[ACID-BASE IMBALANCES IN NEWBORN FOALS AND ADULT HORSES ASSESSED BY THE QUANTITATIVE APPROACH]

PhD Thesis

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CERTIFICA:

Que la tesi doctoral que porta per títol: Acid-Base imbalances in newborn foals and adult horses assessed by the quantitative approach, de la qual és autora la llicenciada en Veterinària **Judit Viu Mella**, s'ha realitzat sota la seva direcció.

I per a que així consti, a efectes de ser presentada com a treball de Tesi per a optar al Grau de Doctor en Veterinària, signo el present certificat a Bellaterra, el 10 de Setembre de 2013.

Eduard Jose Cunilleras

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[INTRODUCTION AND REVIEW OF THE SCIENTIFIC LITERATURE]

The methods of interpretation of acid-base balance are based on chemical and physical laws. In some situations, this could difficult the understanding or application of these systems by physicians or veterinarians not used to apply these principles.

Development of all methods applied to interpretation of acid-base (AB) balance is based on 2 different principles. The traditional concept is based on the principle of electroneutrality (i.e. each positive charge has a negative charge counterpart). In any macroscopic sample of any aqueous solution the sum of all the positively charged ions always equals the sum of all the negatively charged ions. This means that all aqueous solutions are always electrically neutral. Coulomb's law states that "the magnitude of the electrostatic force of interaction between two point charges is directly proportional to the scalar multiplication of the magnitudes of charges and inversely proportional to the square of the distances between them". The application of this law clearly explains the need of this electroneutrality. Small differences in the concentration of positive and negative charges produce enormous electrical effects that living organism cannot resist.

The second principle applied is the conservation of the mass (i.e. each component substance in any aqueous solution remains constant unless: 1) that substance is added or removed or 2) that substance is generated or destroyed by chemical reactions within a solution).

The goal of any approach to the interpretation of acid-base balance is to establish the quantitative relationships that determine H^+ concentration in any solution. Only recently, have two methods been applied to AB equilibrium analyses of biological solutions although a third method has been proposed as a possible approach to AB analyses in polionic monoprotic buffers[1] and polionic polyprotic acids (compounds that are donors of more than one H^+ per molecule)[2], however this method has not been validated in biologic fluids.

1. Evolution of acid-base analyses in human medicine and future directions: Brief explanation of Henderson-Hasselbalch's and Stewart's methods

1.1-. Henderson-Hasselbalch's method

The Henderson-Hasselbalch method (described in 1916) is based primarily on concepts derived from the studies of multiple authors including Arrhenius,[3] Sørenson, Henderson,[4] Hasselbalch,[5] Brønsted and Lowry,[6] Van Slyke , Lewis,[7] Severinghaus,[8] Astrup,[9] Siggaard-Anderson,[10-15] Schwartz,[16] and Relman.[17]

The main conclusions of these investigators are that the activity of H^+ (Acid-base balance) within a given compartment (fluid analyzed) is determined by: 1) the mass balance of H^+ ([H+]), 2) proton transfer reactions mediated by proton donors (weak acids) and proton acceptors (weak bases) that buffer the change in H^+ activity within a certain pH range; and 3) the mass balance of proton donors and proton acceptors (electroneutrality).

The traditional approach is a bicarbonate-centered interpretation in which other proton donors or acceptors are not taken into account. The whole method is based on the following parameters:

a. Carbon dioxide tension (pCO_2)
b. Plasma bicarbonate concentration (HCO_3^-)
c. Negative logarithm of the apparent dissociation constant for carbonic acid in plasma (pK_1')
d. Solubility of CO_2 ($SpCO_2$)

The changes in pH described employing this method are the result of a change in the HCO_3^- concentrations:

$$pH = pK_1' + log\frac{HCO_3^-}{SpCO_2} \quad (1)$$

Therefore, factors that affect HCO_3^- concentration are related with pH changes. The following equation explains how CO_2 fluid concentration can affect the HCO_3^- and consequently the pH value:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (2)$$

Evaluation of acid-base balance, using Henderson-Hasselbalch method, has historically used pH as an indicator of overall acid-base status, pCO_2 as an independent measure of respiratory component, and extracellular Base excess (BE_{ECF}) or real HCO_3^- concentration (standard HCO_3^-) as a measure of non-respiratory component. The controversy between the use of standard HCO_3^- or BE_{ECF} was present during 30 years, but finally real concentration of HCO_3^- was not considered appropriate for interpretation of the metabolic component to acid-base imbalances because it can never be independent from respiratory activity. The BE_{ECF} expresses the amount of strong acid added to titrate the pH of one liter of 100% oxygenated blood to 7.4 at 37°C and at pCO_2 of 40 mmHg. The advantage of BE_{ECF} is that it is independent from pCO_2 and quantifies the effect of non-volatile buffers that otherwise are not taken into account. However, it is considered an inaccurate estimation of the magnitude of the metabolic acidosis or alkalosis when changes in hemoglobin or plasma protein are present. Additionally, the calculation of the anion gap (AG) allows detection of increases in non-measured anions. The AG concept arises from the concept of electroneutrality and represents the difference between unmeasured cations and anions in serum (equations 3, 4). Normally, approximately two-thirds of the AG originates from net negative charge of the plasma proteins and the remaining unmeasured anions reflect phosphate, lactate, sulfate, β-OH butyrate, acetoacetate and anions associated with uremia. A change in plasma protein concentration of 1 g/dL decreases AG value by 3.7 mEq/L, whereas a decrease of 1 g/dL in plasma albumin concentration decreases AG value by 2.5 mEq/L.

$$[Na^+] + [K^+] + [UC] = [Cl^-] + [HCO_3^-] + [UA] \quad (3)$$

$$[UA] - [UC] = AG = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]) \quad (4)$$

Applying BE_{ECF} and AG the Henderson-Hasselbalch method could seem a good approximation for acid-base interpretation in clinical management of critically ill cases, but

it does in fact, present some disadvantages. The main limitations of Henderson-Hasselbalch equation are: 1) it is more descriptive than mechanistic; 2) the cause of acid-base change during disease is hard to establish and 3) it fails to distinguish between the effects of independent and dependent variables on plasma pH. Using this method acid-base disturbances can only be classified as respiratory disorders (acidosis and alkalosis) and metabolic, or BE, disorders (acidosis and alkalosis)

Other problems found in the Henderson-Hasselbalch equation are: the lack of adjustment with temperature, the ignored effect of protein and sodium concentration on pK_1'[18] and the assumption of linear relationship between pH and pCO_2 logarithm. This assumption is clearly incorrect because marked curvilinearity in the log pCO_2-pH relationship is proved *in vitro* and *in vivo* at physiologic pH (7.30).[18]

1.2-. Stewart's method

In 1948, Singer and Hastings proposed that plasma pH was determined by 2 independent factors, pCO_2 and net strong ion charge or strong ion difference (SID)[19]. In 1983, Stewart suggests a third component in acid-base equilibrium: the total plasma concentration of non-volatile weak buffers (A_{TOT}). This component reflexes the effect of weak buffers such as albumin, globulins, and phosphates on plasma pH.[20] Simplified Stewart's method reduces the chemical reactions to simple ion dissolution and is effective because main plasma cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and anions (Cl^-, HCO_3^-, protein, lactate, sulfate and ketoacids) bind each other in saltlike manner.[21] Other ions that take part in more complex reactions such as oxidation-reduction and precipitation reactions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+} and Mn^{2+}) are considered irrelevant to acid-base equilibrium.[21, 22] The ions considered in acid-base equilibrium are categorized into two groups: 1) non-buffer ions (strong ions are fully dissociated at physiologic pH and they do not take part in chemical reactions) and 2) buffer ions (they are derived from plasma weak acids and bases, and are not fully dissociated at physiologic pH neither do they take part in chemical reactions). Strong ions exert an electrical effect (positive charge) because the sum of completely dissociated cations is greater than anions (SID). Buffer ions can be divided into

volatile buffers HCO_3^- (open buffer system in arterial plasma influenced by CO_2 concentrations)

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (2)$$

and non-volatile buffer ions (non-HCO_3^- or buffer ions derived from plasma weak acids closed system).

Buffer ions derived from plasma weak acids and bases are substances in equilibrium with the weak acid/base and their conjugate form:

$$HA \leftrightarrow H^+ + A^- \quad (5)$$

Each of these weak acids has a dissociation constant (K_a) that can be calculated using the following equation:

$$K_a = {[H^+][A^-]}/{[HA]} \quad (6)$$

Weak acids can act as effective buffers at physiologic plasma pH when the pK_a (negative logarithm of the weak acid dissociation constant K_a) lies within pH±1.5.

The plasma weak acids and their conjugates (according to Stewart's assumptions) do not take part in other biological equations. Therefore, the sum of the weak acid concentration and their conjugate base remains constant (law of mass conservation) and receives the name of A_{TOT}:

$$A_{TOT} = [HA] + [A^-] \quad (7)$$

Based on electroneutrality law, Stewart developed the following equation as the center of his methodology of acid-base interpretation:[20]

$$[SID^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] + [H^+] = 0 \quad (8)$$

Using all previous equations, Stewart developed a polynomial equation relating all the independent variables of the method with pH (plasma hydrogen concentration)[20]:

$$[H^+]^4 + ([SID^+] + K_a)[H^+]^3 + [K_a([SID^-] - [A_{TOT}]) - K_W' - K_1' [SP_{CO2}][H^+]^2 - [K_a(K_W' + K_1' SP_{CO2}) - K_3 K_1' SP_{CO2}][H^+] - K_a K_3 K_1' SP_{CO2} = 0 \quad (9)$$

Where K_a is the apparent dissociation constant for plasma non-volatile weak acids, K_W is the apparent dissociation constant for water, K'_1 is the apparent dissociation constant for the Henderson-Hasselbalch equation, K_3 is the apparent dissociation constant for HCO_3^-, and S is the solubility of CO_2 in plasma.

The main limitation of Stewart's method is the difficulty in obtaining an accurate value for SID^+ because it requires the measurement of all strong ions of plasma, including unidentified ions (such as lactate, β-OH butyrate, acetoacetate, and sulfate). Moreover, it will be affected by interlaboratory differences in determination of each ion. As an alternative, a good approximation (measured strong ion difference [SID_m]) can be obtained by using only four strong ions (Na^+, K^+, Cl^- and lactate).

$$[SIDm] = [Na^+] + [K^+] - [Cl^-] - [lactate] \quad (10)$$

Another difficulty of the Stewart's method is the complexity of the equation (9) and the complexity in determining K_a. In the Strong ion approach, pH is function of 8 factors (P_{CO2}, [A_{TOT}], $[SID^+]$ K_1', S, K_a, K_W', and K_3), whereas in the Henderson- Hasselbalch's approach pH is a function of only four parameters (P_{CO2}, $[HCO_3^-]$, K_1' and S).

A criticism of the strong ion approach is the use of hydrogen ion concentration ($[H^+]$) instead of hydrogen ion activity (pH), considering that $[H^+]$ does not exist because it immediately reacts with water

$$[H^+] + [H_2O] \leftrightarrow H_3O^+ \quad (11)$$

On the other hand, Stewart's requirement for electrical neutrality refers to ionic activity of [SID^+], $[A^-]$, $[HCO_3^-]$, CO_3^{2-}, $[H^+]$ and OH^- rather than concentrations. Stewart was aware of this issue, and concluded "when the resulting theory is applied in practice, it may

then become important to use activities to ensure numerical agreement with real system behaviour".[20] This assumption, or error, is one of the main limitations of this method.

1.3-. Simplified strong Ion model

In 1992, Fencl, Rossing and Leith introduced the concept of BE into the quantitative method, dividing it into four different portions: BE due to changes in free water (or sodium concentration; BE_{fw}), BE due to chloride (BE_{Cl}), BE due to unmeasured anions BE_{uma} and BE due to Albumin (BE_{Alb}). [23] When BE components were applied to acid-base interpretation in horses a simplification of BE_{Alb} to Base excess due to total protein (BE_{tp}) was applied.[24] In more recent human studies the effect of BE_{fw} and BE_{Cl} was simplified into the effect of BE_{sid}.[25, 26]

$$BE = 0.9287 \times [HCO_3 - 24.4 + (14.83 \times (pH - 7.4))] \quad (12)$$

$$BE_{uma} = BE - BE_{alb} - BE_{sid} \quad (13)$$

The objective of the adaptation of BE was to easily evaluate metabolic acid-base derangements. When Constable introduced the simplified strong ion method,[18] the BE determination was not routinely used anymore.

Constable simplified the strong ion model in 1997, assuming that plasma ions could be classified as strong ions, volatile buffers ($[HCO_3^-]$), or non-volatile buffer ions ($[A^-]$). Therefore, plasma contained three entities with electric charge: SID+, $[HCO_3^-]$ and $[A^-]$, which must add up to 0 (electroneutrality).

$$[SID^+] - [HCO_3^-] - [A^-] = 0 \quad (14)$$

The differences present between the electroneutrality equation of simplified strong ion method and the Stewart's method are the elimination of the $[CO_3^{2-}]$, $[OH^-]$ and $[H^+]$ due to their small concentration in plasma, and, the fact that the simplified equation is based on ionic activity rather than concentrations. Combining the equation of mass conservation (7), conservation of charge (12) and equations of apparent dissociation of H_2CO_3 (3) and weak acids (5) a logarithmic equation with three independent variables and three constants is obtained (6 factors instead of the 8 present in Stewart model).

$$pH = \log \frac{2SID^{+}}{K_1^{'}SP_{CO2} + K_aA_{TOT} - K_aSID^{+} + \sqrt{(K_1^{'}SP_{CO2} + K_aSID^{+} + K_aA_{TOT})^2 - 4K_a^2SID^{+}A_{TOT}}} \quad (15)$$

All the constants (K_1', K_a, K_a^2) in the above equation are temperature dependent. In addition, some of these are also dependent on ionic strength or the charge of non-volatile plasma buffers. Considering that the effect of temperature on pH is predictable and all the samples are analyzed at 37ºC, the effect of abnormal temperature on pH is removed during acid-base assessment. Moreover, changes in ionic strength are minimal and could be disregarded clinically. These assumptions result in an easier method where pH is mainly dependent on three independent factors $[SID^{+}]$, $[A_{TOT}]$ and pCO_2.

The K_a has to be determined with an experimental method for each animal species. In horses, K_a was determined by CO_2 plasma tonometry by Constable in 1997[18] obtaining the following equation for calculation of $[A_{TOT}]$:

$$[A_{TOT}] = 2.25 \times (Albumin\ g/dL) + 1.40 \times (Globulin\ \ g/dL) + 0.59 \times (Phosphate\ g/dL) \quad (16)$$

The simplified Stewart's method is based on three independent variables ($[SID^{+}]$, $[A_{TOT}]$ and pCO_2) which allows more exact interpretation and determination of the cause of the disturbance in acid-base balance. Metabolic alterations are classified in acidosis or alkalosis due to changes in $[SID^{+}]$ or due to changes in $[A_{TOT}]$.

Changes in unmeasured anions and cations can be taken into account with the strong ion gap (SIG), which is obtained from the following equation.

$$SIG = \frac{A_{TOT}}{1+10^{(pKa-pH)}} - AG \quad (17)$$

Strong ion gap is a more accurate approach to determine the unmeasured strong anions than AG.[22, 27] The main difference between SIG and AG, is that SIG shows the differences between unmeasured strong cations and anions while AG quantifies the differences between all unmeasured cations and anions (including strong ions and non-volatile buffers).

The main disadvantages of the simplified strong ion method are, the difficulty to determine $[SID^{+}]$ and, the greater mathematical complexity of the traditional approach.

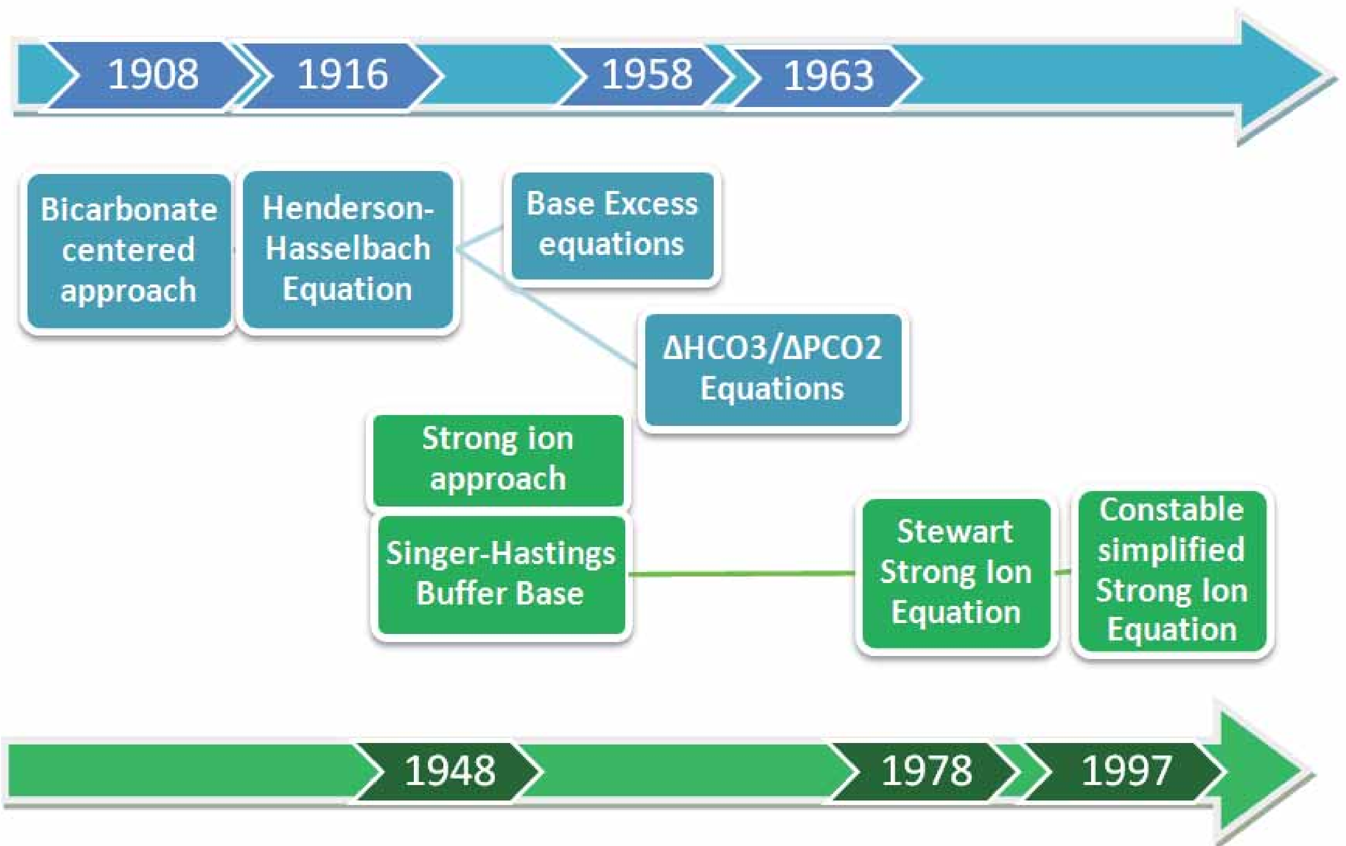

Figure 1.1: Cronology of acid-base interpretation

2. Laboratory Limitations:

As commented in the previous section, exact determination of all the components of acid-base equations is not possible. All the analyzers have intra-assay variations but larger differences can be detected when different devices are used (inter-assay variations). Although determinations can be performed with gold standard techniques, the results obtained will never be the real value due to inter-assay variations.

Reference values of the blood parameters are usually established using the gold standard method of each parameter, but in daily clinic, especially during emergency situations, most of these analyzers are unavailable or the assay time is longer than point-of-care analyzers.

Studies performed, comparing human reference laboratories, highlight differences that could be present in calculated acid base parameters (SID_m and A_{TOT}) due only to different devices employed.[28] Differences in analyzers results are more marked when determination techniques differ between devices. One example of this situation is the use of point-of- care analyzers for electrolyte determination: reference laboratory uses indirect

potentiometry methods and most of handheld devices use direct potentiometry methods. These inter analyzers differences could lead to incorrect acid-base interpretation, therefore specific reference values for each device should be applied in order to reduce mistakes in interpretation. Unfortunately, the reference values for each analyzer are not usually available and, in most cases, interpretations have to be performed with values obtained with gold standard techniques.

3. Brief summary of sports physiology and the main systems involved in acid-base balance

3.1. Human sports physiology

Multiple systems are involved in acid-base equilibrium and most of these systems suffer changes during exercise or as a result of training. The main objective of these adaptations is to improve oxygen delivery and consumption by the difference tissues (VO_2max). Enhanced aerobic metabolism results in diminished lactate production and delayed accumulation in blood, delaying therefore the resultant acidemia and improving athletic performance.

During training, skeletal musculature adapts by increasing capillary density, inducing mitochondrial oxidative enzymes (≈120%) and also increasing mitochondrial volume and number to enhance oxygen uptake.[29] Moreover, muscle fiber types can adapt progressively to the type of workload, changing their metabolism from fast-contraction (anaerobic fibers) to slow-contraction (aerobic fibers) or vice versa.[30] Cardiovascular system enhances oxygen transport by increasing the cardiac output. This increase is obtained by different adaptations: a mild eccentric hypertrophy of the heart (increase in heart chambers dimensions and also in heart weight), increase in plasma volume with increase in central venous pressure and preload (hypervolemia)[31] and increase in red blood cell volume. Humans do not suffer hypoxia during exercise and arterial blood gas concentrations are normal (pCO_2 and O_2). Therefore, the stimulus for respiratory rate increase is a neurological mediated response instead of hypercapnia.[30] The exercise training could lead to improvement in tidal volume and increases in both respiratory rate and breathing volumes.

Electrolyte losses are tightly controlled and the main source is sweat. Human sweat has a hypotonic composition (i.e. low sodium concentration) because sweat glands respond to aldosterone. This hormone enhances sodium and chloride retention but also causes an increase in losses of potassium in sweat and urine.[31] Water losses are also mainly produced by sweat because renal losses are diminished due to the decrease in glomerular filtration rate during exercise.[31, 32] After exercise, a compensatory increase in glomerular filtration rate, plasma volume and plasma sodium concentration is produced between 1 to 6 days. [33]

3.2. Usefulness of acid-base interpretation in human sports medicine

Two main applications of AB balance can be found in the human sports medicine literature: evaluation of fitness and characterization of exercise-induced exhaustion.

Regarding fitness evaluation, plasma lactate concentration has been widely used in human medicine as a measure of training condition. The intensity of exercise resulting in a blood lactate concentration of 4 mmol/L (VLa4) has been used for lactate-guided training methods. VLa4 is considered the anaerobic threshold where lactate begins to accumulate and produces acid-base changes (acidemia) without pathological implications.

The main concerns regarding acid-base balance in human sports medicine are centered in exhaustion syndrome produced by dehydration, acidosis and electrolyte losses. All these alterations are more likely to appear in athletes undertaking great efforts during long periods of time (endurance, marathon runners…).

