Serum lactate in canine babesiosis

by

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DEDICATION

Aan Rudi

Omdat jy in my lewe opgedaag het toe dit makliker gelyk het om op te gee eerder as om aan te gaan.

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ABBREVIATIONS

Ht	Haematocrit
OVAH	Onderstepoort Veterinary Academic Hospital
SIRS	Systemic Inflammatory Response Syndrome
MODS	Multiple Organ Dysfunction
ALT	Alanine Amino Transferase
ALP	Alkaline Phosphatase
ISA	In-saline Agglutination
RR	Relative Risk
P value	Probability value
OR	Odds Ratio

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SUMMARY

Canine babesiosis typically causes a haemolytic anemia and results in hypoxia and sepsis, which can eventually result in multiple organ dysfunction. Human patients with severe injury or disease such as shock, sepsis and malaria often have persistent hyperlactataemia, and there is a correlation between blood lactate and survival rate. There are various similarities between human malaria and canine babesiosis, eg. anaemia, renal failure, cerebral forms, coagulopathy, hepatopathy, pulmonary oedema, and shock. In severe malaria, lactate levels in blood rise in direct proportion to the severity of the disease. Venous lactate concentrations measured at 4 hours after admission appears to be the best prognostic indicator in severe malaria. In dogs blood lactate has been shown to be of prognostic value in patients with gastric dilatation-volvulus and in dogs admitted to intensive care units. Blood lactate has also been shown to be of prognostic value in equine colic.

Blood lactate was determined in ninety dogs with naturally occurring canine babesiosis. Forty-five dogs (50%) presented with hyperlactataemia (blood lactate > 2.5mmol/L) and 20 (22.2%) with hypoglycaemia (blood glucose < 3.3 mmol/L). Measurements significantly associated with mortality were hypoglycaemia on admission, blood lactate > 5mmol/L on admission, blood lactate > 2.5 mmol/L at 8, 16 and 24 hours after admission, and increase or < 50% decrease in blood lactate within 8 and 16 hours after admission. Blood lactate persistently > 4.4 mmol/L indicated a very poor prognosis. The study concluded that serial blood lactate measurements are useful in predicting survival in dogs with severe and complicated canine babesiosis.

OPSOMMING

Babesiose van honde veroorsaak ‘n tipiese hemolitiese anemie en lei tot hipoksie en sepse wat uiteindelik veelvuldige orgaan wanfunksie kan veroorsaak. Menslike pasiente met ernstige siekte of besering soos skok, sepse en malaria toon dikwels volgehoue verhoogde serum laktaat, en daar is ‘n korrelasie tussen bloed laktaat en oorlewing. Daar is verskeie ooreenkomste tussen menslike malaria en babesiose van honde, bv. anemie, nierversaking, serebrale vorms, stollings afwykings, lewerskade, longedeem en skok. In ernstige malaria verhoog serum laktaat vlakke in direkte ooreenstemming met die ergheidsgraad van die siekte. Veneuse laktaat konsentrasies wat 4 ure na opname gemeet word, blyk die beste prognostiese indikator te wees in ernstige malaria. In honde is dit bewys dat bloed laktaat van prognostiese waarde is in pasiente met gastriese dilatasie-volvulus en in honde wat opgeneem is in intensiewe sorgeenhede. Bloed laktaat kan ook gebruik word as prognostiese indicator in perde wat aan koliek ly.

Bloed laktaat is bepaal in negentig honde met natuurlike babesiose. Vyf en veertig honde (50%) het hiperlatatemie (bloed laktaat > 2.5 mmol/L) getoon by opname, en 20 (22.2%) het hipoglisemie (bloed glucose < 3.3 mmol/L) getoon. Metings wat noemenswaardig geassosieer was met mortaliteit was hipoglisemie met opname, bloedlaktaat > 5 mmol/L met opname, bloedlaktaat > 2.5 mmol/L teen 8, 16 en 24 uur na opname, en ‘n verhoging of < 50% verlaging in bloedlaktaat binne 8 en 16 ure na opname. Bloedlaktaat wat voortdurend > 4.4 mmol/L bly dui op ‘n baie swak prognose. Die studie toon dat reeks bloedlaktaat meetings nuttig is om die oorlewing te voorspel van ‘n hond met erge of gekompliseerde babesiose van honde.

CHAPTER 1: LITERATURE REVIEW

1.1. Canine Babesiosis [25, 31, 32]

Babesiosis is a tick-borne disease caused by the haemoprotozoan parasites, *Babesia canis or Babesia gibsoni.* Three subtypes of *Babesia canis* exist of which *Babesis canis rossi* occurs in Southern Africa. Parasites mainly affect erythrocytes, and typically cause a haemolytic anaemia, but can also result in multiple organ dysfunction.

Incubation period following tick exposure is 10 to 21 days, after which intra-erythrocytic parasitaemia leads to both intravascular and extravascular haemolysis. Pyrexia can also occur due to the release of endogenous pyrogens from erythrolysis, parasite destruction and inflammation. The haemolytic crisis that follows, results in anaemic hypoxia, anaerobic metabolism and metabolic acidosis.

Babesiosis can cause severe tissue hypoxia with tissue damage and probable release of inflammatory mediators, which can eventually lead to multiple organ dysfunction syndrome.(MODS)
Systemic inflammatory response syndrome (SIRS) is defined as a syndrome where 2 or more of the following clinical signs appear together: tachycardia, tachypnoea or respiratory alkalosis, hypo- or hyperthermia, leukocytosis, or leukopaenia with a neutrophilic left shift.
In the light of this definition most patients with babesiosis have SIRS. SIRS usually precedes MODS.

Canine babesiosis can be classified as complicated or uncomplicated, the latter classified as mild or severe. Classification is as follows:
Mild, uncomplicated: Ht >15%, no complications.
Severe, uncomplicated: Ht <15% no complications.
In complicated cases one of the following accompanying problems are seen: neurological signs, acute renal failure, acute respiratory distress syndrome, hypotensive shock, haemoconcentration, icterus and hepatopathy, coagulopathy and disseminated intravascular coagulation and immune mediated haemolytic anaemia. Other less common complications include gastrointestinal signs, myalgia, ocular involvement, upper respiratory signs, cardiac signs, necrosis of extremities and ascites and oedema.

1.2. Lactate

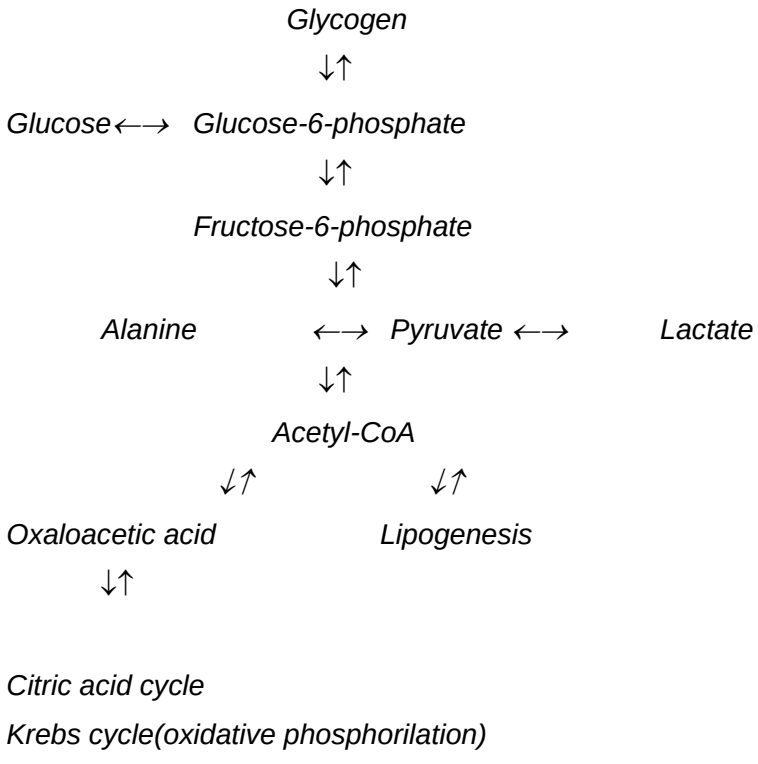
Lactate is a product of carbohydrate metabolism. In the body, lactic acid is a strong acid that is completely ionised to lactate and H^+ at physiologic pH. An elevated blood lactate concentration is termed hyperlactataemia[22].

Lactic acidosis is defined as a metabolic acidosis with significant lowering of blood pH in the presence of increased blood lactate levels[9].

1.3. Normal lactate metabolism

During the breakdown of glucose, pyruvate is formed. Pyruvate then follows one of 5 different pathways: (1) lipogenesis, (2) oxidation via the Kreb's cycle, (3) formation of alanine, (4) gluconeogenesis, or (5) it can be converted to lactate[17]. Figure 1 shows a simplified version of the pathways. Three of the five pathways are aerobic and do not function under anaerobic conditions[56].

Figure 1



During anaerobic conditions pyruvate is converted to lactate in order to provide energy. Lactate that is formed in other tissues, is transported to the liver where it can be used as a substrate for gluconeogenesis to provide further glucose for tissue utilization (Cori cycle)[56]. This will only happen if oxygen is available for gluconeogenesis.

Figure 2*:* CORI CYCLE (simplified)

LIVER	**BLOOD**	**TISSUE(muscle)**
GLYCOGEN		GLYCOGEN
↓↑		↓
GLUCOSE-6-P →	→ GLUCOSE →	→ GLUCOSE-6-P
↓↑		↓
PYRUVATE ←	← (PYRUVATE) ←	← PYRUVATE
↓↑		↓
LACTATE + H^+ ←	← LACTATE + H^+ ←	← LACTATE + H^+

1.4. Lactate metabolism in disease

Under healthy, aerobic conditions, the liver will utilise more lactate than what it will produce, which is what commonly happens after exercise. The increase in lactate is a temporary phenomenon, which corrects itself soon after exercise when the utilization of lactate is adequate to normalise the circulating lactate. In the healthy animal, the liver utilizes lactate in the production of glucose[8, 56]. However, during disease states a pathological accumulation of lactate is seen either due to increased production or decreased utilization.

Increased production is seen with tissue hypoxia and increased rate of metabolism. However, as glycolysis, via lactate formation, can proceed more rapidly than pyruvate oxidation, increased lactate production can occur in the absence of hypoxia in the presence of mechanisms able to speed up glycolysis such as alkalosis, glucose infusion, and sepsis without hypoperfusion[22]. Alkalosis, especially of respiratory origin, stimulates the rate limiting enzyme (phosphofructokinase) in aerobic glycolysis and leads to an increase in lactate production[11].

Decreased utilization can be due to impairment of any of the 3 other pathways by which pyruvate is metabolised. This usually involves tissue hypoxia, and the 3 aerobic pathways don't function due to anaerobic conditions[56].

Most of the lactate is produced in muscle tissue with a small amount also being produced in tissues such as the skin, wounds, white blood cells, red blood cells, and brain[14, 22]. The liver, and to a lesser extent the kidneys, are responsible for lactate metabolism[11]. When lactate production exceeds the capacity of the liver to metabolise it, lactate accumulates.

Hypermetabolism seen in disease includes enhanced glucose uptake and utilization by tissues, hyperlactataemia, increased glucose production, depressed glycogenesis, glucose intolerance and insulin resistance[14, 33]. For the purpose of this review, only hyperlactataemia will be discussed in detail.

It has been shown that humans suffering from severe disease or injury could have persistent hyperlactataemia[33]. The hyperlactataemia has been assumed to be as a result of tissue hypoxia. However, it has been shown that during hypoperfused states, the lactate and pyruvate increase disproportionately, whereas hyperlactataemia of injury or sepsis is usually accompanied by lactate and pyruvate increases that maintain the ratio between the 2 products. This suggests an equilibration phenomenon of hyperlactataemia during disease states[14, 20, 33]. Hyperlactataemia is thought to be due to a combination of tissue hypoxia and/or hypermetabolism. In the light of this statement lactic acidosis can be classified as either type A (due to poor perfusion and hypoxia), or type B (where poor tissue perfusion or poor arterial oxygenation is not obvious)[9, 34].

During progressive organ failure, there is an increase in glucose and fat metabolism. Gluconeogenesis and pyruvate and lactate production progressively increase. Gluconeogenesis appears to be influenced by the increases in pyruvate, lactate, alanine and amino acids as well as hormones such as glucagon, cortisol and epinephrine[14, 33].

In a study on febrile humans suffering from miscellaneous types of infection, pyruvate and lactate levels were found to be increased compared to healthy humans. During the initial acute febrile period, levels were much higher than in the convalescent period[18].

A patient with postoperative polymicrobial bacteraemia, showed increased lactate and pyruvate concentrations, and rapidly developed multiple organ failure[16].

Under normal circumstances gluconeogenesis is inhibited by insulin and glucose in the circulation, but during sepsis there seems to be resistance against these inhibitory mechanisms[33]. This is possibly due to the increased stimuli via the increases in pyruvate, alanine and lactate, which serve as precursors in gluconeogenesis[33].

1.5. Lactate as a prognostic indicator

Studies in human patients have suggested that there is a correlation between blood lactate and survival rate[6, 9, 34].

It was shown that human patients suffering from septic shock, in which the lactate concentration remained high in spite of treatment and in spite of their blood pH normalising, had a higher mortality rate than patients showing decreases in lactate concentration[3].

