APPENDIX A

A CANINE NORMOVOLAEMIC ACUTE ANAEMIA MODEL

T.C. SPOTSWOOD, R.M. KIRBERGER, L.M.P.K. KOMA, F. REYERS and L. VAN DER MERWE

A.1   ABSTRACT

The objective was to develop a non-terminal canine normovolaemic acute anaemia model that has minimal effects on patient well-being. Eleven normal Beagle dogs were used. About 20 % of circulating blood volume was removed from the jugular vein 1-3 times per day over a 3-4 day period until a haematocrit (Ht) of 13-17 % was obtained. Replacing the volume deficit of the red blood cells with Ringer's lactate and re-infusing the plasma maintained normovolaemia. Full blood count and Ht were monitored twice daily. The 13-17 % Ht was reached within 3 – 4 days with the number of phlebotomies ranging from four to seven. The model was primarily developed to determine echocardiographic values as well as Doppler abdominal splanchnic blood flow parameters in anaemic dogs as part of a study that will compare these results to similar studies in babesiosis-induced anaemia. The model may also be used for comparative studies in other conditions such as immune-mediated anaemia or ehrlichiosis or to study the effect of haematinics or cardiovascular drugs in anaemic patients.

A.2   INTRODUCTION

Acute and sub-acute to chronic anaemia is a common presentation in humans and animals. There are many causes of such anaemia which vary from blood loss and its associated iron deficiency, toxicosis (such as rodenticide and drug-induced), hereditary, chronic renal failure, immune mediated and blood parasites such as canine babesiosis and falciparum malaria in man. The latter is very important with 300-500 million clinical cases of malaria occurring each year worldwide with approximately 2 million of these being fatal (Artavanis-Tsakonas, Tongren & Riley 2003). In dogs, immune-mediated haemolytic anaemia, particularly the idiopathic form (Reimer, Troy & Warnick 1999), is the most common cause except in countries with babesiosis, like South Africa, where canine babesiosis is caused by a particularly severe strain (*Babesia canis rossi*) and can account for up to 12 % of all cases presented to veterinary practices (Reyers, Leisewitz, Lobetti, Milner, Jacobson & van Zyl 1998). Many of these patients suffer from severe anaemia necessitating hospitalisation and may die if untreated. The roles of anaemia and haemodynamic change in the pathophysiology of diseases such as babesiosis has not been clearly elucidated and are being investigated in this Department. The research program prompted the *inter alia* development of this anaemia model in an attempt to create normotensive, normovolemic tissue hypoxia. In addition it is intended to use the model to assess the effect of red cell reduction and the associated reduction in viscosity, at selectable levels of anaemia, during the recovery period, on a number of parameters (such as blood flow and erythrocyte regeneration) without the possible added effect of parasite-host generated inflammatory, haemodynamic and potential parasite-associated toxic suppression.

A.2.1   Experimental anaemia models - Numerous anaemia models, in a variety of animals, have been developed. Models for human malaria have used Japanese monkeys infected with

Plasmodium coatneyi (Kawai, Aikawa & Kano 1993) and squirrel monkeys infected with *Plasmodium falciparum (*Contamin, Behr, Mercereau-Puijalon & Michel 2000). Erythrocyte metabolism has been studied in an acute blood loss model in the horse (Smith & Agar 1976). The horses were bled at 16 mℓ/kg daily and reached a haematocrit (Ht) of <15 % after 3-4 days. The circulatory effects of anaemia-induced hypoxia have also been studied in pigs (Schou, Perez de Sa, Sigurdardottir, Roscher, Jonkarker & Werner 1996). A number of models have been developed in rats to study iron deficiency anaemia. These include being fed iron deficient diets (Gambling, Charania, Hannah, Antipatis, Lea & McArdle 2002) or phlebotomy at regular intervals (Bhargave & Gabbe 1984).

In dogs, experimental anaemia models have mainly been developed for human cardiovascular studies (Fowler & Holmes 1971; Fowler & Holmes 1975; Szlyk, King, Jennings, Cain & Chapler 1984). Invasive experimental techniques in animals have documented a hyperdynamic cardiovascular response to severe acute normovolaemic anaemia (haemodilution), characterized by increased cardiac output and reduced systemic vascular resistance (Fowler, Franch & Bloom 1956; Vatner, Higgins & Franklin 1972). In dogs and rats, invasive methods have been used to compute cardiac output and systemic vascular resistance in order to demonstrate the hyperdynamic state in anaemia (Donald, Ferguson & Milburn 1968). The methods include cardiac and great blood vessel catheterisation with indicator dilution, pressure transducers or electromagnetic flow meters and intraoperative echocardiography and indwelling Doppler flow meters (Donald *et al.* 1968). These studies did not always document exactly how the model was created. In some cases the dogs were euthanised at the end of the trial (Habler, Kleen, Podtschaske, Hutter, Tiede, Kemming, Welte, Corso & Mesmer 1996) and in others nothing was stated on experimental animal survival. (Fowler & Holmes 1971; Fowler & Holmes 1975; Fowler *et al* 1956). In these studies normovolaemia was maintained by a