Dehydration can impair aerobic exercise performance, increasing lactate production, hampering thermoregulation and increasing effort perceived during exercise. Due to the composition of human sweat, dehydration results in an increase in plasma osmolarity. However, the most common problem reported in marathon runners is hyponatremia probably because of excessive intake of hypotonic solutions. Although human sweat glands are sensitive to aldosterone (enhance sodium retention and promote potassium losses) no significant tendency to hypokalemia has been found in these athletes.[34] Excessive rehydration with water and inappropriate fluid retention have been linked to hyponatremia which is the responsible of adverse events such as altered sensorium, seizures, pulmonary edema, and even death.[34, 35]

In order to avoid the hyponatremia when exercise lasts more than 2 hours, sodium should be included in fluids consumed during exercise. Moreover, rehydratation after exercise should be done slowly.[34]

Dehydration also impairs adequate tissue oxygen supply and enhances anaerobic metabolism. Accumulation of blood lactate contributes to acidosis and also the increase in SID produced by hemoconcentration. It is also known that dehydration in combination with myoglobinuria, that could be present during intense exercise, produces transient acute kidney injury in 40% of marathon runners and complicates the correct regulation of AB balance.[36]

3.3. Equine sports medicine (horse's adaptations to exercise): main differences and similarities with human beings

Horses are animals adapted to exercise. When horses are compared with other terrestrial mammals, they are considered to be one of the most athletic animals. Aerobic capacity or maximal oxygen uptake (VO_2max) is considered an excellent indicator of performance. Due to their adaptations horses easily beat animals of the same size or even some smaller species. Muscular and cardiovascular systems are the most adapted to exercise and are also the systems with more plasticity to increase their capacity during training in contrast to respiratory system, which has very low adaptability.

Regarding muscular function, horses have greater muscle mass relative to their weight (50 vs. 40%) compared to humans. Horses present muscle adaptations to increase oxygen uptake and consumption, they have better capillary flow in skeletal muscles and greater mitochondrial density than humans. These differences allow improved tissue oxygenation and enhanced aerobic metabolism. Moreover, in both species training induces changes to improve oxygen consumption such as muscle fiber adaptations (type of fibers), an increase in both number and volume of muscle mitochondria, an increase in capillary density and also an increase in oxidative enzymes.[37] Most of these adaptations to training are marked more in horses than in humans (Table 1.1).

Table 1.1: Comparative muscle training adaptation of humans and horses.

	Human[29]	Horse[38, 39]
Muscle diffusion capacity		
Cross-sectional fiber	119%	130%
Capillary density	107%	113%-136%
Oxidative enzymes	133%	200%
Mitochondrial volume	272%	175-300%

The equine cardiovascular system is also highly adapted to exercise. Horses have a larger heart than other species, relative to their weight and lower basal heart rate. These cardiac adaptations result in greater cardiac reserve than in human beings. Horses also have a characteristic adaptation that allows a marked increase of their circulating blood volume during exercise: the splenic contraction. The spleen acts as red blood cell reservoir and its contraction enhances oxygen transport to tissue by releasing stored red cells and increasing circulating red cell mass. During exercise, both humans and equines suffer a shift of fluids toward circulatory system to enhance oxygen consumption of tissues and delay dehydration. During training, the cardiovascular system suffers additional adaptations such as hypervolemia and eccentric cardiac hypertrophy. These changes can be found in both trained horses and human beings.

One of the most important impairments of athletic performance in horses is the respiratory system. Horses suffer a coupling between stride and breathing frequency (locomotor-respiratory coupling) that limits their respiratory capacity during high intensity exercise. In consequence, although tissues have the capacity of extract and consume more oxygen than humans, the equine respiratory system is unable to satisfy the oxygen demand. The result is hypoxemia and mild hypercapnia during intense exercise with a rapid increase in lactate concentration and acidemia. These phenomena do not occur in humans and athletes at high level intensity and do not therefore suffer hypoxia.

Other important differences between humans and horses are their thermoregulatory system and sweat capacity and composition. Thermal energy produced during exercise is proportional to weight and oxygen consumption of tissues, but the capacity to eliminate

this thermal energy is proportional to skin surface. The horse has greater VO_2max (200 ml/kg/min vs. 80 ml/kg/min) and weighs six times more than a human, but has only 2.5 times more surface than human being for heat elimination. As a consequence, the horse had to adapt to enhance heat loss by increasing sweat production and evaporation. This explains why exercise induced dehydration is more common in horses than humans. Another difference is sweat composition. Horses have hypertonic sweat (Table 1.2) because their sweat glands are not sensitive to aldosterone (in contrast to those of human beings). During exercise, horses can lose large amounts of fluids and electrolytes due to sweat production and lead to important acid-base disturbances not usually seen in human beings.

Table 1.2: Main sweat and plasma components (humans and horses).[30, 40]

(mEq/L)	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	Osmolarity
Horse sweat composition	117-134	26-42	2-3	5	142-156	290-320
Horse plasma composition	132-146	3-5	2.8-3.4	0.5-2	98-110	270-300
Human sweat composition	60-80	4.5	1.5	3.3	40-90	170-220
Human plasma composition	140	4.5	2.5	15-2.1	110	300

Comparing fluids and electrolytes losses of human beings and horses (Figure 1.2) the amount of salts and fluids lost by horses cause more severe changes in blood composition than the losses in human beings.

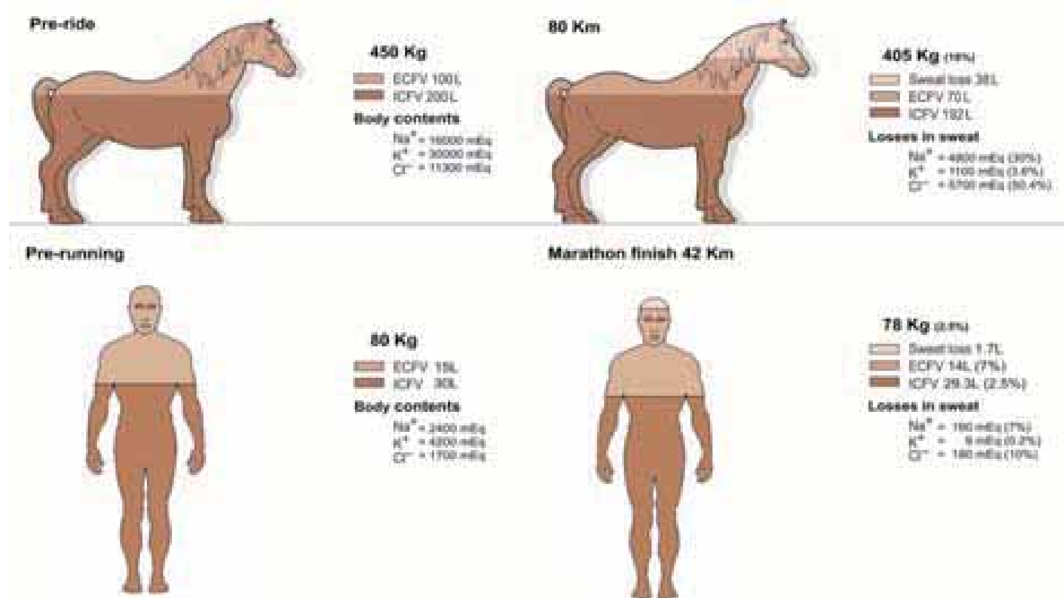

Figure 1.2: Comparison of fluids and electrolytes losses of human and horses during intense exercise.[34, 40] (ECFV extracellular fluid volume; ICFV intracellular fluid volume)

Figure adapted with permission of Jose-Cunilleras[40]

3.4. Reported applications of acid-base parameters to equine sports medicine

Multiple studies of some of AB parameters in sport horses have been performed. Acid-base balance has been assessed in different events such as show jumping,[41] polo competitions,[42, 43] endurance races[44, 45] or three day events.[46]

Most of the studies are centered in endurance horses. This discipline is one of the hardest ones, especially due to long distance, time employed and thermal stress that horse may endure during competitions.

Multiple studies performed regarding AB parameters in equine sports medicine are performed using traditional approach. This method is more descriptive than mechanistic and is only accurately applied to plasma samples with approximately normal protein, albumin and hemoglobin concentrations.[18] The interpretation of changes in acid-base balance occurring during athletic events is complicated because the main variables change simultaneously, often in opposite directions. These complex alterations require a method that evaluates acid-base equilibrium using independent variables (such as partial venous pressure of CO_2, SID and A_{TOT}) and not one that evaluates acid-base alterations focused on dependent variables (pH, bicarbonate) as the traditional approach does.

Recent studies of acid-base balance in endurance horses have determined the alterations of some variables and electrolyte disturbances using a quantitative approach,[44,47,48] but according to this method no interpretation of acid-base balance was made according to this method.

4. Brief summary of neonatal physiology of the main systems involved in acid-base balance

4.1. Human neonates (maturation of organic systems):

Human neonates suffer multiple adaptations during the neonatal period. During the first weeks of life several physiological systems present differences with adults (generally due to a relative immaturity), making neonates more susceptible than adults to suffer certain conditions. Some of these immature systems are profoundly related to acid-base balance and, therefore, differences between adults and neonates are observed.

Regarding the respiratory system, the chest wall in neonates is highly compliant offering little support to uncompliant lungs, which facilitates airway collapse. To counteract this, a positive end expiratory pressure is maintained physiologically by increasing resistance of nasal airway and partial closure of vocal cords. Due to these relative uncompliant lungs, the inspiratory reserve volume is limited and an increase of minute volume has to be achieved increasing the respiratory rate. Moreover, an estimate of total shunt of 24% of cardiac output at birth has been reported in human neonates that progressively decreases to 10% at one week of age.[49] All of these facts, in addition to an increased metabolic rate and oxygen consumption, result in a higher respiratory rate in neonates. Also, peripheral chemoreceptors in the aortic and carotid bodies are functional at birth but are initially silent due to the high postdelivery oxygen content. Receptors need a 48 hours period for adaption to extrauterine gas concentrations, and the response to hypercarbia is therefore the same as in adults, but faster because of the lower resting carbon dioxide level.

Most of the enzymatic pathways of the liver are present in the neonate, but inactive at birth. Hepatic function becomes fully active at 3 months of age.[49] Glomeruli and nephrones are immature at birth, resulting in a lower glomerular filtration rate and limited concentration capacity. There is also a lack of renal medullary osmotic gradient and limited capacity of the tubules to concentrate. Therefore, glycosuria and aminoaciduria are common. During the first hours of life (12-24 hours) urine output is also limited due to poor renal perfusion, which improves with circulatory adaptations and posterior to this time of oliguria there is a phase of natriuresis. Isotonic fluid is lost from extracellular compartments at a rate of 1-2% of the bodyweight per day during the first 5 days.[49]

Carbohydrate metabolism at birth can also influence acid-base status (i.e. aerobic vs. anaerobic metabolism). Glycogen stores are depleted after 12 hours from birth and after this period energy is obtained by lipid oxidation. A balanced dietary intake of protein and carbohydrate will avoid induction of the catabolic pathways responsible for proteolysis and release of gluconeogenic substrates. In cases of depletion of carbohydrates stores, proteolysis is stimulated resulting in aminoacidaemia and therefore altered acid-base balance.[49]

4.2. Usefulness of acid-base interpretation in human neonatal intensive care unit (ICU)

The application of Stewart's methodology to pediatric acid-base disturbances has highlighted differences and similarities with adults. Human neonates have a few partially developed organs (gut, liver and kidneys) and this could lead to different handling of acidifying anions, differences in causes of multiorgan failure or predisposition to some illnesses presented only in the neonatal period. The physiological differences between neonates and adults result in different acid-base alterations when suffering the same pathologies. Two approaches of Stewart's methodology are applied in pediatric acid-base interpretation:

Standard approach: quantifying abnormalities in terms of Strong Ion Gap (SIG) via the complete unmodified Stewart-Fencl's equations.

Approximation using BE by partitioning the latter using abridged Stewart's equations. Base excess represents the influence of weak acids (mainly albumin BE_{alb}), strong ions (mainly difference between sodium and chloride; BE_{sid}) and unmeasured anions (BE_{uma}).

Unfortunately, there is a lack of standardization in calculation of BE components and four different equations are proposed

Gilfix[14]:

$$BE_{uma} = BE - BE_{alb} - BE_{sid} \quad (18)$$

$$BE_{sid} = 0.3 \times (Na - 140) + (102 - Cl_{corr}) \quad (19)$$

$$BE_{alb} = 3.5 \times (4.5 - Albumin\ g/dL) \quad (20)$$

BE= standard base excess; Na sodium; Cl= Chloride; Cl_{corr}= chloride corrected to sodium given by the formula $Cl_{corr} = [Cl^-] \times 140 / [Na^+]$

Story[25]:

$$BE_{uma} = BE - BE_{alb} - BE_{sid} \quad (18)$$

$$BE_{sid} = Na^+ - Cl^- - 38 \quad (21)$$

$$BE_{alb} = (42 - Albumin\ g/dL) \times 0.25 \quad (22)$$

Taylor[50]:

$$BE_{uma} = BE - BE_{alb} - BE_{sid} \quad (18)$$

$$BE_{sid} = Na^{+} - Cl^{-} - 32 \quad (23)$$

$$BE_{alb} = (42 - Albumin\ \ g/dL) \times 0.25 \quad (22)$$

Hatherill[26]:

$$BE_{uma} = BE - BE_{alb} - BE_{sid} - (1.5 - lactate) \quad (24)$$

$$BE_{sid} = 0.3 \times (Na^{+} - 140) + (108 - Cl^{-}_{corr}) \quad (25)$$

$$BE_{alb} = (0.123 \times pH - 0.631)\,(42 - Albumin\ \ g/dL) \quad (26)$$

Out of all equations for BE partitioning, the most recommended is that of Taylor's, which has a reasonable approximation of the true [SIG] effect. The advantage of BE partitioning method compared with the SIG calculation is that of its easy application to daily clinic and the proved usefulness of BE_{uma} as predictor of outcome.[51]

In some studies based on general pediatric ICU population, half of all metabolic acidosis is associated with [SIG] increase (52%) but not always correlated with lactate increase. The commonest isolated alteration causing metabolic acidosis is hyperchloremia (38%). Lactic acidosis, as a unique cause of acidosis, is rare (10% of all metabolic acidosis). It therefore, has to be taken into account that the most common fluid therapy in pediatric ICU is by using saline isotonic solutions, which may contribute to the large incidence of hyperchloremia in the pediatric ICU population.[52]

4.3. Equine neonates: differences and similarities with human neonates

Foals also suffer multiple adaptations to extra uterine life during first weeks of age. Horses are precocial species and the physiological systems usually are more mature than in altricial species (humans). Although equine neonates have a greater degree of maturity compared to altricial species, foals also had relatively immature systems at birth when compared to adult horses. Some of these neonatal characteristics have a direct influence on plasma electrolytes, proteins or pCO_2 and therefore in AB balance.

Neonatal foals, similar to human neonates, have a very compliant thorax and they have to make active respiratory movements for inspiration and expiration. Lungs also collapse easily (relative uncompliant lungs) compared to adults. Due to this relationship between highly compliant thorax and relatively uncompilant lungs, the blood oxygenation and pCO_2 levels are highly influenced by the position of the foal (lateral recumbency vs.

sternal recumbency). The minute volume of the foal is more than twice of that of an adult horse due to the need to move air into a higher dead-space volume and the high metabolic rate of the newborns. Although this higher minute volume requires higher respiratory rates no great differences in pCO_2 levels between neonates and adult horses have been found.

Regarding renal function, kidneys of equine neonates are not fully mature for secretory function of tubular cells. Although glomerular filtration rate and effective renal plasma flow are comparable to adult horses, variations in electrolyte concentrations are particularly prevalent during the first 4 days of life,[53] when neonatal foals show differences regarding electrolytes concentrations (i.e decreased fractional excretions of [K^+] or calcium) and creatinine excretion compared to adults.[54, 55]

Liver function is efficient from birth but not fully mature until 4-6 weeks of age, when it achieves adult capacities.[56] Fibrinogen concentration is lower in foals at birth than in adults and increase until 5 months of age.[57, 58]

Another difference between human and equine neonates is the IgG concentration at birth. Inmunoglobulins are transferred transplacentary in human beings, but neonatal foals have to ingest and absorb them with colostrum. This fact causes a marked difference in Albumin:Globulin ratio between human and equine neonates, especially during the first hours of life. Foals also suffer marked changes in serum IgG concentrations during the first months of life. The lowest IgG concentrations have been reported at 2 months of age, just before the endogenous synthesis is able to cover IgG consumption. At 8 months of age, IgG concentrations are still about half of that measured in mature horses.[59]

4.4. Reported applications of acid-base parameters to the equine neonatal unit

Some parameters of quantitative acid-base balance analyses have been employed in neonatal equine intensive care units (neICU). Evaluation of electrolytes, lactate, pCO_2, pH and protein is used routinely in neICU but evaluation of the calculated variables of quantitative analyses of acid base balance is less commonly used. One of the most interesting applications of these parameters is to obtain a diagnostic or outcome predictor marker for ill foals.

The most commonly used outcome predictor in foals is plasma L-lactate concentration. The L-lactate concentration on admission [60-62] or, its progression during the first 24 hours of treatment, has shown useful to be predict survival in both humans[63] and neonatal foals.[61] Although others parameters of acid-base balance have been determined in ill foals (BE_{uma}, BE, AG, SIG), no correlation with lactate concentration was found and no evaluation of each one of these parameters, as outcome predictors, has been carried out.[62] Other parameters such as venous pCO_2 pressure have also been correlated with outcome.[64]

5. Lack of application of some acid-base parameters to veterinary and equine medicine

Quantitative methods of assessment of AB balance have been demonstrated as a better approximation to AB disorders in human medicine. Although most of the parameters (or their components) needed for this interpretation have been studied in equine sports medicine, no complete interpretation of AB disturbances, using Stewart's method, has been performed. Similarly, blood pH, electrolytes, proteins, and pCO_2 are parameters used often in equine critically ill neonate assessment but no reference ranges of the calculated variables for foals had been reported. However, there is lack of information regarding alterations of parameters used in the quantitative method of AB balance during illness, and also whether or not these alterations could be used as prognostic markers on hospital admission of ill neonate foals. Some of the more useful AB parameters used as predictors of survival in human neonatal ICU have not been evaluated in foals.

[HYPOTHESES]

1. The quantitative analyses of acid-base balance is a better option than the traditional bicarbonate centered approach in critical care situations. The former allows an improved assessment of the clinical state of equine patients in the same way as in human sport medicine and pediatric intensive care unit (pICU).

2. The quantitative method used to assess acid-base balance in sick adult horses could be useful for the assessment of changes suffered by sport elite horses and neonatal foals.

3. The quantitative method to evaluate acid-base balance is applicable in equine medicine under emergency situations.

4. Reference values of the quantitative approach to acid-base balance could be different in healthy neonatal foals from those in adult horses. Reference values in foals could also change with age.

5. The parameters used for the quantitative approach to acid-base balance could be adapted to sick foals and could be useful as predictors of survival.

[OBJECTIVES]

- GENERAL OBJECTIVES

  - To determine the accuracy of the traditional and the quantitative methods for acid-base balance analyses applied to equine sport medicine.
  - To assess the usefulness of the quantitative method to determine acid-base balance in equine healthy and ill neonatal foals under field/emergency situations.
  - To determine the reference values and any necessary adaptations for the use of the quantitative method in foals.
  - To assess the usefulness of acid-base balance parameters used in human neonatal medicine to predict outcome in neonatal foals.

To achieve these general objectives four different studies have been proposed detailing specific objectives in each one of them.

Chapter Four

Chapter Four

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[STUDY ONE]

[Acid-base imbalances during a 120 km endurance race compared by traditional and simplified strong ion difference methods]

Summary

Reasons for performing study: Acid-base disturbances are traditionally assessed using the Henderson-Hasselbach equation. The simplified strong ion approach describes more accurately the complex acid-base and electrolyte abnormalities present in endurance horses.
Objective: To describe acid-base and electrolytes changes in fit horses competing in a FEI*** 120-km endurance race, and to compare traditional versus strong ion approach.
Methods: Thirty horses were initially enrolled in the study. Venous blood samples were obtained before the race (n=25), at the second (n=29; 65.4 km) and third vet-gates (n=23, 97.4 km) and upon race completion (n=17). Blood gas analyses was performed to determine pH, pCO_2, pO_2, Na^+, K^+, iCa^{++}, and calculate HCO_3^-, base excess and tCO_2. Haematocrit, total protein, globulins, albumin, lactate, phosphate, glucose, and creatinine concentrations, as well as muscle enzymes activities were also determined. Calculated variables included strong ion difference (SID_m), strong ion gap (SIG), and non-volatile buffer concentration (A_{TOT}). A Longitudinal Linear model using the General Estimating Equation (GEE) methodology was used for statistical analyses.
Results: Mild but significant increases in pCO_2, SID_m, lactate, plasma protein, globulins, and A_{TOT}, as well as a decrease in potassium concentrations were observed from the 2nd vet-gate to race finish when compared to pre-race values ($P<0.05$). Using the strong ion approach, 67% samples showed acid-base disturbances versus 70% when using the traditional method, but their interpretations only matched in 24% of measurements.
Conclusions: A complex acid-base imbalance characterized by a mild strong ion alkalosis (hypochloremia attenuated by hyperlactatemia), non-volatile buffer acidosis and compensatory mild respiratory acidosis were present in most horses, although pH did not significantly change during a 120-km endurance race. The strong ion approach to interpretation of acid-base balance should be favoured over the traditional approach in endurance horses, given the frequent and complex alterations in pCO_2, SID_m and A_{TOT} during a race.

Introduction

Acid-base and electrolyte disorders have been described in horses associated with prolonged exercise and competitive endurance races.[1-4] Two different methods have been used during the last couple of decades to describe these alterations in horse plasma: the traditional approach uses the Henderson-Hasselbach equation and is clinically adequate to describe acid-base imbalances. However, it is more descriptive than mechanistic and only is accurately applied to plasma with approximately normal protein, albumin, and haemoglobin concentrations.[5] On the other hand, the simplified strong ion model offers a quantitative in depth insight into the pathophysiology of acid-base disorders. This quantitative approach explains how pH can be affected by alterations in plasma protein and phosphate concentrations, as well as by changes in the concentration of strong ions like sodium and chloride,[5] which can be very useful to decide specific treatments if horses develop metabolic disorders.

The interpretation of changes in acid-base balance occurring during athletic events is complicated because the main variables change simultaneously, often in opposite directions. These complex alterations require a method that evaluates acid-base equilibrium not focused on dependent variables (pH, bicarbonate) like traditional approach, but an alternative method that evaluates alterations using independent variables (such as partial venous pressure of CO_2, strong ion difference and non-volatile weak buffers concentration).

Recent studies of acid-base balance in endurance horses have determined the alterations of some variables using a quantitative approach[6-7] or electrolyte disturbances,[4] but no interpretation of acid-base balance was made according to this method. To the authors' knowledge, no comparison between the traditional and the quantitative methods has been described in endurance competitions or a comprehensive characterization of acid-base disorders by the quantitative approach in an endurance race setting. The purpose of this study was to compare changes detected in acid-base balance and electrolyte status using traditional and quantitative approaches and to describe the observed alterations during a 120 km FEI*** endurance race.

Horses and study protocol:

The study was performed in 120 km regional championship race equivalent to a FEI*** endurance race with mean environmental temperature of 10ºC (9-14.5ºC) and relative humidity of 96% (73-100%). After being informed about the purpose and requirements of the study, 30 competitors consented to participate. Horses were Arabians, or Arabian-crossbreds, had been transported varying distances and arrived at a common stable between 12 to 24 h before the race. Sex distribution was 19 geldings, 5 mares and in 6 cases the information was not registered. The mean ±SD age was 9 ±1.3 years. Horses enrolled in this study received various types of electrolytes supplements and nutrition before and during the race. A standardized FEI veterinary examination was performed previous to the race, at 30 km, 65.4 km, 97.4 km, and at the end of the race (120 km). The mean ±SD velocity of horses was 15.2 ±1.6 km/h.

