—decrease in apparent strong ion difference (acidosis) and decrease in total weak acids (alkalosis). The semi-quantitative method identified the free water and chloride effects as variables causing acidosis and the albumin effect causing alkalosis.

Conclusions and clinical relevance In an experimental haemorrhage and resuscitation model in cats, blood pH was similar among treatments over time regardless of severe haemorrhage and resuscitation with LRS or Voluven or mild haemorrhage and no resuscitation.

Keywords Henderson-Hasselbalch approach, lactated Ringer's solution resuscitation, semi-quantitative approach, Stewart approach, tetrastarch 130/0.4 resuscitation.

Introduction

The blood acid-base status of animals is regulated in order to maintain the pH within a narrow range; in healthy cats, this pH range is 7.34–7.43 (Hopper et al. 2014a). The essence of this tight regulation of pH range is to ensure optimal enzyme activity that is required for metabolism and regulation of organ function. Enzyme functional activity decreases or halts when the blood or tissues undergo extreme acid-base changes, which lead to altered organ function and potentially failure (Mitchell et al. 1972; Crimi et al. 2012). Acidaemia has been reported in 49% of all dogs and cats admitted to a teaching hospital (Hopper & Epstein 2012). In humans, acidaemia alters the cardiovascular function by causing a decrease in systemic vascular resistance and cardiac output (Crimi et al. 2012). Also, in acidaemic humans, the oxygen

binding capacity of haemoglobin is decreased (Mitchell et al. 1972). The decreases in organ function in dogs and cats during acidaemia are thought to be similar to those in humans and could contribute to mortality (Hopper & Epstein 2012). The routine measurement of acid-base status, especially in emergency and critical care of ill or traumatized cats, is becoming increasingly relevant to modern case management (Elliott et al. 2003; Lee & Drobatz 2003; Drobatz & Cole 2008; Hopper & Epstein 2012; Ha et al. 2013; Hopper et al. 2014a,b); however, there are scant reports of acid-base balance in anaesthetized cats (Souza et al. 2005) and a paucity of information in cats undergoing severe haemorrhage and fluid resuscitation.

Understanding changes in acid-base status in cats is complicated by the use of different methods of assessment. Berend (2013) surmises that understanding the pathophysiology of acid-base balance in human medicine is confusing, illogical and controversial. This is because there are many methods that can be used to analyse acid-base status and no standardized approach to analysis. In veterinary medicine, there are few reports detailing different methods of evaluating acid-base status. The three frequently used methods in companion animals are the 1) Henderson-Hasselbalch, 2) Stewart, and 3) semi-quantitative methods (Hopper et al. 2014a,b).

The Henderson-Hasselbalch method has been the cornerstone of interpreting blood pH for a century and incorporates information from the bicarbonate (HCO_3^-) buffer system (Henderson 1908; Hasselbalch 1917). The Henderson-Hasselbalch method describes the acidosis or alkalosis as originating from the respiratory system (partial pressure of carbon dioxide: PCO_2) and/or through metabolic processes based on the measurement of bicarbonate (HCO_3^-). The descriptive nature of this method does not provide sufficient insight into the origin of derangements outside the HCO_3^- buffer system. Thus, changes in base excess (BE) and the anion gap have been incorporated into this traditional method of pH analysis (Hopper et al. 2014a). Stewart's method was based on physical chemistry principles which included electroneutrality, conservation of mass and dissociation of electrolytes (Russell et al. 1996). He proposed that overall pH is controlled by three independent variables: PCO_2, the strong ion difference (SID) and total week acids (Atot). The hydrogen and HCO_3^- concentrations are dependent upon these independent variables (Stewart 1983). The Stewart method aimed to describe the mechanisms and quantify acid-base disorders more completely than the Henderson-Hasselbalch method. Furthermore, the Stewart method has been used to theoretically predict and describe changes in acid-base status during fluid resuscitation (Muir 2017). Fencl et al. (2000) combined concepts from these two methods to derive the semi-quantitative method. The semi-quantitative method quantifies the effects of individual acid-base processes on the BE and focuses only on metabolic processes. These processes include the effects of albumin, chloride, phosphate, sodium and lactate concentrations (Fencl et al. 2000).

These acid-base analysis methods rely on the measurement or calculation of variables; and these variables could change during anaesthesia, haemorrhage and fluid resuscitation (Hopper et al. 2014a,b; Muir 2017). Therefore, the aim of this study was to describe and compare blood acid-base status during three different resuscitation treatments using these three different methods of analysis in a feline acute haemorrhage-resuscitation model. It was hypothesized that there would be no change in the variables over time or among treatments.

Material and methods

Animals, study design and treatments

The study was approved by the animal ethics committees of the Universities of Pretoria (v006-15) and Witwatersrand (2017-10-68-C-AREC) prior to data collection. This study was part of a larger haemorrhage-resuscitation project and only data relevant to the present study are reported here. A total of six neutered domestic cats (three males and three females), aged mean ± standard deviation 21 ± 1 months and weighing 4.9 ± 1.2 kg, from a research colony that was housed in a communal indoor-outdoor cattery were used. The sample size was based on availability, the study budget and previous research experience (Zeiler et al. 2020). The cats were cared for by experienced animal technicians following a routine animal husbandry protocol for research animals which included daily human interaction (Griffin & Baker 2002). Environmental enrichment was achieved by providing interactive toys and building outdoor climbing structures.