Blood lactate has been shown to be of prognostic value in equine colic[10, 15, 35, 38, 40, 41, 45]. In all of these studies lactate was found to be a good indicator of survival, with horses showing the highest lactate levels having the highest mortality rates. It has also been implemented in a colic scoring system to predict survival[15].

A study in human septic shock showed a distinctive pattern of increased lactate related to survival. When blood lactate was above 3 mmol/L, either on admission, or during the stay in the ICU, the prognosis proved extremely grave, despite therapy. Increase in blood lactate was found to be significantly related to survival in both the rat and human studies with circulatory shock[51].

Vincent [50] showed that, in ICU patients, blood lactate levels started dropping within the first hour of starting appropriate therapy in patients responding to therapy. Patients who did not respond to therapy showed no significant changes in blood lactate. It was suggested that hourly lactate measurements be done. In these studies it was found that lactate alone serves as the best prognostic indicator of survival during circulatory shock.

The study concluded that in patients who did not show significant decreases in blood lactate after one hour of aggressive therapy, change in therapy should be considered.

A study using serial lactate measurements in patients with septic shock showed that measurements were correlated with survival and organ failure. Initial lactate were not much different between survivors and non-survivors, but in the survivor group lactate levels decreased within the first 24 hours and lactate levels remained high for much shorter times compared to non-survivors[3]. Another study also showed that lactate levels can serve as a bedside parameter to identify patients with a high risk of mortality[4]. It was shown that oxygen-derived variables were not useful when trying to predict outcome in human septic shock. Lactate levels were closely related to ultimate survival of these patients, and could also serve as a reliable clinical guide to therapy[2].

A study on ventilated neonates to investigate the prognostic implications of blood lactate and acid-base parameters was done in 1997[11]. Because of the size of the patients, the authors were looking for less invasive ways to predict tissue perfusion and hypoxia. They found that patients with increased blood lactate showed increased mortality rates. Infants showing only slight increase or substantial decrease in lactate had better survival rates than infants showing persistently high blood lactate levels. They also concluded that serial determinations of blood lactate were of value in predicting outcome and evaluating response to treatment in seriously ill patients. Blood lactate concentrations increased before clinical deterioration was observed, thus lactate could serve as an early warning system of possible organ failure.

Abramson [1] found that the ability to clear lactate within 24 hours correlated well with survival in patients. In a study of children undergoing open-heart surgery, outcome and organ failure could also accurately be predicted using serum lactate levels[48].

There are various similarities between human malaria and canine babesiosis, eg. anaemia, renal failure, cerebral forms, coagulopathy, hepatopathy, pulmonary oedema, and shock[25].
In severe malaria, lactate levels in arterial and venous blood and CSF fluid rises in direct proportion to the severity of the disease. Venous lactate concentrations measured at 4 hours after admission appears to be the best prognostic indicator in severe malaria[42, 52].

Several studies in malaria patients showed increased blood lactate levels, which were well correlated to survival rate[26, 42, 43, 44, 53].

1.6. Measuring methods for blood lactate

A hand-held portable lactate analyser (Accusport®)[a] is available from Boehringer-Mannheim for determination of blood lactate concentrations[47]. Blood is applied to a test strip and seeps through the yellow protective mesh into a glass fibre fleece where the erythrocytes are retained. Only the plasma reaches the detection film. Lactate is measured using reflectance photometry, which takes 60 seconds to measure the colour developed by a drop of blood or plasma placed on the dry reagent strip. Lactate in the sample is then converted via a lactate-oxidase mediator reaction to molybdenum blue. It has a measuring range of 0.8 to 22.0 mmol/L. It is easy to use and only one drop of blood is needed to determine blood lactate concentrations[47, 49].

In a human study the Accusport® analyzer was found to be accurate, linear (up to at least 18.7 mmol/L), has good reliability at both low (1.7 mmo/L) and high (14.4 mmol/L) blood lactate concentrations, and is able to analyze whole blood up to 15 minutes after sample application to the test strip[13]. At values less than 20 mmol/L, the difference between the Accusport® and the reference method (Kodak Ektachem E250) is less than 1.1 mmol/L. The mean difference between the two methods is 0.26 mmol/L with a standard deviation of 1.17 mmol/L, and all values up to 16.1 mmol/L fall within one standard deviation of the mean difference. No significant difference exists between lactate values for haematocrits of 35% and 45% [13]. It was found to be a simple and reliable method to determine blood lactate concentrations in cord arterial blood immediately after birth, using small blood samples[55]. When using the Accusport® analyzer, a sample of 20 microlitres or more is required for accurate measurement of blood lactate[36].

In equine studies the Accusport® analyser has been used to measure blood lactate. A study in horses taking part in endurance rides has shown the Accusport® analyser to be suitable for quick measuring of blood lactate concentrations under field conditions[30].

[a] Roche Diagnostics, South Africa

Lindner [29] compared the Accusport® to laboratory wet methods for measuring lactate in horses and showed that there was a strong correlation ($p<0.001$) between the 2 methods. Simmons et al. [49] also compared the 2 methods in horses and found them to be highly correlated ($p< 0.001$ and $r = 0.988$) at blood lactate levels less than 13 mmol/L. At levels above 13 mmol/L samples were diluted, and there was a significant correlation between the 2 methods ($p< 0.05$).

Evans et al [12] compared the accuracy of Accusport® in equine blood and plasma. They found that at lactate concentrations greater than 10 mmol/l and PCV greater than 53%, blood lactate was underestimated when using whole blood. However, when heparinised samples were centrifuged and plasma was applied to the test strip, plasma lactate was accurately measured for concentrations ranging from 0.8-20 mmol/L. They concluded that this difference could be due to the fact that the machine was designed to analyse human blood, and that human and animal erythrocytes possibly played different roles in blood lactate concentrations. Another explanation would be that at very high PCV, not enough plasma seeps through onto the measuring strip to obtain an accurate reading. In this study, heparin was used as an anticoagulant, as the Accusport® manufacturer instructs that sodium fluoride should not be added to samples.

Williamson [54] showed that using Accusport® is an acceptable and convenient method for measuring blood lactate concentrations in horses. In the light of these findings, the Accusport® may serve as a valuable clinical and research tool.

1.7. Sampling methods

In equine studies serum lactate was determined using blood obtained from jugular venipuncture[10, 15, 35, 38, 40, 41, 45, 49].

Most human studies made use of arterial samples[1, 3, 5, 11, 48, 51], although venous blood has been used[18].

In the canine gastric dilatation-volvulus study, venous serum samples obtained from either jugular or cephalic venipuncture were used[39].

In a study done in dogs to determine the incidence of hyperlactataemia in patients admitted for emergency care, venous blood obtained from the jugular vein, was used to determine blood lactate levels[27].

1.7.1.Suggested sampling sites:

In a study of normal dogs, plasma lactate concentration was highest in cephalic vein samples (1.57 ± 0.47 mmol/L), lower in arterial samples from the femoral artery (1.43 ± 0.52 mmol/L), and lowest in the samples from the jugular veins (1.25 ± 0.49 mmol/L). However, these differences were minor and not of any clinical significance to indicate that any site would be preferable for sampling. This has also been shown to be true in hypoperfused humans[23].

Orringer [37] did a two- part study in healthy pigs that were bled into hypovolemic shock, and human patients undergoing open-heart surgery to compare various sample sites. Serial lactate measurements were done making use of arterial, central venous and peripheral venous sites. He found that blood lactate concentration in a peripheral vein shows little difference from arterial blood lactate values.

The area drained by a particular vein can influence venous blood lactate concentrations[46]. If serial samples are to be collected, it is important to use the same collection site for repeated sampling. In the dog, free-flowing jugular blood can be used to reflect values of mixed venous blood.

Haskins [21] suggested that central venous blood might provide a better indication of the status of the interstitial fluid than does arterial blood.

It can also be argued that in order to determine specific organ perfusion or oxygenation deficits, only blood from a particular organ should be evaluated. As this is not practically possible, whole body lactate production is used[19].

1.7.2. Sampling intervals:

A human study has suggested 4 to 8 hourly sampling[51]. Mizock [34] measured blood lactate every 6 hours for a period of 24 hours. Another study [50] noted changes in blood lactate within the first hour of therapy and the author suggested hourly measurements. In

malaria patients lactate was measured at 4 hours post admission[42, 52]. Abramson et al[1] collected samples at admission and 8, 16, 24, 36 and 48 hours after admission. In a study on ventilated human neonates [11] blood was collected at approximately 12 hourly intervals for a maximum of five serial measurements per baby. Bernardin[4] made use of a series of five consecutive measurements.

1.8. Normal serum lactate values

In humans values greater than 7 mmol/L are seen as "high"[9], with the normal value in humans set to be below 2 mmol/L[11].

In dogs, the normal value is set to be between 0.2 and 2.5mmol/L[20, 22, 39].

Blood lactate levels >5 mmol/L are usually associated with acidaemia[22].

In dogs values of 3-5 mmol/L are seen in mild systemic hypoperfusion; moderate hypoperfusion shows levels of between 5 and 10 mmol/L; and severe hypoperfusion >10mmol/L. If blood lactate levels fail to drop below 10 mmol/L in spite of adequate treatment or if sharp rises in lactate levels occur, then the prognosis appears to be poor regardless of the underlying cause of hyperlactataemia[22].

According to Roche Diagnostics normal lactate values measured in dogs were below 1 mmol/L.

1.9. Factors influencing serum lactate measurements

Gross haemolysis can depress results, intravenous drugs that alter acid-base status could alter lactate levels, and epinephrine, exercise, glucose administration and hyperventilation could lead to increased blood lactate levels [24].

When using the Accusport® analyser, lactate could be underestimated at PCV exceeding 55%, and after intravenous infusion of ascorbic acid[47].

It has been said that "lactate washout" can occur immediately after perfusion is restored. A study investigating this statement found that in a pig model of cardiac arrest and resuscitation, this only happened for the first 2.6 ± 0.3 minutes post resuscitation. This study proved that lactate measurements are not invalidated because of a washout phenomenon immediately post treatment[28].

1.10. Blood lactate in canine babesiosis

Because babesiosis is a disease during which animals could suffer from anaemia, resulting in hypoxia as well as the fact that babesiosis is known to cause sepsis in severe illness [7, 25, 31], it is expected that lactate concentrations would follow the same pattern as seen in other studies as discussed above.

In a study by Button [7] it was shown that the metabolic acidosis seen in severe babesiosis is principally due to generation of lactic acid. All infected dogs in this study showed metabolic acidosis and increased blood lactate levels. It was also noted that lactate in non-survivors was higher than in controls and survivors. Blood lactate value in healthy dogs was 1.53 ± 0.84 mmol/L, for survivors of disease it was 4.33 ± 4,9 mmol/L, and for fatally infected dogs it was 16.1 ± 2.87 mmol/L. The use of lactate as a prognostic indicator was not studied.

1.11. Blood lactate in canine gastric dilatation-volvulus

A study of blood lactate in dogs with gastric dilatation-volvulus showed that venous lactate is a good predictor of gastric necrosis and outcome. Survival rate for dogs with lactate concentrations < 6 mmol/L was significantly higher than the survival rate of dogs with serum lactate > 6 mmol/L[39].

1.12. Blood lactate in canine emergency care

A preliminary study was done in dogs to determine the incidence of hyperlactataemia in patients admitted for emergency care[27]. In this study venous blood was collected from the jugular vein of dogs at admission to the ICU. Diseases included gastro-intestinal problems, major and minor trauma, neurological disease, pulmonary disease, intoxications, urinary tract disease, haematological problems, metabolic diseases and cardiovascular disease. It was documented that there was an association between the severity of the hyperlactataemia and outcome of the disease. The higher the blood lactate value above normal, the more likely the dog was to die. The authors suggested that serial samples be evaluated in future, as serial measurements might be of greater prognostic value.

CHAPTER 2: HYPOTHESIS

2.1.Research Questions

- Do dogs with severe and complicated Babesiosis have increased blood lactate levels?
- Are serial blood lactate measurements useful as a prognostic indicator in severe and complicated canine babesiosis?

CHAPTER 3: BENEFITS

3.1.Benefits arising from the study

- We need to be able to predict whether a patient with canine babesiosis is being treated adequately, or whether more intensive therapy should be instituted early to prevent further organ damage.
- We need to be able to accurately predict the outcome of dogs suffering from babesiosis in order to give the owner an accurate prognosis.

CHAPTER 4: STUDY OBJECTIVES

4.1. Objectives

- To investigate the incidence of increased blood lactate in severe and complicated canine babesiosis cases.
- To investigate the usefulness of serial blood lactate measurements in predicting the outcome in severe and complicated canine babesiosis cases.

CHAPTER 5: MATERIALS AND METHODS

5.1. Models system

We conducted a prospective cross-sectional observational study.

5.2. Experimental design

The Animal Use and Care Committee and the Research Committee of the University of Pretoria approved this study. Ninety dogs with naturally occurring severe uncomplicated and complicated canine babesiosis infection were used in this study. Inclusion criteria were a positive identification of *B. canis rossi* parasites on a stained thin capillary blood smear using Cams Quick Stain[b], and admission to the Onderstepoort Veterinary Academic Hospital (OVAH) due to either severe anemia (haematocrit below 15%) and/or complications such as respiratory distress, acute renal failure (urine production < 1 ml/kg/h that does not respond to rehydration), hepatic involvement (elevated alanine amino transferase(ALT), alkaline phosphatase(ALP), icterus), pancreatitis, haemoconcentration, hypotensive shock, electrolyte imbalances (changes in sodium or potassium), hypoglycaemia, coagulopathy, and immune mediated haemolytic anaemia.