Sample collection and analyses:

Blood samples were taken within 1-2 h of starting the race from 25 of 30 horses, at the second vet-gate (65.4 km) from 29 horses, at the third vet-gate (97.4 km) from 23 horses and within 10 min of finishing the race (120 km) from 17 horses. Blood samples were obtained anaerobically by jugular venipuncture into 10 mL evacuated heparinized tubes (lithium heparin).[a] Blood gas determination was performed immediately after sample collection. Samples were kept in ice up to 20 minutes until PCV, blood glucose and lactate were determined. The remaining heparinized blood samples were centrifuged at 1500 x g for 15 minutes to obtain plasma samples and these were divided into 3 aliquots of 1-1.5 mL and frozen at –20ºC until further analyses. Plasma samples were maintained in solid carbon dioxide (dry ice) for shipping. Rectal temperature was recorded for each horse at the time of venous sampling. Blood gas analyses was performed using a portable analyzer[b] to determine pH, partial venous pressure of CO_2 (pCO_2) and partial venous pressure of O_2 (pO_2) corrected by temperature; and concentrations of sodium [Na^+], potassium [K^+], ionized calcium [iCa^{++}], and haemoglobin (Hb). The hand-held device calculated bicarbonate [HCO_3^-] and total CO_2 (tCO_2) concentrations using pH and pCO_2 values and

the Henderson-Hasselbach equation.[8] Blood glucose and lactate concentrations were determined using portable hand-held devices.[c,d] Packed cell volume was determined using a microhaematocrit centrifuge and total plasma protein (TP) by direct refractometry. Plasma chloride concentration (Cl^-), creatinine, creatine kinase (CK), aspartate aminotransferase (AST), albumin, globulins, and phosphates (P_i) were determined by standard colorimetric biochemical procedures.

Calculated parameters:

Traditional analyses was completed calculating anion gap (AG) from the equation described by Emmett and Narrins[9] and base excess (BE) was obtained from the Henderson-Hasselbach formula in conjunction with Siggaard-Anderson equation:

$$AG= (Na^+ + K^+) - (Cl^- + HCO_3^-) \quad (27)$$

$$BE= 0.02786 \times pCO_2 \times 10^{(pH-6.1)} + 13.77 \times (pH - 124.58) \quad (28)$$

Values of HCO_3^- and tCO_2 were calculated by the hand-held device using the following equations:

$$HCO_3^- = S_{CO2} \times pCO_2 \times 10^{(pH-pK'1)} \quad (29)$$

$$tCO_2 = HCO_3^- + 0.03 \times pCO_2 \quad (30)$$

where pK'_1 is the apparent dissociation constant which has an estimated value of 6.1 and is obtained from the sum of pKs (6.038 at 37º) and the negative logarithm of the activity coefficient of the hydrogen ion (0.091) generating a value of 6.129; and the value used for CO_2 solubility (S_{CO2}) in plasma at 37 °C was 0.03 mEq/L.[5]

Quantitative analyses of acid-base balance was assessed using the method described by Stewart[10] and simplified by Constable.[5] Measured strong ion difference (SID_m), strong ion gap (SIG), and total non-volatile buffers (A_{TOT}) were calculated using the following formulas:

$$SID_m= (Na^+ + K^+) - (Cl^- + Lactate) \quad (10)$$

$$A_{TOT}= 2.25 \times Albumin + 1.4 \times Globulines + 0.59 \times P_i \quad (16)$$

$$SIG = \frac{Atot}{1+10^{(pKa - pH)}} - AG^{10} \quad (17)$$

The value used for K_a of plasma (2.22 x10^{-7} Eq/L; pK_a= 6.65) is the experimentally determined for horses by Constable.[5]

Finally, plasma osmolarity was calculated as:

$$Osmolarity = 2(Na^{+} + K^{+}) + Glucose + \frac{Urea \times 0{,}47}{2{,}8} \quad (31)$$

Interpretation of acid-base status was performed using a bicarbonate based traditional approach and the quantitative strong ion difference based analyses, and the agreement between the two methods of interpretation was assessed. In order to categorize acid-base status by the traditional and quantitative methods the measured values of pH, pCO_2, HCO_3^-, blood lactate concentration, SID_m and A_{TOT} were compared to the reference range detailed on Table 1. For interpretation of SID_m and A_{TOT}, the mean ±2 standard deviations of pre-race values were used as reference range due to the lack of reported values in endurance horses.

Statistical analyses:

Data are shown as mean ±Standard Error (SE) for all dependent variables, except for CK and AST which are reported as median (25th; 75th percentiles). The study variables (i.e., pH, [K^+], [Na^+], [Cl^-], [iCa^{++}], [HCO_3^-], tCO_2, PCV, SID_m, SIG, A_{TOT}, lactate, CK, AST, creatinine, glucose, BE, albumin, globulins, total protein (TP) and calculated osmolarity) were analyzed by means a longitudinal linear model using the general estimating equation (GEE) methodology to account for intra-subject correlations for phases completed with the assumption of unstructured correlation matrix, and in the case of non-convergence, first degree dependence of data was assumed. Of the 30 horses enrolled in the study, pre-race blood samples were not obtained from 5 horses (Horse 1, 15, 23, 25, 27), and we assumed the average pre-race values of the other 25 horses was an adequate approximation when performing the statistical analyses. The last observation carried forward (LOCF) method was applied to impute the missing values on the dependent variables of eliminated horses at the second or third vet-gates. Bonferroni correction was

used when performing multiple comparisons. All data were analyzed with statistical analyses software (SPSS version 15) and values of $P \leq 0.05$ were considered statistically significant.

Seventeen horses completed the race, 10 horses were eliminated for lameness (4 in the second vet-gate and 6 in the third vet-gate) and three for metabolic problems (2 in second control and 1 at the end race, all due to mild dehydration). One of the thirty horses was eliminated for the purpose of this study (horse 22) because it was retired for lameness before the race mid-point and a second blood sample was not obtained. Mean speed of finishers was 15.6 km/h with a range of 9.5-21 km/h.

Parameters used in the quantitative acid-base balance evaluation are shown in Figure 4.1, and the available data plus imputed missing values for all dependent variables are presented also in Table 4.1. A mild but significant ($P= 0.047$; Table 4.1) decrease in blood pH was detected with increasing distance (Figure 4.1), but no statistical significant differences were found between phases; whilst pCO_2, HCO_3^-, tCO_2 and calculated BE showed an initial increase (Table 4.1; $P<0.001$) with a tendency to decrease from the race mid-point to the end. Endurance exercise resulted in a mild but significant increase in blood lactate that reached a plateau from mid-race onwards (Table 4.1; $P= 0.04$). Distance completed had an effect on plasma or blood electrolyte concentrations ($P<0.001$; Table 4.1), characterized by a modest and sustained decrease in sodium, chloride and ionized calcium, and a slight decrease in potassium concentrations that recovered by the end of the race. Measured strong ion difference showed a significant increase (Figure 4.1 and Table 4.1; $P<0.001$) due to combined effects of a moderate decrease in Cl^- and mild increase of blood lactate concentrations. Packed cell volume, TP and creatinine concentrations increased significantly (Table 4.1; $P<0.001$) with increasing distance. Similarly, albumin, globulin, phosphate and A_{TOT} concentrations initially increased significantly (Table 4.1; $P<0.001$) but these stabilized during the second half of the race. Strong ion gap increased significantly ($P<0.001$; Table 4.1) with distance until third phase, returning to resting values by the end of the race. Blood glucose concentrations remained stable except for a ~20% decrease detected at the third vet-gate ($P<0.001$; Table 4.1). Finally, no significant changes in calculated plasma osmolarity along the race were detected.

The interpretation of acid-base balance of each horse at the end of the race (or at the time of elimination) showed a poor agreement between the traditional and quantitative

approaches given that it only matched in 3 cases (Table 4.2 and Figure 4.2). All 94 blood gas determinations performed in this study were evaluated using a quantitative and traditional approach and the interpretation only matched in 33 of 94 of these. Using a traditional approach 66/94 (70%) determinations of acid-base status presented detectable alterations (25 lactic metabolic acidosis, 2 respiratory acidosis and 5 metabolic alkalosis) and the remaining 34 of these 66 had complex imbalances (18 had a combination of the three above mentioned derangements and the other 16 had combinations of 2 of these conditions). Using a quantitative evaluation of acid-base balance, 63/94 (67%) blood gas analyses presented alterations of which 12 were non-volatile buffers (A_{TOT}) metabolic acidosis, 1 metabolic acidosis due to decrease in SID_m, 4 metabolic alkalosis due to SID_m, and 7 respiratory acidosis; and the remaining 39 of these 63 analyses showed mixed acid-base disturbances (9 respiratory acidosis with metabolic alkalosis due to SID_m and acidosis due to A_{TOT}, 14 metabolic alkalosis due to SID_m with metabolic acidosis due to A_{TOT} and other mixed disorders).

The traditional and quantitative methods are used to evaluate acid-base balance in horses. Recent studies in endurance horses evaluated changes in acid-base balance and electrolyte concentrations using a quantitative approach,[6-7] but a detailed description of quantitative acid-base alterations was not provided. The main findings of the present study of acid-base balance in fit endurance horses during a 120-km FEI*** endurance race are: (1) the presence of a complex acid-base imbalance in most horses characterized by a mild strong ion alkalosis (hypochloremia) attenuated by mild lactic acidosis, non-volatile buffer ion acidosis and compensatory mild respiratory acidosis, (2) poor agreement between the interpretation of acid-base balance using the traditional and quantitative approaches.

The traditional or Henderson-Hasselbach approach has been used applied to horse plasma since 1964.[12] This is a simple method of interpretation which does not require determination of a large number of parameters, but it has four important limitations: 1) inexact results when protein or electrolytes alterations are present, 2) the approach is based on 2 dependent variables (pH and HCO_3^-) and an independent variable (pCO_2), 3) disorders are classified into a limited number of categories compared with the quantitative method, and 4) it does not help guide a fluid therapy regime, should it be necessary. For these reasons the Henderson-Hasselbach approach to acid-base disturbances is not the most appropriate method when there are alterations in protein, Na^+ or Cl^- concentrations.[5] These derangements are common in endurance horses where large changes in electrolyte and protein concentrations can occur due to sweat losses and hemoconcentration.

Using the traditional approach to acid-base assessment the most consistent alterations found in this study were mild metabolic lactic acidosis and complex changes characterized by mild metabolic alkalosis plus mild compensatory respiratory acidosis.

Previous studies in endurance horses have shown changes in pH, pCO_2, Na^+, K^+, iCa^{++}, Cl^-, lactate, glucose, PVC, CK, AST, TP, albumin and creatinine concentrations as a function of speed,[4,6] reporting more severe disturbances in horses running at higher speed (15-20 km/h) when compared to lower speed (8-12 km/h). In the present study, performed with high level competition horses and high mean velocity (15.6 km/h) alterations of the different variables were evaluated for disturbances along the distance instead of speed.

Changes observed in this study were similar to those found in studies that correlate the evaluated variables with high velocity. However, our study was performed in favourable environmental conditions at fast speed and only mild changes were observed in the studied variables.

The first variable used for traditional assessment is pH. In the present study statistical but not clinically significant changes in pH were detected with increasing distance, as it was expected from previously reported positive association with speed.[6-7]

The resting pCO_2 values in this study were slightly high compared with reference values for horses in some[13] but not all studies.[14] The initial decrease of pCO_2 described in the literature[15] due to increased alveolar ventilation was not found in our study likely because the first blood sample during exercise was delayed to the second vet-gate at 65.4 km and at this point of the race the initial hyperventilatory response was exceeded and pCO_2 had began to accumulate.

The third variable used in traditional approach to acid-base balance is HCO_3^-. Plasma concentration of HCO_3^- is determined by pCO_2 and should decrease to compensate for a lactate increase.[16] In the present study, an increase of pCO_2 was detected, which would lead to an increase in HCO_3^-, but the simultaneous increase in lactate blunted this elevation in HCO_3^-. Base excess is used to quantify the non-respiratory aspect of acid-base balance determinations. In this study it had a tendency to increase indicating a slight increase of HCO_3^- not clearly detected with direct determinations.

Finally, in the traditional approach an increase in anion gap is used as indirect evidence of increased blood lactate concentration when direct measurement is not possible. In the present study blood lactate concentration was directly measured with a portable analyzer, which has been previously validated in horses[17] and used in studies of exercise induced changes in blood lactate.[18]

Evaluation of acid-base balance using traditional approach only takes into a count pH, pCO_2, BE, HCO_3^- and AG. This provides limited information and only allows to classify alterations into respiratory alkalosis or acidosis, and metabolic alkalosis or acidosis. In endurance horses it is necessary to evaluate the magnitude of electrolyte changes and the effect of hemoconcentration (increase in protein concentration). Most of the endurance

horses in the present study showed mixed alterations that did not result in significant changes in pH, but important alterations can be present and not detected using traditional approach. In contrast, quantitative analyses allows differentiating metabolic acidosis or alkalosis due to changes in electrolytes (SID_m), lactate or due to changes in non-volatile buffers concentration (A_{TOT}) and respiratory alkalosis or acidosis.

Simplified stewart's approximation to acid-base balance has been previously used in man before and after exercise using venous blood samples[19] and has also been evaluated for show jumper horses.[16] To the authors' knowledge, there are no studies comparing the quantitative and traditional assessment of acid-base analyses in endurance horses. The quantitative approach uses three independent (pCO_2, SID_m and A_{TOT}) and one dependent variable (pH) for the assessment of acid-base equilibrium. This approach has important advantages for the evaluation of endurance horses: 1) it takes into account the contribution of non-volatile weak buffers like proteins (albumin and globulins) and of strong ions on pH, and 2) complex alterations of acid-base balance are easier to detect. The quantitative approach allows an improved interpretation of acid-base equilibrium when electrolytes or protein concentrations are altered, but requires measurement of multiple parameters and use of complex equations. In the quantitative method, pH and pCO_2 are interpreted as in the traditional approach. The difference between both methods is focused on assessment of metabolic disturbances. In the present study, the most frequent alteration found using the quantitative method was metabolic alkalosis due to increased SID_m, as well as complex alterations characterized by metabolic alkalosis due to SID_m plus metabolic acidosis due to A_{TOT}. Hoffman *et al.*[6] reported that the strongest determinant of plasma pH in an 80 km race was SID_m. Changes in SID_m values in exercising horses are due to electrolyte losses in sweat and increases of blood lactate concentration. Horse sweat is iso- or slightly hypertonic with the same concentration of sodium than plasma, but higher chloride concentration relative to plasma. Due to electrolyte concentrations of sweat, chloride losses are more severe than others ions.[15,20] Plasma chloride concentration also decreased in the initial minutes of jumping exercise due to an influx into muscle and red cell.[16] The combination of these conditions produces a fast decrease in plasma Cl^-. In the present study, a greater decrease of Cl^- concentration was detected (6 mmol/L) compared with values reported in

previous studies,[21] but similar to that reported in horses competing in a 160 km race.[4] The decrease of Cl^- can lead to metabolic alkalosis, but usually an increased lactate concentration will attenuate this.

Decreased sodium concentration was likely associated with a combination of prolonged sweating and addition of water to extra cellular fluid (ECF) space by voluntary drinking of water or hypotonic electrolytes solutions (more important from midpoint race until end), or a shift of intra cellular fluid (ICF) poor in Na^+ and Cl^- to the ECF space, specially from tissues that are less metabolically active during prolonged exercise.[22] Hypotonic fluid shifts or water consumption also contributes to dilution of other electrolytes and plasma components.[22]

Plasma K^+ concentration showed a mild tendency to decrease along the race that recovered at the race finish-line. Previous studies report an increase of plasma K^+ concentration beginning at 4 m/s (14 km/h) and explain the decrease in plasma K^+ concentration detected after the race due to rapid redistribution (3 minutes) of this electrolyte during recovery inside red blood cells and muscle cells.[7]

A slight increase in SID_m values was detected due to a decrease in Cl^- despite a mild decrease in Na^+ and mild increase in lactate concentrations. Strong ion difference was higher during exercise suggesting that the main determinant of it was Cl^- loss leading to metabolic alkalosis (increase in SID_m). Usually when SID_m increases pH does increase as well (i.e. tendency towards alkalosis) but in the present study no changes in pH were observed. During the race a complex derangement developed in which pH did not clearly change due to the combined effects of: (1) mild increase in SID_m (tendency towards alkalosis), (2) an increase in pCO_2 (leading to acidosis) and (3) an increase in A_{TOT} (it also causes a tendency toward acidosis).

Plasma proteins (mainly albumin) and phosphates are weak acids that are not fully dissociated at physiologic pH, and as such are capable of buffering hydrogen ions. In the simplified strong ion approach [A_{TOT}] represents the total plasma concentration of non-volatile weak buffers. Another important contribution to pH imbalances in endurance horses is the increase of A_{TOT} due to increased plasma proteins as a consequence of dehydration. An increase of TP has been documented during long distance endurance rides

(120-160 km) but values at the end of the ride generally returned to the resting values.[21] This finding suggests that hemoconcentration is greater during the first 60-80 km of exercise and the return to pre-ride results could be explained by a decrease in exercise intensity, due to losses of proteins from vascular space or addition of water to extra cellular fluid. A marked increase in TP (12%) was observed during the initial 65 km in the present study and a tendency to stabilization from midpoint to race finish. This tendency was more evident for plasma creatinine, which had an initial increase of 42% during the first half of the race and no statistical changes were detected from midpoint to the end. Similar changes of ~14% were detected in albumin and globulins concentrations. All these alterations were indicative of more marked dehydration tendency during first half of race and a posterior plateau maintained until race end, likely due to lack of drinking during the first half of the race. This is consistent with previous studies reporting that thirst is not stimulated during the first stages of an endurance race leading to sustained dehydration during and after the event.[23-24] Non-volatile buffer concentration also followed a tendency to increase because 2/3 of A_{TOT} is albumin, but the former increased more than expected due to higher increase of P_i concentration. Pre-race A_{TOT} was slightly lower than previously reported values,[13] likely associated with a mild hypoglobulinemia as seen in dog athletes during periods of endurance training.[25]

The interpretation of 33 out of 94 analyses matched using the two methods. The agreement between the traditional and quantitative methods of interpretation of acid-base balance was not as poor as expected, given that large alterations in pCO_2, SID_m or A_{TOT} were not found in this study. However, the concordance was poor when the interpretation was compared for each horse at the race finish (or at the time of elimination), and the authors suggest to use the quantitative approach whenever possible. The most common acid-base disorder observed was a mild SID_m alkalosis due to mild hypochloremia combined with mild non-volatile weak buffer acidosis due to mild increase in plasma protein concentrations. These derangements are detected using the quantitative approach but would be missed using the traditional approach to acid-base status.

In addition to the limitations inherent in field investigations, there were additional limitations in this study that warrant mention. First, no complete data collection and

detailed list of management factors were recorded. No information about supplement or electrolyte administration was obtained during the race. This fact made impossible to determine whether the mild electrolyte derangements seen in these hoses were related to administration of supplements by riders or exercise induced. The use of vacutainers for analyses of blood gases is currently not considered as the best sampling method according to clinical and laboratory standards institute[26] given that an anaerobic sample cannot be maintained. However, the authors believe minimal changes are expected due to our sampling method (samples for blood gas analyses were immediately performed) and the same sampling method has previously been used in other equine studies.[13-14,16]

Finally, accuracy of point-of-care blood analyzers may not be as precise as bench top analyzers. However, the hand-held analyzers used in this study have been validated for use in field studies of exercising horses[27-28] and also are currently used by team and treating veterinarians. But it was demonstrated that these analyzers underestimate blood potassium concentration (by ~0.4 mEq/L) and overestimates plasma Cl^- (by ~3 mEq/L),[29] as well as blood lactate concentrations (by 0.6 mmol/L for values lower than 5 mmol/L).[17] This deviation may explain the observed lower prerace SID_m values reported in this study.

Recently, a new quantitative method was suggested for acid-base interpretation in human beings. In contrast to traditional and Stewart's methods this new approach is not based on the principle of electroneutrality, but instead on the principles of mass conservation and pre-equilibrium proton concentration. The predictive value of pH using this new formula has a very good correlation with measured values,[30] but these equations have only been applied in protein-free multiple-buffered aqueous solutions and not yet in plasma.

In conclusion, traditional analyses should not be used for evaluation of endurance horses because mild alterations in pH are mainly caused by electrolyte and protein (A_{TOT}) changes that are only taken into account using Stewart's method of assessment of acid-base balance. Although pH did not significantly change in fit endurance horses during a 120-km race, a complex acid-base imbalance characterized by mild strong ion alkalosis (hypochloremia) attenuated by mild lactic acidosis, non-volatile buffer acidosis and compensatory mild respiratory acidosis was present in most horses. Using quantitative

approach these disturbances were detected and a more rational treatment could be elaborated. Complex acid-base alterations are present frequently in endurance horses and the quantitative method is more suitable to detect these kinds of disturbances.

Manufacturers' address

[a] Venoject, Terumo Europe, Leuven, Belgium.

[b] i-STAT® with EG7+ cartridges, Abbot, Illinois, USA.

[c] Accucheck Glucose, Roche Diagnostics, Basel, Switzerland.

[d] Accutrend Lactate, Roche Diagnostics, Basel, Switzerland.

Table 4.1: Mean ±SE values (except for CK and AST reported as median and 25th-75th percentiles) before, during and at the end of a 120 km FEI ride for all horses. Summarized data are all available observations plus missing values imputed by LOCF to perform statistical analyses by GEE models.

	Reference range†	Pre-race n=25	2nd Vet-gate n=29‡	3rd Vet-gate n=23‡	Final n=17‡
pH	7.31-7.45	7.40 ±0.01	7.40 ±0.01	7.39 ±0.01	7.39 ±0.01
SID_m (mmol/L)	35-38.5	37 ±0.2	39 ±0.5*	39 ±0.8*	38 ±0.5*
pCO_2 (mmHg)	41-53	47 ±0.5	52 ±0.9*	52 ±0.9*	50 ±0.9*
HCO_3^- (mEq/L)	24-30	29 ±0.3	32 ±0.7*	31 ±0.6*	30 ±0.6
Na^+ (mEq/L)	134-144	137 ±0.33	137 ±0.6	135 ±0.8	134 ±1.0
K^+ (mEq/L)	3.5-4.5	3.5 ±0.1	3.1 ±0.1*	3.1 ±0.1*	3.2 ±0.1*
Cl^- (mEq/L)	90-100	102 ±0.4	99 ±0.1*	97 ±1.3*	96 ±1.3*
Lactate (mmol/L)	<2	1.2 ±0.1	2.6 ±0.1*	2.7 ±0.1*	2.6 ±0.2*
P_i (mg/dL)	1.5-4.5	2.7 ±0.1	3.3 ±0.2*	3.7 ±0.2*	3.5 ±0.2*
BE (mEq/L)	-6-+6	4.4 ±0.3	7.2 ±0.8*	6.2 ±0.7*	5.3 ±0.7
tCO_2 (mmHg)	28-35	30.4 ±0.3	33.3 ±0.7*	32.5 ±0.7*	31.1 ±0.7
PCV (%)	36-44	38.2 ±0.8	45.2 ±0.9*	46.5 ±1*	45.8 ±1.1*
TP (g/dL)	6.5-7.5	6.6 ±0.1	7.4 ±0.1*	7.5 ±0.1*	7.5 ±0.1*
Albumin (g/dL)	3.4-4.7	3.5 ±0.04	4.0 ±0.06*	4.0 ±0.07*	4.0 ±0.06*
Globulin (g/dL)	2.6-3.6	3.0 ±0.07	3.4 ±0.09*	3.3 ±0.09*	3.3 ±0.09*
A_{TOT} (mEq/L)	12-15	12.9 ±0.14	15.6 ±0.3*	15.8 ±0.3*	15.7 ±0.3*
SIG	-2-+6	1.8 ±0.2	3.4 ±0.5*	4.4 ±0.7*	2.6 ±0.5
Glucose (mmol/L)	5.45-5.98	6.2 ±0.1	6.4 ±0.21	5.4 ±0.3*	6.3 ±0.3
iCa^{++} (mmol/L)	1.4-1.6	1.6 ±0.01	1.5 ±0.03*	1.4 ±0.03*	1.5 ±0.03*
Creatinine (mg/dL)	<2	1.2 ±0.03	1.6 ±0.06*	1.7 ±0.08*	1.7 ±0.07*
CK (IU/L)	100-300	250 (181.3-299.5)	594 (460-1,705)	1,035 (767-2,943)	1,649 (1,254-3,037)*
AST (IU/L)	150-400	325 (276-379)	399 (334-542)	450 (370-630)	450 (416-603) *
Osmolarity (mOsm/kg)	270-300	292.5 ±0.6	293.9 ±1.15	290.9 ±1.5	290 ±1.7

*Significant difference compared to pre-race values; †[5,13-14,31]; ‡ number of horses at the end of each phase.