The cats were allocated randomly (balanced single block design; www.randomization.com) to undergo three treatments at 2 month intervals. The treatments were divided into two phases, haemorrhage phase and resuscitation phase, as follows:

- Treatment NoPHR: cats underwent no purposeful haemorrhage (aside from blood sampling) and no resuscitation where only maintenance fluids were administered.
- Treatment LRS: cats underwent a purposeful haemorrhage followed by an isotonic crystalloid (lactated Ringer's solution, LRS; Fresenius Kabi, RSA) infusion at 60 mL kg^{-1} $hour^{-1}$ for 120 minutes.
- Treatment Voluven: cats underwent a purposeful haemorrhage followed by a 6% tetrastarch 130/0.4 suspended in 0.9% saline (Voluven; Fresenius Kabi) infusion at 20 mL kg^{-1} $hour^{-1}$ for 120 minutes.

During the purposeful haemorrhage phase, blood was drawn manually into a semi-closed system using 20 mL syringes primed with citrate-phosphate-dextrose (4 mL; JMS blood bag, 450 mL; JMS Singapore, Singapore) at a targeted rate of 2 mL

kg^{-1} $minute^{-1}$ until one of two end points was reached first. The end point was either a 1) maximum blood withdrawal of 30 mL kg^{-1}; or 2) mean arterial blood pressure of < 48 mmHg that persisted for at least 3 minutes (Zeiler 2020).

Study procedures

A health check comprising a clinical examination and blood collection from the jugular vein was performed 1 week prior to treatments. Exclusion criteria included any cat that was unwell or had haematology and biochemistry profile values outside of normal reference intervals for cats. Food, but not water, was withheld 8 hours prior to treatment. On the morning of treatment, the cat was premedicated with buprenorphine hydrochloride (0.02 mg kg^{-1}, Temgesic; Reckitt Benckiser Healthcare, RSA) intramuscularly and left undisturbed for 45 minutes. A catheter (22 gauge, Jelco; Smith Medical International, UK) was aseptically inserted into one of the cephalic veins and secured. Alfaxalone (Alfaxan-CD RTU; Afrivet, RSA) was administered intravenously (IV) to induce anaesthesia. The trachea was intubated with a cuffed endotracheal tube (4 mm internal diameter; Teleflex Incorporated, RSA), which was secured in place and connected to a paediatric circle breathing system (15 mm internal diameter, Compact paediatric breathing system; Intersurgical; RSA). General anaesthesia was maintained by delivering isoflurane (Isofor; Safeline Pharmaceuticals, RSA) in oxygen at a fixed flow rate of 80 mL kg^{-1} $minute^{-1}$. The vaporiser was initially set at 2% to achieve and maintain an end-tidal isoflurane concentration of 1.7% which is a referenced minimum alveolar concentration for surgery throughout the procedures (Shaughnessy & Hofmeister 2014). The ventral neck region was clipped and surgically scrubbed before the cat was transferred to the procedure table and positioned in dorsal recumbency. An isotonic crystalloid fluid (lactated Ringer's solution; Fresenius Kabi) was infused by an electronic device (Infusomat Space; B-Braun, Germany) at 5 mL kg^{-1} $hour^{-1}$ via a y-port administration set (Infusomat Space Set; B-Braun) connected to the cephalic catheter for peri-anaesthetic maintenance fluids (maintenance fluids).

Physiological variables were monitored by placing probes and leads and connecting them to a multiparameter machine (Datex-Ohmeda Cardiocap 5; GE Healthcare, Finland), as follows: heart rate by electrocardiogram, peripheral oxyhaemoglobin saturation by pulse oximetry, end-tidal carbon dioxide and respiratory rate by capnography, and oesophageal temperature (reusable temperature thermistor probe; GE Healthcare). A catheter (22 gauge, 50 mm, Arrow arterial catheterization set; Arrow International, PA, USA) was inserted into the carotid artery using a cut-down technique to enable invasive blood pressure measurement. Another catheter (22 gauge, 50 mm, Arrow arterial catheterization set; Arrow International) was inserted percutaneously into the left jugular vein for intermittent blood sampling and facilitation of purposeful haemorrhage later. Both catheters were inserted using the Seldinger technique (Seldinger 1953).

Once instrumentation was completed, one of the three randomly allocated treatments commenced. During the purposeful haemorrhage phase, blood was drawn from the jugular catheter for LRS and Voluven. Whereas, when there was no haemorrhage, only blood samples were drawn, and a 15 minute waiting period was observed. During the resuscitation phase, the treatment fluid was infused using a second electronic device (Infusomat Space; B-Braun), and the administration set was connected to the y-port of the maintenance fluid administration set. The maintenance fluids were administered simultaneously with LRS or Voluven, but only maintenance fluids were administered to NoPHR cats.

On completion of the study procedures, the monitoring equipment and catheters, except the jugular catheter, were removed and the cats were then transferred to the intensive care unit of the hospital for recovery and overnight observation. The cats were given a single subcutaneous injection of meloxicam (0.2 mg kg^{-1}; Petcam; CiplaVet, RSA) and an IV injection of buprenorphine (0.03 mg kg^{-1}). The following day, blood was sampled, and the jugular catheter was removed before the cats were returned to the cattery. The cats were monitored for 2 days after the procedures by daily clinical examinations and observation of their social, drinking and eating behaviours. All cats were adopted and rehomed 1 month after conclusion of the project.

Data collection and analysis

Data relevant to the present study were collected by measuring variables or calculating them where necessary using the three acid-base analysis methods. Data were collected during the health check, 15 minutes before haemorrhage (T−15), immediately after haemorrhage phase (T0), at 30 minute intervals during the resuscitation phase until 120 minutes (T30, T60, T90, T120) and 24 hours later (T24h).