5.3. Ethical considerations

The research did not include any invasive procedures. Repeated blood sampling for micro-haematocrit determination was done routinely(approximately 8 hourly) in all cases of canine babesiosis. These samples were also anti-coagulated using heparin and were used to determine lactate at the same time.

As samples obtained were part of a routine database, and did not adversely affect the patients, there were no significant ethical considerations in this study. However, owners were requested to complete a consent form that allowed participation of the patient in this study. (AppendixB)

5.4. Experimental procedures

5.4.1. Sampling and lactate measurement

Approximately 1 ml of blood was collected from the jugular vein into a heparinized syringe prior to treatment. Animals were subsequently treated with the standard therapy for canine babesiosis at the OVAH, which included an anti-babesial drug and blood transfusion as needed. All complicated cases were treated for the specific complication as deemed necessary by the attending clinician. Attending clinicians were blinded to the results of blood lactate measurements performed for this study. Follow up blood samples were obtained at 8 hourly intervals for a period of 24 hours (4 samples per patient). Owners of dogs discharged from hospital were contacted 2 weeks after the date of discharge to obtain information regarding survival and progress after discharge. At the end of the study period, two weeks after discharge from the OVAH, dogs were classified as survivors or non- survivors.

Blood lactate was measured immediately after collection using the Accusport® blood lactate analyzer[c], according to manufacturers instructions. Blood glucose was measured on a Technicon RA XT system[d] using the hexokinase method[e]. The microhematocrit was measured using a microhematocrit centrifuge[f] and microhematocrit tubes[g]. Tubes were sealed with vitrex plasticene and centrifuged at 11800 rpm (14000 G) for 5 minutes. The haematocrit was measured using a Hawksley hematocrit reader.

An in-saline-agglutination test was performed by mixing one drop of blood with 6 drops of saline. One drop of the mixture was placed on a glass slide and covered with a cover slip and examined under a microscope using a 10X magnification. The test was deemed positive when red blood cells were clumping together in spite of dilution with saline.

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[b] CA Milsch P.O. Box 943, Krugersdorp, Johannesburg, 1740, South Africa

[c] Roche Products, Africa Region, P.O. Box 129, Isando, 1600, South Africa

[d] Technicon Instruments Corporation, Tarrytown, USA

[e] Bayer Health Care Division, P.O. Box 198, Isando, 1600, South Africa

[f] Jouan Hema-C microhaematocrit centrifuge, Hawksley and Sons, Ltd, Sussex, U.K.

[g] A&M Link Stiftung, Wertheim, Germany

5.4.2. Performance checks

Performance checks were performed on the Accusport® analyzer according to manufacturers instructions.

The following two standard solutions are available from Boehringer-Mannheim for performance checks:

BM- Control lactate (mmol/L)	Solution 1	Solution 2
Blood	1.6 – 3.1	4.6 – 8.1
Plasma	1.5 – 3.2	5.5 – 9.7

5.4.3. Reasons for using Accusport®

- It is easy to use even in practice conditions where large laboratories are not easily available to all practitioners.
- Results are obtained immediately at the "bedside", so decisions can be made right away regarding treatment.
- Low cost, thus affordable to all veterinarians and clients.

5.5. Observations

The following data was collected:

Micro-haematocrit, blood lactate values (0,8,16 and 24 hours), blood glucose, presence of neurological signs, presence of respiratory difficulty, capillary refill time, habitus (mental status), heart rate and duration of disease prior to presentation. (Appendix A)

Owners of dogs discharged from hospital, were contacted 2 weeks after discharge to obtain information regarding survival and progress after discharge.

5.6. Statistical Analysis:

Data analysis was done using NCSS 2001[h] and Epicalc 2000[i] statistical software programs. Univariable analysis of the effects of haematocrit, blood glucose concentration and serial blood lactate concentrations on mortality was done using the

[h] NCSS, Kaysville, Utah

[i] EpiCalc 2000 v1.02, Brixton Books, UK

Wilcoxon rank-sum test for differences between medians and the Fisher's exact test for categorical data. Variables with p<0.3 on univariable analysis were selected for testing using multivariable logistic regression. Two models were tested: one including lactate concentration on admission and the other including lactate concentration eight hours after admission. The models were then developed by backward elimination; variables remained in the model if they were significant (Wald *P*<0.01) or if their removal resulted in >10% change in the effect of other variables.

CHAPTER 6: RESULTS

6.1. Signalment

Ninety dogs (47 males and 43 females) were included in the study. The median age of the dogs was 1 year (range 1 month to 13 years). Breeds included sixteen Boerboels, eleven cross breeds, eight German shepherd dogs, seven Labrador Retrievers, six Rottweilers, six Staffordshire terriers, five Maltese dogs, five Chow-chows, four Dachshunds, three each of American Pitt Bull Terriers, Jack Russell Terriers and Fox Terriers, two each of Rhodesian Ridgebacks and Boston Terriers, and one each of Saint Bernard dog, Bouvier des Flandres, Spaniel, Mastiff, Chihuahua, Boxer, Bull Terrier, Border Collie and Pekingese.

6.2. Duration of disease

Approximate duration of disease prior to presentation was two days.

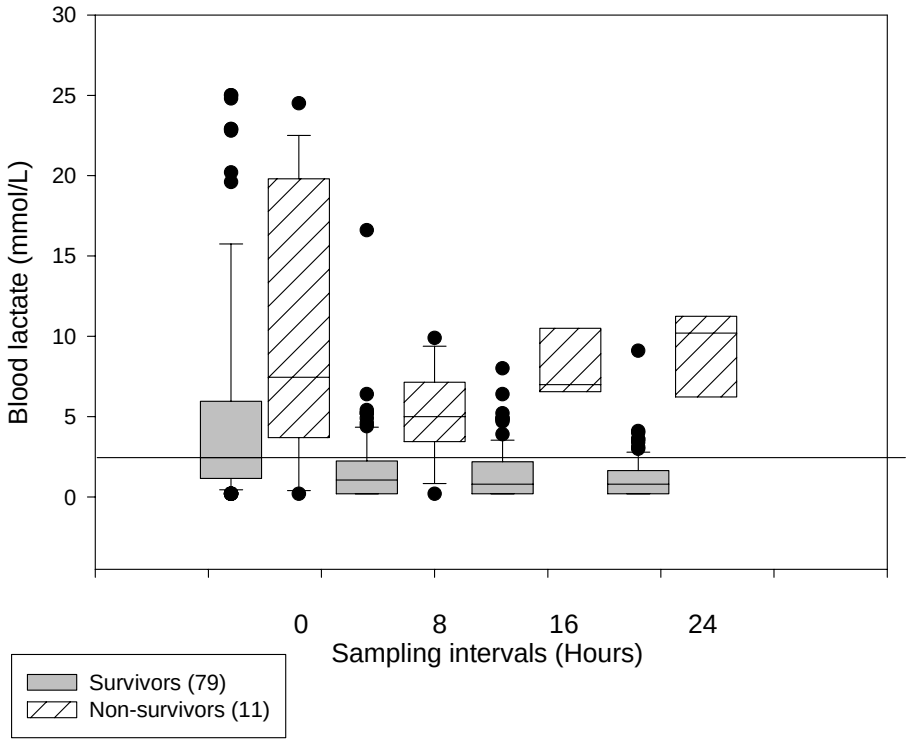
6.3. Complications of disease

Three dogs (3.3%) presented with neurological signs including seizures in one dog, and coma in the other two. Fourty-seven dogs (52.2%) showed respiratory signs such as tachypnoea, polypnoea and dyspnoea. Fifteen dogs (16.6%) were alert, 38 dogs (42.2%) were depressed, and 37 dogs (41.1%) were non-responsive to stimuli. Twenty dogs (22.2%) showed icterus at presentation. In-saline-agglutination was positive in 17 dogs (18.8%) and negative in 73 dogs (81.1%). Microhaematocrit values ranged from 5 to 58% with a median of 10%.

6.4. Death and survival

Eleven dogs (12%) died, and 79 dogs (88%) survived. Blood lactate values of these 2 groups are shown in Graph 1.

Graph 1. Mean blood lactate values of survivors were lower at each sampling interval when compared to non-survivors.
Lines within boxes indicate the median values with the line across the graph indicating the cut off value 2.5 mmol/L.
Dots represent outlying values.
Whiskers represent the tenth and ninetieth percentiles.



6.5. Hyperlactataemia in babesiosis

Forty-five dogs (50%) presented with hyperlactataemia (blood lactate > 2.5 mmol/L). Seven dogs died before the end of the 24 hour sampling period. Five of these seven dogs were hyperlactataemic at presentation. Twenty-four hours after admission, 13 dogs were still hyperlactataemic, all of which had been hyperlactataemic on admission. Of these 13 dogs, nine (69.2%) survived and four (30.8%) died. All nine surviving dogs had shown a decrease in blood lactate at the 8-hour interval, with seven of them showing a >50% decrease. All four dogs with blood lactate > 4.4 mmol/L at 24 hours after admission, died. All four had shown persistently raised blood lactate (> 4.4 mmol/L) at all sampling times and in two of them blood lactate was higher at 24 hours than on

admission. On the other hand, all (n = 75) dogs with blood lactate < 4.4 mmol/L at 24 hours after admission, survived.

Two dogs were euthanased during the study. They included one dog that was euthanazed after 16 hours, and one dog that was euthanazed after 24 hours. The first dog was euthanazed after developing severe disseminated intravascular coagulation with widespread haemorrhages and bleeding from the nose, eyes, and gastrointestinal tract. The second dog developed neurological signs and became comatose. As the presence of neurological signs indicates a poorer prognosis[20], the owner decided not to spend any further money on treatment of the patient and the dog was euthanazed. The second dog that was euthanazed showed an initial blood lactate value of 0.2 mmol/L, but by the 8-hour sampling period, lactate had risen to 3.4 mmol/L and by 16 hours it was 7 mmol/L. The other non-surviving dog that presented with a normal lactate value of 0.6 mmol/L died within 30 minutes of admission. The dog was comatose, severely icteric, and hyperglycaemic (9.8 mmol/L). Unfortunately the owner did not give permission for a post mortem examination.

6.6. Survival analysis

Haematocrit, glucose and serial lactate measurements of survivors and non-survivors are compared in Table 1. Non-survivors had significantly higher blood lactate concentrations than survivors at each interval.

Table 1. Median haematocrit and blood glucose concentration on admission, and serial blood lactate concentrations in survivors and non-survivors of canine babesiosis.

	Ht (%)	Glucose (mmol/L)	Lactate (mmol/L)			
			0 hours	8 hours	16 hours	24 hours
Survivors	10 [a]	4.6 [a]	2.5 [a]	1.1 [a]	0.8 [a]	0.8 [a]
Non-survivors	11 [a]	3.4 [a]	8.5 [b]	5.7 [b]	7 [b]	9.7 [b]
TOTAL	10.5	4.45	2.6	1.2	0.9	0.9

[a,b] Values within columns with differing superscripts differ significantly (Wilcoxon rank-sum test, $P<0.05$)

Univariable associations between each variable and mortality are shown in Table 2, expressed in terms of the relative risk of mortality compared with a reference category.

Table 2. Association of haematocrit, blood glucose and blood lactate with mortality in canine babesiosis: univariable analysis

Variable	Category	Mortality		RR *	95% confidence interval	*P* §
		Yes	No			
Haematocrit (%)	<10	3	27	0.40	0.05 to 3.0	0.4
	10-14	5	38	0.47	0.07 to 3.1	0.4
	15-19	2	5	1.1	0.15 to 9.0	1.0
	20-39	1	3	1 †		
	≥40	0	6	0	0 to -	0.4
Glucose 0 hours (mmol/L)	<3.3	5	15	3.2	1.0 to 9.9	0.05
	3.3 to 6.6	5	59	1 †		
	>6.6	1	5	2.1	0.30 to 15	0.4
Lactate 0 hours (mmol/L)	≤2.5	2	43	1 †		
	2.6 to 5	1	14	1.5	0.15 to 15	1.0
	>5	8	22	6.0	1.4 to 26	0.01
Lactate 8 hours (mmol/L)	≤2.5	1	62	1 †		
	2.6 to 5	3	14	11	1.2 to 100	0.03
	>5	4	3	36	4.7 to 279	0.0002
Lactate 16 hours (mmol/L)	≤2.5	0	65	1 †		
	2.6 to 5	0	9	-	-	1.0
	>5	5	2	-	-	<0.0001
Lactate 24 hours (mmol/L)	≤2.5	0	66	1 †		
	2.6 to 5	1	9	-	-	0.1
	>5	3	0	-	-	<0.0001
TOTAL		11	79			

* Relative risk of mortality compared to reference category
† Reference category
§ *P*-value for Fisher's exact test

In dogs admitted with hyperlactataemia, an increase or a less than 50% decrease in blood lactate at 8 hours and at 16 hours were significantly associated with mortality, compared to cases in which lactate decreased by more than 50% (Table 3). There was no evidence of an association between haematocrit on admission and survival.