Table 4.2. Interpretation of acid-base balance of all horses at their last time point (at the time of elimination or the race finish, n=29) and of all finishing horses (n=17) using the traditional and the quantitative approaches.

	Competitors (n=29)	Finishers (n=17)	Interpretation
Traditional approach	11	6	Lactic metabolic acidosis
	6	2	Metabolic alkalosis + Lactic metabolic acidosis
	1	1	Respiratory acidosis
	5	2	Respiratory acidosis + Metabolic alkalosis + Lactic metabolic acidosis
	3	3	Respiratory acidosis + Lactic metabolic acidosis
	3	3	Normal
Quantitative approach	3	0	Lactic metabolic acidosis
	2	2	SID_m metabolic acidosis
	1	1	SID_m metabolic alkalosis
	2	1	SID_m metabolic alkalosis + Lactic metabolic acidosis
	4	3	A_{TOT} metabolic acidosis + Lactic metabolic acidosis
	8	4	A_{TOT} metabolic acidosis + SID_m metabolic alkalosis + Lactic metabolic acidosis
	3	2	Respiratory acidosis + Lactic metabolic acidosis
	1	1	Respiratory acidosis + A_{TOT} metabolic acidosis + Lactic metabolic acidosis
	1	1	Respiratory acidosis + A_{TOT} metabolic alkalosis + Lactic metabolic acidosis
	1	1	Respiratory acidosis + A_{TOT} metabolic acidosis + SID_m metabolic alkalosis
	3	1	Respiratory acidosis + A_{TOT} metabolic acidosis + SID_m metabolic alkalosis + Lactic metabolic acidosis

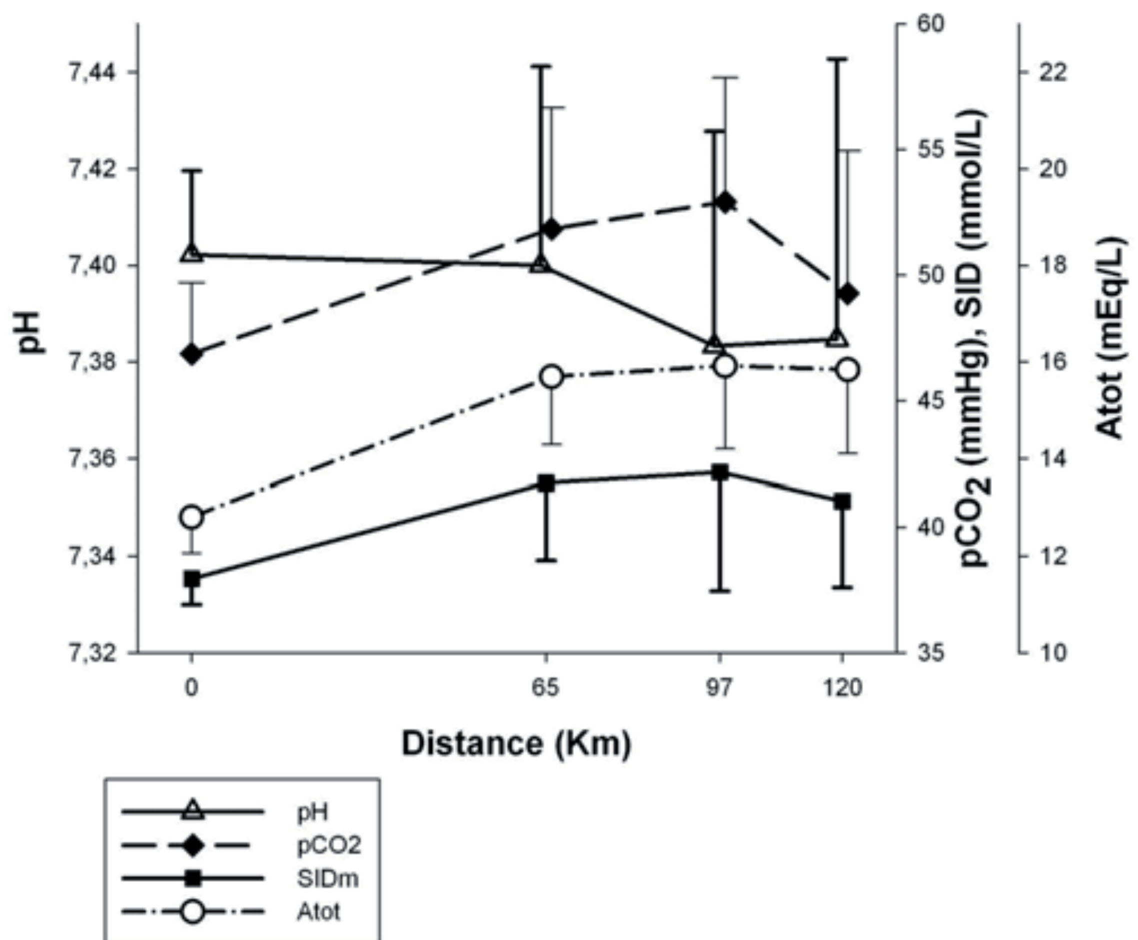

Figure 4.1. Mean ±SE temperature corrected pH, strong ion difference (SID_m), pCO_2 and non-volatile weak buffer concentration (A_{TOT}) in horses during a 120-km FEI*** endurance race. All available data are presented without imputation of missing values by LOCF.

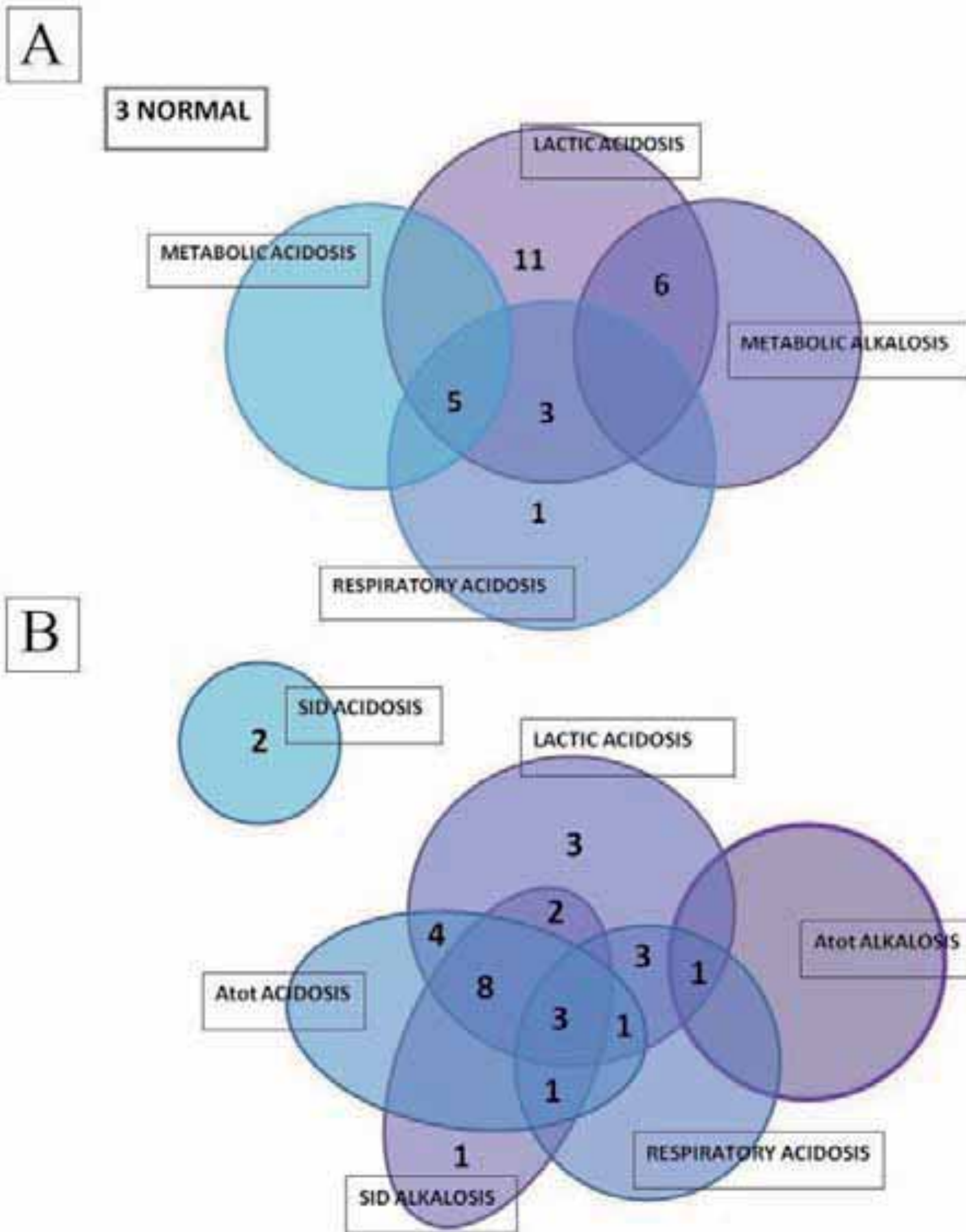

Figure 4.2. Interpretation of acid-base balance at the race finish (or at the vet-gate that were eliminated) of each horse using the traditional (A) or the quantitative approaches (B).

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Chapter Four

Article under revision for the Journal of Veterinary Emergency and Critical Care . Accepted for publication with major revisions to combine study 2 and study 3.

[STUDY TWO]

[Reference values of A_{TOT} and SID_m in healthy neonatal foals]

Summary

Objective – To determine strong ion difference (SID_m) and total non-volatile weak buffers (A_{TOT}) in healthy neonatal foals and compare calculated variables obtained from reference biochemistry laboratory with those calculated from results obtained with analyzers usually available during emergency hours.

Design – Observational study performed during 2011-12 foaling season

Setting – Study performed at *Unitat Equina-Fundació Hospital Clínic Veterinari*

Animals – 33 healthy neonatal foals (<21 days) from stud farms nearby hospital were used.

Interventions – Blood samples (EDTA, lithium heparin and plain tubes) were obtained by direct vein puncture. Blood L-lactate concentration was determined after collection using a handheld analyzer. Further determinations in plasma (sodium, potassium, chloride and albumin) were performed using 3 different afterhours analyzers and reference laboratory. Reference ranges of SID_m and A_{TOT} for each of the analyzers under comparison was established using mean±2×SD and 2.5^{th} - 97.5^{th} percentile, respectively. In both cases, Bootstrap method (1000 re-sampling) was applied. Agreement between devices for each one of the parameters determined was evaluated using Bland-Altman and Lin's concordance coefficient methods.

Measurements and Main Results – Reference ranges for SID_m and A_{TOT} were obtained for each of the analyzers tested. Regarding cross-comparison of results obtained with different devices, agreement in numerical values was poor, and the differences between afterhours analyzers and the "gold-standard" biochemistry analyzer were considered clinically relevant for SID_m in more than half of the samples. No clinically relevant differences in A_{TOT} were detected when comparing afterhours and the "gold-standard" analyzer.

Conclusions – Reference ranges obtained in this study were wider and lower than those for adult horses. Regarding different devices, all handheld analyzers could be useful to assess acid-base balance, but numerical agreement between analyzers is poor and it is recommended to apply normal specific device reference ranges.

Introduction

In human medicine, some acid-base (AB) imbalances are associated with certain neonatal conditions. These imbalances differ between neonates and adults even when both suffer the same conditions.[1] Application of the simplified Stewart methodology to pediatric AB disturbances has shown similarities but also important differences between adult and neonatal human patients, probably due to immaturity of organs involved in maintenance of AB status in pediatric patients.[1] A better understanding of these alterations may allow detection and characterization of the differences in mechanisms leading to multi organ failure in neonates and decide their therapy more appropriately. On the other hand, normal values of AB balance (SID_m, A_{TOT}, pH, BE) in healthy human neonates differ from adults.[1]

Although multiple studies performed in horses and neonatal foals have determined most of the parameters required for the quantitative method of AB balance interpretation (i.e. concentrations of sodium [Na^+],[2, 3] potassium [K^+],[2, 3] chloride [Cl^-],[2, 3] L-lactate (Lac),[4] Albumin (Alb),[3, 5] Globulins (Gb),[5] phosphates (P_i)[2] or partial venous pressure of CO_2 (pCO_2)[2, 3]) the reference range for calculated variables has not been determined in healthy or ill neonatal foals. Due to the complex balance present between the principle variables calculated of AB balance employing the quantitative method (strong ion difference (SID_m) and non-volatile buffers (A_{TOT})), an estimation using normal values of their components is not accurate enough for a correct establishment of reference range. Multiple studies comparing adults and neonatal foals have shown significant differences of biochemical parameters[6] and standard values of SID_m and A_{TOT} published for adult horses are not therefore necessarily applicable to equine neonates. Determination of a reference range of SID_m and A_{TOT} in neonatal foals is essential for a correct quantitative AB interpretation.

It is important to point out that in daily clinical veterinary practice and during emergency hours most of the reference laboratories are not available. Fortunately, multiple handheld analyzers validated for equine species or user-friendly analyzers are available afterhours. These point–of–care analyzers use direct potentiometry methods that could differ from reference laboratory results (indirect potentiometry) especially when certain interference substances are present in samples.[7] The variability of the results obtained with

these types of analyzers in relation to a reference laboratory could be significant in some cases, especially when the clinician has to use an equation combining results obtained from different analyzers. Each value obtained by these devices has slight deviations from results obtained with gold standard methods. Due to these, the accumulated error in complex calculations is sufficient to lead to an incorrect interpretation of AB balance as it has been reported in human medicine.[8]

The objectives of the present study were 1) to determine reference values for calculated parameters of quantitative AB balance interpretation in neonatal foals 2) to determine the agreement between commonly used analyzers and the gold standard biochemistry method for foals, and 3) suggest a reference range for each type of analyzer in order to avoid errors in acid-base interpretation.

Materials and methods

Healthy foals from 1 to 21 days of age from stud farms near the hospital were included in the study. Healthiness was assessed by physical examination and determination of IgG concentrations. Blood samples (EDTA, Lithium heparin and plain tubes) were obtained by direct vein puncture. L-lactate was determined immediately with blood obtained in lithium heparin using a horse validated handheld analyzer[a].

Plasma and serum were obtained from lithium heparin and plain tubes respectively by centrifugation (1500 rpm for 30 minutes) within 2 hours after collection. Samples were maintained refrigerated (4ºC) until being processed. Serum was used to determine IgG concentrations using a semiquantitative turbidimetric method ($ZnSO_4$ test) and plasma was frozen (-20ºC) until further determinations (Na^+, K^+, Cl^-, Alb and P_i) were performed.

Ionized sodium, K^+ and Cl^- concentrations were determined in all foals using Analyzer 1[b] (Ana-1) by ion selective electrode potentiometry (Nerst equation). These electrolytes were also measured with Analyzer-2 (Ana-2)[c] and reference biochemistry laboratory using ion selective electrode method [e]. In addition, Alb and P_i concentrations were measured in the reference biochemistry laboratory using green bromocresol method and molybdate method[e], respectively. Albumin concentration was determined also using Analyzer-3 (Ana-3)[d] by dry-slide technology (bromocresol green method).

Blood EDTA samples were used to determine PCV (microcentrifugation) and total protein measured by refractometry[f] (TP).

The globulin (Gb) value was obtained using the following formula:

$$Gb = TP - Alb \quad (32)$$

Comparison of devices

Results obtained with Ana-1, Ana-2 and Ana-3 were compared with the results obtained using the same samples determined in the reference biochemistry laboratory as described above (n=33).

For the purpose of this study, in order to determine if differences between analyzers were considered clinically significant, a tolerable difference for each parameter was set (Na^+ =6mEq/L, K^+ =0.5mEq/L , Cl^- =6mEq/L, Alb =0.5g/dL, SID_m =3 mEq/L, A_{TOT}

=0.3mEq/L).

Acid-Base Analyses

Quantitative analyses of AB balance was assessed using the method described by Stewart (1983) and simplified by Constable (1997). Measured strong ion difference (SID_m) and total non-volatile buffers (A_{TOT}) were calculated using the following formula:

$$SID_m = ([Na^+] + [K^+]) - ([Cl^-] + Lac) \quad (10)$$

$$A_{TOT} = 2.25 \times Alb + 1.4 \times Gb + 0.59 \times P_i \quad (16)$$

Statistical analyses

Descriptive values are shown as mean ± standard deviation (SD), unless otherwise stated. Determination of the reference range values for SID_m and A_{TOT} was performed by the calculation of mean ± 2×SD for normal distributed variables and 2.5^{th}-97.5^{th} percentiles for non-normal distributed variables. With the aim to assess more accurately the variability, Bootstrap procedure was performed with n=1000 samples for estimating SD and percentiles. The evaluation of the agreement between the gold standard analyses and different analytical devices was performed by means of Lin's concordance coefficient with 95% confidence interval (95%CI). [9, 10] The Lin's concordance coefficient measures the accuracy and precision to determine whether data observed significantly diverges from the line of perfect concordance, i.e. a lineal regression at origin (0.0) and 45 degrees (i.e. the slope of the line equals one). This value increases from 0 to 1 as the accuracy and precision of the observed data improve.

In addition, a graphical Bland and Altman[11] analyses was used to compare results between different methods and to evaluate the causes of disagreement between pairs of methods. This graphical representation is complementary to Lin's analyses.

All data was analyzed with statistical analyses software[g] and values of $p \leq 0.05$ were considered statistically significant.

Thirty three healthy foals (10 colts and 23 fillies) were included in the study. The breeds of foals were Andalusian (n=13) and Arabian (n=20), with a median age of 4 days (range 1-20 days).

Descriptive values and resulting reference ranges for Na^+, K^+, Cl^-, Lac, Alb, Gb, P_i, SID_m and A_{TOT} variables (using Ana-1, Ana-2 and reference laboratory) are presented in Table 4.3.

Comparison between analyzers

Initial analyses in the field was made with handheld analyzers (Ana-1 and L-lactate analyzer[a]) and further analyses (Ana-2, Ana-3 and reference laboratory) were performed simultaneously. The mean±SD of Na^+, K^+, Cl^- and Alb for each analyzer are shown in Table 4.3. Significant differences were found between values obtained in each analyzer. Lin's concordance coefficients are shown in Table 4.4. No agreement (close to 0) was observed between gold standard analyses and Ana-1 for Na^+ and Cl^- concentrations and SID_m calculation, between gold standard and Ana-2 for Na^+ and Cl^- concentrations and between gold standard and Ana-3 for Alb concentration. Poor concordance (<0.65) was detected between gold standard and Ana-2 for K^+ concentration and SID_m calculation. Moderate concordance (0.65-0.8) was found between gold standard and Ana-1 for K^+ concentration and almost perfect correlation (>0.9) was observed between gold standard and Ana-3 for A_{TOT} calculation.

A tolerable difference among results from different analyzers for each parameter was set. The highest number of discordances between the reference biochemistry laboratory and other analyzers were found for Na^+ (12/33 Ana-1 and 20/33 Ana-2) and Alb (21/33 Ana-3) concentrations and SID_m calculation (18/33 Ana-1 and 17/33 Ana-2). An important number of cases with discordant results were found for Cl^- concentration (6/33 Ana-1 and 22/33 Ana-2) and small number of cases with discordant results were found for K^+ concentration (6/33 Ana-1 and 3/33 Ana-2) and A_{TOT} calculation (0/33 Ana-3). Considering Ana-1 results, several animals had clinically significant abnormal values.

Precisely, 5/33 foals for Na^+ concentration, 2/33 for K^+ concentration, and 6/33 for Cl^- concentration would have required clinical intervention. When Ana-2 was used, 2/33 for Na^+ concentration 3/33 for K^+ concentration and 9/33 for Cl^- concentration animals would have required treatment. In case of Ana-3, only one foal would have required treatment. Regarding laboratory reference results, 6/33 animals for Na^+ concentration, 1/33 for K^+ concentration, 2/33 for Cl^- concentration and 0/33 for Alb concentration would have required treatment.

Bland Altman plots comparing reference laboratory values with each of the 3 afterhours devices are presented in Figures 4.3, 4.4 and 4.5.

Discussion

Clinicopathological differences observed between neonates and adults of any species are well known. Adaptation to extra uterine life, a higher proportion of total body water in neonates, different diet and immaturity of certain organs explain some of the differences detected between adults and neonates.[1, 12] Establishment of reference values in neonatal period is essential for correct clinical evaluation of critically ill patients and have been performed for most of the clinically relevant parameters. Regarding AB interpretation, Constable's simplification[13] of the Stewart method[14] makes the last one easier to use. Since the late 90's, the description of normal AB values for horse plasma allows the evaluation of critically ill horses using the quantitative method. Despite the early application of quantitative AB assessment in equine practice, reference values of all the main variables are not well established for animals of all ages. This is especially important during the neonatal period because ill foals usually require intensive care treatments and accurate AB interpretations. Calculated variables (SID_m and A_{TOT}) are composed of ions and buffers that are in complex equilibrium. Therefore, reference range values for these calculated variables are not equivalent to the calculated reference ranges from their individual components. In the present study, different reference range values for calculated variables are obtained from a healthy group of foals using both reference laboratory and handheld analyzers.

Reference values for calculated variables SID_m and A_{TOT} in neonatal foals were likely to be different from adult horses as occurs in human medicine.[1, 15, 16] In the present study, SID_m reference ranges obtained for all the analyzers are slightly wider and include lower values than reported for adult horses. These results were foreseen because electrolyte concentrations previously reported for equine neonates show a wide variation especially regarding Na^+ concentration,[2, 17] with normal published reference ranges as wide as 123-159 mEq Na^+/L during first days of life.[2] Although renal function is relatively mature in a foal at birth, variations in electrolyte concentrations are particularly prevalent during first 4 days of life,[18] when neonatal foals show differences regarding electrolytes concentrations (i.e decreased fractional excretions of K^+, Ca^{2+}) and creatinine excretion[6, 19] compared to adults.

Considering A_{TOT} and its components, several differences are observed in neonatal foals relative to adult horses. The reference range of A_{TOT} in this study was numerically wider and included lower values. Albumin and Gb concentrations were lower in neonatal foals, as previously described.[5] Decreased Alb and Gb concentrations resulted in lower values of A_{TOT}. Higher concentrations of plasma P_i could not totally offset lower values of Alb and Gb compared to adults, due to the lower contribution of P_i to the electrical charge of non-volatile weak buffers (A_{TOT}).

Since all the animals used in the present study had more than one day of life, reference range values obtained could not be applied to younger foals. Compared to the results herein reported, during the first 8-12 hours of life, small differences could be expected in A_{TOT} due to immunoglobulin absorption, and in SID_m due to potentially increased L-lactate concentration.