Venous blood samples were drawn from the jugular vein using a needle and syringe and decanted into serum vacuum storage tubes (serum BD Vacutainer tube; Becton Dickinson, UK) during the health check; or via the jugular catheter into a syringe and immediately decanted into serum vacuum storage tubes during the treatment period and 1 day later. The first 1.5 mL of blood was always discarded prior to drawing the actual blood samples via the jugular catheter. Blood samples were submitted immediately to the onsite laboratory for analyses of serum biochemistry (serum albumin, lactate) and electrolyte (sodium, chloride, potassium, phosphate, ionized calcium) concentrations using daily-calibrated machines by experienced veterinary clinical pathologists. The venous blood samples for

acid-base determination were collected into heparinized syringes (BD A-line; Becton Dickinson) and analysed within 10 minutes using a daily-calibrated benchtop blood gas analyser (interpreted at a fixed temperature of 37 °C; Rapidlab 500 System; Siemens, RSA).

The blood gas analyser measured the pH and venous partial pressure of carbon dioxide ($PvCO_2$) by a potentiometric method using standard ionselective electrode technology and modified potentiometric method based on the principles of the Severinghaus electrode, respectively. The actual and standard bicarbonate ion concentrations [HCO_3^-(act) and HCO_3^-(std), respectively] and extracellular fluid and blood base excess [BE (ecf) and BE(B), respectively] were calculated using standard equations provided by the Clinical Laboratory Standards Institute C46-A2 (Rapidlab 500 Operator's Manual; Siemens). The measured variables were used in the formulae to evaluate acid-base status (Table 1).

The total blood removed at each time point was cumulative and included the purposeful haemorrhage volume (treatments LRS and Voluven), all blood samples for analysis for this study and the entire larger haemorrhage-resuscitation project. The cumulative blood loss was calculated over time for each cat for each treatment and converted to a value in mL kg^{-1}. The blood loss in treatment NoPHR was defined as mild haemorrhage (<10 mL kg^{-1}). A severe haemorrhage was defined when more than 22 mL kg^{-1} (>40% assumed circulating volume) of blood was lost (Zeiler et al. 2020).

Statistical analysis

Data were tested for normality by plotting histograms, evaluating descriptive statistics and performing the Anderson-Darling test for normality. Data were normally distributed and presented as using mean (± standard deviation). All variables used within each acid-base analysis method were compared among treatments and over time using a general linear mixed model. Significant findings underwent *post-hoc* pairwise comparisons using Bonferroni correction. Model fits were assessed by inspecting residual plots to assess linearity, homogeneity of variances, normality, and outliers. Frequently reported criteria of three acid-base analysis methods were used to describe the acid-base status in the cats (Table 2; Hopper et al. 2014a). The acid-base statuses were described with the aid of tables and figures. Data were compared using commercially available software (MiniTab 18; Minitab, PA, USA) and significance interpreted at $p < 0.05$.

Table 1 Equations used to calculate various variables required for the interpretation of acid-base status using the Henderson-Hasselbalch, Stewart and semi-quantitative methods of analysis. Atot, total quantity of weak acids; BE(B), base excess of the blood; BE (ecf), base excess of the extracellular fluid; Cl^-, chloride concentration; Hb, haemoglobin concentration; HCO_3^-(act), actual bicarbonate ion concentration; HCO_3^-(std), standard bicarbonate ion concentration; K^+, potassium concentration; Na^+, sodium concentration; SID, strong ion difference.[1]

Variable	Equations
Anion gap	$([Na^+] + [K^+]) - ([HCO_3^-(act)] + [Cl^-])$
SIDapparent	$([Na^+] + [K^+] + [Ca^{2+}]) - [Cl^-]$
Albumin contribution	Measured albumin × [(0.123 × pH) − 0.631] × 10
Phosphorus contribution	Measured phosphorus × 0.323 × [(0.309 × pH) − 0.469]
Atot	Albumin contribution + phosphorus contribution
SIDeffective	$[HCO_3^-(act)]$ + albumin contribution + phosphorus contribution
Strong ion gap	SID apparent − SID effective
Free water effect	0.22 ($[Na^+]$ − mid-normal $[Na^+]$)
Corrected chloride	Measured $[Cl^-]$ × (mid-normal $[Na^+]$/measured $[Na^+]$)
Chloride effect	Mid-normal $[Cl^-]$ − corrected $[Cl^-]$
Phosphate effect	0.58 (Mid-normal [phosphorus] − measured [phosphorus])
Albumin effect	3.7 (Mid-normal albumin − measured [albumin])
Lactate effect	−1 × [lactate]
Sum of effects	Free water effect + chloride effect + phosphate effect + albumin effect + lactate effect
Unmeasured ions effect	Base excess − sum of effects
Blood gas machine equations	
HCO_3^-(act)	$pH + \log (PvCO_2 \times 0.0307) - 6.105$
HCO_3^-(std)	$24.5 + 0.9A + (A - 2.9)^2 \times (2.65 + 0.31 \times Hb)/1000$
	Where: $A = BE(B) - (0.2 \times Hb \times (100 - SvO_2))/100$
BE (ecf)	$HCO_3^-(act) - 24.8 + 16.2\ (pH - 7.40)$
BE(B)	$(1 - 0.014 \times Hb)[(HCO_3^-(act) - 24.8) + (1.43 \times Hb + 7.7)\ (pH - 7.40)]$

[1]Mid-normal values were determined as the central value of the variable calculated from pooled values obtained during the health checks. Albumin and Hb measured in g dL^{-1}; phosphate measured in mg dL^{-1}; electrolytes and lactate measured in mmol L^{-1}.