Table 3. Association between change in blood lactate concentration and mortality in canine babesiosis patients with hyperlactataemia on admission

Variable	Category	Mortality		RR *	95% confidence interval	*P* §
		Yes	No			
Decrease in lactate 0 to 8 hours	≤50%	5	6	7.3	1.6 to 32	0.008
	>50%	2	30	1 †		
Decrease in lactate 0 to 16 hours	≤50%	3	7	8.4	1.0 to 72	0.05
	>50%	1	27	1 †		
TOTAL		7	36			

* Relative risk of mortality compared to reference category
† Reference category
§ *P*-value for Fisher's exact test

Blood lactate concentrations on admission and at eight hours after admission were the only predictor variables that remained significant in the two logistic regression models (Tables 4 and 5).

Table 4. Multiple logistic regression model of risk factors for mortality in canine babesiosis: blood lactate on admission

Variable	Category	β	OR *	95% confidence interval	*P*
Lactate 0 hours (mmol/L)	≤2.5	0	1 †		
	2.6 to 5	0.429	1.5	0.13 to 18	0.7
	>5	2.06	7.8	1.5 to 40	0.01

* Odds ratio relative to reference category
† Reference category

Table 5. Multiple logistic regression model of risk factors for mortality in canine babesiosis: blood lactate 8 hours after admission

Variable	Category	β	OR *	95% confidence interval	*P*
Lactate 8 hours (mmol/L)	≤2.5	0	1 †		
	2.6 to 5	2.59	13	1.3 to 137	0.03
	>5	4.41	83	6.9 to 986	0.0005

* Odds ratio relative to reference category
† Reference category

Both measurements were significantly associated with mortality, but the 8-hour measurement showed a much stronger association (odds ratios of 13 and 83 for lactate concentrations >2.5 and >5 mmol/L respectively).

Although hypoglycaemia (blood glucose < 3.3 mmol/L) on admission was associated with an increased risk of mortality in the univariable analysis, it was eliminated from the logistic regression models because hypoglycaemia and hyperlactataemia were highly associated (chi-squared test, *P*< 0.0001). Of the 20 dogs that were hypoglycaemic on admission, 19 (95%) were hyperlactataemic and 18 (90%) had blood lactate > 5 mmol/L.

CHAPTER 7: DISCUSSION

Babesiosis is a disease that results in hypoxia and sepsis[7,25,31]. In dogs with severe babesiosis it has been shown that the metabolic acidosis was principally due to generation of lactic acid[7]. It was also shown that lactate in non-survivors was higher than in controls and survivors. Blood lactate value in healthy dogs in that study was 1.5 mmol/L, for survivors of disease it was 4.3 mmol/L, and for fatally infected dogs it was 16.1 mmol/L.

In dogs, the normal reported value for blood lactate (amperometric autoanalyzer) is between 0.2 and 2.5 mmol/L[20,22,39]. According to the manufacturers of the Accusport® analyzer normal dogs have blood lactate values of up to 1 mmol/L. Values of 3-5 mmol/L, 5-10 mmol/L and >10 mmol/L are seen in mild, moderate and severe hypoperfusion, respectively [22]. Blood lactate levels 5 mmol/L are usually associated with acidaemia[22].

Hyperlactataemia seen in humans suffering from severe disease or injury has been attributed to hypoxia[33]. However, during hypoperfused states, the lactate and pyruvate increase disproportionately, whereas hyperlactataemia of injury or sepsis is usually accompanied by lactate and pyruvate increases that maintain the ratio between the 2 products. This suggests an equilibration phenomenon of hyperlactataemia during disease states[14,20,33]. Hyperlactataemia is now thought to be due to a combination of tissue hypoxia and/or hypermetabolism. Lactic acidosis can be classified as either type A (due to poor perfusion and hypoxia), or type B (where poor tissue perfusion or poor arterial oxygenation is not obvious)[9,34].

Serial lactate measurements are recommended in the human literature, as pre-treatment lactate values do not differ between survivors and non-survivors. Survivors in studies investigating blood lactate in ventilated babies and patients suffering from septic shock showed a decrease in blood lactate within the first 24 hours and lactate levels remained high for much shorter times compared to non-survivors[3,11]. In this study we found that lactate concentration differed between survivors and non-survivors at every time period, including pre-treatment. However, this difference tended to become greater at each subsequent measurement. The best prediction of survival was obtained at 24 hours, when blood lactate > 4.4mmo/L and <4.4mmol/L correctly predicted death and survival respectively, in every case.

In human studies blood lactate concentrations increased before clinical deterioration was observed, thus lactate could serve as an early warning system of possible organ failure[11]. An observation made in this current study is that clinical signs accompanied changes in blood lactate. Although attending clinicians were blinded to blood lactate values, the authors who were responsible for collecting the blood samples(MN and NK) observed that dogs showing decreasing blood lactate values showed clinical improvement, whereas dogs showing constantly increased or rising blood lactate values continued to deteriorate clinically. This was only a subjective observation, and grading of clinical signs will need to be evaluated in further studies to confirm this observation.

CHAPTER 8: CONCLUSIONS

We conclude that blood lactate values can serve as a predictor of outcome in dogs suffering from severe or complicated canine babesiosis. Although pre-treatment hyperlactataemia indicates a poorer prognosis, subsequent serial lactate values show a much stronger association with mortality. Dogs with greater post-treatment decreases in blood lactate have a higher survival rate, whereas serial blood lactate persistently remaining above 4.4 mmol/L indicates a very poor prognosis. Although hypoglycaemia on admission was also associated with an increased risk of mortality, hypoglycaemia and hyperlactatemia tended to occur together and blood lactate alone can serve as a prognostic indicator in dogs with severe or complicated canine babesiosis.

REFERENCES

1. Abramson D, Scalea TM., Hitchcock R. et. al., 1993. Lactate clearance and survival following Injury. *Journal of Trauma 35*: 584-589.
2. Bakker J, Coffernils M., Leon M., Gris P., Vincent J-L., 1991. Blood lactate levels are superior to oxygen-derived variables in predicting outcome in human septic shock. *Chest 99*: 956-962.
3. Bakker J, Gris P., Coffernils M., Kahn RJ, Vincent J-L., 1996. Serial blood lactate levels can predict the development of multiple organ failure following septic shock. *American Journal of Surgery. 171*: 221-226.
4. Bernardin G, Pradier C., Tiger F., Deloffre P., Mattei M., 1996. Blood pressure and arterial lactate levels are early indicators of short-term survival in human septic shock. *Intensive Care Medicine. 22*: 17-25.
5. Blair E, Adams Cowley R., Tait MK., 1965. Refractory septic shock in man : Role of lactate and pyruvate metabolism and acid-base balance in prognosis. *American Journal of Surgery. 31*: 537-540.
6. Broder G, Weil M., 1964. Excess lactate: An index of reversibility of shock in human patients. *Science 143*: 1457-1459.
7. Button C, 1979. Metabolic and electrolyte disturbances in acute canine babesiosis. *Journal of the American Veterinary Medical Association 175*: 475-479.
8. Center S, 1996. Pathophysiology of liver disease: Normal and abnormal function. *Strombeck's Small Animal Gastroenterology.* Philadelphia: W.B.Saunders Company: 553-556.
9. Cohen R, Woods HF., 1976. The clinical presentations and classification of lactic acidosis. *Clinical and Biochemical Aspects of Lactic Acidosis.* Oxford London Edinburgh Melbourne: Blackwell Scientific Publications., 41-84.
10. Dabareiner RM, White NA, 1995. Large colon impaction in horses: 147 cases (1985-1991). *Journal of the American Veterinary Medical Association 206*: 679-685.
11. Desphande S, Ward Platt MP., 1997. Association between blood lactate and acid-base status and mortality in ventilated babies. *Arch. Disease Child. 76*: F15-F20.

12. Evans D, Golland LC., 1996. Accuracy of Accusport for measurement of lactate concentrations in equine blood and plasma. *Equine Veterinary Journal. 28*: 398-402.
13. Fell J, Rayfield JM., Gulbin JP., Gaffney PT., 1998. Evaluation of the Accusport lactate analyser. *International Journal of Sports Medicine 19*: 199-204.
14. Frank B, Cerra MD., 1987. Hypermetabolism, organ failure, and metabolic support. *Surgery 101*: 1-14.
15. Furr MO, Lessard P, White NA, 1995. Development of a colic severity score for predicting the outcome of equine colic. *Veterinary Surgery 24*: 97-101.
16. Gammaitoni C, Nasraway SA., 1994. Normal lactate/pyruvate ratio during overwhelming polymicrobial bacteremia and multiple organ failure. *Anesthesiology 80*: 213-216.
17. Ganong.W.F, 1975. Endocrinology and metabolism. Ganong.W.F, ed. *Review of medical physiology*. San Francisco,California: Lange Medical Publications, 206-221.
18. Gilbert V, 1968. Blood pyruvate and lactate during human febrile infections. *Metabolism 17*: 943-951.
19. Guttierrez G, 1995. Tissue oxygenation and high-energy phosphate metabolism. Shoemaker W, Ayres,SM., Grenvik, A., Holbrook, P.R., ed. *Textbook of Critical Care*. Philadelphia London Toronto Montreal Sydney Tokyo: W.B.Saunders Company., 300-303.
20. Hardie. EM, 2000. Therapeutic management of sepsis. Kirk R, ed. *Current Veterinary Therapy XIII Small Animal Practice.* Philadelphia: W.B.Saunders Company, 272-275.
21. Haskins S, 1977. Sampling and storage of blood for pH and blood gas analysis. *Journal of the American Veterinary Medical Association 170*: 429-433.
22. Hughes D, Bonagura JD, 2000. Lactate measurement: diagnostic, therapeutic, and prognostic implications. *Kirk's current veterinary therapy XIII: small animal practice*: 112 - 116.
23. Hughes D, Rozanski ER., Frances S., et.al., 1999. Effect of sampling site, repeated sampling, pH, and pCO2 on plasma lactate concentration in healthy dogs. *American Journal of Veterinary Research. 60*: 521-524.

24. Jacobs D, Kasten BL., DeMott WR., Wolfson WL., 1990. Lactic acid, blood. Jacobs D, Wolfson WL., ed. *Laboratory Test Handbook*. Baltimore, Hong Kong, London, Sydney: Williams and Wilkens, 245-246.

25. Jacobson L, Clark IA., 1994. The pathophysiology of canine babesiosis: new approaches to an old puzzle. *Journal of the South African Veterinary Association. 65*: 134-145.

26. Kawo N, Msengi AE., Swai ABM., et.al., 1990. Specificity of hypoglycaemia for cerebral malaria in children. *The Lancet. 336*: 454-457.

27. Lagutchik M, Ogilvie GK., Hackett TB., Wingfield, WE., 1998. Increased lactate concentrations in ill and injured dogs. *The Journal of Veterinary Emergency and Critical Care 8*: 117-127.

28. Leavy J, Weil MH, Rackow EC., 1988. "Lactate washout" following circulatory arrest. *Journal of the American Veterinary Medical Association 260*: 662-664.

29. Lindner A, 1996. Measurement of plasma lactate concentation with Accusport. *Equine Veterinary Journal. 28*: 403-405.

30. Lindner A, Guhl A., Mallison J., 1995. Measurement of blood lactate concentration in horses during endurance rides using Accusport pocket analyser. *Pferdeheilkunde 11*: 393-398.

31. Lobetti R, 1998. Canine babesiosis. *Compendium of Continuing Education for the Practicing Veterinarian. 20*: 418-430.

32. Lobetti R, Mohr AJ., Dippenaar T., Myburgh E., 2000. A preliminary study on the serum protein response in canine babesiosis. *Journal of the South African Veterinary Association. 71*: 38-42.

33. Mizock.B.A., 1995. Alterations in carbohydrate metabolism during stress:A review of the literature. *Am.J.Med. 98*: 75-84.

34. Mizock.B.A. Falk JL., 1992. Lactic acidosis in critical illness. *Critical Care Medicine. 20*: 80-93.

35. Moore J, Owen R ap R., Lumsden JH., 1976. Clinical evaluation of blood lactate levels in equine colic. *Equine Veterinary Journal. 8*: 49-54.

36. Nordatrom L, Chua S., Roy A., Arulkumaran S., 1998. Quality assesment of two lactate strip test methods suitable for obstetric use. *Journal of perinatal medicine. 26*: 83-88.

37. Orringer R, Carey LC., 1973. A comparison of arterial and venous lactate levels in low flow states. *Wisconsin Medical Journal 72*: 254-256.

38. Orsisi J, Elser AH., Galligan DT., 1988. Prognostic index for acute abdominal crisis(colic) in horses. *American Journal of Veterinary Research. 49*: 1969-1971.

39. Papp Ed, Drobatz KJ, Hughes D, de Papp E, 1999. Plasma lactate concentration as a predictor of gastric necrosis and survival among dogs with gastric dilatation-volvulus: 102 cases (1995-1998). *Journal of the American Veterinary Medical Association 215*: 49-52.

40. Parry B, 1986. Prognostic evaluation of equine colic cases. *Compendium of Continuing Education for the Practicing Veterinarian. 8*: 98-104.

41. Parry B, 1987. Use of clinical pathology in the evaluation of horses with colic. *Veterinary Clinics of North America.(Equine Practice) 3*: 529-542.

42. Phillips R, 1989. Hypoglycaemia as an important complication of falciparum malaria. *Quarterly Journal of Medicine. 71*: 477-483.