The second objective of the present study was to evaluate concordance of different analyzers available afterhours with the reference biochemistry laboratory. Unfortunately, poor concordance was found between the reference laboratory and devices usually used in emergencies. The differences observed between analyzers for most of the parameters assessed were greater than what was considered to be a tolerable difference, according to the criteria established by these authors in the materials and methods section. This disagreement was previously reported for Ana-1 regarding Na^+, K^+ and Cl^- determination in adult horses.[20,21] Only K^+ determination with Ana-1 and A_{TOT} with Ana-3 had low number of values out of the accepted variation. One proved source of variability between analyzers [7] is the different technology used in point-of-care analyzers (direct potentiometry) and reference laboratory analyzers (indirect potentiometry). Another possible cause of variation, in the present study, could be the time taken to process samples. In case of Ana-1, samples were analyzed immediately but in cases of Ana-2, Ana-3 and reference laboratory samples had to be transported and processed before determination. Storage at 4ºC slows Na^+-K^+ ATP'ase pump and causes an extracellular movement of potassium.[22] However, as it has previously been reported[23] potassium concentrations do not change significantly during the first 2 hours in samples kept at 4ºC, so therefore, in the present study, sample processing should not have a significant change SID_m. When compared with reference laboratory

results, ome of the results obtained with the different analyzers (false positive results) indicate that a clinical intervention would be required. This is concerning because all the animals used for the present study were healthy foals. Instead of using a general reference range, the application of the specific reference range values for each one of the analyzers would reduce the risk of false positive results and improve the accurancy of interpretation. In the present study, a different SID_m reference range for the gold standard biochemistry laboratory and another for two afterhours analyzers (Ana-1 and Ana-2) were set in order to facilitate a more accurate evaluation. On the other hand, these results were obtained with a sample of convenience of healthy animals and the reference range obtained should be validated using an external population of healthy neonatal foals.

In conclusion, we suggest that normal range of SID_m in neonatal foals is 34-46 mEq/L for reference laboratory, 34-50 mEq/L for Ana-1 and 31-47 mEq/L for Ana-2. Regarding A_{TOT}, reference range obtained for reference laboratory is 11-17 mEq/L and 11-17.5 mEq/L for Ana-3. Concordance between afterhours analyzers and the reference biochemistry laboratory is poor for some parameters. It is advisable therefore, to use only one device when serial determinations are performed over time on the same patient, and specific device reference range should be used in order to minimize interpretation errors. In cases where more than one device has to be used, only K^+ concentration obtained from Ana-1 and A_{TOT} obtained with Ana-3 are similar enough to the reference biochemistry laboratory results to be used interchangeably.

Footnotes:

[a]Accutrend lactate, F. Hoffmann-La Roche, France

[b] iSTAT, Abbott laboratories, IL, USA

[c] Vetlyte, Idexx laboratories, Inc, Netherlands

[d] Catlyst, Idexx laboratories, Inc, Netherlands

e Olympus AV400, Olympus corp, Germany

[f]Atago Corporation (Atago Ltd, Tokyo, Japan)

[8]PASW Statistics 18 for Windows, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com).

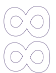

Table 4.3: Mean, standard deviation (SD) and reference range (Mean ± 2 × SD) for normally distributed and median, [P25th-P75th] and reference range [P2.5th-P97.5th] for non-normal distributed variables obtained from 33 healthy foals to assess AB balance by Stewart's method.

		Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Lac (mmol/L)	Alb (g/dL)	Gb (g/dL)	P_i (mg/dL)	SID_m (mEq/L)	A_{TOT} (mEq/L)
Ref Range published[13]		130-154[2]	3.8-5.8[2]	94-110[2]	<2.0[17]	2.7-3.4[2]	1.6-3.9[2]	5.4-9.4[2]	37.0-43.0[*13]	13.0-16.5[*24]
Reference laboratory	Mean± SD	134±5	4.1±0.4	95±4	2.6±1[a]	2.8±0.2	3.3 [2.6-3.7]	6.4 [5.2-7.5]	40.3±3.1	14.9 [13.0-15.8]
	Ref Range	(124-144)	(3.3-4.9)	(87-103)	(0.6-4.6)[a]	(2.4-3.2)	[1.4-4.6]	[2.9-8]	(34.1-46.5)	[10.9-17.0]
Ana-1	Mean± SD	135±6	4.0±0.5	94±5	-	-	-	-	42.5±4.5	-
	Ref Range	(123-147)	(3.0-5.0)	(84-104)	-	-	-	-	(33.5-51.5)	-
Ana-2	Mean± SD	141±6	4.2±0.7	103±4	-	-	-	-	39.2±3.9	-
	Ref Range	(129-153)	(2.8-5.6)	(95-111)	-	-	-	-	(31.4-47.0)	-
Ana-3	Mean± SD	-	-	-	-	2.3±0.2	3.9 [3.2-4.1]	-	-	14.7§ [13.2-15.3]
	Ref Range	-	-	-	-	(1.9-2.7)	[2.4-5.1]	-	-	[11.1-17.5]§

* Adult horses[13,24] one week foal reference [2,17]; [a]Accutrend lactate, Ana-1 (iSTAT®), Ana-2 (Vetlyte®), Ana-3 (Catalyst®); §A_{TOT} calculated using P_i from reference laboratory.

Table 4.4: Lin's concordance coefficient (95% confidence interval) between reference laboratory and different devices obtained from 33 healthy neonatal foals.

	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	SID_m (mEq/L)	Alb (g/dL)	A_{TOT} (mEq/L)
Ana-1	0.06 (-0.27 - 0.38)	0.72 (0.53 - 0.84)	0.26 (-0.05 - 0.53)	0.12 (-0.16 - 0.39)	-	-
Ana-2	0.11 (-0.09 - 0.31)	0.53 (0.32 - 0.70)	0.04 (-0.07 - 0.14)	0.57 (0.32 - 0.75)	-	-
Ana-3	-	-	-	-	0.10 (0.01 - 0.20)	0.96 (0.93 - 0.97)

Ana-1 (iSTAT®), Ana-2 (Vetlyte®), Ana-3 (Catalyst®)

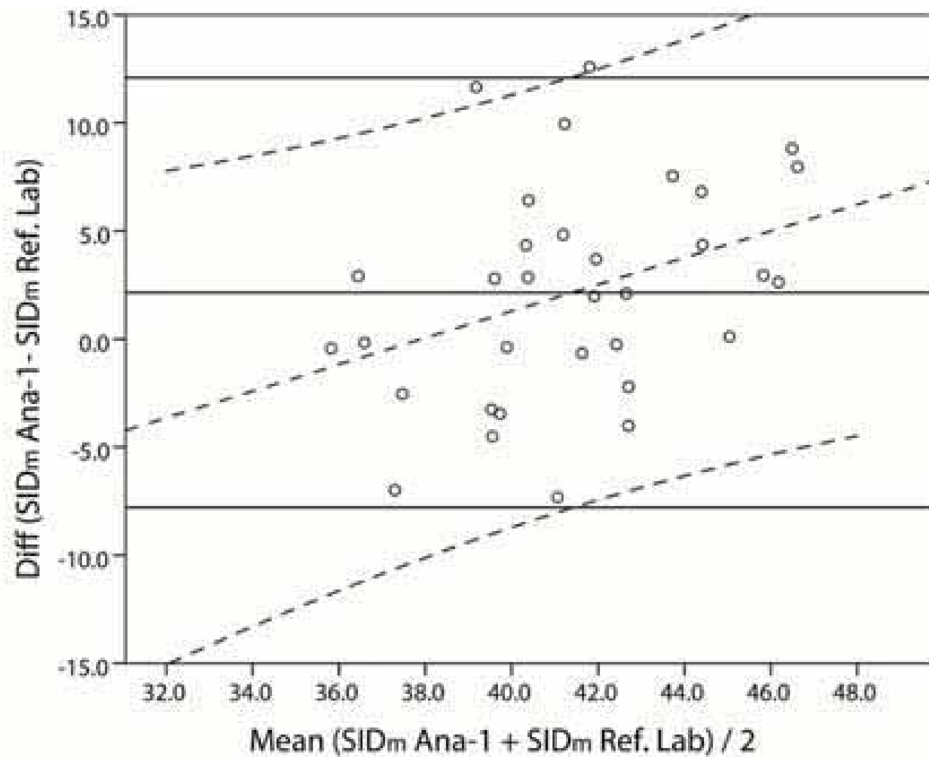

Figure 4.3: Bland Altman plot, with mean and mean ± 1.96×SD, comparing SID_m obtained from results of Ana-1 (iSTAT) and SID_m obtained from results of reference laboratory. Dashed lines represent regression line and 95% confidence intervals of the individual data.

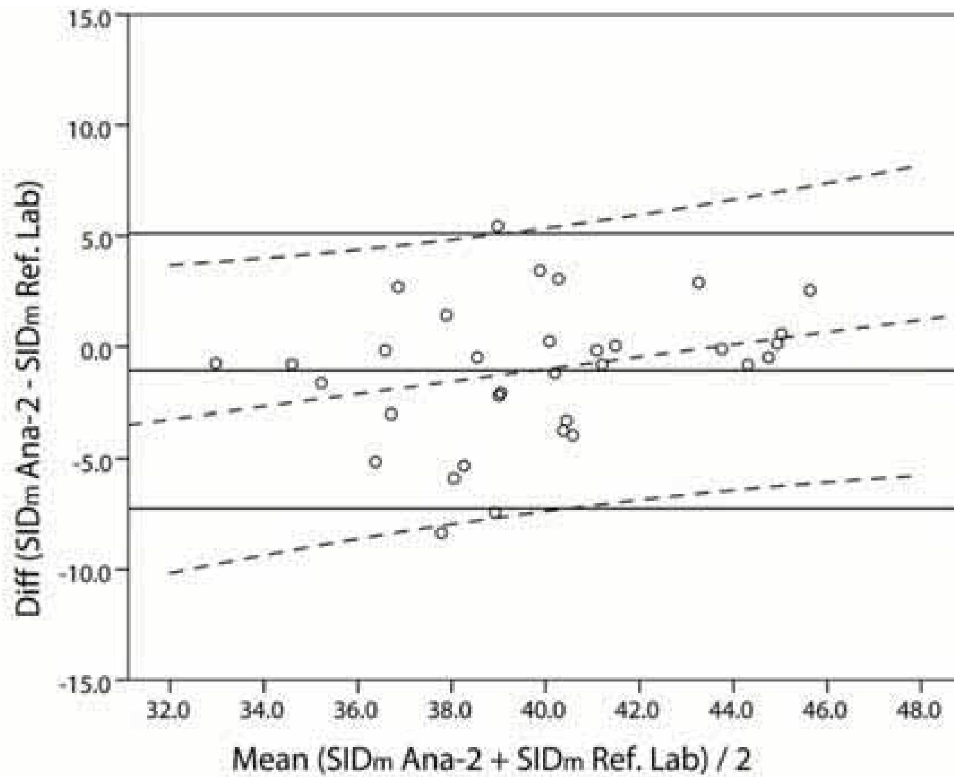

Figure 4.4: Bland Altman plot, with mean and mean ± 1.96×SD, comparing SID_m obtained from results of Ana-2 (Vetlyte®) and SID_m obtained from results of reference laboratory. Dashed lines represent regression line and 95% confidence intervals of the individual data.

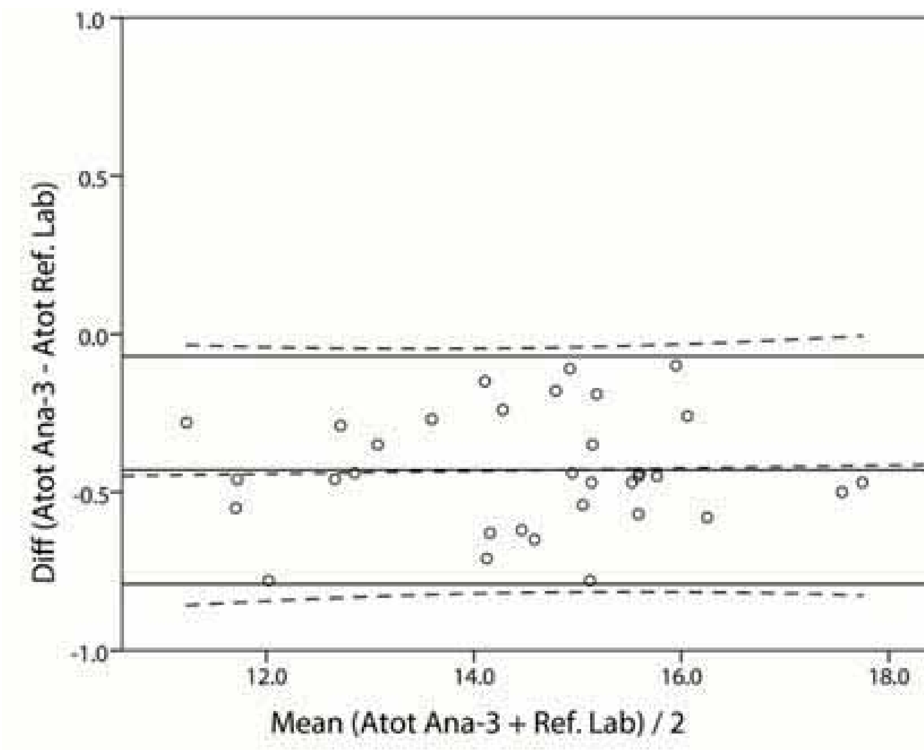

Figure 4.5: Bland Altman plot, with mean and mean ± 1.96×SD, comparing A_{TOT} obtained from Ana-3 (Catalyst®) and A_{TOT} obtained from results of reference laboratory. Dashed lines represent regression line and 95% confidence intervals of the individual data

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[STUDY THREE]

[Simplified strong ion difference approach to acid-base balance in healthy foals: effect of age]

Objective–To determine the effect of age on strong ion difference (SID_m) and non-volatile weak buffers (A_{TOT}) in healthy foals (<1 year) and provide a new simplified equation to estimate A_{TOT} from plasma protein concentration in neonatal foals.
Design– Observational study performed over 2 years (2010-12)
Setting– Study performed at *Unitat Equina-Fundació Hospital Clínic Veterinari* and , Equine Sport Medicine Center, CEMEDE, School of Veterinary Medicine, University of Cordoba .
Animals– 236 healthy foals distributed in 6 groups: A (21 days-2 months old; n=38), B (2-3 months old; n = 42), C (3-6 months old; n = 41), D (6-9 months old; n = 42), E (9-12 months old; n = 40) and 33 healthy neonatal foals (<21 days-old).
Interventions–Blood samples were obtained to determine L-lactate, sodium, potassium, chloride, total protein measured by refractometry and albumin concentrations in plasma or serum. Globulin concentration was estimated by the difference between total protein and albumin.

Constable equations were used to obtain calculated acid-base parameters. Age effect was evaluated using one-way ANOVA analyses. Agreement between A_{TOT} calculation methods was evaluated using Lin's concordance coefficient and Bland-Altman plots.
Measurements and Main Results– A significant age effect was detected for A_{TOT} and SID_m. In all foals younger than 6 months, A_{TOT} values were lower than older foals. A clinically significant difference in SID_m was detected only in the neonatal period. The equation to estimate A_{TOT} from plasma adjusted for neonatal foal protein is A_{TOT}=2.4× plasma total protein.
Conclusions–Reference values of A_{TOT} should be considered different from adults during the first 6 months of life. Regarding SID_m values should be considered different only during first 21 days of life. The new simplified A_{TOT} calculation for neonatal foals appears to be a better estimate of A_{TOT}, but it should only be applied in foals older than 1 day.

Introduction

During the neonatal period and first months of life multiple hematologic and biochemical changes occur in foals. The age effect on these results must be recognized before one can interpret alterations of plasma biochemical parameters in ill foals. Small variations observed due to age could be especially important when complex equations are applied to comprehensively assess a patient. In acid-base balance, quantitative interpretation relies on calculated variables (strong ion difference (SID_m) and total non-volatile weak buffers (A_{TOT})). These independent variables are calculated from ions and buffers that change during the neonatal period and the first months of life (total protein, albumin [Alb], [1, 2] Globulins (Gb), L-lactate [Lac] or phosphate [P_i][3, 4]) as well as other parameters without significant differences due to age (sodium [Na^+], potassium [K^+] and chloride [Cl^-]).[2] A tight balance is present between electrolytes and proteins to maintain plasma pH within a narrow physiologic range. The respiratory and renal functions adjust pCO_2, SID_m and A_{TOT} making it impossible to estimate the reference values of these variables from the normal values of their components.

Some clinicians consider the simplified Stewart method of interpretation of acid-base equilibrium difficult to apply in field conditions, regarding especially the determination of A_{TOT}. Several determinations are necessary, some of them not easily performed in ambulatory practice or during emergency hours, when only handheld analyzers are available. In order to facilitate the quantitative approach to daily clinic, a simplified approximation to estimate A_{TOT} was developed by Constable.[5] Doubt regarding the application of these approximations was presented by the authors in cases when albumin and globulin ratio (Alb:Gb) were not maintained. This situation is a special concern in neonatal foals in which Alb:Gb ratio is not equal to adult horses but, at the same time, rapid evaluation of patients is essential.

The objectives of the present study were; 1) to determine the influence of age in the calculated variables of the simplified Stewart method of acid-base balance equilibrium, 2) to obtain reference values for SID_m and A_{TOT} variables during different stages of the first year

of life and 3) to determinate a new equation to estimate A_{TOT} from total protein adjusted to neonatal foals.

Materials and methods

Neonatal Foals

Thirty three healthy foals from 1 to 21 days old from stud farms near to the hospital were included in the study. Healthiness was assessed by physical examination and determination of IgG concentrations. Blood samples (EDTA, lithium heparin and plain tubes) were obtained by direct vein puncture. Immediate determination of [Lac] was performed with blood obtained in lithium heparin using a horse validated handheld analyzer[a].

Plasma and serum were obtained from lithium heparin and plain tubes respectively by centrifugation (1500 rpm for 30 minutes) within first 2 hours after sample collection. Samples were maintained chilled (<4ºC) until processed. Serum was used to determine IgG levels using a semiquantitative turbidimetric method ($ZnSO_4$ test). Plasma was frozen (-20ºC) until further determinations ([Na^+], [K^+] , [Cl^-], [Alb] and [P_i]) were performed.

Determinatinon of [Na^+], [K^+] , [Cl^-] concentrations were performed in all foals using ion selective electrode[c]. Albumin concentration was determined using green bromocresol method[b] and [Pi] by the molybdate method[b].

Ethylenediaminetetraacetic acid samples were used to determine PCV (microcentrifugation) and total protein measured by refractometry[d] (TP). Globulin ([Gb]) concentration was obtained using the following formula:

$$[Gb]= [TP]-[Alb] \quad (32)$$

Quantitative analyses of acid-base balance was assessed using the method described by Stewart (1983) and simplified by Constable (1997). Measured strong ion difference (SID_m) and total non-volatile buffers (A_{TOT}) were calculated using the following formulas:

$$SID_m=([Na^+]+[K^+])-([Cl^-] +[Lac]) \quad (10)$$

$$A_{TOT}=2.25\times[Alb]+1.4\times[Gb]+0.59\times[P_i] \quad (16)$$

Results obtained for A_{TOT} were compared with those obtained using the simplified equation proposed by Constable for adult horses.

$$A_{TOT}=2.24\times Plasma\ total\ proteins \qquad (33)$$

Foals

A second group of foals between 21 days up to 12 months old was studied. All the animals were found to be healthy on physical examination, hematology, and biochemical determinations, including fibrinogen concentrations. According to their age, the foals were divided into 5 groups: A (21days-2 months old; n = 38), B (2–3 months old; n = 42), C (3–6 months old; n = 41), D (6–9 months old; n = 42) and E (9–12 months old; n = 40). Foals were weaned at 6 months of age (groups D and E). Furthermore, all the horses were sampled in the same month of the year (June). Fasting samples were taken in the foals older than 6 months. In the foals younger than 6 months, no attempt was made to control the time of the withdrawal of the blood samples in relation to the feeding time, as they were still nursing. Venous jugular blood samples were divided into 3 fractions and poured into tubes with EDTA, with citrate and plain tubes, respectively. EDTA tubes were used for hematological studies, citrate tubes for fibrinogen determination and plain tubes for clinical biochemistry. Citrate and plain tubes were centrifuged within the first 15 min after withdrawal. Citrated plasma and serum were harvested, placed in plastic tubes, kept refrigerated until their transport to the laboratory and frozen at -80 °C until assays were performed. All the analyses were performed within 3 months after extraction.

The following parameters were determined by spectrophotometry, using specific reagents: total serum proteins, [Alb], fibrinogen (Fib) and [Lac]. Globulins were calculated by adding fibrinogen to total serum proteins and subtracting [Alb]. Plasma total proteins were calculated by adding total serum proteins and fibrinogen. Serum [Na^+], [K^+] , and [Cl^-] concentrations (mEq/L) were measured using an analyzer with selective electrodes for each electrolyte (Analyzer-1[c]). The same equations described above were used to calculate SID_m and simplified equation for A_{TOT} calculation.

Statistical analyses

Descriptive analyses was performed using mean and standard deviation (SD). With the aim to assess more accurately the variability, Bootstrap procedure was performed with n= 1000 samples for estimating SD. To establish the reference range mean±2 SD were used for normal distributed variables and 2.5^{th}; 97.5^{th} percentiles for non-normal distributed variables in order to include 95% of healthy animals values.

Evaluation of normal distribution was performed by means of graphical methods. One way ANOVA analyses was used to detect differences in calculated parameters due to age, using Student-Newman-Keuls method for post-hoc analyses. In non-normal distributed variables a non-parametric approach by rank ANOVA analyses with the same post-hoc method was employed.

In neonatal foals, the agreement between different A_{TOT} calculations was evaluated using Lin's concordance index and Bland-Altman graphical method as complementary analyses. A_{TOT} calculated by means of [Gb], [Alb] and [P_i] was used as reference value. Linear regression using total solids was performed in order to evaluate a new constant for neonatal A_{TOT} simplified formula. The agreement of these new results was evaluated also with Lin's concordance method.

All data was analyzed with statistical analyses software[e] and a value of $p \leq 0.05$ was considered statistically significant.

Mean, minimum and maximal age for neonatal foals were 6 days (1 to 20 days), for group A were 39 days (30 to 60 days), for group B were 78 days (63 to 90 days), for group C were 134 days (93 to 178 days), for group D were 218 days (180 to 264 days) and for group E were 316 days (250 to 358 days). There were 23 females and 10 males in the neonatal group; 20 females and 19 males in group A; 24 females and 18 males in group B; 25 females and 16 males in group C; 19 females and 23 males in group D; and finally 25 females and 15 males in group E. Twenty Arabians foals were included in the neonatal group and the remaining foals were Andalusian bred foals.

Significant differences ($p<0.01$) in A_{TOT} and SID_m were found between groups due to age (Figure 4.6). Reference range obtained for each group of age (mean ± 2×SD) for normal distributed variables (SID_m) and percentile 2.5^{th}; 97.5^{th} for non-normal distributed variables, using Bootstrap (1000 re-sampling method) for both types of variables are represented in table 4.5.

Strong ion difference of neonatal foals differed from all other age groups except for group C (3-6 month; $p<0.001$). No statistical differences were found between the remaining groups.

Regarding A_{TOT}, neonatal foals were statistically different from all other groups ($p<0.003$). No significant differences were found between foals included in groups A, B, and C (1-6 months) but these 3 groups were significantly different from groups D and E (6-12 months; $p<0.001$).

In the 33 neonatal foals, A_{TOT} results obtained from both published equations ($A_{TOT}= 2.25 \times [Alb] + 1.4 \times [Gb] + 0.59 \times [P_i]$; and $A_{TOT}=2.24 \times PTP$) were compared using the Bland Altman plot (Figure 4.8) of A_{TOT} calculated from [Alb], [Gb] and $[P_i]$ equation and estimated from plasma total protein (PTP).

The resulting formula to estimate A_{TOT} from total solids adjusted for neonatal foals in the present study was

$$A_{TOT}=2.4 \times \text{Plasma total proteins} \quad (34)$$

When this equation was employed an improvement in A_{TOT} estimation was confirmed by Lin's concordance index. When using the equation A_{TOT}=2.24 × PTP a substantial agreement was observed (0.7 [0.6-0.8]), whereas almost perfect agreement (0.9 [0.8-0.95]) was observed for the new equation provided herein (A_{TOT}= 2.4 × PTP).[6] Bland Altman plot of A_{TOT} calculated with reference laboratory results and A_{TOT} obtained with new correction factor and traditional simplified A_{TOT} are presented in Figure 4.8. The resulting regression line for new A_{TOT} estimation (A_{TOT}= 2.4 × PTP) is shown in Figure 4.7.