Table 2 Criteria used to interpret acid-base status based on the reference ranges of variables required for the Henderson-Hasselbalch, Stewart and semi-quantitative methods of analysis. HCO_3^-, bicarbonate ion concentration; $PvCO_2$, venous partial pressure of carbon dioxide

Analysis variable	Interpretation	Criteria
Traditional acid-base analysis method (Henderson-Hasselbalch)		
Simple disturbances	Metabolic acidosis	pH < 7.34, HCO_3^- < 18 mmol L^{-1}
	Metabolic alkalosis	pH > 7.43, HCO_3^- > 26 mmol L^{-1}
	Respiratory acidosis	pH < 7.34, $PvCO_2$ > 39 mmHg
	Respiratory alkalosis	pH > 7.43, $PvCO_2$ < 34 mmHg
Mixed disturbances	Abnormalities present for both $PvCO_2$ and HCO_3^- then the cats were reported to have two co-existing abnormalities	
Metabolic acidosis further classified by anion gap (AG)	Metabolic acidosis associated with increased AG	AG > 20 mmol L^{-1}
	Metabolic acidosis not associated with i ncreased AG	AG < 20 mmol L^{-1}
Stewart acid-base analysis method		
Apparent strong ion difference (SIDa)	Increased SIDa metabolic alkalosis	SIDa > 44 mmol L^{-1}
	Decreased SIDa metabolic acidosis	SIDa < 40 mmol L^{-1}
Total weak acids (Atot)	Increased Atot metabolic acidosis	Atot > 17 mmol L^{-1}
	Decreased Atot metabolic alkalosis	Atot < 8 mmol L^{-1}
Unmeasured anions	Increased SIG	SIG > 9 mmol L^{-1}
Semi-quantitative acid-base analysis method		
Free water effect	Dilutional acidosis	Free water effect < −1.0 mmol L^{-1}
	Contraction alkalosis	Free water effect > 0.7 mmol L^{-1}
Chloride effect	Acidosis	Chloride effect < −4.0 mmol L^{-1}
	Alkalosis	Chloride effect > 5.0 mmol L^{-1}
Phosphorus effect	Acidosis	Phosphorus effect < −1.0 mmol L^{-1}
	Alkalosis	Phosphorus effect > 1.0 mmol L^{-1}
Albumin effect	Acidosis	Albumin effect < −3.0 mmol L^{-1}
	Alkalosis	Albumin effect > 3.0 mmol L^{-1}
Lactate effect	Acidosis	Lactate effect > −2.0 mmol L^{-1}
Unmeasured ions effect	Unmeasured acids	Unmeasured ions effect < −0.5 mmol L^{-1}
	Unmeasured alkalis	Unmeasured ions effect > 0.5 mmol L^{-1}

Results

All cats completed the study. The cumulative blood loss at T0 was 3.4 ± 0.8, 22.3 ± 7.7 and 21.6 ± 4.7 mL kg^{-1} for treatments NoPHR, LRS and Voluven, respectively; at T120, the blood loss was 10.2 ± 2.3, 29.3 ± 9.0 and 29.1 ± 6.3 mL kg^{-1}, respectively. The total volumes of LRS and Voluven administered by T120 were 120 and 40 mL kg^{-1}, respectively. The haemoglobin concentration decreased with all treatments during general anaesthesia and the concentration decreased even further with treatments LRS and Voluven from T30 to T120 compared with T0 and treatment NoPHR (treatment × time: $p < 0.001$). The blood volumes withdrawn, total resuscitation fluids administered and variables used in acid-base calculations obtained over time are reported in Table S1.

All cats were acidaemic during anaesthesia regardless of the treatment. When data were pooled and averaged, venous blood pH values decreased significantly from the health check value of 7.35 (±0.06) to 7.25 (±0.06). The haemorrhage phase did not alter the pH, which was 7.25 (±0.06) at T0, but it increased during the resuscitation phase with all treatments; with treatment NoPHR, pH increased to 7.32 (±0.08) at T120. The pH trends among the treatments over time are presented in Fig. 1 (time: $p < 0.001$).

A mixed acid-base disturbance was diagnosed during anaesthesia using the traditional Henderson-Hasselbalch method of interpretation. The $PvCO_2$ was greater than 39 mmHg (5.2 kPa) for the entire duration of general anaesthesia in cats administered treatments LRS and Voluven; however, in cats administered treatment NoPHR, it returned to values between 34 and 39 mmHg (4.5 to 5.2 kPa) by T30 (Fig. 2 & Table S2; time: $p < 0.001$). Therefore, there was a persistent respiratory acidosis with treatments LRS and Voluven, but only a transient respiratory acidosis in treatment NoPHR. The HCO_3^-(act) was between 18 and 26 mmol L^{-1} at all time

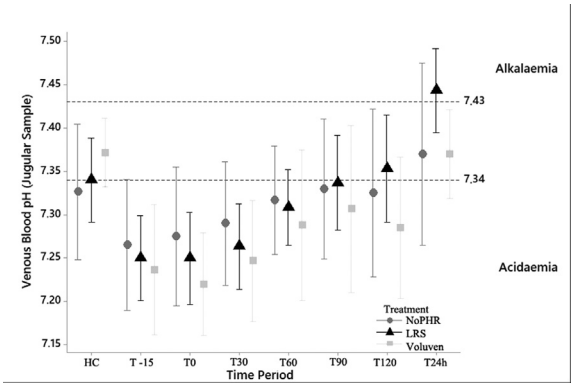

Figure 1 The venous blood pH obtained from a jugular vein sample in six cats undergoing three treatments during general anaesthesia. The treatments were: 1) no purposeful haemorrhage (aside from blood sampling) and no resuscitation (NoPHR), or purposeful haemorrhage followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Voluven) infusions for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (T−15), after haemorrhage (T0) and then at 30 minute intervals (T30, T60, T90, T120) during fluid resuscitation and 24 hours later (T24h). The plot represents the mean and 95% confidence interval.