43. Phillips R, Looareesuwan S., Molyneux ME, Hatz C., Warrell DA., 1993. Hypoglycaemia and counter regulatory hormone responses in severe falciparum malaria: treatment with sandostatin. *Quarterly Journal of Medicine. 86*: 233-240.

44. Pukrittayakamee S, Davis TME, Levy J., et.al., 1991. The matabolic response to rapid intravenous glucose injection in acute falciparum malaria. *Transactions of the Royal Society of tropical medicine and hygiene. 85*: 189-193.

45. Reeves M, Curtis CR., Salman MD. et.al., 1989. Prognosis in equine colic patients using multivariable analysis. *Canadian journal of Veterinary Research. 53*: 87-94.

46. Robertson S, 1989. Simple acid-base disorders. *Veterinary Clinics of North America.(Small Animal Practice) 19*: 289-306.

47. Roche, Accusport lactate system. South Africa: Roche Products.

48. Siegel L, Dalton HJ., Hetrzog JH., Hopkins RA., Hannan RL., Hauser GJ., 1996. Initial post- operative serum lactate levels predict survival in children after open heart surgery. *Intensive Care Medicine. 22*: 1418-1423.

49. Simmons D, Stewart AW., Stewart C., Pedler P., Roberts P., 1999. A comparative evaluation of the Accusport vs. conventional assay methods for determination of lactate in equine plasma. *Journal of equine veterinary science. 19*: 402-407.

50. Vincent J-L, Dufaye P., Berre J., Leeman M., Degaute J-P., Kahn RJ., 1983. Serial lactate determinations during circulatory shock. *Critical Care Medicine. 11*: 449-451.

51. Weil M, Abdelmonen AA., 1970. Experimental and clinical studies on lactate and pyruvate as indicators of severity of acute circulatory failure(shock). *Circulation XLI*: 989-1001.

52. White N, 1996. Malaria. Cook G, ed. *Manson's Tropical Diseases.* Philadelphia: WB Saunders Company, 1104.

53. White N, Warrell DA., Chanthavanich P. et.al., 1983. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. *The New England Journal of Medicine. 309*: 61-66.

54. Williamson C, James EA., James MP., May CD., Casey PJ., 1996. Horse plasma lactate determinations: Comparison of wet and dry chemistry methods and the effect of storage. *Equine Veterinary Journal. 28*: 406-408.

55. Yam J, Chua S., Razvi K., Arulkumaran S., 1998. Evaluation of a new portable system for cord lactate determination. *Gynecological and obstetrical investigations. 45*: 29-31.

56. Zilva J, Pannall PR., Mayne PD., 1988. Lactate production and lactic acidosis. *Clinical Chemistry in Diagnosis and treatment*. London Baltimore Melbourne, Auckland: Edward Arnold A division of Hodder and Stoughton, 205-209.

APPENDICES

Case no:____

"L" no :____

HYPOGLYCAEMIA and HYPERLACTATAEMIA IN CANINE BABESIOSIS HISTORY AND CLINICAL EXAMINATION FORM

Date:……

Owner:
Surname: ……Owner number:…….

Patient: Name:…… Number:……..

Age:……Sex:…… Breed:…….

History Questionnaire

1.How long ago did you first notice that your dog was not well?

1day	2days	3days	>3 days

2.When was the last time your dog ate a full meal?

<1day	1-3days	4-6days	7+days

3. Did your dog vomit?

Yes	No

4. Does your dog have diarrhoea?(runny tummy)

Yes	No

5. Is your dog sterilised?

Yes	No

6. If your dog is a female, is she pregnant?

Yes	No

7. If yes – how far? _____

8. If your dog is a female, does she have puppies at the moment?

Yes	No

9. How many puppies? _____

10. How old are the puppies?

< 2 weeks	2 – 4 weeks	4 – 6 weeks	> 6 weeks

11.When last did you de-worm your dog?

Days ago	Weeks ago	Months ago	Years ago

12. Does your dog suffer from any medical condition:

Yes	No

13. If yes – specify: _____

14.Is your dog on any medication?

Yes	No

15. If yes – specify (drug, dose and route): _____

_____

Case no:____

"L" no: _____

Clinical findings

1.Habitus:

Alert	Depressed	Collapsed

2.Cerebral signs:

Yes	No

3. If yes – specify: _____

4.Visisble Icterus:

Yes	No

5.Respiratory rate:_____ Respiratory component:

Abdominal	Thoracic	Both

6.Micro-haematocrit:_____

7.In-saline-agglutination:

Pos.	Neg.

Case no: _____

"L" no: _____

Blood lactate at presentation:_____mmol/L Hb:___g/l

Blood Glucose at presentation:_____mmol/L

Glucose level: 8hr: …… mmol/l

16hr: …… mmol/l

24hr: …… mmol/l

Lactate level: 8hr: …… mmol/l Hb:____g/l

16hr: …… mmol/l Hb:____g/l

24hr: …… mmol/l Hb:____g/l

Complications:

	YES	NO	Not assessed
Insaline agglutanation pos.:			
Respiratory distress:			
Cerebral:			
Icterus:			

Discharged: Date:…… Time:…….

Died: Date:…… Time:……

Euthanased: Date:…… Time:……..

Reason:

Cost	Poor prognosis	Other

Specify:…….

Follow-up phone call: Date:……

Alive:

Yes	No

If no – specify:……

Case no: ____

"L" no: ____

HYPOGLYCAEMIA and HYPERLACTATAEMIA IN CANINE BABESIOSIS HISTORY AND CLINICAL EXAMINATION FORM

Date:……

Owner:

Surname: ……Owner number:…….

Patient: Name:……. Number:……..

Admitted: Time:……

Treatment: Diminazine, imidocarb, bloodtransfusion, ringers lactate, KCl, glucose, etc.

Date:	**Time:**	**Treatment:**	**Dose and route:**

CONSENT FORM

Your dog has been diagnosed with *B. canis* (babesiosis/ tick fever/ biliary). At present we are conducting studies to evaluate the blood sugar (glucose) and blood lactate levels in dogs with babesiosis. This entails the collection of blood samples (±2ml of blood/ half a teaspoon) taken at the time of diagnosis as well as during the stay in hospital. At no time will the studies interfere with the treatment of your pet.

The costs of these tests will not be added to your account. We will be paying for the extra tests. However, you will still be responsible for other tests and the treatment of your dog.

This study has been passed by the Ethics Committee of the Faculty of Veterinary Science, University of Pretoria.

Thank you for your willingness to allow your animal to be entered into these studies. We hope that the information we gain will improve our understanding and treatment of babesiosis. Should you require more information please contact:

Dr N Keller or Dr M Nel
Companion Animal Medicine
Onderstepoort Veterinary Academic Hospital
Tel: 529-8000 or 529-8366 or 529-8094

I,……hereby give permission that my dog (name)……, a (colour)……, (sex)……, (breed)……, may participate in the clinical studies at the Onderstepoort Veterinary Academic Hospital. I understand that the studies will in no way harm my dog. Furthermore I understand that no additional costs will be incurred by me in respect of the trial for blood sampling and testing.

Signed at Onderstepoort on the …….. day of ……2001/2002.

Signature owner/authorised person ……..

Home tel: ……
Work tel: …….
Cell: ……

TOESTEMMINGS VORM

U hond is gediagnoseer met *Babesia canis* (babesiose/ bosluiskoors/ galkoors). Op die oomblik is ons besig met studies wat die bloedsuiker (glukose) en bloed laktaat vlakke ondersoek in honde met babesiose. Dit genoodsaak die neem van bloedmonsters (±2ml bloed/halwe teelepel) ten tye van diagnose asook gedurende hospitaal verblyf. Op geen stadium sal die studies inmeng met die behandeling van u hond nie.

Daar sal geen ekstra kostes by u rekening wees nie. Ons betaal vir die ekstra toetse. U sal wel verantwoordelik wees vir alle ander toetse en die behandeling van u hond.

Die studies is goedgekeur deur die Etiese Kommittee van die Fakulteit Veeartsenykunde, Universiteit van Pretoria.

Dankie vir u bereidwilligheid om u hond in die studies toe te laat. Ons hoop dat die inligting wat ons gaan insamel, ons sal help om die siekte beter te verstaan en te behandel. As u meer wil weet kan u ons kontak by:

Dr N Keller of Dr M Nel
Geselskapsdier Geneeskunde
Onderstepoort Veterinere Akademiese Hospitaal
Tel: 529-8000 of 529-8366 of 529-8094

Ek,…….gee hiermee toestemming dat my hond (naam)…….., ‘n (kleur)……., (geslag)…….., (ras)…… mag deelneem aan die kliniese studies by die Onderstepoort Veterinere Akademiese Hospitaal. Ek verstaan dat die studies geensins my hond sal benadeel nie. Verder verstaan ek dat daar geen ekstra kostes sal wees vir die neem en toets van die bloed vir die studies nie.

Geteken te Onderstepoort op die …… dag van ……..2001/2002.

Handtekening eienaar/gemagtigde persoon ……
Huis tel: ……
Werk tel: …….
Selfoon: ……

Bab no	Age	Breed	Sick	Anorex	Habitus	Neuro	Icterus	RR	Type	Ht	ISA	Gluc 0	Lact 0	Lact 8	Lact 16	Lact 24	Survive
L01 26	2 years	GSD	2	1-3 days	Collapsed	No	Yes	44	Both	9	Pos	2.2	19.8	5	6.1	4.9	*
L02 27	7 years	Lab X	1	<1	Depressed	No	No	28	Thorax	47		3.8	0.2	Low	Low	Low	Yes
L03 28	1.5 years	Chow	>3	4-6 days	Collapsed	No	No	44	Abdominal	8	Neg	6.5	3.1	3	1.8	2.3	Yes
L04 29	7 months	Boxer	2	1-3 days	Collapsed	No	No		Thorax	11	Neg	6.2	2.2	Low	0.9	Low	Yes
L05 30	2 months	Lab X			Depressed	No	No	50	Both	10	Neg	2.1	19.6	3.9	2.6	0.8	Yes
L06 32	8 months	Dach X	3	1-3 days	Depressed	No	No	60	Thorax	10	Neg	4.6	1.2	1	Low	Low	Yes
L07 34	6 years	Chih	2	1-3 days	Depressed	No	No	32	Thorax	55	Neg	2.5	0.7	2.7	1.1	0.7	
L08 35	8 months	Boerboel	3	1-3 days	Depressed	No	No	24	Thorax	9	Neg	4.9	1	1.8	Low	Low	Yes
L09 39	3.5 months	Boerboel	3	1-3 days	Collapsed	Yes	No	52	Both	10	Neg	4.3	10.8	0.8	Low	Low	Yes
L10 47	3 months	GSD	2	1-3 days	Collapsed	Yes	No	80	Both	10	Neg	0.5	14.3	1.4	1.3	1.3	Yes
L11 48	1.5 years	RRB	1	<1	Depressed	No	No	40		14	Pos	3.1	11.1	9.9	14	11.6	No
L12 50	10 months	Dach	>3	7+ days	Depressed	No	Yes	20	Thorax	12	Neg	3.7	1.7	1.4	1.4	1.5	
L13 51	6 months	Fox T.	2	1-3 days	Depressed	No	No	36		10	Neg	5.2	0.2	Low	Low	Low	
L14 54	6 months	Am.Pittb.	3	<1	Collapsed	No	No	34	Thorax	10	Neg	4.9	1.2	Low	Low	Low	
L15 55	3 years	CB	1	1-3 days	Collapsed	No	Yes	60	Both	5	Neg	3.1	5.5	2.6	2.3	1.5	Yes
L16 56	4.5 months	Boerboel	1	<1	Depressed	No	No	60	Both	13	Neg	6.2	1.2	0.7	Low	Low	Yes
L17 57	9 months	Am. Pittb.	2	1-3 days	Collapsed	No	No	40	Both	9	Neg	4.5	2.1	Low	Low	Low	Yes
L18 65	4 months	Boerboel	3	<1	Depressed	No	No	72	Thorax	7	Neg	7	3.1	1.1	Low	0.9	Yes
L19 66	4 months	GSD	1	<1	Depressed	No	No	70	Thorax	11	Neg	5.4	1.5	Low	Low	Low	Yes
L20 67	2 months	Malt X	1	<1	Depressed	No	No		Thorax	8	Neg	6	1.7	Low	Low	1	Yes
L21 71	3 months	Chow	1	<1	Depressed	No	No	28	Abdomen	8	Neg	1.7	17	4.9	no sample	Low	Yes
L22 75	4 years	Rott	1	<1	Collapsed	No	No	40	Both	25	Neg	3.5	2.7	1.6	2.2	0.7	Yes
L23 78	7 months	CB	2	1-3 days	Collapsed	No	Yes		Abdomen	5	Neg	2.2	25	3.5	2.4	1	Yes
L24 82	9 months	CB	3	<1	Depressed	No	Yes		Thorax	13	Pos	3.8	6.4	3.6	*	*	No
L25 87	1 year	Boerboel	2	1-3 days	Collapsed	No	Yes			7	Neg	5.6	0.2	3.4	7	Euthanase	No
L26 89	4 months	Mastiff	2	<1	Depressed	No	No	34	Thorax	11	Neg	4.2	2.5	Low	Low	Low	Yes
L27 96	3 years	Boston T.	2	1-3 days	Depressed	No	No			15	Neg	6.7	1.3	Low	0.8	Low	Yes
L28 97	3 years	Boston T.	1	1-3 days	Depressed	No	No			12	Neg	4.2	0.7	Low	0.7	Low	Yes
L29 100	2 years	CB	>3	4-6 days	Collapsed	No	No		Both	9	Neg	2.5	22.9	Low	Low	Low	
L30 102	7 months	Boerboel	>3	<1	Collapsed	No	No			9	Neg	6	3.1	Low	Low	Low	Yes
L31 91	4 years	Spaniel	2	<1	Collapsed	No	No	60	Both	11	Neg	2.3	9.2	1.9	2.9	1	Yes
L32 104	1 year	Boerboel	>3	<1	Collapsed	No	No	64	Both	10	Neg	0.7	20.2	5.4	4.8	2.7	Yes
L33 106	1 year	Dasch	>3	<1	Depressed	No	No	30	Thorax	9	Neg	4.2	1.3	Low	0.7	Home	
L34 111	9 years	Malt	1	<1	Alert	No	No	40	Thorax	53	Neg	3.9	1.5	Low	Low	Low	Yes
L35 113	3 years	Chow	Month	1-3 days	Alert	No	No	Panting	Both	11	Neg	4.8	1	2.8	Aggressive	Aggressive	
L36 114	1.6 years	Boerboel	2	1-3 days	Depressed	No	No	40	Thorax	15		4.8	0.8	1	Low	Low	Yes
L37 115	4 weeks	GSD	3	<1	Depressed	No	No	Panting	Thorax	11	Neg	7	0.7	1.2	2.3	Home	Yes
L38 116	5 years	Foxt	3	Unknown	Collapsed	No	No		Both	24	Neg	2	4.8	Low	2.9	2.8	
L39 178	3 years	Rott	2	1-3 days	Depressed	No	No		Thorax	16	Neg	4.3	2.5	1.9	2.2	1.9	*
L40 122	10 weeks	GSD	3	4-6 days	Depressed	No	No	40	Both	10	Neg	4.8	3.4	Low	Low	Low	
L41 126	1 year	Boerboel X	>3	1-3 days	Depressed	No	No		Thorax	12	Neg	4.3	6.9	16.6	2.5	1.5	
L42 131	5 yrs	Staffie	1	<1	Depressed	No	No	80	Both	11	Neg	4.2	1.1	0.2	0.2	0.2	
L43 130	1 year	CB	1	<1	Depressed	No	No	60	Both	9	Neg	3.4	0.2	3.8	4.9	2.5	
L44 131	8 months	St.Bernard	>3	<1	Depressed	No	No	80	Both	11	Neg	4.2	1.1	0.2	0.2	0.2	*
L45 132	5 months	Malt	2	1-3 days	Collapsed	No	No	Panting	Abdomen	11	Neg	1.2	13.5	1.8	1.6	1.2	*