Discussion

Quantitative assessment of an ill neonatal or young foal is essential for a correct approach to treatment and accurate interpretation; especially in critically ill animals.[7] Traditionally, some biochemical parameters are considered age dependent in young foals but changes related to age in calculated variables, used in simplified Stewart's acid-base interpretation, have not been evaluated previously. The approximation of a reference range for SID_m and A_{TOT} in foals using the parameters from which these are calculated is not possible because a tightly regulated equilibrium exists between the different elements of acid-base calculated parameters. The results of the present study can provide more detailed information about age related changes and also interpretation of acid-base balance disorders in young animals. The main results obtained herein suggest: 1) SID_m and A_{TOT} are related with age and this must be considered for acid-base interpretation in young animals; 2) Estimates of A_{TOT} using total protein are not accurate enough to be applied in neonatal foals in which Alb:Gb ratio is not the same as in adults; 3) An alternative simplified equation for A_{TOT} estimation from total protein adjusted for neonatal foals could be $A_{TOT} = (2.4 \times PTP)$.

Non-volatile weak buffer concentrations obtained in this study showed 4 statistically different groups: neonatal foals, 1-2 months animals, foals up to 6 months and older animals. This is probably related to the progression of total solids described in foals over the first year of life. Reported serum [Alb] concentration in neonates is usually lower than in adult horses[7, 8] and progressively increase during the first months of life.[1] Moreover, liver function is efficient from birth but not fully mature until 4-6 weeks when it achieves adult capacities.[9] Fibrinogen concentration is lower in foals at birth than in adults and increases until 5 months of age.[2, 10] Finally, marked changes in serum IgG concentrations during the first months of life have been reported in foals. The lowest IgG concentrations have been reported at 2 months of age, just before endogenous synthesis of IgG increases sufficiently to equal the rate of consumption, and at 8 months IgG concentrations are still approximately half of that measured in mature horses.[11] Changes in [Gb] and [Alb] concentrations with age suggest that Alb:Gb ratio in young foals (1-6 months old) is not

exactly the same as in adult horses.[1, 2] Results obtained in the present study are similar to those previously reported in the literature and confirm changes in protein values around the first and 6 months of life. Moreover, observed results in this group of foals suggested that further studies might be needed to validate adult A_{TOT} simplified equation in foals from 1 to 6 months old.

Regarding SID_m, neonatal values differ from foals of other age groups except in foals between 3-6 months of age. In the remaining age groups, differences statistically significant but not clinically relevant were found and had reference values (SID_m 39-46 mmol/L) similar to adult horses.[5, 12-14] Values obtained for [Na^+] in 3-6 months group were between neonate and previously reported adult concentrations.[15] When mean values for each age (Table 4.5) were compared no clinically significant differences were found between groups except for neonatal foals. Moreover, mean values of [Lac] obtained at 3-6 months of age are higher than in adult horses (Table 4.5). Increased [Lac] values could be explained by exercise performed to catch foals of 3-6 months on pasture just before venous sampling.

Changes in both components of SID_m ([Na^+] and [Lac]) resulted in a statistically decreased level in foals 3-6 months of age but this decrease was not clinically relevant. Other studies performed in foals of the same age confirmed that [Na^+] and [Lac] levels are similar to adult values.[1, 10] In order to discriminate differences between devices and due to age, electrolytes were determined in neonatal foals with the same analyzer used for the remaining foals. Although the device[c] employed to obtain electrolyte values uses the same technique (ion selective electrode method) as the gold standard biochemical laboratory, results could present wide variations especially in [Na^+] and [Cl^-] (Study two, pages 75-96). The results presented in this study should therefore be interpreted taking into account the inherent slight differences and reliability between electrolyte analyzers. Although results were not obtained from a reference laboratory, the value of neonatal SID_m was lower than oldest foals evaluated in the present study and reported adult.value.[5] Strong ion difference measured values in the remaining foals in this study were more similar to published reference but presents a wider range and slightly higher values than adult horses although adult values were obtained with a similar or less number of animals.[5, 12-14] These differences

could be attributed to a small number of samples or variations between analyzers. Slightly lower SID_m values in neonates have also been reported in human beings and could be explained by a higher [Lac] concentrations and relative high values of [Cl^-] (high [Cl^-] /[Na^+] ratio and low [Na^+] [Cl^-] difference).[15, 16]

Regarding estimation of A_{TOT} from TP concentration in adult horses, Constable suggested an adjustment factor of 2.24 ± 0.42.[5] The factor obtained in the present study to estimate A_{TOT} from TP concentration in neonatal foals is within the 95% confidence interval of that for adult horses. The small difference between adult horses and foals could be related to the higher P_i concentration of young animals previously described.[17] When A_{TOT} is calculated solely from total protein concentration a normal proportion between A_{TOT} components is assumed (Alb, Gb and P_i). The simplified equation should not therefore be used in animals with altered P_i or Alb:Gb ratio.[5] This exception includes protein losing disorders, marked acute or chronic inflammations and neonatal foals. The most marked differences in Alb:Gb ratio in newborn foals will be found during the first hours of life, due to immunoglobulin absorption. In this period A_{TOT} values obtained are probably lower than those obtained in this study due to low plasma [Gb] concentrations. Neonatal foals included in the present study were older than 1 day of age. The authors do not recommend the use of the new equation to estimate A_{TOT} from PTP or the A_{TOT} reference range, herein reported, in foals younger than one day or those that do not have the same Alb:Gb ratio than the studied population. Further studies focused on the first hours of life could be useful to establish a reference range in the youngest neonatal foals.

Footnotes:

[a]Accutrend lactate, F. Hoffmann-La Roche, France.

[b] Olympus AV400, Olympus corp, Germany.

[c] Vetlyte, Idexx laboratories, Inc, Netherlands.

[d] Atago Corporation (Atago Ltd, Tokyo, Japan)

[e]PASW Statistics 18 for windows, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com).

Table 4.5: Strong ion difference (SID_m) and total non-volatile weak buffers (A_{TOT}) reference range for each group of age (Mean±2 standard deviation (SD) for normal distributed variables and 2.5th and 97.5th percentiles for non-normal distributed variables). SID_m significant and clinically relevant differences marked with * and A_{TOT} significant differences between groups marked with a, b, c, d. Normal distributed variables were presented as Mean±SD and non-normal distributed as Median [P25th;P75th].

	Adult reference[5, 12]	Neonate	1-2 month	2-3 month	3-6 month	6-9 month	9-12 month
SID_m (mEq/L) Mean ± SD	39.0-46.0[5, 12]	31.4-47.0* 39.2±3.9	37.4-49.4 43.4±3.0	37.0-47.4 42.2±2.6	34.8-46.4* 40.6±2.9	37.5-50.3 43.9±3.2	37.8-46.6 42.2±2.2
Sodium (mEq/L)	135-142[18]	133±5	141±3	143±2	139±3	142±3	142±2
Potassium (mEq/L)	3.5-4.6[13]	4.2 [4.0;4.6]	4.4 [4.3;4.6]	4.6 [4.4;4.8]	4.7 [4.4;4.9]	4.2 [4.0;4.4]	4.5 [4.3;4.7]
Chloride (mEq/L)	97-103[18]	103±4	100±2	102±2	100±2	100±2	102±1
L-lactate (mmol/L)	< 2 [18]	2.6±1.2	1.8±0.5	2.7±0.7	3.1±0.5	2.7±0.8	1.9±0.5
A_{TOT} (mEq/L)[5] § Median [P25th;P75th]	13.0-16.5[12]	11.3-18.0 15.0 [13.7;15.8]	-	-	-	-	-
Albumin (g/dL)	2.3-3.6[13]	2.8±0.2	-	-	-	-	-
Globulin (g/dL)	1.7-4.7[13]	3.3 [2.7;3.7]	-	-	-	-	-
Phosphate (mg/dL)	3.1-5.6[13]	6.3 [5.3;7.5]	-	-	-	-	-
Simpl. A_{TOT} (mEq/L)[5]|| Median [P25th;P75th]		10.7-17.01[a] 13.9 [13.0;14.3]	13.1-16.9[b] 15.8 [15.0;16.3]	14.2-17.1[c] 15.4 [14.9;15.9]	13.6-16.0[c] 15.0 [14.6;15.6]	13.7-17.6[d] 16.7 [16.2;17.0]	14.7-17.5[d] 17.2 [16.2;17.4]
Total Protein (g/dL)	5.8-7.7[13]	6.3±0.7	6.9±0.4	6.9±0.3	6.7±0.3	7.3±0.4	7.5±0.4
New simpl. A_{TOT} (mEq/L)¶ Median [P25th;P75th]		11.5-18.2 14.9 [13.0;14.3]	-	-	-	-	-

P25th=percentile 25th ; P75th=Percentile 75th; §A_{TOT}= 2.24×[Alb] + 1.4×[Gb] + 0.59×[P_i]; ||A_{TOT}= 2.24×Total protein ;¶A_{TOT}=2.4×Total protein

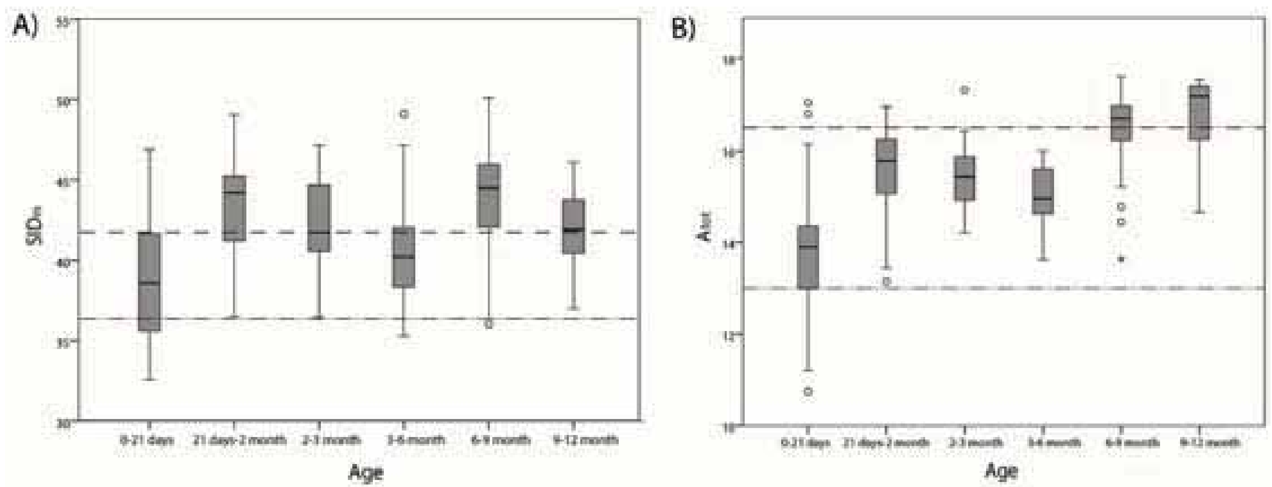

Figure 4.6: Effect of age on A) Strong ion difference (SID_m) and B) Total non-volatile weak buffers (A_{TOT}). Dashed lines represent reported reference range in adult horses. Open circles and asterisk depict outlier values.

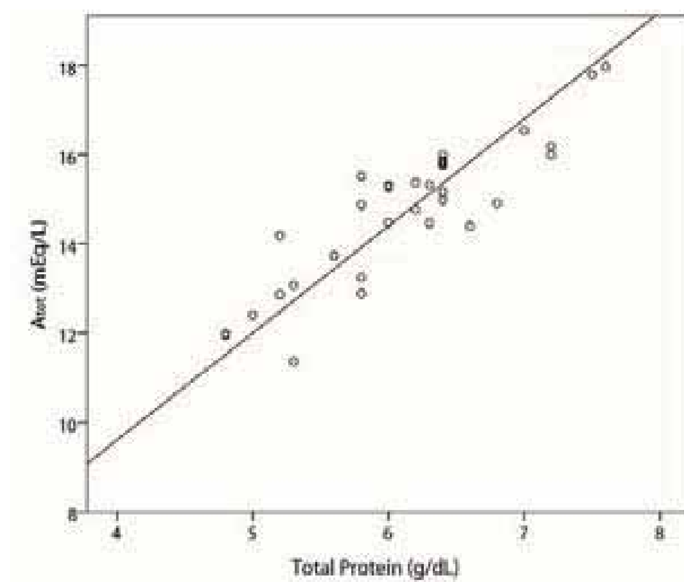

Figure 4.7: Regression line of total non-volatile weak buffers (A_{TOT}) calculated from Albumin, Globulin and Phosphate over total plasma protein obtained for neonatal foals.

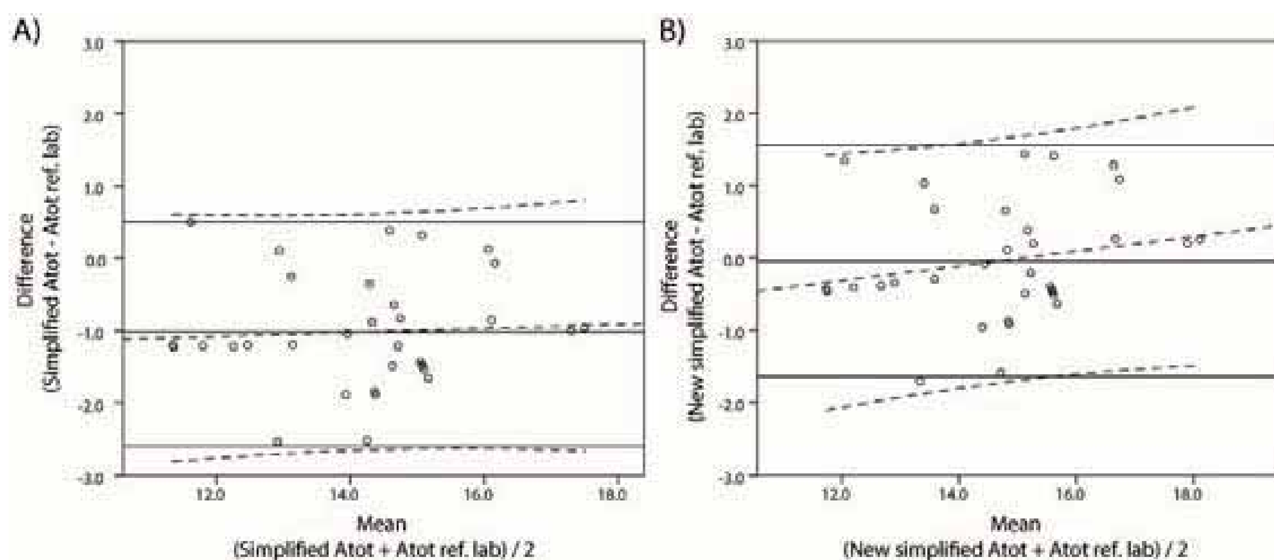

Figure 4.8: Bland Altman, with mean and mean±1.96×SD, comparison between total non-volatile weak buffers (A_{TOT}) calculation methods. Dashed lines represent linear regression and his 95% confidence interval (calculated over individual data). A) Comparison between A_{TOT} calculated from total proteins using factor described by Constable (Simplified A_{TOT}= 2.24×PTP) and A_{TOT} calculated from results obtained in reference laboratory (A_{TOT} ref. lab). B) Comparison between A_{TOT} calculated from total proteins using factor obtained in the present study (New simplified A_{TOT}=2.4 × PTP). and A_{TOT} calculated from results obtained in reference laboratory (A_{TOT} ref. lab).

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Results partially presented at the 11th Congress of the European Veterinary Emergency and Critical Care Society (June 15-17th 2012, Barcelona, Spain)

[STUDY FOUR]

[Acid-base imbalances in critically ill neonatal foals and their association with survival]

Abstract

Background- In pediatric medicine acid-base imbalances are useful for prognosis and they differ from alterations presented by adults.

Hypothesis/objectives- To determine acid-base imbalances by the quantitative method and to assess their association with diagnosis and prognosis in ill neonatal foals.

Animals- Foals <21 days-old admitted to *Unitat Equina-Fundació Hospital Clínic Veterinari* which had acid-base parameters determined upon admission were included (33 non-septic and 32 septic foals) and a control group of healthy animals from nearby stud farm (33 foals).

Methods- Observational study performed during 2005-2011 foaling seasons. Blood pH, pCO_2, sodium, potassium, chloride, L-lactate, plasma albumin and phosphate concentrations were determined. Calculated parameters were bicarbonate, globulin, strong ion difference (SID_m), non-volatile weak buffer concentrations (A_{TOT}), base excess and its components. ANCOVA statistical analyses was performed. Data are summarized as mean ±SD for normally distributed variables and median [25-75th quartiles] for non-normally distributed ones.

Results - Compared to healthy foals, ill foals had lower SID_m (non-septic 31.6±6.3 ($p<0.01$) and septic 32.0±6.4 ($p<0.01$) vs. control 40.3±3.1 mmol/L), potassium (non-septic 3.5 [3.3-3.8] ($p<0.01$) and septic 3.6 [3.2-4.3] ($p=0.01$) vs. control 4.2 [3.8-4.5] mEq/L) and higher L-lactate (non-septic 5.1±4.2 ($p=0.01$) and septic 5.0±3.7 ($p=0.03$) vs. control 2.5±1.3 mmol/L). Significantly higher levels of L-lactate and $pvCO_2$ were found in non surviving foals (6.4±3.5 mmol/L ($p=0.04$) and 51±13 mmHg ($p<0.01$)) compared to surviving foals.

Conclusions and clinical importance- The most common acid-base imbalances observed in ill foals were respiratory alkalosis and mixed respiratory alkalosis with acidosis due to low SID_m. Increased $pvCO_2$ and blood L-lactate concentration were associated with poor outcome in the present study.

In human medicine, some acid-base (AB) imbalances are often associated with certain neonatal diseases. These alterations differ between neonates and adults even when both are suffering the same conditions.[1] Application of the simplified Stewart's methodology to pediatric AB disturbances in humans has shown similarities but also large differences between adults and neonates, probably due to immaturity of organs involved in maintenance of AB status.[1] The knowledge of these different alterations of AB balance in neonates allows an improved detection and understanding of important differences in the mechanisms leading to multiorgan failure.[1]

Some studies in healthy and sick neonatal foals have determined normal and altered values of certain parameters used for quantitative AB interpretation (i.e. concentrations of sodium [Na^+], potassium [K^+], chloride [Cl^-], L-lactate, albumin (Alb), phosphates (P_i) partial venous pressure of CO_2 (p_vCO_2) or Base Excess (BE)).[2-9] A recent study performed by our group describes the normal values of calculated variables (Strong ion difference (SID_m) and total non-volatile buffers (A_{TOT})) in healthy foals. (Study two, pages 75-96) Regarding ill neonatal foals, a comprehensive description of alterations in AB balance using the quantitative method has not been reported.

Changes in AB balance, or, its components, are widely studied and used in human medicine for the prediction of survival in neonatal intensive care units.[1] In equine neonatal medicine, blood L-lactate concentrations on admission have been correlated with outcome.[10] However, some other AB parameters or components used in human medicine have not been evaluated in foals regarding survival.

For the above mentioned reasons the main objectives of the present study were: 1) to describe the frequency and type of AB imbalances in septic and non-septic foals and 2) to assess the association of AB variables with diagnosis (septic vs. non-septic vs. control foals) and prognosis in critically ill foals (survivors vs. non survivors).

Animals

All critically ill foals up to 21 days-old referred to *Unitat Equina-Fundació Hospital Clínic Veterinari*, from January 2005 until December 2011 were included in this study when a sample for blood gas analyses could be obtained before any in-hospital treatment. Animals were classified into 2 groups according to diagnosis: septic and critically ill non-septic foals. Newborn foals were included in the septic group when at least one of the following conditions occurred: positive blood culture, sepsis score >14[11] or post-mortem findings consistent with sepsis. Ill foals were included in the non-septic group when criteria for sepsis were not met. A control group of healthy newborn foals (<21 days old) from a stud farm near the hospital was also included. Healthiness of these foals was assessed by physical examination and determination of serum IgG concentrations. Ill foals were also classified by outcome in surviving foals (discharged) and non surviving foals. Non surviving foals (n=22) included animals euthanized due to poor prognosis (n=16) and those euthanized due to financial constraints in addition to poor life prognosis (n=6).

Samples obtained and measurements

Venous blood samples were obtained in EDTA, lithium heparin and plain tubes. An additional arterial or venous sample with a heparinized syringe was obtained for immediate determination of blood pCO_2, pH, [Na^+], [K^+], [Cl^-], bicarbonate[a] (calculated) and L-lactate[b] concentrations with devices validated for equine blood.[12, 13] Arterial or venous sampling was decided by the attending clinician in each case. Heparinized blood samples were centrifuged within 4 hours after blood collection at 1500 × g for 15 minutes to obtain plasma that was divided into 2 aliquots of 1-1.5 mL and frozen at −20ºC until further analyses were performed. In case that [Cl^-] could not be determined by hand-held analyzer, it was determined by ion-selective electrode technique[c]. The EDTA sample was used to determine PCV by microhematocrit centrifuge, fibrinogen concentration (heat precipitation technique)[14] and total plasma protein (TP) by direct refractometry[d]. Hematology was also performed with the EDTA sample using a hematology service analyzer[e] or an out-of-hours in-hospital hematology analyzer[f]. Lithium heparin plasma was

used to determine albumin by bromocresol green method[f] and phosphates (P_i) by molybdate procedure[g]. Globulin (Gb) concentration was calculated as the difference between TP and Alb. Serum obtained from plain tubes was used to determine immunoglobulin G concentration.

Calculated parameters of AB balance:

Quantitative analyses of AB balance was assessed using the method described by Stewart[15] and simplified by Constable.[16] Measured strong ion difference and A_{TOT} were calculated using the following formulas:

$$SID_m = ([Na+] + [K+]) - ([Cl^-] + L\text{-}lactate) \quad (10)$$

$$A_{TOT} = 2.25 \times Alb + 1.4 \times Gb + 0.59 \times P_i \quad (16)$$

Values of bicarbonate [HCO_3^-] and total CO_2 (tCO_2) were calculated by the hand-held device[a] using the following equations[17]:

$$HCO_3^- = S_{CO2} \times pCO_2 \times 10^{(pH - pK'1)} \quad (29)$$

$$tCO_2 = HCO_3^- + 0.03 \times pCO_2 \quad (30)$$

where pK'_1 is the apparent dissociation constant which has an estimated value of 6.1 and is obtained from the sum of pKs (6.038 at 37ºC) and the negative logarithm of the activity coefficient of the hydrogen ion (0.091), generating a value of 6.129; and the value used for CO_2 solubility (S_{CO2}) in plasma at 37 °C was 0.03 mEq/L.[16]

Standard BE (BE) and their different components of Base Excess (BE unmeasured anions (BE_{uma}), BE albumin (BE_{alb}), BE strong ion difference (BE_{sid})) were calculated using the following equations adapted from human pediatric medicine:

$$BE_{uma} = BE - BE_{alb} - BE_{sid} \quad (18)$$

$$BE = 0.9287 \times [HCO_3 - 24.4 + (14.83 \times (pH - 7.4))] \quad (12)$$

$$BE_{alb} = [(28^{\ddagger} - Alb) \times 0.22^{f}] \quad (35)$$

$$BE_{sid} = [([Na^+] - [Cl^-] - 38^*)] \text{ (Story equation)} \quad (21)$$

ǂMean normal value of Albumin in healthy foal in the present study

ʄ effective dissociation constant for equine plasma albumin[16]

*Difference between mean values of $[Na^+]$ and $[Cl^-]$ in control neonatal foals in this study

Strong ion gap (difference between apparent and effective strong ion difference) was also calculated adapting the human formula to equine plasma using the following equation:

$$SIG = SID_m - \frac{A_{tot}}{(1+10^{pKa-pH})} - HCO_3^- \quad (36)$$

Reference values used for interpretation of AB balance were: arterial pCO_2=45-50 mmHg [18], venous pCO_2=44-60 mmHg[19], SID_m= 33.5-51.5 mmol/L, A_{TOT}= 11-17 mmol/L (authors' unpublished data, second study) and pH=7.30-7.46.[19]

Statistical analyses:

Data is shown as mean ± standard deviation (SD) for all variables, except for non-normal distributed variables ($[K^+]$, $BE_{standard}$ and BE_{alb}) which are reported as median (25th; 75th percentiles). Qualitative variables are shown as absolute frequencies and percentages.