periods; furthermore, the HCO_3^-(act) and HCO_3^-(std) were greater following treatment LRS compared with treatments NoPHR and Voluven ($p = 0.001$). The BE (ecf) was more negative than −5 mmol L^{-1} for all treatments, which indicated a metabolic acidosis, except for treatment LRS in which the BE (ecf) was greater than −5 mmol L^{-1} at T120 and T24h after treatments (treatment × time: $p < 0.001$). The HCO_3^-(std) (treatment: $p = 0.003$; time: $p < 0.001$) and BE(B) (treatment × time: $p < 0.001$) had similar trends over time to the HCO_3^-(act) and BE (ecf), respectively. The anion gap was 20 mmol L^{-1} or less for all treatments over time, but the decrease in anion gap over time was less with treatment NoPHR than with treatments LRS and Voluven (treatment × time: $p < 0.001$).

The Stewart method diagnosed acidotic respiratory and metabolic contributing factors and alkalotic metabolic contributing factors. The SIDapparent was consistently less than 40 mmol L^{-1} for all treatments over time, thus indicating metabolic acidosis (Fig. 3 & Table S3; treatment × time: $p = 0.003$). However, the Atot was less than 8 mmol L^{-1} for treatments LRS and Voluven from T30 to T120. This indicated metabolic alkalosis, but otherwise the values were within the 8−17 mmol L^{-1} range, which was comparable to treatment NoPHR, during the health check and T24h (treatment × time: $p < 0.001$). The strong ion gap was normal for all treatments over time, but the decrease was not as severe with treatment NoPHR compared with treatments LRS and Voluven (treatment × time: $p < 0.001$).

The semi-quantitative method diagnosed acidotic and alkalotic metabolic contributing factors. The free water effect indicated a dilutional acidosis for all treatments from T−15 to T120 (Fig. 4 & Table S4; time: $p < 0.001$), and the chloride effect indicated an acidosis from T30 to T120 with all treatments (treatment × time: $p < 0.001$). The lactate effect was more negative than −2 mmol L^{-1} in treatment LRS predominantly, from T30 to T120, but with all treatments, values were less than −2 mmol L^{-1} at T24h (treatment × time: $p < 0.001$). Unmeasured acids were also detected, using the unmeasured ions effect, with all treatments during the health check and early stages of general anaesthesia until T30 and T24h (treatment: $p = 0.004$; time: $p < 0.001$). The phosphate effect only detected an acidosis during T−15 and T0 in cats given treatments LRS and Voluven, but this effect was before the resuscitation phase (treatment × time: $p = 0.001$). A metabolic alkalosis was diagnosed by the albumin effect for treatments LRS and Voluven from T30 to T120 (treatment × time: $p < 0.001$), and by the unmeasured ions effect that indicated the presence of unmeasured alkalis from T30 to T120 in treatments LRS and Voluven, but not in treatment NoPHR (treatment: $p = 0.004$; time: $p < 0.001$).

Discussion

In an experimental haemorrhage and resuscitation model in cats, blood pH was similar among treatments over time regardless of severe haemorrhage and resuscitation with LRS or Voluven or mild haemorrhage and no resuscitation. The Henderson-Hasselbalch, Stewart and semi-quantitative methods of acid-base analysis allowed for variable interpretations of the cause of these pH changes. The Henderson-Hasselbalch method, being descriptive in nature, indicated that the acidaemia was mostly due to metabolic processes reflected by BE. However, in treatments LRS and Voluven, a persistent respiratory acidosis, based on the $PvCO_2$, was present. This method indicated that cats undergoing severe haemorrhage and resuscitation while anaesthetized manifest a mixed acidosis. The Stewart method identified shifts in electrolyte and total weak acid concentrations which allowed quantification of the metabolic processes. The two counter metabolic processes that contributed to the overall pH was a decrease in SIDapparent (acidosis) and decrease in Atot (alkalosis). The semi-quantitative method focuses on the metabolic contributing factors that influence BE. The major contributing factors causing acidosis were the free water effect and chloride effect and the major contributing factor causing alkalosis was the albumin effect.

The Henderson-Hasselbalch and Stewart methods use $PvCO_2$ as a variable to indicate respiratory processes that contribute to the pH. During treatment NoPHR, the $PvCO_2$ was within a normal reference interval indicating that the cats were normocapnic. The normocapnia indicated that there was