Bab no	Age	Breed	Sick	Anorex	Habitus	Neuro	Icterus	RR	Type	Ht	ISA	Gluc 0	Lact 0	Lact 8	Lact 16	Lact 24	Survive
L46 134	1 year	JRT	>3	4-6 days	Alert	No	No	Panting		13	Neg	4.4	0.2	0.2	0.9	0.9	
L47 136	2 years	Lab	1	<1	Alert	No	No	Panting	Thorax	6	Neg	6.8	2.7	1.3	1.1	1.1	*
L48 139	1 year	Foxt	3	1-3 days	Collapsed	No	Yes	40		14	Neg	4.2	3.8	2.5	2.5	2.7	*
L49 135	3 years	RRB	2	1-3 days	Alert	No	No	48	Thorax	58	Neg	3.9	0.2	0.9	0.2	0.2	*
L50 143	4.6 years	GSD	2	<1	Alert	No	No			10	Neg	4.2	4.6	1.4	0.2	0.8	*
L51 147	9 years	Malt	3	1-3 days	Depressed	No	No	42	Thorax	12	Pos	5.3	1.3	1.5	0.2	0.2	*
L52 150	2 years	StaffX	1	<1	Depressed	No	No		Thorax	9	Neg	5.1	3.1	0.9		0.8	*
L53 228	7 months	JRT	2	<1	Depressed	No	Yes	40	Thorax	10	Pos	4.7	1.3	0.2	0.7	0.8	*
L54 156	6 years	Rottw	3	1-3 days	Collapsed	No	No			41	Neg	3.6	3.8	4.3	2.2	1.7	*
L55 155	3 months	CB	1	<1	Collapsed	No	No		Abdomen	9	Neg	5.6	1.7	1.4	0.2	1.5	*
L56 161	8 weeks	Boerboel	1	<1	Alert	No	No	30	Abdomen	16	Neg	5.5	0.2	1.1	0.2	0.2	*
L57 159	4 years	Bouv.	1	<1	Alert	No	No	Panting	Both	55	Neg	3.3	1	0.8	0.2	0.7	*
L58 160	2 years	CB	3	4-6 days	Collapsed	No	Yes			8	Neg	1.9	5.7	4.4	3.7	3	*
L59 162	4 months	Rottw	1	<1	Depressed	No	No	40	Thorax	12	Neg	5.2	2.2	0.8	0.7	Home	*
L60 163	2 years	SBT	1	<1	Depressed	No	No	32	Thorax	17	Neg	4.7	6.2	1.8	1.6	2	*
L61 173	8 years	Staff	3	1-3 days	Depressed	No	No	40	Thorax	13	Pos	6	7.6	3.3	2.5	3.1	Yes
L62 174	3 months	GSD	1	<1	Depressed	No	No	36	Thorax	13	Neg	5.3	1.1	0.2	0.2	0.2	Yes
L63 175	11 months	Boerboel	>3	4-6 days	Depressed	No	No	48	Thorax	9	Neg	4.8	1.8	0.8	1.7	1.6	*
L64 179	10 years	GSD	2	4-6 days	Collapsed	No	Yes	36	Both	11	Neg	9.8	0.6	Died	Dead	Dead	No
L65 180	5 months	Boerboel	1	<1	Collapsed	No	No		Thorax	32	Neg	5.7	3.7	0.2	Died	Dead	No
L66 182	2 years	Am Pitbull	2	<1	Collapsed	No	Yes	52	Thorax	7	Neg	3.4	9.5	1.8	2.8	4	Yes
L67 184	1 year	Boerboel	1	<1	Collapsed	No	Yes	120	Thorax	10	Pos	5	2.5	2.1	1.5	1.7	Yes
L68 245	9 weeks	Chow	>3	4-6 days	Collapsed	No	Yes	72	Thorax	5	Neg	5.6	2.4	0.7	0.8	0.8	Yes
L69 189	9 years	Border C.	1	1-3 days	Collapsed	No	No	34	Thorax	8	Pos	4.4	9.3	4.6	1	1	Yes
L70 192	4 months	Boerboel	1	<1	Collapsed	Yes	No	36	Both	9	Pos	0.3	22.8	4.4	5.2	3.6	Yes
L71 193	7 years	Malt	2	1-3 days	Depressed	No	Yes	44	Thorax	13	Pos	6.2	1	0.2	1	1.2	Yes
L72 194	4 months	Lab	2	<1	Depressed	No	No	48	Both	8	Neg	4.1	0.2	0.2	0.2	0.2	Yes
L73 198	5 years	Pekeng	1	2	Alert	No	No	Panting	Both	27	Pos	5.5	2.8	0.2	0.2	0.2	Yes
L74 199	1 year	Staff	2	<1	Alert	No	No	54	Thorax	11	Neg	5	1.2	0.8	0.2	2	Yes
L75 203	3 years	Bull T.	>3	3	Collapsed	No	Yes	48	Thorax	7	Neg	3.8	25	5.2	4.7	4.1	*
L76 205	2 years	Lab	1	<1	Alert	No	No	Panting	Thorax	12	Pos	5	3.5	1.3	1.3	1.7	Yes
L77 213	2 years	ChowX	2	2	Collapsed	No	Yes	80	Both	8	Neg	2.1	14.5	0.9	8!!	1.2	Yes
L78 220	8 months	Rott	2	<1	Alert	No	No	Panting	Thorax	11	Neg	5.4	1.6	1.1	1.1	0.2	Yes
L79 221	13 years	Staff	3	3	Depressed	No	Yes	54	Thorax	10	Pos	6.4	8.2	2.4	1.6	2.1	Yes
L80 222	4 months	CB	1	<1	Collapsed	No	No	80	Thorax	11	Neg	1.6	8.5	7.3	7	10.2	No
L81 225	4 months	Rott	1	<1	Collapsed	No	No	36	Thorax	19	Neg	3.4	5.5	6.7	Dead	Dead	No
L82 226	8 years	Lab	>3	3	Alert	No	No	Panting	Both	13	Pos	5	1.5	0.7	0.2	1.6	Yes
L83 229	5 years	CB	3	3	Collapsed	No	No	72	Thorax	10	Neg	5.3	4	0.2	0.2	0.2	Yes
L84 230	3 years	Dach	3	<1	Depressed	No	Yes	48	Thorax	10	Neg	4.6	0.2	0.8	0.2	0.2	Yes
L85 232	4 months	CB	>3	2	Collapsed	No	Yes	44	Thorax	6	Pos	2.2	24.8	3.3	3.9	3.4	Yes
L86 234	5 weeks	CB	1	<1	Collapsed	No	No	28	Thorax	10	Neg	0	24.5	Died	Dead	Dead	No
L87 235	6 years	Lab	>3	36987	Alert	No	Yes	Panting	Thorax	15	Pos	4.6	10.4	6.4	6.4	9.1	No
L88 237	4 years	Boerboel	3	2	Alert	No	No	Panting	Thorax	9	Neg	4.7	1	0.9	0.2	0.7	
L89 238	4 months	JRT	1	<1	Collapsed	No	No	42	Both	7	Neg	0.5	20.5	Died	Dead	Dead	No
L90 241	4 years	Boerboel	>3	<1	Collapsed	No	No	28	Thorax	8	Pos	4	2.5	1.4	0.2	0.2	Yes

APPENDIX D:

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Prognostic value of blood lactate, blood glucose and hematocrit in canine babesiosis

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Abstract
Canine babesiosis typically causes hemolytic anemia, but also can result in multiple organ dysfunction. Human patients with severe disease often have persistent hyperlactatemia, and blood lactate concentration is correlated with survival rate. In dogs, blood lactate concentration has been shown to be of prognostic value in patients with gastric dilatation-volvulus and in dogs admitted to intensive care units. Serial blood lactate and glucose concentrations and hematocrit on admission were determined in 90 dogs with naturally-occurring, severe or complicated canine babesiosis. Forty-five dogs (50%) had hyperlactatemia (blood lactate concentration > 22.5 mg/dL) and 20 (22.2%) had hypoglycemia (blood glucose concentration < 59.4 mg/dL) at presentation. Measurements significantly associated with mortality were hypoglycemia on admission, blood lactate concentration > 45 mg/dL on admission, blood lactate concentration > 22.5 mg/dL at 8, 16 and 24 h after admission, and increase or < 50% decrease in blood lactate concentration within 8 and 16 h after admission. Blood lactate concentration persistently > 40 mg/dL indicated a very poor prognosis. We conclude that serial blood lactate measurements are useful in predicting survival in dogs with severe and complicated canine babesiosis.

Key words
Dog, *Babesia canis rossi*

Canine babesiosis is a tick-borne disease caused by the hemoprotozoan parasites, *Babesia canis* or *B. gibsoni*. Three subtypes of *B. canis* exist of which *B. canis rossi* occurs in South Africa [1-3]. The disease typically causes hemolytic anemia, but also can result in multiple organ dysfunction[4]. The hemolytic crisis that develops can result in anemic hypoxia, anaerobic metabolism, and metabolic acidosis[5].

During carbohydrate metabolism, glucose is metabolized to pyruvate. Pyruvate then follows one of 5 different pathways: (1) lipogenesis, (2) oxidation via the Kreb's cycle, (3) formation of alanine, (4) gluconeogenesis, or (5) conversion to lactate[6] (Figure 1). During anaerobic conditions, pyruvate is converted to lactate as an energy substrate. Lactate is transported to the liver, which is primarily responsible for lactate metabolism[7]. When lactate production exceeds the capacity of the liver to metabolize it, hyperlactatemia occurs[8, 9]. During disease states, a pathological hyperlactatemia is seen either due to increased production or decreased utilization[10, 11]. Increased production is seen with tissue hypoxia and increased rate of metabolism[7], whereas decreased utilization of lactate usually involves tissue hypoxia, leading to a decreased functioning of the 3 aerobic pathways due to anaerobic conditions[10].

Hyperlactatemia in severe disease has been ascribed to a combination of tissue hypoxia and increased metabolism [9]. Human patients suffering from severe injury or infection can have persistent hyperlactatemia[12]. Studies investigating blood lactate concentrations in shock have shown a correlation between blood lactate concentration and survival rate[13-15], in which human patients who were in septic shock and in whom the lactate concentration remained high despite treatment, had a higher mortality rate compared to patients who experienced a decrease in lactate concentration[16]. One study in humans suffering from circulatory shock[17], showed that blood lactate concentrations started to decrease within the first h of starting appropriate therapy, whereas patients who did not respond to therapy showed no significant changes in blood lactate concentration. Lactate alone served as the best prognostic indicator of survival, and it was concluded that in patients who did not experience significant decreases in blood lactate concentration after 1 h of aggressive therapy, alternative therapy should be considered. Also, the ability to clear lactate within 24 h of starting appropriate therapy correlated well with survival in patients suffering from multiple trauma[18]. Several studies in human malaria patients suffering from acute, cerebral or severe malaria showed that increased blood lactate concentrations affected survival rate[19-23], and patients with persistent hyperlactatemia were far more likely to die when compared to patients who experienced a decrease in blood lactate concentration after initiation of therapy.