variety of methods. Exchange transfusions were performed with a range of molecular weights and concentrations of dextran solutions (mainly 6 % to simulate the viscosity of plasma and maintain oncotic pressure) (Fowler & Holmes 1975; Szlyk *et al.* 1984; Fowler *et al.*1956; Fahim & Singh 1992), dextran in physiologic saline (Fowler & Holmes 1971), hydroxyethyl starch (Habler *et al.* 1996), Ringer's lactate (Geha 1976), and harvested plasma with dextran (Vatner *et al.* 1972). In most of these models the experimental data were recorded immediately after the exchange infusion while the dogs were under general anaesthesia with their haemodynamic parameters rigorously controlled. An experimental anaemia model using non-anesthetized dogs has been developed (Lobetti, Reyers & Nesbit 1996), specifically for veterinary related research, but these dogs were euthanised afterwards.

In humans, acute normovolaemic haemodilution (ANH) is a technique used to eliminate or decrease the need for homologous blood transfusions in surgical patients (Stehling & Zauder 1991). The advantages of ANH include avoiding disease transmission and transfusion reactions, and the acquisition of fresh autologous blood for transfusion (Stehling & Zauder 1991). Reduction in red blood cell (RBC) loss during surgery is limited: for example, a patient with an Ht of 40 % who loses a litre of blood loses 400 mℓ of RBCs. By inducing ANH and reducing the Ht to 25 % preoperatively, the patient will only lose 250 mℓ of RBCs per litre blood loss. In practice, ANH has been used extensively in elderly patients and small children, especially for cardiac surgery (Stehling & Zauder 1991). Briefly, the technique of ANH involves the removal of blood before surgery and the simultaneous replacement with a crystalloid or colloid. Several formulae for the calculation of the volume of blood to be removed for achieving the desired Ht in man have been developed (Bourke & Smith 1974; Gross 1983). The following formula has been developed (Bourke & Smith 1974):

$$Lt = V (\ln Ho - \ln Ht)$$

Where Lt = blood removed, V = circulating blood volume, ln = natural logarithm, Ho = initial Ht and Ht = final Ht.

This formula takes into account the progressive haemodilution and therefore the exponential reduction of the red blood cell number per unit volume blood during extraction. The investigators showed that their formula was accurate in clinical practice in predicting the final Ht, with a standard deviation of only 1.46 volume percent of the mean difference between actual and predicted Ht. To the best of the authors' knowledge, this predictive formula has not been verified in dogs.

A.3   OBJECTIVES

To develop and describe, in detail, a non-lethal canine normovolaemic acute anaemia model (Ht range 13-17 %) that has minimal effects on patient well-being. Developing such a model would permit, *inter alia*, the evaluation of the effect of anaemia on echocardiographic and duplex Doppler parameters of abdominal splanchnic blood flows as well as the marrow response to varying degrees of anaemia that could be applied in studies on the pathophysiology of babesiosis-induced anaemia and possibly apply correction factors to some of these parameters, based on the degree of anaemia. It is envisaged to develop a haemolytic anaemia model to complement the model presented here.

The Animal Use and Care Committee of the Faculty of Veterinary Science, University of Pretoria, approved this study (reference 36-5-562).

A.4   MATERIALS AND METHODS

A.4.1   **Animals** - One intact male, three neutered males and seven non-pregnant female Beagle dogs, on loan from the Onderstepoort Veterinary Academic Research Unit (OVARU), were used. The ages of the dogs were all between two and three years. The general health status of each dog was evaluated by a complete physical, faecal and urine examination performed 2-4 weeks before the trial. In addition, peripheral blood smear examinations (for blood parasites), full blood count (FBC); Ht as well as biochemical profiles for kidney and liver function, liver cell integrity and electrolytes were performed. These included serum levels of total proteins, albumin, urea, creatinine, sodium, potassium, total and ionised calcium, phosphate, alanine amino transferase and alkaline phosphatase. All dogs then underwent echocardiography, general abdominal ultrasonography and thoracic radiography. Only dogs in good physical condition, and clinically healthy with the above values and findings within normal limits were included. All dogs were then dewormed and received a long acting ectoparaciticide. For the active trial period, the dogs were transferred to and housed at the Onderstepoort Veterinary Academic Hospital. They were fed a high protein and calorie commercial dog food. On Day 0, prior to the first phlebotomy, and before the first (baseline) experimental ultrasonographic examinations, the physical examination, peripheral blood smear, FBC and Ht were repeated. The above biochemical profiles, excluding total calcium, phosphate and urea were also repeated. Peripheral blood smear examinations were conducted weekly to ensure that haematozoon parasites did not contribute to, or interfere with, the experimentally induced anaemia. Habitus and appetite were monitored daily on a scale from Level 1 to Level 4. For habitus, 1 was severely depressed and moribund ranging to 4 which was bright and active. For appetite, 1 was anorexic ranging to 4 for a good appetite. Every morning during the trial the temperature and heart rate were monitored and a physical examination was performed.