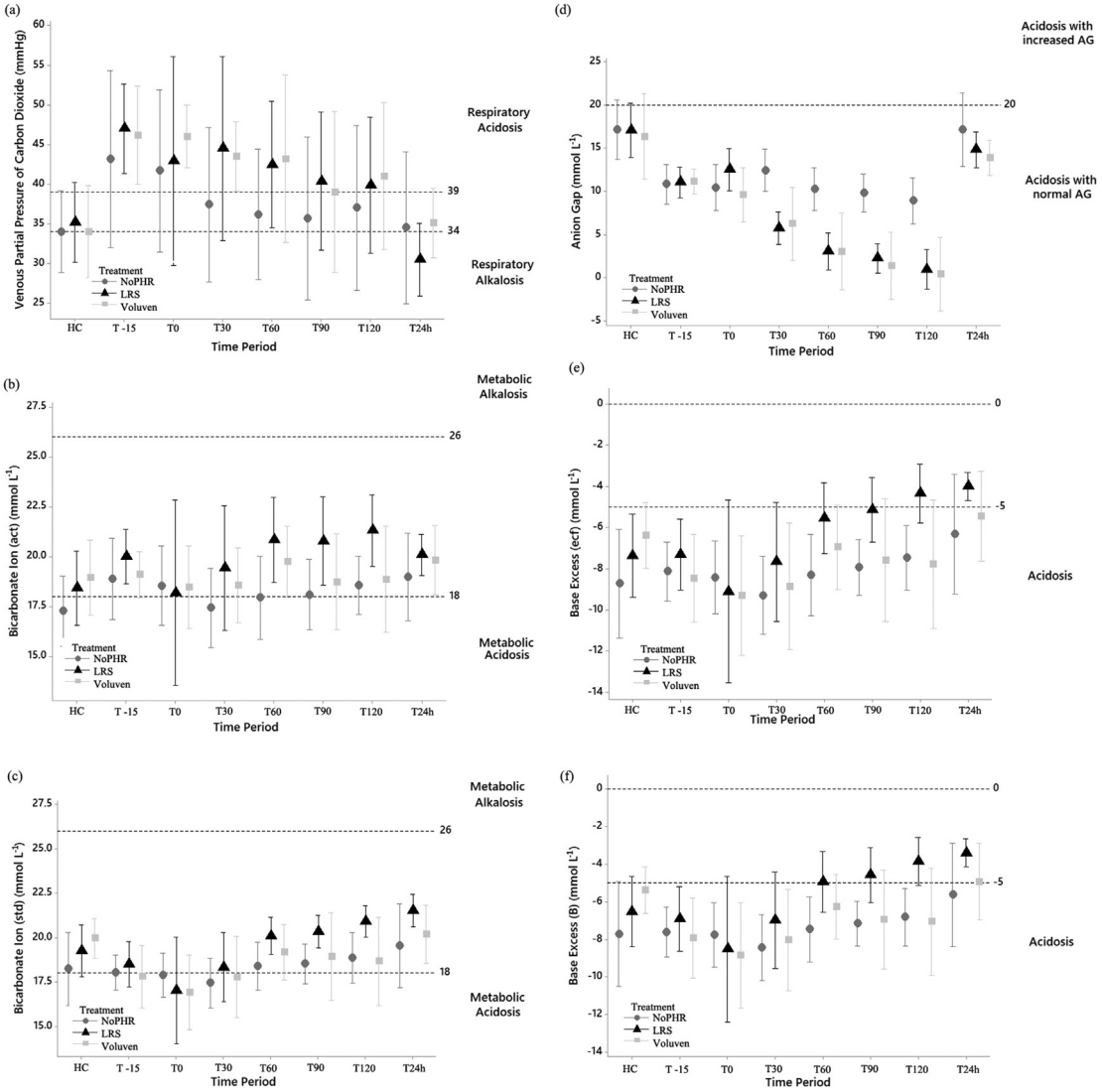

Figure 2 The venous partial pressure of carbon dioxide (a), actual (b) and standard (c) bicarbonate ion concentration, anion gap (AG) (d), extracellular fluid (e) and blood (f) base excess variables used to interpret blood pH using the Henderson-Hasselbalch method, anion gap and base excess in six cats undergoing three treatments during general anaesthesia. The treatments were: 1) no purposeful haemorrhage (aside from blood sampling) and no resuscitation (NoPHR), or purposeful haemorrhage followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Voluven) infusions for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (T−15), after haemorrhage (T0) and then at 30 minute intervals (T30, T60, T90, T120) during fluid resuscitation and 24 hours later (T24h). The plot represents the mean and 95% confidence interval.

no hypoventilation related to the effects of anaesthesia. The increase in $PvCO_2$ observed during treatments LRS and Voluven could have arisen from an increased plane of anaesthesia, whereby the low cardiac output enhanced the uptake of the isoflurane making the cats more profoundly anaesthetized. This effect has been described in dogs anaesthetized with isoflurane and undergoing haemorrhage-induced hypovolemia (Mattson et al. 2006). Furthermore, the gap between the arterial and the $PvCO_2$ is usually less than 5 mmHg in anaesthetized or conscious dogs and cats (Dubin et al. 1990). However, during low cardiac output states, the gap tends to widen during the low flow state (Benjamin et al. 1987). This widening gap could also explain the higher $PvCO_2$ in the two treatments where the cats underwent haemorrhage and resuscitation compared with treatment NoPHR, reported in another study (Zeiler et al. 2020).