Blood lactate measurement has been shown to be of prognostic value in equine colic[24-30], and horses with the highest lactate concentrations had the highest mortality rates.

In dogs with gastric dilatation-volvulus, venous lactate concentration was a good predictor of gastric necrosis and outcome[31]. Another study in dogs admitted for emergency care for various diseases showed an association between the severity of the hyperlactatemia at admission and outcome, and dogs with the highest blood lactate concentrations were more likely to die[32].

To date, the serial measurement of lactate in severely ill dogs and its use as a prognostic indicator have not been reported. The aim of this study was to determine whether dogs with severe or complicated canine babesiosis suffer from hyperlactatemia, and whether blood lactate and blood glucose concentrations and hematocrit could be used as prognostic indicators.

Materials and Methods

The Animal Use and Care Committee and the Research Committee of the University of Pretoria approved this study. Ninety dogs with naturally-occurring, severe, uncomplicated and complicated canine babesiosis infection were used in this prospective study. Inclusion criteria were a positive identification of *B. canis rossi* parasites on a stained thin capillary blood smear using Cams Quick Stain[a], and admission to the Onderstepoort Veterinary Academic Hospital (OVAH) due to either severe anemia (hematocrit < 15%) or complications such as respiratory distress, acute renal failure (urine production < 1 ml/kg/h that did not respond to rehydration), hepatic involvement (abnormally high alanine amino transferase [ALT], alkaline phosphatase [ALP], icterus), pancreatitis, hemoconcentration, hypotensive shock, electrolyte imbalances (abnormal sodium or potassium concentrations), hypoglycemia, coagulopathy or immune-mediated hemolytic anemia.

Approximately 1 ml of blood was collected from the jugular vein into a heparinized syringe before treatment. Animals then were treated by standard OVAH protocol for canine babesiosis, which included an anti-babesial drug and blood transfusion as needed. All dogs with complications were treated for the specific complication as deemed necessary by the attending clinician. Attending clinicians were blinded to the results of blood lactate measurements performed for this study. Follow up blood samples were obtained at 8 hourly intervals for a period of 24 hours (4 samples per patient). Owners of dogs discharged from the hospital were contacted 2 weeks after the date of discharge to obtain information regarding survival and progress after discharge. At the end of the study period (2 weeks after discharge from the OVAH) dogs were classified as survivors or non- survivors. Blood lactate concentration was measured immediately after collection using the Accusport® blood lactate analyzer[b], according to the manufacturer's instructions. Blood glucose concentration was measured on a Technicon RA XT system[c] using the hexokinase method[d]. The microhematocrit was measured using a microhematocrit centrifuge[e] and microhematocrit tubes[f]. Tubes were sealed with vitrex plasticene and centrifuged at 11,800 rpm (14,000 g) for 5 min. The hematocrit was measured using a Hawksley hematocrit reader.

An in-saline-agglutination test was performed by mixing one drop of blood with 6 drops of saline. One drop of the mixture was placed on a glass slide, covered with a cover slip and examined under a microscope using 10X magnification. The test was deemed positive when red blood cells clumped together despite dilution with saline.

Data collected at presentation included signalment, mental status (alert, depressed or non-responsive), presence of neurological signs (seizures, coma, disorientation, confusion, vocalization, pacing, circling, head pressing, vision abnormalities, abnormal pupillary light responses, abnormal gait), presence of respiratory difficulty (dyspnea, tachypnea, Kussmaul's breathing [paroxysmal breathing], polypnoea, hypopnoea), microhematocrit, blood glucose concentration, and blood lactate concentration.

Data analysis

Data analysis was done using NCSS 2001[g] and Epicalc 2000[h] statistical software programs. Univariable analysis of the effects of hematocrit, blood glucose concentration and serial blood lactate concentrations on mortality was done using the Wilcoxon rank-sum test for differences between medians and the Fisher's exact test for categorical data. Variables with $P<0.3$ on univariable analysis were selected for testing using multivariable logistic regression. Two models were tested: one including lactate concentration on admission and the other including lactate concentration 8 h after admission. The models then were developed by backward elimination; variables remained in the model if they were significant (Wald $P<0.1$) or if their removal resulted in >10% change in the effect of other variables.

Results

Ninety dogs (47 males and 43 females) were included in the study. The median age of the dogs was 1 year (range, 1 month to 13 years). Breeds included 16 Boerboels, 11 mixed breeds, 8 German shepherd dogs, 7 Labrador Retrievers, 6 Rottweilers, 5 Maltese dogs, 5 Chow-chows, 5 Staffordshire terriers, 4 Dachshunds, 3 each of American Pitt Bull Terriers, Jack Russell Terriers and Fox Terriers, 2 each of Saint Bernard dogs, Rhodesian Ridgebacks and Boston Terriers, and 1 each of Bouvier des Flandres, Spaniel, Mastiff, Chihuahua, Boxer, Bull Terrier, Border Collie and Pekingese.

Median duration of disease before presentation was 2 days. Three dogs (3.3%) presented with neurological signs including seizures in 1 dog, and coma in the other 2. Forty-seven dogs (52.2%) showed respiratory signs such as tachypnea, polypnea and dyspnea. Fifteen dogs (16.6%) were alert, 38 dogs (42.2%) were depressed, and 37 dogs (41.1%) were non-responsive to stimuli. Twenty dogs (22.2%) were icteric at presentation. In-saline-agglutination was positive in 17 dogs (18.8%) and negative in 73 dogs (81.1%). Microhematocrit values ranged from 5 to 58% with a median of 10%. Eleven dogs (12%) died, and 79 dogs (88%) survived. Blood lactate concentrations of these 2 groups are shown in Figure 2.

Forty-five dogs (50%) presented with hyperlactatemia (blood lactate concentration > 22.5 mg/dL). Seven dogs died before the end of the 24 h sampling period. Five of these 7 dogs were hyperlactatemic at presentation. Twenty-four h after admission, 13 dogs were still hyperlactatemic, all of which had been hyperlactatemic on admission. Of these 13 dogs, 9 (69.2%) survived and 4 (30.8%) died. All 9 surviving dogs experienced a decrease in blood lactate concentration at the 8 h interval, with 7 of them showing a > 50% decrease. All 4 dogs with blood lactate concentration > 40 mg/dL at 24 h after admission died. All 4 had shown persistently high blood lactate concentrations (> 40mg/dL) at all sampling times and in 2 of them blood lactate concentration was higher at 24 h than on admission. On the other hand, all (n = 75) dogs with blood lactate concentration < 40 mg/dL at 24 h after admission survived.

Two dogs were euthanized during the study. They included 1 dog that was euthanized after 16 h, and 1 dog that was euthanized after 24 h. The first dog was euthanized after developing severe disseminated intravascular coagulation with widespread hemorrhages and bleeding from the nose, eyes, and gastrointestinal tract. The second dog developed neurological signs including coma and tremors. As the presence of neurological signs indicates a poorer prognosis[33], the owner decided not to spend any further money on treatment of the dog and it was euthanized. The second dog that was euthanized had an initial blood lactate concentration of 1.8 mg/dL, but by the 8 h sampling period, lactate concentration had risen to 30.6 mg/dL and by 16 h it was 63 mg/dL. The other non-surviving dog had a normal lactate concentration of 5.4 mg/dL but died within 30 min of admission. The dog was comatose, severely icteric, and hyperglycemic (176.4 mg/dL). Unfortunately, the owner did not give permission for a post-mortem examination.

Hematocrit, glucose and serial lactate measurements of survivors and non-survivors are compared in Table 1. Non-survivors had significantly higher blood lactate concentrations than survivors at each interval. Univariable associations between each variable and mortality are shown in Table 2, expressed in terms of the relative risk of mortality compared with a reference category.

In dogs admitted with hyperlactatemia, an increase or a < 50% decrease in blood lactate concentration at 8 h and at 16 h were significantly associated with mortality, compared to dogs in which lactate concentration decreased by > 50% (Table 3). There was no evidence of an association between hematocrit on admission and survival.

Blood lactate concentrations on admission and at 8 h after admission were the only predictor variables that remained significant in the 2 logistic regression models (Tables 4 and 5). Both measurements were significantly associated with mortality, but the 8 h measurement showed a

much stronger association (odds ratios of 13 and 83 for lactate concentrations >22.5 and >45 mg/dL, respectively).
Although hypoglycemia (blood glucose < 59.4 mg/dL) on admission was associated with an increased risk of mortality in the univariable analysis, it was eliminated from the logistic regression models because hypoglycemia and hyperlactatemia were highly associated (chi-squared test, $P < 0.0001$). Of the 20 dogs that were hypoglycemic on presentation, 19 (95%) were hyperlactatemic and 18 (90%) had blood lactate concentrations > 45 mg/dL.

Discussion

Babesiosis is a disease that results in hypoxia and sepsis[1, 2, 5]. In dogs with severe babesiosis, metabolic acidosis is principally due to generation of lactic acid[5] and lactate concentrations in non-survivors were higher than in controls and survivors. The mean blood lactate concentration in healthy dogs in that study was 13.8 mg/dL, for survivors of disease 39 mg/dL, and for fatally infected dogs it was 145 mg/dL.
In dogs, the normal blood lactate concentrations (amperometric autoanalyzer) are between 1.8 and 22.5 mg/dL[9, 31, 33]. According to the manufacturers of the Accusport® analyzer, normal dogs have blood lactate concentrations of up to 9 mg/dl. Values of 27-45 mg/dL, 45-90 mg/dL and > 90 mg/dL are seen in mild, moderate and severe hypoperfusion, respectively [9]. Blood lactate concentrations > 45 mg/dL usually are associated with acidemia[9].
Hyperlactatemia seen in humans suffering from severe disease or injury has been attributed to hypoxia[12]. During hypoperfused states, however, the lactate and pyruvate increase disproportionately whereas hyperlactatemia of injury or sepsis usually is accompanied by lactate and pyruvate increases that maintain the normal ratio between the 2 products. This observation suggests an equilibration phenomenon of hyperlactatemia during disease states[8, 12, 33]. Hyperlactatemia now is thought to be due to a combination of tissue hypoxia and hypermetabolism. Lactic acidosis can be classified as either type A (due to poor perfusion and hypoxia), or type B (in which poor tissue perfusion or poor arterial oxygenation is not apparent)[14, 15].
Serial lactate measurements are recommended in the human medicine, because pre-treatment lactate concentrations do not differ between survivors and non-survivors. Survivors in studies investigating blood lactate concentrations in ventilated babies and patients suffering from septic shock showed a decrease in blood lactate concentration within the first 24 h and lactate concentrations remained high for much shorter times compared to non-survivors[7, 16]. In this study, we found that lactate concentration differed between survivors and non-survivors at every time period, including pre-treatment. However, this difference tended to become greater at each subsequent measurement. The best prediction of survival was obtained at 24 h, when blood lactate concentrations > 40 mg/dL and < 40 mg/dL correctly predicted death and survival respectively in every case.
In studies of humans, blood lactate concentrations increased before clinical deterioration was observed, and thus lactate could serve as an early warning system of possible organ failure[7]. An observation made in our study is that clinical signs accompanied changes in blood lactate concentrations. Although attending clinicians were blinded to blood lactate concentrations, the investigators who were responsible for collecting the blood samples (MN and NK) observed that dogs showing decreasing blood lactate concentrations showed clinical improvement whereas dogs showing persistently high or rising blood lactate concentrations continued to deteriorate clinically. This observation was subjective, and grading of clinical signs should be evaluated in future studies to confirm this observation.
We conclude that blood lactate concentrations can serve as a predictor of outcome in dogs suffering from severe or complicated canine babesiosis. Although pre-treatment hyperlactatemia indicates a poorer prognosis, subsequent serial lactate concentrations show a much stronger association with mortality. Dogs with greater post-treatment decreases in blood lactate

concentrations have a higher survival rate whereas serial blood lactate concentrations persistently remaining > 40 mg/dL indicate a very poor prognosis. Although hypoglycemia on admission also was associated with an increased risk of mortality, hypoglycemia and hyperlactatemia tended to occur together and blood lactate concentration alone can serve as a prognostic indicator in dogs with severe or complicated canine babesiosis.