Analyses of variance adjusted by age in days (ANCOVA) was used for comparison between septic, non-septic and control foals or survivors vs. non-survivors. Dunnet test was used as post-hoc analyses in order to compare both ill groups from control group. A Chi-square test was used to compare differences in relative frequencies of AB imbalances between septic and non-septic and surviving and non surviving foals. Evaluation of acid-base interpretations of pH, SID_m, A_{TOT}, and pCO_2 as independent risk factors of death was made by estimation of odds ratio (OR) and their 95% confidence interval (95%CI) by binary logistic univariate models. For statistically significant factors in the univariate approach, adjusted models by the other factors were made in order to discard confounding factors.

All data was analyzed with statistical analyses software[h] and p≤0.05 was considered statistically significant.

Sixty-five ill neonatal foals were included in the present study. Forty-three newborns were Andalusian, 10 Arabian, 7 crossbred foals and 5 were animals of others breeds. Of these 65 foals, 35 were males and 30 females. Thirty-two foals were included in the septic group and 33 were classified as non-septic. In addition, 33 healthy foals, 23 females and 10 males, were also included as the control group. Thirteen foals were Andalusian and 20 Arabian-bred foals.

Twenty-one arterial samples and 44 venous samples were obtained from ill foals and all blood samples from control foals (n=33) were venous.

The septic group included 10 animals with additional diagnoses not related to sepsis. Clinical signs or diagnoses related with possible causes of acid-base imbalances in septic and non-septic neonatal foals and surviving and non surviving neonatal foals are listed in table 4.6.

Age was 2±2 days in the septic group, 4±5 days in the non-septic group and 6±1 days in the control group. Apart from septicemia, the most frequent diagnoses in both groups of ill foals were perinatal asphyxia syndrome, immaturity and myopathy. Forty-three out of 65 ill foals were discharged and 22/65 died or were euthanized due to poor prognosis. Sixteen out of 22 non surviving foals were septic foals and the remaining 6 neonates were non-septic (more detailed results shown in Table 4.7 and Table 4.8).

No statistical differences were detected between arterial and venous samples for the assessed variables except for significant differences in pCO_2 values ($p<0.01$).

No statistically significant differences were detected between septic and non-septic foals (Table 4.7), but significant differences were found in septic and non-septic groups compared to control foals in SID_m (lower values in septic ($p<0.01$) and non-septic ($p<0.01$)), L-lactate (higher values in septic ($p=0.03$) and non-septic ($p=0.01$)) and $[K^+]$ (lower values in septic ($p=0.01$) and non-septic ($p<0.01$)). Significant differences between the control and the non-septic groups were detected ($p<0.01$) for $[Cl^-]$ ($p<0.01$) and between the control group and the septic group for albumin ($p=0.01$).

Regarding surviving and non surviving foals, significantly higher L-lactate (4.4±4.0 vs. 6.4±3.5 mmol/L; $p<0.01$), $[K^+]$ (3.5±4.0 vs. 4.6±1.5 mmol/L; $p<0.01$) and venous

pCO_2 concentrations (40.4±6.5 vs. 50.7±13.0 mmHg; $p<0.01$) were found in non surviving animals (Table 4.8). Calculated SIG had a tendency to be higher in surviving foals (-3.3±4.55 vs. -6.08±6.5; $p=0.051$).

Differences in pH statistically significant but not clinically relevant were detected between surviving and non surviving foals (7.39±0.08 vs. 7.31±0.10; $p<0.01$).

In the univariate logistic regression models, only respiratory alkalosis was statistically significant as a good prognostic factor with an OR=0.5; 95%CI (0.1-0.7), $p=0.012$. The interpretation of remaining variables was not significantly associated with foals' outcome. Adjusted models of SID_m, A_{TOT} or pH for the effect of pCO_2 on survival shows minimal changes in relation to univariate models, therefore a diagnosis of respiratory alkalosis would be an independent prognostic factor.

Taking into account the acid-base interpretation, the most common alterations found in this study were respiratory alkalosis, with or without metabolic acidosis due to decreased SID_m. No differences were detected in the relative proportion of alterations in A_{TOT}, SID_m or L-lactate concentration in septic and non-septic foals ($p=0.22$). Frequent AB imbalances detected in septic foals were respiratory alkalosis and metabolic acidosis due to decreased SID_m. These findings were the same in the non-septic group. There were alterations in blood pH in 24 foals and the most common disturbance presented was acidemia (n=13). In foals with respiratory acidosis (n=6), the number of non surviving foals (83%; n=5) was significantly higher ($p<0.01$) than surviving ones (17%; n=1), in contrast to 8 (19%) non surviving foals out of 42 foals with respiratory alkalosis.

Several studies have been performed in neonatal foals to detect prognostic markers associated with sepsis. Some of the most useful parameters used in human neonates to predict prognosis are included in AB interpretation.[20] To the authors' knowledge, this is the first study in which all AB variables of simplified Stewart's method are determined in critically ill foals. The main findings of this study are: (1) the most common AB imbalance found in critically ill neonatal foals was respiratory alkalosis with or without SID_m acidosis, (2) in non surviving foals the most common AB imbalance was metabolic acidosis due to decreased SID_m without compensatory respiratory alkalosis, (3) L-lactate concentration, p_vCO_2 and pH were statistically different between survivors and non survivors, and (4) chloride, SID_m and L-lactate concentrations were significantly altered in ill foals but no significant differences were found between septic and non-septic foals.

In the present study, high concentrations of blood L-lactate on admission were correlated with poor outcome. This association has previously been reported in multiple studies and is one of the proved and widely used predictor linked to AB balance in human and equine neonates.[3,7,10,21-25] However, the progression of L-lactate concentration during the first 24 hours of treatment has been shown to be more useful to predict survival in both humans[25] and foals.[7]

Other important prognostic markers in human babies are BE and BE_{uma}[1] These parameters are considered to be so important that they are included in multiple neonatal disease severity scoring systems.[20] Changes of BE in human neonates were mainly due to BE_{uma} representative of ketoacids, citrate, D-lactate and Krebs cycle intermediates.[26, 27] As it is reported in previous studies,[23] in this group of ill foals no relationship between outcome and BE was found. To the authors' knowledge, this is the first study that assesses various components of BE in neonatal foals. In contrast to human neonatal medicine, BE and BE_{uma} do not seem to be related with outcome in neonatal foals. This difference might be due to liver and gut immaturity of human babies at birth, which may be unlike that in full term foals. Another reason could be the equations used to calculate BE components. The only approximation to BE components proposed in the literature for adult equine plasma[29]

include $BE_{freewater(fw)}$, $BE_{chloride\ (Cl)}$, $BE_{total\ protein(TP)}$, but this approach would be inaccurate in neonatal foals. On one hand, in human literature BE_{fw} and BE_{Cl} have been later simplified to BE_{sid}.[30] On the other hand, the variables used to adjust BE_{TP} to equine plasma should be used only in animals with Alb:Gb ratio similar to adult healthy horses[31], which is not the case of neonatal foals.[6, 32] Because of this, the authors propose the use of BE_{alb} adjusted for equine plasma instead of BE_{TP} and BE_{sid} instead BE_{fw} and BE_{Cl}. Albumin is the main contributor to BE[29] and BE_{alb} is extensively used in human pediatric intensive care units.[1, 33] The adjustment needed for equine albumin could be carried out by using the effective dissociation constant for plasma albumin determined by Constable.[31] Other studies should be performed in order to determine which approximation of BE_{sid} is the most accurate for foals and validate the alternative calculation of BE_{alb} proposed herein. For the purpose of this study, the Story equations[30] for assessment of BE in human neonatal medicine were used due to better approximation to the difference between [Na^+] and [Cl^-] observed in control foals. Other equations available in human medicine (Taylor [34], Gilfix[35] or Hatherill[33]) were not assessed.

Another predictor of outcome used in human medicine is the SIG, the difference between apparent and effective SID. The SIG has been traditionally used in horses to estimate lactate concentration,[16] but nowadays modern point-of-care L-lactate analyzers are routinely used in equine clinical settings. Traditional arterial SIG calculation has been evaluated as an outcome predictor in critically ill foals, but no association has been detected[21] despite a significant relationship between lactate concentration and outcome found in other studies.[3, 7, 10, 21] In human patients, a SIG calculation including lactate is used to estimate intermediate products of the Krebs cycle or other non-measured negative ions. In horses, this new SIG formula value has not been described. For the purpose of this study, the SIG human formula including lactate has been adapted taking into account horses' proteins pKa[31] for calculation of the dissociated component of A_{TOT}. In the present study, calculated SIG from ill foals had a tendency ($p=0.051$) to be lower in non surviving foals than in surviving ones. This differs from human studies using the equivalent formula, in which high SIG values are related with poor outcome.[36-38]

In the present study, a difference in pH on admission was detected between surviving and non surviving foals, but this difference (7.39 *vs.* 7.31) may not be considered clinically relevant as both values lie within the reference range reported in the literature.[19] This situation also occurs in human medicine, where similar pH is found in surviving and non surviving patients.[33]

Regarding AB interpretation in ill foals of this study, the presence of respiratory alkalosis was associated with better prognosis relative to those without it. Respiratory alkalosis in these ill foals probably was present to compensate a metabolic acidosis. In the quantitative analyses of p_vCO_2, higher partial pressures of CO_2 (i.e. respiratory acidosis) had a tendency to be associated with poor outcome. This was probably due to a failure of the respiratory compensation mechanism or to a marked increase in CO_2 production in ill tissues. In fact, Hatherill *et al.*[33] showed that aetiology of acidosis was a better prognostic marker than its magnitude in children with shock, and this situation may also occur in neonatal foals. A previous study reported the usefulness of p_vCO_2 as a prognostic tool in a group of ill equine neonates.[9] However, the increase in p_vCO_2 was always considered a consequence of lung disease. In the studied population, not all the foals reported with respiratory acidosis had respiratory problems and, therefore, lung function may not be considered the only cause of increased p_vCO_2 pressure in ill equine neonates.

The association of AB parameters with diagnosis has not been described in the literature for neonatal foals. In the present study, none of the evaluated variables showed significant differences between septic and non-septic foals. This could be due to similar clinical presentations of septic and non-septic foals in this study (i.e. diarrhea) and also to the fact that some septic cases had other diagnosis apart from septicemia. A larger number of cases could increase the chances of finding differences between septic and non-septic ill foals. Nevertheless, in human medicine, none of the AB variables are used as a diagnostic marker of septicemia.[39]

In the population of this study, the AB alteration most often observed in septic foals was respiratory alkalosis with or without SID_m acidosis. In human medicine, an association between sepsis and respiratory alkalosis has been reported in patients with or without metabolic acidosis or complex AB disorders.[40,41] Regarding outcome, acidemia is a useful

marker of poor prognosis in critically ill human patients.[42] Causes of acidemia are usually hyperlactatemia, hyperchloremia or an increase in unmeasured anions.[41] In the present study, most ill foals had normal blood pH (n=41) and only 13 showed acidemia, caused by hyperlactatemia with or without a decrease in SID_m.

This study was performed in order to provide information applicable to AB interpretation during daily clinical work. For this purpose, analyzers employed herein are devices commonly available during emergency hours. Although certain studies of acid-base and electrolyte disorders are performed with gold standard techniques, the results obtained are not always applicable to hand-held or user friendly devices due to methodological differences. In the present study, TP was assessed by direct optic refractometry, which is the method most commonly used for TP determination in daily clinical work although considered to be an inexact technique.[43] L-lactate determination was performed on blood instead of plasma samples with a horse validated device in order to be comparable with an emergency situation. In previous studies performed in adult horses this device had an excellent correlation using plasma and good correlation using whole blood when compared to a gold standard technique,[12] with larger differences being found for L-lactate concentrations higher than 5 mmol/L and hematocrit higher than 53%. Although some foals could be out of these limits, most of the animals were below these values.

In order to minimize interventions, in cases where arterial samples were obtained no additional venous samples were taken. Authors compared results from all venous and arterial samples and only significant differences were found in blood gases, but a comparison of simultaneously taken samples of arterial and venous samples has not been performed in the present study. In previous studies with simultaneous extraction of both samples no clinical relevant differences between electrolytes [44], L-lactate[45] and proteins were detected between arterial and venous results. Regarding calculated parameters (SID_m and A_{TOT}) no differences were found in resting horses between arterial and venous samples. [46]

Finally, the results of this study should be interpreted taking into account the above mentioned limitations and the following ones: 1) only critically ill neonatal foals were included in this study and may not therefore be representative of all sick neonatal foals admitted to an equine referral hospital, 2) differences in the time to referral to the hospital,

treatment before referral or age in either the septic or non-septic groups, and 3) differences between gold standard and point-of-care electrolyte and AB analyzers.

In conclusion, the most common AB disturbances in critically ill foals of this study were respiratory alkalosis with or without SID_m acidosis. No differences between septic and non-septic foals were detected. Increased p_vCO_2 pressure and metabolic lactic acidosis were related to poor outcome in critically ill foals. Other biomarkers of AB balance used in human medicine (BE, BE_{uma} or SIG) do not seem to be useful as prognostic markers in ill neonatal foals. Acid-base balance formulas adapted from human medicine to equine neonates in this study would require further validation.

Footnotes

[a] EC8+, iSTAT, Abbott laboratories, IL, USA

[b]Accutrent lactate, F. Hoffmann-La Roche, France.

[c] Vetlyte, Idexx laboratories, Inc, Netherlands.

[d] Atago Corporation (Atago Ltd, Tokyo, Japan)

[e]ADVIA 1200, Siemens Healthcare, Munich, Germany.

[f] LaserCyte, Idexx laboratories, Inc, Netherlands.

[g] Olympus AV400, Olympus corp, Germany.

[h]PASW Statistics 18 for windows, Release Version 18.0.0 (=D3 SPSS, Inc., 2009, Chicago,IL, www.spss.com).

Table 4.6: Clinical signs or diagnoses related with possible causes of acid-base imbalances in septic and non-septic neonatal foals and surviving and non surviving neonatal foals

	Septic (n=33)	Non-septic (n=32)	Surviving (n=43)	Non Surviving (n=22)
Pneumonia	14/33	9/32	12/43	11/22
Diarrhea	7/33	9/32	10/43	6/22
Reflux	4/33	0/32	0/43	4/22
Enteritis	7/33	10/32	10/43	7/22
Myositis	5/33	3/32	4/43	4/22
Renal insufficiency	2/33	2/32	3/43	1/22
FPTI	20/33	13/32	18/43	15/22
PAS	6/33	10/32	10/43	6/22

PAS Perinatal asphyxia syndrome; FPTI failure of passive transfer immunity; Enteritis diagnosed by ultrasonography compatible images and/or presence of clinical signs (diarrhea and/or reflux).

Table 4.7: Mean ± SD for normal distributed variables. Not normally distributed variables were presented as Median [25th -75th percentiles] and marked with ⨍. Significance values (p-values) are presented below for the comparison of septic or non-septic versus control.

	Control	Septic		Non-septic	
			p-value		p-value
pH	-	7.37±0.10		7.37±0.10	
SID_m(mmol/L)	40.3±3.1	32.0±6.4	<0.01	31.6±6.3	<0.01
Sodium (mEq/L)	133±8.3	132±8	0.31	134±6	0.68
Potassium⨍ (mEq/L)	4.2 [3.8-4.5]	3.6 [3.2-4.3]	0.01	3.5 [3.3-3.8]	<0.01
Chloride (mEq/L)	95±4	100±9	0.07	101±5	<0.01
L-Lactate (mmol/L)	2.5±1.3	5.0±3.7	0.03	5.1±4.2	0.01
A_{TOT} (mEq/L)	14.9 [13.0-15.8]	13.4 [11.3-15.4]	0.96	14.3 [12.7-15.0]	0.87
Albumin (g/dL)	2.8±0.2	2.6±0.5	0.01	2.7±0.4	0.19
Globulin (g/dL)	3.1±0.7	3.3±1.1	0.06	3.2±0.8	0.23
Phospate (mg/dL)	6.0±1.4	5.4±1.9	0.56	5.8±2.2	0.79
Total protein (g/dL)	6.3±0.7	5.9±1.2	0.30	5.9±0.8	0.43
PCO_2 venuos (mm Hg)	-	44.6±6.9		44.5±14.5	
PCO_2 arterial (mm Hg)	-	37.9±7.0		36.6±9.6	
HCO_3^- (mEq/L)	-	25±5		24±6	
BE_{sid} (mEq/L)	-	1.1±5.9		1.0±5.1	
BE_{uma} (mEq/L)	-	-4.9±6.6		-5.7±5.6	
BE_{alb}⨍ (mEq/L)	-	4.0 [3.4-4.5]		3.8 [3.0-4.2]	
$BE_{standard}$ (mEq/L)⨍	-	1.6 [-1.2-3.0]		1.5 [-3.2-3.8]	

Table 4.8: Mean ± SD for normal distributed variables. Non-normally distributed variables were presented like Median [25th -75th percentiles] and marked with ⨍. Significance values (p-values) are presented below for the comparison of survivors versus non survivors.

	Survivors	Non Survivors	p-value
pH	7.4±0.1	7.3±0.1	<0.01
SID$_m$ (mEq/L)	32.4±5.9	30.5±7.9	0.47
Sodium (mEq/L)	134±5	132±10	0.70
Potassium⨍ (mEq/L)	3.4 [3.2-3.7]	4.1 [3.8-5.2]	<0.01
Chloride (mEq/L)	100±6	100±10	0.98
L-Lactate (mmol/L)	4.4±4.02	6.4±3.5	0.04
A_{TOT}⨍ (mEq/L)	13.5 [12.1-14.7]	14.6 [12.4-15.8]	0.02
Albumin (g/dL)	2.6±0.4	2.6±0.5	0.70
Globulin (g/dL)	3.1±0.8	3.5±1.2	0.07
Phospate (mg/dL)	5.3±1.4	6.2±2.9	0.08
Total protein (g/dL)	5.8±0.9	6.1±5.8	0.06
PCO_2 venous (mm Hg)	40±6	51±13	<0.01
PCO_2 arterial (mm Hg)	37±8	36±11	0.31
HCO_3 (mEq/L)	24.4±5.7	24.7±5.9	0.70
BEsid (mEq/L)	1.4±4.3	0.3±7.4	0.67
BE_{uma} (mEq/L)	-5.5±5.6	-5.1±7.1	0.64
BE_{alb}⨍ (mEq/L)	3.9 [3.2-4.4]	3.7 [3.2-4.5]	0.87
$BE_{standard}$⨍ (mEq/L)	2.3 [-2.5-3.7]	0.6 [-1.5-2.3]	0.55
SIG (mEq/L)	-3.3±4.5	-6.1±6.5	0.051

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[DISCUSSION]

1. Similarities and differences in acid-base balance between human and horses

1.1. In human sports medicine

The traditional, or, Henderson-Hasselbalch approach has been applied to human plasma since the forties[65] and horse plasma since the sixties.[66] This is a simple method of acid-base interpretation that does not require determination of a large number of parameters, but has 4 important limitations: 1) inexact results when protein or electrolytes alterations are present; 2) the approach is based on 2 dependent variables (pH and HCO_3^-) and an independent variable (pCO_2); 3) disorders are classified into a limited number of categories compared with the quantitative method and 4) it does not help guide a fluid therapy regime when necessary. For these reasons the Henderson-Hasselbalch approach to acid-base disturbances is not the most appropriate method when there are alterations in protein, [Na^+] or [Cl^-] concentrations.[13] Blood [Na^+] alterations and, in minor measure, other electrolytes derangements are the main concerns in human sports medicine. These facts make less useful the traditional method than quantitative approach in AB interpretation. Electrolytes imbalances, dehydration and renal consequences are common concerns in human and equine sports medicine and the quantitative approach, instead of the traditional, take into account all these issues.

In the first study, poor agreement between quantitative and traditional methods was observed. Multiple mixed AB disorders were detected using the quantitative method with no pH change during competition. The horses included suffered changes mainly in SID_m, attenuated by changes in opposite directions of their components (increase in lactate and decrease of Cl^-), but also in A_{TOT} (due to hemoconcentration). Both alterations are not taken into account when the traditional approach is applied and both are known causes of AB disturbances that may require emergency treatment during exercise.

Regarding the respiratory component of AB analyses, horses suffer more often than human beings respiratory acidosis due to pCO_2 accumulation. This fact has been previously reported and also detected in study one. Human beings do not have this tendency to

respiratory acidosis, and horses may suffer pCO_2 accumulation during exercise due to locomotor-respiratory coupling. In both species, an increase of blood lactate produces lactic acidosis but changes in SID_m are mitigated by electrolyte changes. Lactate increase is offset in humans by an increase in sodium concentrations and in horses changes in SID_m are mitigated by a simultaneous decrease in chloride concentrations. Main electrolyte changes during exhausting exercise are different in humans and horses. In the field of human exercise physiology concerns about AB are focused on Na^+ accumulation and incorrect rehydration (with hypotonic solutions) that lead to a marked hyponatremia. In horses, concerns are focused on SID_m decrease due to chloride loss (more marked in horses than in humans, due to sweat composition) and metabolic alkalosis produced by this decrease as it was seen in study one. Dehydration suffered in competing animals can also have consequences in horse's thermoregulatory system. A_{TOT} increase due to hemoconcentration was often present in horses in study 1 and it produces a tendency toward acidosis (which attenuates the alkalosis induced by an increased SID_m). Although A_{TOT} is one of the variables to take into account in AB equilibrium, the effect on blood pH is less than SID_m.[67] For this reason metabolic alkalosis due to increased SID_m is the predominant concern in horses that needs veterinary treatment during an endurance race.

1.2. Comparison with other studies in sport horses

Few references in the scientific veterinary literature perform a complete analyses using the quantitative approach to acid-base balance in sports medicine. Studies performed in horses during sprint exercise demonstrate a tendency to increase in A_{TOT}, SID_m and p_vCO_2.[68] In show jumping horses, a decrease in p_vCO_2, increase in SID_m and A_{TOT} were observed.[41] Studies performed in endurance races evaluated changes in AB balance and electrolyte concentrations using a quantitative approach.[47, 48] These studies focused on SID_m and A_{TOT} suggest an increase of these variables during exercise but detailed description of quantitative acid-base alterations was not provided. Previous studies focused on components of the different variables of quantitative analyses (SID_m, A_{TOT} and pCO_2) have shown changes in pH, pCO_2, Na^+, K^+, iCa^{2+}, Cl^-, lactate, glucose, CK, AST, TP, albumin and creatinine concentrations as a function of speed,[44, 47] reporting more severe disturbances in horses running at higher speed. In the study one, performed with high level

competition horses and high mean velocity, alterations of the different variables were evaluated for disturbances related to distance rather than speed. Changes observed in this study were similar to those found in studies that correlate the evaluated variables with high velocity. Most of the animals presented mixed AB disturbances without pH change and these alterations were not easily detected by the traditional method. The most common AB imbalance detected was mild strong ion alkalosis (hypochloremia) attenuated by mild lactic acidosis, non-volatile buffer acidosis and mild respiratory acidosis.