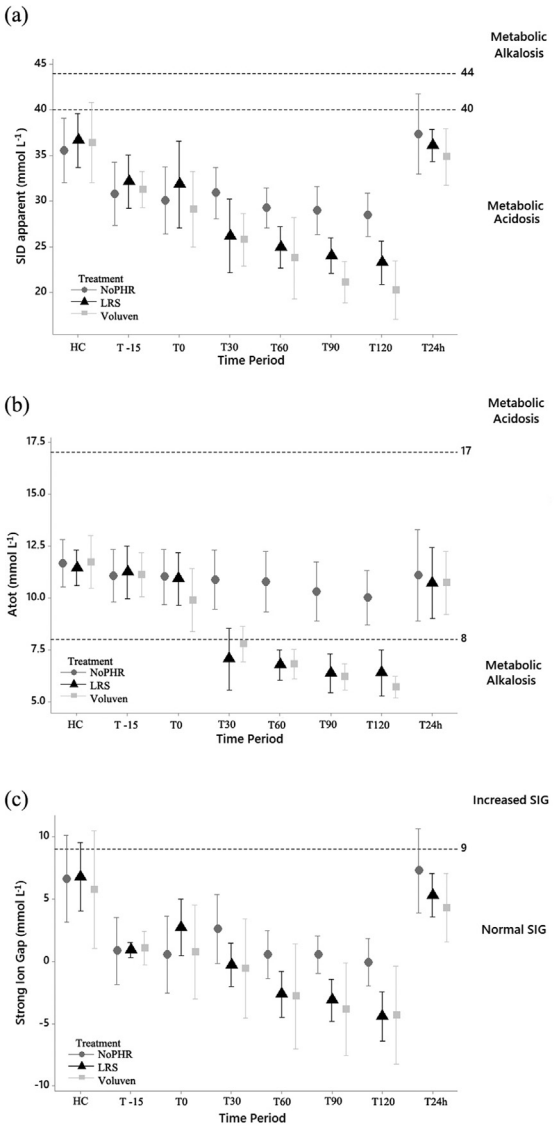

Figure 3 The strong ion difference (SID) apparent (a), total weak acids (Atot) (b) and strong ion gap (SIG) (c) variables used to interpret blood pH using the Stewart method in six cats undergoing three treatments during general anaesthesia. The treatments were: 1) no purposeful haemorrhage (aside from blood sampling) and no resuscitation (NoPHR), or purposeful haemorrhage followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Voluven) infusions for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (T−15), after haemorrhage (T0) and then at 30 minute intervals (T30, T60, T90, T120) during fluid resuscitation and 24 hours later (T24h). The plot represents the mean and 95% confidence interval.

The BE is descriptively evaluated when the Henderson-Hasselbalch method of analysis is used. If its value is greater or less than the reference interval, the animal has a metabolic alkalosis or acidosis, respectively. Both the calculated BEs indicated a metabolic acidosis was present with all treatments, except for treatment LRS at 90 and 120 minutes and the next day (Hopper et al. 2014a). Both calculations incorporate the HCO_3^-(act) and pH as variables which could explain the increase in both BE (ecf) and BE(B) for LRS (Russell et al. 1996). For the semi-quantitative method, the free water effect indicated a dilutional acidosis for all time periods during general anaesthesia. This finding could be attributed to the overall low sodium concentration reported in our cats, as previously described for SIDapparent. The chloride effect was different between treatment NoPHR and the haemorrhage and resuscitation treatments, and this difference could be because of the difference in haemoglobin concentration. However, the haemoglobin concentration decreased profoundly, and the corrected chloride increased over time during the resuscitation phase. This result suggests that the chloride concentration was in excess relative to the erythrocyte count (haemoglobin concentration), especially with treatments LRS and Voluven when compared with treatment NoPHR. The albumin effect was similar to the Atot of the Stewart method, which has already been discussed.

The Stewart method includes the analysis of electrolyte concentrations to calculate the SIDapparent, which was less than 40 mmol L^{-1} at all time periods and indicated metabolic acidosis (Hopper et al. 2014a). The acidosis was present mostly during the general anaesthetic period and not during conscious states. We made use of frequently reported SIDapparent ranges for interpretation (Hopper et al. 2014a). However, perhaps the acidosis threshold in cats should be reduced to a SIDapparent of 35 mmol L^{-1}. This change would reflect the pH measured in our cats during the health check and the following day during the two time periods when there were theoretically no derangements in acid-base status. Another explanation for the overall low SIDapparent value is that our cats had a lower sodium ion concentration throughout the study compared with normal published ranges (Hopper et al. 2014a). The Stewart method includes an analysis of Atot which indicates an alkalinizing effect during the resuscitation phase with treatments LRS and Voluven, which might have been caused by hypoalbuminaemia (Russell et al. 1996). However, the hypoalbuminaemia did not contribute to a difference in the overall pH among treatments and over time in our cats, which agrees with the finding that the albumin concentration does not influence blood pH (Berend 2013). Despite a similar pH among treatments, the Steward method demonstrated clear differences in SIDapparent and Atot between treatment NoPHR and the two haemorrhage and resuscitation treatments.

Finally, despite finding differences in the variables among treatments and over time within treatments using each method of analysis, the acidosis was not considered life threatening, even after severe haemorrhage and large-volume fluid resuscitation.