[a] CA Milsch P.O. Box 943, Krugersdorp, Johannesburg, 1740, South Africa
[b] Roche Products, Africa Region, P.O. Box 129, Isando, 1600, South Africa
[c] Technicon Instruments Corporation, Tarrytown, USA
[d] Bayer Health Care Division, P.O. Box 198, Isando, 1600, South Africa
[e] Jouan Hema-C microhaematocrit centrifuge, Hawksley and Sons, Ltd, Sussex, U.K.
[f] A&M Link Stiftung, Wertheim, Germany
[g] NCSS, Kaysville, Utah
[h] EpiCalc 2000 v1.02, Brixton Books, UK

REFERENCES:

1. Jacobson L, Clark IA. The pathophysiology of canine babesiosis: new approaches to an old puzzle. J S Afr Vet Assoc. 1994; 134-145.
2. Lobetti R. Canine Babesiosis. Comp Cont Educ Pract Vet. 1998; 418-430.
3. Lobetti R, Mohr AJ, Dippenaar T, Myburgh E. A preliminary study on the serum protein response in canine babesiosis. J S Afr Vet Assoc.2000; 38-42.
4. Welzl C., Leisewitz A.L., Jacobson L.S., Vaughn-Scott T., Myburgh E. Systemic inflammatory response syndrome and multiple-organ damage/dysfunction in complicated canine babesiosis. J S Afr Vet Assoc. 2001; 158-162.
5. Button C. Metabolic and electrolyte disturbances in acute canine babesiosis. J.Am Vet Assoc. 1979; 475-479.
6. Ganong WF. Endocrinology and Metabolism. *In* Ganong WF, ed. Review of Medical Physiology. San Francisco,California.: Lange Medical Publications, 1975. pp. 206-221.
7. Desphande S, Ward Platt MP. Association between Blood Lactate and Acid-Base status and Mortality in Ventilated Babies. Arch. Disease Child. 1997; F15-F20.
8. Frank B, Cerra MD. Hypermetabolism, Organ Failure, and Metabolic Support. Surgery 1987; 1-14.
9. Hughes D,. Lactate measurement: diagnostic, therapeutic, and prognostic implications. *In* Kirk R., Bonagura JD., ed. Current Veterinary Therapy XIII: Small Animal Practice. Philadelphia: WB Saunders Company, 2000. pp. 112-116.
10. Zilva J, Pannall PR, Mayne PD. Lactate production and lactic acidosis. Clinical Chemistry in Diagnosis and Treatment. London: Edward Arnold: A division of Hodder and Stoughton, 1988. pp. 205-209.
11. Center S. Pathophysiology of Liver Disease: Normal and Abnormal Function. *In* Strombeck, ed. Strombeck's Small Animal Gastroenterology. Philadelphia: W.B.Saunders Company, 1996. pp. 553-556.
12. Mizock BA. Alterations in carbohydrate metabolism during stress: A review of the literature. Am J Med. 1995; 75-84.
13. Broder G, Weil M. Excess Lactate: An Index of Reversibility of Shock in Human Patients. Science 1964; 1457-1459.
14. Cohen R, Woods HF. The Clinical Presentations and Classification of Lactic Acidosis. Clinical and Biochemical Aspects of Lactic Acidosis. Oxford: Blackwell Scientific Publications, 1976. pp. 41-84.
15. Mizock BA. Lactic acidosis in critical illness. Crit Care Med. 1992; 80-93.
16. Bakker J, Gris P, Coffernils M, Kahn RJ, Vincent J-L. Serial Blood Lactate Levels Can Predict the Development of Multiple Organ Failure Following Septic Shock. Am J Surg.1996; 221-226.
17. Vincent J-L, Dufaye P, Berre J, Leeman M, Degaute J-P, Kahn RJ. Serial lactate determinations during circulatory shock. Crit Care Med. 1983; 449-451.
18. Abramson D, Scalea TM, Hitchcock R, et.al. Lactate Clearance and Survival following Injury. J Trauma 1993; 584-589.
19. Kawo N, Msengi AE, Swai ABM, et.al. Specificity of hypoglycaemia for cerebral malaria in children. The Lancet. 1990; 454-457.
20. Phillips R. Hypoglycaemia as an important complication of falciparum malaria. Q J Med. 1989; 477-483.
21. Pukrittayakamee S, Davis TME, Levy J, et.al. The metabolic response to rapid intravenous glucose injection in acute falciparum malaria. Trans. Royal Soc. Trop. Med. Hyg. 1991; 189-193.
22. White N, Warrell DA, Chantavanich P, et.al. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. New Engl J Med. 1983; 61-66.

23. Phillips R, Looareesuwan S, Molyneux ME, Hatz C, Warrell DA. Hypoglycaemia and counterregulatory hormone responses in severe falciparum malaria: treatment with sandostatin. Q J Med. 1993; 233-240.
24. Parry B. Prognostic Evaluation of Equine Colic Cases. Comp Cont Educ Pract. 1986; 98-104.
25. Reeves M, Curtis CR, Salman MD, et.al. Prognosis in Equine Colic Patients using Multivariable Analysis. Can J Vet Res. 1989; 87-94.
26. Orsisi J, Elser AH, Galligan DT. Prognostic Index for Acute Abdominal Crisis (colic) in Horses. Am J Vet Res. 1988; 1969-1971.
27. Parry B. Use of Clinical Pathology in the Evaluation of Horses with Colic. Vet Clin N Am-Eq 1987; 529-542.
28. Moore J, Owen R ap R, Lumsden JH. Clinical Evaluation of Blood Lactate Levels in Equine Colic. Eq Vet J. 1976; 49-54.
29. Dabareiner RM, White NA. Large colon impaction in horses: 147 cases (1985-1991). J Am Vet Med Assoc. 1995; 679-685.
30. Furr MO, Lessard P, White NA. Development of a colic severity score for predicting the outcome of equine colic. Vet Surg. 1995; 97-101.
31. Papp ED, Drobatz KJ, Hughes D, de Papp E. Plasma lactate concentration as a predictor of gastric necrosis and survival among dogs with gastric dilatation-volvulus: 102 cases (1995-1998). J Am Vet Med Assoc.1999; 49-52.
32. Lagutchik M, Ogilvie GK, Hackett TB, Wingfield WE. Increased lactate concentrations in ill and injured dogs. J Vet Emerg Crit Care. 1998; 117-127.
33. Hardie EM. Therapeutic Management of Sepsis. *In* Kirk R., ed. Current Veterinary Therapy XIII Small Animal Practice. Philadelphia: WB Saunders Company, 2000. pp. 272-275.

FIGURES, GRAPHS AND TABLES

Figure 1. Glucose metabolism.

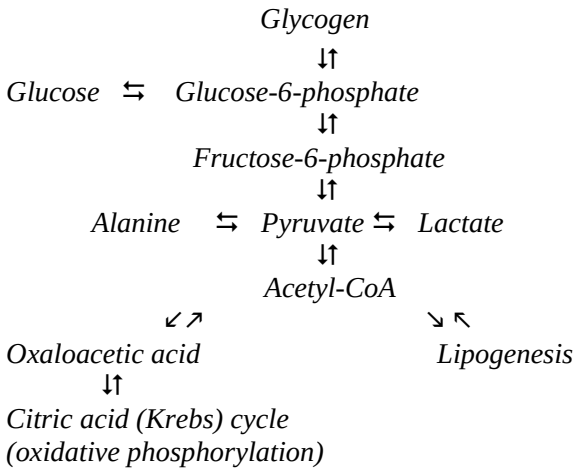

Figure 2. Mean blood lactate concentrations of survivors at each sampling interval compared to non-survivors. Lines within boxes indicate median values. The normal cut off value is 25 mg/dL. Dots represent outlying values. Whiskers represent the 10th and 90th percentiles.

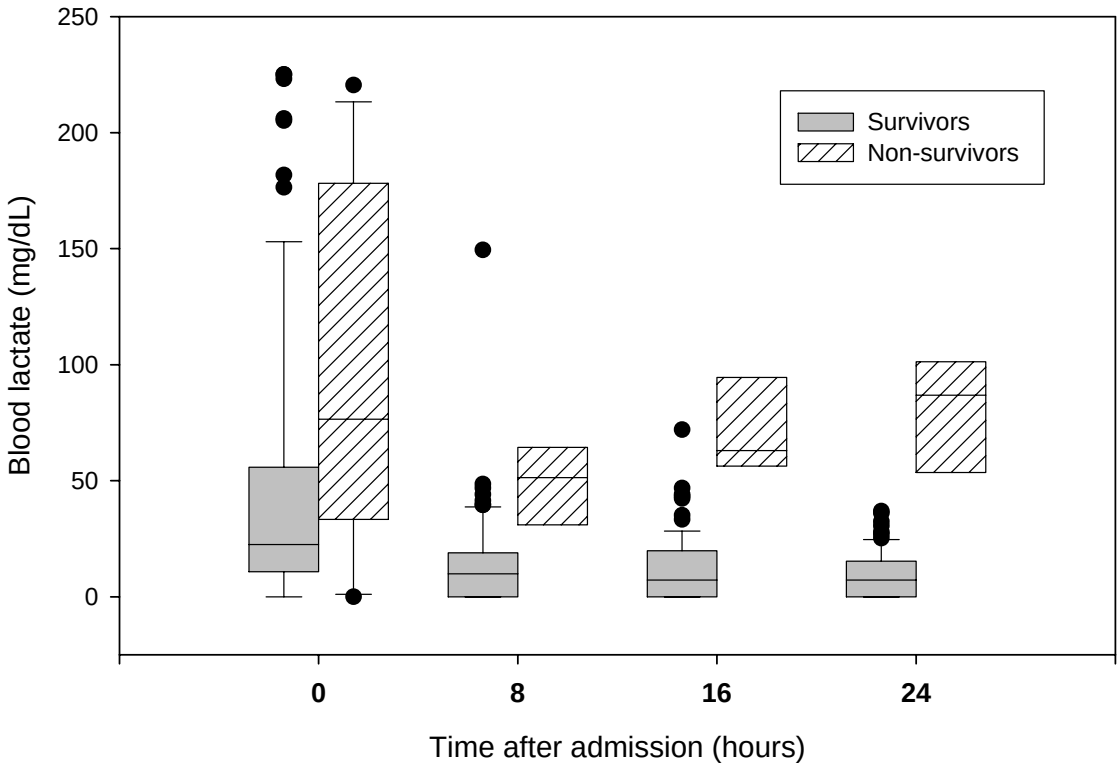

Table 1. Median hematocrit and blood glucose concentration on admission, and serial blood lactate concentrations in survivors and non-survivors of canine babesiosis.

	Ht (%)	Glucose (mg/dL)	Lactate (mg/dL)			
			0 hours	8 hours	16 hours	24 hours
Survivors	10 [a]	82.8 [a]	22.5 [a]	9.9 [a]	7.2 [a]	7.2 [a]
Non-survivors	11 [a]	61.2 [a]	76.5 [b]	51.3 [b]	63 [b]	86.9 [b]
TOTAL	10.5	80.1	23.4	10.8	8.1	8.1

[a,b] Values within columns with differing superscripts differ significantly (Wilcoxon rank-sum test, $P<0.05$)

Table 2. Association of hematocrit, blood glucose and blood lactate with mortality in canine babesiosis: univariable analysis

Variable	Category	Mortality		RR *	95% confidence interval	*P* §
		Yes	No			
Hematocrit (%)	<10	3	27	0.40	0.05 to 3.0	0.4
	10-14	5	38	0.47	0.07 to 3.1	0.4
	15-19	2	5	1.1	0.15 to 9.0	1.0
	20-39	1	3	1 †		
	≥40	0	6	0	0 to -	0.4
Glucose 0 hours (mg/dL)	<59.4	5	15	3.2	1.0 to 9.9	0.05
	59.4 to 118.8	5	59	1 †		
	>118.8	1	5	2.1	0.30 to 15	0.4
Lactate 0 hours (mg/dL)	≤22.5	2	43	1 †		
	22.6 to 45	1	14	1.5	0.15 to 15	1.0
	>45	8	22	6.0	1.4 to 26	0.01
Lactate 8 hours (mg/dL)	≤22.5	1	62	1 †		
	22.6 to 45	3	14	11	1.2 to 100	0.03
	>45	4	3	36	4.7 to 279	0.0002
Lactate 16 hours (mg/dL)	≤22.5	0	65	1 †		
	22.6 to 45	0	9	-	-	1.0
	>45	5	2	-	-	<0.0001
Lactate 24 hours (mg/dL)	≤22.5	0	66	1 †		
	22.6 to 45	1	9	-	-	0.1
	>45	3	0	-	-	<0.0001
TOTAL		11	79			

* Relative risk of mortality compared to reference category
† Reference category
§ *P*-value for Fisher's exact test

Table 3. Association between change in blood lactate concentration and mortality in canine babesiosis patients with hyperlactatemia on admission

Variable	Category	Mortality		RR *	95% confidence interval	*P* §
		Yes	No			
Decrease in lactate 0 to 8 hours	≤50%	5	6	7.3	1.6 to 32	0.008
	>50%	2	30	1 †		
Decrease in lactate 0 to 16 hours	≤50%	3	7	8.4	1.0 to 72	0.05
	>50%	1	27	1 †		
TOTAL		7	36			

* Relative risk of mortality compared to reference category
† Reference category
§ *P*-value for Fisher's exact test

Table 4. Multiple logistic regression model of risk factors for mortality in canine babesiosis: blood lactate on admission

Variable	Category	β	OR *	95% confidence interval	*P*
Lactate 0 hours (mg/dL)	≤22.5	0	1 †		
	22.6 to 45	0.429	1.5	0.13 to 18	0.7
	>45	2.06	7.8	1.5 to 40	0.01

* Odds ratio relative to reference category
† Reference category

Table 5. Multiple logistic regression model of risk factors for mortality in canine babesiosis: blood lactate 8 hours after admission

Variable	Category	β	OR *	95% confidence interval	*P*
Lactate 8 hours (mg/dL)	≤22.5	0	1 †		
	22.6 to 45	2.59	13	1.3 to 137	0.03
	>45	4.41	83	6.9 to 986	0.0005

* Odds ratio relative to reference category
† Reference category