1.3. In healthy foals

Variables used for AB quantitative analyses (A_{TOT}, SID_m) are calculated from ions and buffers that change during the neonatal period and the first months of life (total protein, albumin [Alb],[58, 59] Globulins (Gb), L-lactate [Lac][69, 70] or phosphate [P_i][71]) as well as other parameters without significant differences due to age (Na^+], [K^+] and [Cl^-]).[58] Although reference values for the components of A_{TOT} and SID_m for different ages are published, the tight balance between electrolytes and proteins to maintain plasma pH makes it impossible to estimate A_{TOT} and SID_m reference ranges when using reference ranges of each one of their components. In studies two and three significant differences were found in SID_m in the neonatal period and A_{TOT} values until 6 months of age, and reference ranges for each age group were provided. Slightly lower SID_m values detected in these studies have also been reported in human beings and it could be explained by a higher [Lac] concentrations and relative high values of [Cl^-] (high [Cl^-]/[Na^+] ratio and low [Na^+]-[Cl^-] difference).[72, 73]

Non-volatile weak buffer concentrations obtained in these studies showed 4 statistically different groups: neonatal foals, 1-2 months animals, foals up to 6 months and older animals. Differences in A_{TOT} values during the firsts 6 months of life could be explained by changes in [Alb] concentration,[59, 74, 75] fibrinogen concentration,[57, 58] serum IgG,[76] [Pi] concentration [71] and maturity of liver function.[56]

Changes in [Gb] and [Alb] concentrations with age suggest that Alb:Gb ratio in young foals (1-6 months) is not exactly the same as that in adult horses.[53, 59] For this reason the approximation for A_{TOT} calculation from total protein used in adult horses could not be accurate enough for neonatal foals. In study three, a new adjustment factor for neonatal foals has been obtained (within 95% confidence interval of the previously suggested

Constable's factor for adult horses [18]) but observed results in the group of foals of the study three suggested that further studies might be needed to validate adult A_{TOT} simplified equation in foals from 1 to 6 months. When A_{TOT} is calculated solely from total protein concentration a normal proportion between A_{TOT} components is assumed (Alb, Gb and P_i). Therefore, the simplified equation should not be used in animals with altered P_i or Alb:Gb ratio.[18] This exception includes protein losing disorders, marked acute or chronic inflammations and neonatal foals. The most marked differences in Alb:Gb ratio in newborn foals will be found during first hours of life, during immunoglobulin absorption. During this period A_{TOT} values obtained are probably lower than those obtained in this study due to low plasma [Gb] concentrations. Neonatal foals included in studies two and three were older than 1 day of age. It is not therefore recommended the use of the new equation to estimate A_{TOT} from total proteins, or, the A_{TOT} reference range reported on these studies in foals younger than one day, or, those that do not have the same Alb:Gb ratio than the studied.

1.4. Comparison of the prognostic usefulness of acid-base parameters

Multiple parameters of AB equilibrium have been used in human pediatric medicine as outcome predictors. Some of these parameters have been demonstrated to be associated with survival in ill foals, such as L-lactate.[60-62, 70] On the other hand, some parameters have not been evaluated or applied to equine neonatal foal medicine. Study four assesses these parameters in foals as outcome predictors and diagnostic markers.

Outcome predictors evaluated in this study were SID_m, A_{TOT}, SIG, BE, BE_{uma}, BE_{sid}, BE_{alb}, lactate, pH, p_vCO_2, p_aCO_2 and components of all calculated variables.

In human medicine, lactate, pH, SIG, BE and BE_{uma} are related to outcome. In study four, as seen in other human and equine studies; a significant relationship of high admission lactate,[60-62, 70] lower blood pH[26] and high $pvCO_2$[64] with poor outcome was also found.

Strong Ion Gap has been traditionally used in horses to estimate lactate concentration.[77] Although lactate is related with outcome, when SIG has been evaluated in critically ill foals no relation with poor prognosis has been detected.[62] In human patients, a

SIG calculation including lactate is used to estimate intermediate products of the Krebs cycle or other non-measured negative ions but this new calculation formula have not been used in horses. In study four, the SIG human formula was adjusted to equine plasma (using equine pKa[18] for calculation of the dissociated component of A_{TOT}) and evaluated as an outcome predictor in ill foals. In non surviving foals a tendency ($p=0.051$) to lower levels was found in contrast to studies in human medicine, in which higher levels are related with poor prognosis.

Other important prognostic markers in human babies are BE and BE_{uma}.[78] Previous studies in horses evaluated BE[79] as a prognostic marker but not their components. The approximations to BE components for equine plasma found in the literature[24] include BE_{fw}, BE_{Cl}, and BE_{TP}. These calculations are not appropriate for foals due to different Alb:Gb ratio present in equine neonates. In human medicine, the former calculations had been simplified to BE_{sic}[25] and BE_{alb}. In the study four, approximations of human formulas adapted to horse plasma were used. No relation of BE_{uma}, BE, BE_{alb} or BE_{sid} with outcome was detected using the Story approximation adapted to equine plasma. [25]

Lactate and p_vCO_2 were correlated with outcome but other AB evaluated parameters were not useful as prognostic markers in this group of foals.

The usefulness of AB as a diagnostic marker of septicemia was also evaluated in study four, but no characteristic alterations in acid-base balance due to sepsis were found. The most common alteration in ill foals (septic and non-septic foals) was respiratory alkalosis with or without SID_m acidosis. These alterations have also been reported in human septic patients.[80,81]

2. Comparison between analyzers

In the second study, a comparison between analyzers used on emergency hours and the gold-standard reference laboratory techniques were performed to assess the accuracy and the reliability of AB interpretation based on results of hand-held devices. Unfortunately, poor concordance was found between them. Results obtained by both methods are not interchangeable. This low concordance is probably due to the different technology used in point-of-care analyzers (direct potentiometry) and reference laboratory

analyzers (indirect potentiometry). The same device should therefore be used when serial determinations are performed over time on the same patient. In order to allow a correct AB interpretation during emergency hours, a reference range for each of the analyzers was proposed in the second study.

This disagreement was previously reported for iSTAT® regarding Na^+, K^+ and Cl^- determination in adult horses.[82,83]

The application of reference values obtained with gold standard assays for results of portable analyzers could also lead to inexact interpretations in adult horses. In order to avoid these kind of mistakes in interpretation of results, reference values of SID_m and A_{TOT} used in study one are obtained from prerace samples using point-of-care analyzers.

3. Determination of reference values

In studies one, two and three horses were evaluated under conditions in which the previous literature reports differences on components of AB quantitative calculated variables. The results of these three studies allowed assessment of horses under these specific situations and reduce the chance of incorrect interpretation.

In study one, reference values obtained for SID_m and A_{TOT} are based on prerace values of elite equine athletes. There are certain adaptations of trained horses and humans athletes that may influence SID_m or A_{TOT}. For this reason published values of untrained horses may not be accurate enough for AB interpretation in endurance horses. In fact, SID_m reference values obtained in study one had a tendency to be lower than those published for normal adult horses (35-38.5 vs. 39-46).[84, 85] Reference values obtained for A_{TOT} also had a tendency to be lower than published ones (12-15 vs. 13-16.5).[84, 85] These reference values were obtained with a low number (n=25) of horses and should be validated with an external population. These results do, however, suggest that endurance sport horses may have different resting values of SID_m and A_{TOT} due to training adaptations.

In studies two and three, the effect of age on SID_m and A_{TOT} was evaluated. A clear difference between neonates and older foals was found in both parameters showing lower reference for SID_m and A_{TOT} values as occurs in human medicine.[72, 73, 78]After the first month of life, SID_m values are more similar to adult values, but A_{TOT} does not achieve adult

values until 6 months of life. The progression of A_{TOT} with age defines 4 groups during the first months of life: neonatal foals (<21 days), foals until 2 months, foals under 6 months and older foals. Non-volatile weak buffer concentration had a tendency to increase until 6 months, with the largest differences being found between the neonatal and adult periods.

4. Application of the simplified quantitative method to acid-base interpretation (usefulness and limitations)

The use of simplified equations for AB interpretation allows application of the quantitative method to daily clinic but it has to be taken into account that it cannot be applied under all circumstances. The main simplifications applied to Stewart's equation are proposed by Constable[18] and are focused on simplifying the application of quantitative method.

Strong ion difference is simplified to main strong positive and negative electrolytes found in plasma and all the electrolytes with low concentrations had been deleted from this equation in order to diminish the number of determinations needed for SID_m calculation. This simplification does not suppose a major problem since marked changes of the strong ions eliminated from the calculation are not common.

The simplification for A_{TOT} calculation using total protein can be applied in animals with normal Alb:Gb ratio and no alterations of phosphate concentration.[18] The approximation depends on the maintenance of normal proportions between all A_{TOT} components and changes, mainly on Alb:Gb ratio, could lead to erroneous results using A_{TOT} simplification. Young foals, specially neonatal foals, do not have the same Alb:Gb proportion as adult healthy horses and the approximation used was adapted. In the third study an alternative simplified equation for neonatal foals was obtained in a group of healthy neonatal foals. However, it should be validated on an external population before being used in general practice. In the third study the Alb:Gb ratio and A_{TOT} are not similar to adult published values until 6 months of life. The same procedure that has been carried out in neonatal foals should therefore be performed in the different age groups detected. The obtained approximation using this procedure will provide a more accurate A_{TOT} approximation from total proteins than adult formula for young foals. The simplified equation could be applied to AB interpretation when protein losing disorders are not

suspected or taking into account that values obtained with protein approximation would be inexact in cases with low albumin.

The correct use of the simplified equations described herein and the knowledge of their limitations could improve the interpretation of AB balance in critically ill patients. Moreover, the correct reference values used in each situation, taking into account the animal age, training status or the analyzer employed, should allow a better approximation to daily clinical work and emergency situations.

[CONCLUSIONS]

Conclusions:

1. Traditional approximation is not accurate enough to detect AB imbalances in equine sports medicine.

2. Poor agreement is present between traditional and quantitative methods in endurance horses during competition.

3. Quantitative analyses of acid-base balance allows identification and treatment (in necessary cases) of complex alterations in AB equilibrium.

4. During endurance races, the most common AB imbalances identified are mild strong ion alkalosis (hypochloraemia), attenuated by mild lactic acidosis, non-volatile buffer ion acidosis and mild respiratory acidosis.

5. Differences in hand-held analyzers could influence the reference ranges for SID_m and A_{TOT}.

6. Horses performing endurance exercise, as well as sick foals, may present alterations in acid-base balance characterized better by using the quantitative method rather than the traditional approach.

7. Neonatal foals had different SID_m and A_{TOT} values than adult horses.

8. SID_m and A_{TOT} are age-dependent and this has to be considered for acid-base interpretation in young animals

9. Adult A_{TOT} values are not achieved until 6 months of age.

10. Quantitative method formulas used in human neonates could be applied to foals with adaptations.

11. Estimates of A_{TOT} from total protein values used in adult horses are not accurate enough to be applied in neonatal foals given that the Alb:Gb ratio is not the same.

12. An alternative simplified equation for A_{TOT} estimation from total protein adjusted for neonatal foals could be $A_{TOT} = (2.4 \times PTP)$.

13. Constable simplified approximation for quantitative method is applicable on clinical emergency situation and daily clinic.

14. Devices used during emergency situation could be an important source of error and adequate reference ranges should be applied.

15. Results obtained with different afterhours analyzers are not interchangeable. The same device should be used when serial determinations are performed over time on the same patient, and device specific reference range should be used in order to minimize interpretation errors.

16. The most common AB disturbance found in critically ill foals was respiratory alkalosis with or without SID_m acidosis.

17. No differences in AB imbalances were found between septic and non-septic ill foals.

18. Increased p_vCO_2 pressure and metabolic lactic acidosis were related to poor outcome in critically ill foals.

19. Other biomarkers of AB balance used in human medicine (BE, BE_{uma} or SIG) do not appear to be as useful as prognostic markers in neonatal foals.

[SUMMARY]

Traditional and quantitative methods have been applied to both equine sports medicine and veterinary clinic. One of the problems of quantitative analyses are the complicated equations and large number of determinations needed for its application versus traditional approach to AB analyses. After Constable's[18] simplification, the equations applied to quantitative analyses are easier and need less parameters, thus allowing the application on daily clinic.

Although simplified equations are easier to use, other adaptations are required in order to apply human equations to horse plasma. The main difference is due to different horse pKa of A_{TOT} and was taken into account during the recalculation of the equations.

Published differences of the AB variables components (electrolytes and proteins) during different physiological states could lead to differences in AB calculated parameters (SID_m and A_{TOT}). The present work evaluates changes of SID_m and A_{TOT} in elite sport endurance horses and the effect of aging during the first year of life. Lower values of A_{TOT} and SID_m were found in neonate foals and elite horses compared to normal adult horses. Adult values of A_{TOT} are not achieved until 6 months of life while SID_m adult values are found after neonatal period. Due to differences observed in neonatal values of A_{TOT}, the published simplification used in adult horses is not adequate and a new equation for neonatal foals is provided in this work.

Another possible source of errors on AB interpretation in emergency situations is the point of care analyzers or afterhours analyzers available in veterinary hospitals. These devices use different determination methods from those of the reference laboratory (direct potentiometry vs. indirect potentiometry) and a large difference could be found on results of both types of analyzers. The present work provides normal reference values for neonatal foals, foals during first year of life and endurance horses using these kind of point-of-care analyzers. Concrete reference values for analyzers diminish interpretation mistakes on AB equilibrium during emergency hours.

Moreover, the present work also evaluates AB parameters such as prognostic/diagnostic markers in ill foals and the frequency of AB imbalances in these foals.

The most common AB disturbance in critically ill foals was respiratory alkalosis with or without SID_m acidosis. Increased p_vCO_2 and metabolic lactic acidosis were related with poor outcome in critically ill neonatal foals, but other prognostic markers used in human medicine (BE, BE_{uma} or SIG) were not useful. No specific differences on AB imbalances between septic and non-septic foals were observed.

Acid-base imbalances were also evaluated on endurance horses using traditional and quantitative analyses. Poor agreement between both methods was observed and complex AB disturbances were detected with quantitative analyses. The most common alteration detected in endurance horses was mild strong ion alkalosis (hypochloremia), attenuated by mild lactic acidosis, non-volatile buffer ion acidosis and mild respiratory acidosis.

Human quantitative AB equations could be applied to horse plasma taking into account the necessary adaptation depending on the species. For correct AB interpretation during emergency situations, adequate reference values had to be used in different physiologic situations or depending on the analyzer used.

[RESUM]

Els mètodes tradicional i quantitatiu per l' interpretació de l'equilibri àcid-base (AB) s'han aplicat a la medicina esportiva i la clínica veterinària equina. Els problemes argumentats pels detractors del mètode quantitatiu són la complexitat de les equacions emprades i el major nombre de determinacions necessàries per dur a terme l'anàlisi. Després de les simplificacions fetes per Constable, l'aplicació de les formules és més senzilla i requereix menys determinacions, el que permet l'aplicació a la clínica diària. Encara que les equacions simplificades són més fàcils d'emprar, es necessiten d'altres adaptacions per aplicar les equacions de medicina humana al plasma de cavall. La principal diferència es deu a la diferent pKa de l'A_{TOT} (espècie específica) i ha de tenir-se en compte en fer aquestes adaptacions.

En publicacions anteriors s'han constatat diferències en els components de les variables emprades en el mètode quantitatiu (electròlits i proteïnes) durant els diferents estats fisiològics. Aquestes diferències podrien donar lloc a canvis en els valors de normalitat dels paràmetres calculats de l'AB (SID_m i A_{TOT}). El present treball avalua els canvis en el SID_m i A_{TOT} en cavalls de raid d'elit i l'efecte de l'edat durant el primer any de vida. Els valors de referència obtinguts en poltres nounats i cavalls d'esport d'elit són més baixos que els valors considerats normals en cavalls adults. A més s'ha observat que els poltres no assoleixen els valors adults d'A_{TOT} fins als 6 mesos de vida, mentre que el SID_m assoleix el valor dels adults després del període neonatal. A causa de les diferències observades en els valors de A_{TOT} en nounats, la simplificació publicada per cavalls adults utilitzant les proteïnes plasmàtiques no és l'adequada per nounats i en aquest treball es proposa una nova equació més acurada per a poltres durant el període neonatal.

Una altra possible font d'errors en la interpretació AB en situacions d'emergència són els analitzadors disponibles durant les urgències. Aquests dispositius utilitzen mètodes de determinació diferents del laboratori de referència (potenciometria directa *vs.* indirecta) i poden donar-se grans diferències en els resultats obtinguts. El present treball proporciona els valors normals de referència per a poltres nounats, poltres durant el primer any de vida i cavalls de raid utilitzant aquest tipus d'analitzadors. Els valors de referència concrets per cada analitzador permeten disminuir errors d'interpretació de l'equilibri AB.

A més aquest treball també avalua els paràmetres d'AB com a marcadors de pronòstic/diagnòstic i la freqüència dels desequilibris AB en poltres malalts. Les alteracions AB més comuns detectades varen ser l'alcalosi respiratòria amb o sense acidosi deguda al SID_m. L'augment de la pressió venosa de CO_2 i acidosi làctica metabòlica es van relacionar amb un mal pronòstic, però altres marcadors de pronòstic utilitzats en medicina humana (BE, BE_{uma} o SIG) no varen ser útils. No es van observar diferències específiques en els desequilibris AB entre poltres sèptics i no sèptics.

Els desequilibris AB també es van avaluar en cavalls de raid mitjançant l'anàlisi tradicional i quantitativa. La concordança entre ambdós mètodes va ser pobre. L'alteració més freqüentment detectada durant el raid va ser una lleu alcalosi deguda al SID_m (hipoclorèmia), emmascarada per acidosi làctica lleu, acidosi deguda a l'A_{TOT} i acidosi respiratòria lleu.

Les equacions de l'anàlisi de l' AB quantitatiu de medicina humana poden ser aplicades al plasma de cavall tenint en compte les adaptacions necessàries degudes a l'espècie. Per a la interpretació correcta AB en situacions d'emergència, han d'emprar-se els valors de referència adequats a les diferents situacions fisiològiques (nivell d'entrenament o edat de l'animal) i també als analitzadors utilitzats.

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[EQUATIONS]

Equations:

(1) $pH = pK\,'1 + log \frac{HCO_3^-}{SpCO_2}$

(2) $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$

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

(4) $[UA] - [UC] = AG = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$

(5) $HA \leftrightarrow H^+ + A^-$

(6) $K_a = {[H^+][A^-]}/{[HA]}$

(7) $A_{tot} = [HA] + [A^-]$

(8) $[SID^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] + [H^+] = 0$

(9) $[H^+]^4 + ([SID^+] + K_a)[H^+]^3 + [K_a([SID^-] - [A_{TOT}]) - K_W' - K_1'[SP_{CO2}][H^+]^2 - [K_a(K_W' + K_1' SP_{CO2}) - K_3K_1' SP_{CO2}][H^+] - K_aK_3K_1'SP_{CO2} = 0$

(10) $[SIDm] = [Na^+] + [K^+] - [Cl^-] - [lactate]$

(11) $[H^+] + [H_2O] \leftrightarrow H_3O^+$

(12) $BE_{uma} = BE - BE_{alb} - BE_{sid}$

(13) $BE = 0.9287 \times [HCO_3 - 24.4 + (14.83 \times (pH - 7.4))]$

(14) $[SID^+] - [HCO_3^-] - [A^-] = 0$

(15) $pH = \log \frac{2SID^+}{K_1'SP_{CO2} + K_aA_{TOT} - K_aSID^+ + \sqrt{(K_1'SP_{CO2} + K_aSID^+ + K_aA_{TOT})^2 - 4K_a^2SID^+A_{TOT}}}$

(16) $[A_{TOT}] = 2.25 \times (Albumin\ g/dL) + 1.40 \times (Globulin\ g/dL) + 0.59 \times (Phosphate\ g/dL)$

(17) $SIG = \frac{A_{TOT}}{1+10^{(pKa-pH)}} - AG$

(18) $BE_{uma} = BE - BE_{alb} - BE_{SID}$

(19) $BE_{SID} = 0.3 \times (Na^{+} - 140) + (102 - Cl^{-}_{corr})$

(20) $BE_{alb} = 3.5 \times (4.5 - Albumin\ g/dL)$

(21) $BE_{SID} = Na^{+} - Cl^{-} - 38$

(22) $BE_{alb} = (42 - Albumin\ g/dL) \times 0.25$

(23) $BE_{SID} = Na^{+} - Cl^{-} - 32$

(24) $BE_{uma} = BE - BE_{alb} - BE_{SID} - (1.5 - lactate)$

(25) $BE_{SID} = 0.3 \times (Na^{+} - 140) + (108 - Cl^{-}_{corr})$

(26) $BE_{alb} = (0.123 \times pH - 0.631)(42 - Albumin\ g/dL)$

(27) $AG = (Na^{+} + K^{+}) - (Cl^{-} + HCO_3^{-})$

(28) $BE = 0.02786 \times pCO2 \times 10^{(pH-6.1)} + 13.77 \times (pH .7124.58)$

(29) $HCO_3^{-} = S_{CO2} \times pCO_2 \times 10^{(pH-pK'1)}$

(30) $tCO_2 = HCO_3^{-} + 0.03 \times pCO_2$

(31) $Osmolarity = 2(Na^{+} + K^{+}) + Glucose + \frac{Urea \times 0,47}{2,8}$

(32) $Gb = TP - Alb$

(33) $A_{TOT} = 2.24 \times Plasma\ total\ proteins$

(34) $A_{TOT} = 2.4 \times Plasma\ total\ proteins$ (Neonate foals)

(35) $BE_{alb} = [(28 - Alb) \times 0.22]$

(36) $SIG = SID_m - \frac{A_{tot}}{(1+10^{pKa-pH})} - HCO_3^{-}$

[ABBREVIATIONS]

- $[A^-]$……………………………… Conjugated form A_{TOT}
- AB ……………………..……….. Acid-base
- AG ……………………………… Anion Gap
- Alb…..…………………………. Albumin
- Ana-1…………….,……………. Analyzer 1 (Istat®)
- Ana-2 …………………………… Analyzer 2 (Vetlyte®)
- Ana-3 …………………………… Analyzer 3 (Catalyst®)
- A_{TOT} …………………………….. Total non-volatile weak buffers
- BE …………………..…………… Standard Base Excess
- BE_{alb} ……………………………. Base Excess due to albumin
- BE_{ECF} ………………...……….. Standard Base Excess of extracellular fluid
- BE_{fw} …………….…………….... Base Excess due to free water
- BE_{sid} …………………………….. Base Excess due to strong ion difference
- BE_{tp}………………….………... Base Excess due to total protein
- BE_{uma} …………………………… Base excess due to unmeasured anions
- $[Cl^-]$……………………….…….. Chloride
- Cl^-_{corr} …………..……………….. Corrected by sodium Chloride concentration
- Fib ……………………………… Fibrinogen
- Gb ………………………………. Globulin
- HCO_3^- ………………...……….. Bicarbonate
- $[K^+]$………………………………. Potassium
- K_1' ……………………………….. Apparent dissociation constant the Henderson-Hasselbalch equation
- K_3………………………………… Apparent dissociation constant for HCO_3^-
- K_a………………………………… Apparent dissociation constant for plasma non-volatile weak acids
- K_w…………..…………………….. Apparent dissociation constant for water
- Lac …………………..………….. Lactate

- $[Na^+]$ ……………………..……….. Sodium
- neICU…………………………….. Neonatal equine intensive care unit
- pCO_2 .... Partial pressure of CO_2
- P_i ………………….…………….. Phosphates
- pK_1' ………………..…………… Negative logarithm of apparent dissociation constant for carbonic acid in plasma
- pK_a ………………..…………… Negative logarithm of apparent dissociation constant for apparent dissociation constant for plasma non-volatile weak acids.
- PTP…………………………….. Plasma total protein
- p_vCO_2 ……………………...… Partial venous pressure of CO_2
- $[SID^+]$……………………..…….. Strong ion difference
- SID_m …………………….....…… Measured strong ion difference
- SIG…………………………….. Strong ion gap.
- $SpCO_2$ ………………………… The solubility of CO_2 in plasma
- TP…………………..…………… Total plasma protein

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