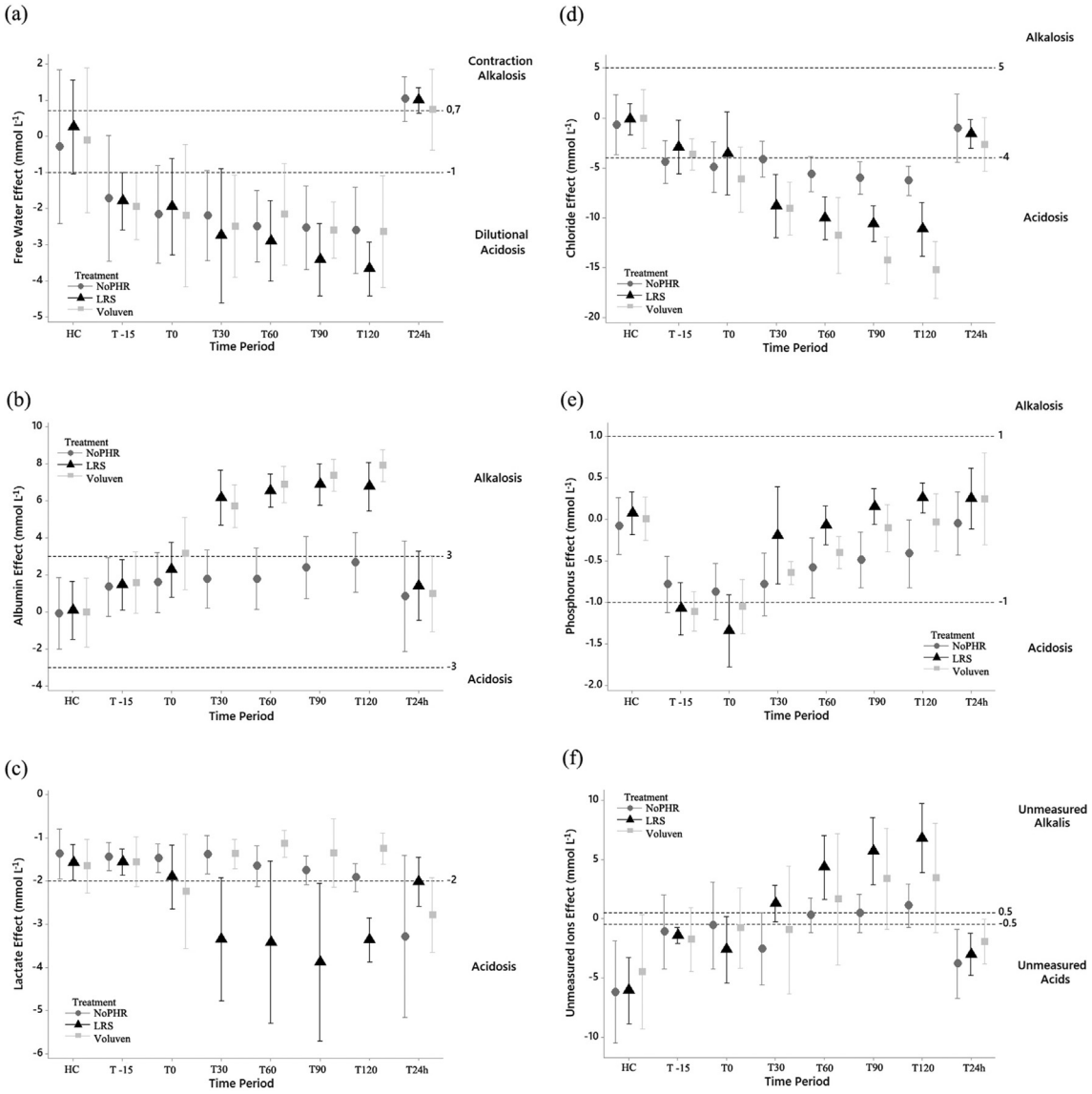

Figure 4 The free water effect (a), albumin effect (b), lactate effect (c), chloride effect (d), phosphorus effect (e) and unmeasured ions (f) effect variables used to interpret blood pH using the semi-quantitative method in six cats undergoing three treatments during general anaesthesia. The treatments were: 1) no purposeful haemorrhage (aside from blood sampling) and no resuscitation (NoPHR), or purposeful haemorrhage followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Voluven) infusions for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (T−15), after haemorrhage (T0) and then at 30 minute intervals (T30, T60, T90, T120) during fluid resuscitation and 24 hours later (T24h). The plot represents the mean and 95% confidence interval.

The study had notable limitations. The sample size was small and thus had the power to reliably detect only large effects. This study is the first of its kind in cats and the observations herein will assist future researchers in estimating adequate samples sizes to validate these observations under various clinical conditions. The study was conducted in anaesthetized cats under controlled conditions. Therefore, extrapolation of our findings to traumatized or conscious feline patients or those receiving different drugs during anaesthesia will require further investigation. Lastly, treatment NoPHR was not a true control treatment and thus it is difficult to know how these acid-base variables would differ when compared to real control groups.

Conclusions

Blood pH in cats was similar among treatments over time regardless of severe haemorrhage and resuscitation with LRS or Voluven or mild haemorrhage and no resuscitation. The Henderson-Hasselbalch, Stewart, and semi-quantitative methods of acid-base analysis allowed for variable interpretations of the cause of these pH changes. However, the clinical relevance of any of these findings or the utility of the different methods of acid-base analysis (for diagnostic or treatment purposes) is unknown.

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Authors' contributions

GEZ study design, data collection and data analysis. BTD and AF: study design. RKB and FP: data collection. PK: data analysis. All authors contributed with manuscript editing.

Conflict of interest statement

Authors declare no conflict of interest.

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Supplementary information

Additional Supporting Information may be found in the online version of this article: https://doi.org/10.1016/j.vaa.2021.07.004.

Table S1. The venous blood pH, variables measured or calculated for use in acid-base analysis and cumulative blood loss and resuscitation fluid volumes in six isoflurane anaesthetized cats undergoing three treatments.

Table S2. The variables used for the Henderson-Hasselbalch and traditional methods of acid-base analysis in six isoflurane anaesthetised cats undergoing three treatments.

Table S3. The variables used for the Stewart method of acid-base analysis of six isoflurane anaesthetized cats undergoing three treatments.

Table S4. The variables used in the semi-quantitative method of acid-base analysis of six isoflurane anaesthetized cats undergoing three treatments.