The study was self-controlled with each dog being compared with its own pretrial and Day 0 Ht data. Sedation was used for bleeding uncooperative dogs. The use and choice of sedatives depended on the temperament of the dog and clinician preference and was one of the following: diazepam (Pax, Bayer Isando, RSA) and morphine sulphate (Bodene Pty. Ltd., Port Elizabeth RSA) combination; acepromazine (Aceprom 2, Bayer Isando RSA) and butorphenol (Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa USA) combination; and medetomidine (Domitor, Novartis, Kempton Park RSA) which was reversed with atipamazole (Antisedan, Novartis, Kempton Park RSA) after phlebotomy).

A.4.2   **Induction of anaemia** - The phlebotomy procedure was a modification of the method previously described (Lobetti *et al.* 1996). It has been shown that 20 % of the estimated blood volume may be safely removed at one time without inducing shock (Knottenbelt & Mackin 1998). Based on the assumption that circulating blood volume is about 90 mℓ /kg in the dog (Knottenbelt & Mackin 1998), the formula for the maximum volume of blood that could be removed is:

Blood volume to be removed (mℓ) = 20 % X weight (kg) x 90

Multiple phlebotomies were performed at least 4 h apart on a daily basis until an Ht of 14-17 % was obtained. The blood was collected in a Fenwal® triple blood-pack with Adsol red cell preservation solution (Adcock Ingram, Aeroton, RSA) by a vacuum technique using a suction pressure of 125 – 175 mm Hg. A proportion of the 70 mℓ citrate anticoagulant in the bag was removed just prior to phlebotomy to maintain the correct ratio with the volume of blood collected.

A cephalic over-the-needle catheter was placed in the cephalic vein prior to phlebotomy to ensure venous access for re-infusion and possible emergency treatment. Catheter venous access was maintained for the duration of the active bleeding phase of the trial, and catheters were replaced after three days. The dogs were manually restrained in lateral recumbency with the head and neck in moderate extension. At least two people, (usually three) were required for the phlebotomy, two for restraint of the dog (one holding the head and one the limbs), and the third to insert the needle into the jugular vein and control the needle. The jugular vein region was aseptically prepared. If a subcutaneous haematoma developed, an attempt was made to draw blood from a different site on the same vein. As a last resort the contralateral vein was used for collection. The Onderstepoort Animal Blood Bank vacuum collection technique weighed the collecting bag blood during collection allowing reasonably accurate determination of the removed blood volume. The volume of blood to mass of blood ratio was assumed to be 1 mℓ:1g, for practical purposes. However, the SG of blood is 1.053 therefore the ratio of blood volume: blood mass is 1: 1.05, for example, 200 g of blood = 190 mℓ of blood (Mathews 1998).

A.4.3   **Maintaining normovolaemic status** - To ensure normovolaemia after bleeding, the volume deficit of the packed cells (determined from multiplying the Ht by the volume removed) was accounted for by infusing an equal amount of Ringer's lactate, using a 15 drop/mℓ blood infusion administration set connected to the cephalic catheter, immediately after completion of phlebotomy. The blood bag was then centrifuged for 10 min in a refrigerated Sigma® automatic centrifuge at 4 °C and 3500 revolutions per minute. After centrifuging, the plasma was separated from the packed cells and sealed, and the Adsol preservative added to the remaining packed red blood cells. The plasma was then immediately infused back into the same dog over a period of an hour, simultaneously with the Ringer's, if this was not finished yet. The packed cell bag was labelled and stored at 4 °C for the duration of the study in the

event that it was needed for re-infusion. The packed cells were discarded at the end of the project as the blood type had not been established and thus precluded its use in other dogs.

The individual animal's response to the phlebotomy was monitored by the Ht readings prior to each bleed. Once the predetermined Ht range of between 14-17 % was reached, and verified by the laboratory-calculated value, dogs were allowed to recover naturally from their anaemia with daily Ht monitoring.

A.4.4   Additional procedures - Microhaematocrit PCV was monitored twice daily just prior to phlebotomy and daily or twice daily (if recovery was more rapid than anticipated) during the recovery phase. The results were compared with the analyser-calculated value when an accurate value was necessary, such as when haemodynamic data were to be captured. The Ht was done as close to the same time of day as possible. Each dog was weighed daily during the trial, and then later whenever experimental data were captured. In the first week of the study, one dog was introduced to the trial followed by two dogs each additional week. This limitation was to accommodate the additional experimental procedures that had to be performed on each dog. These were an echocardiographic examination, which was completed within 45-60 min, followed by an abdominal splanchnic vascular Doppler examination lasting 90-120 min. At the end of the trial each animal, having recovered sufficiently from the induced anaemia, was returned to OVARU. All echocardiographic and Doppler parameters were measured immediately before and after induction of severe, acute anaemia (Ht 14-17 %). Measurements were repeated during recovery in moderate chronic (Ht 25-27 %) and mild chronic anaemia (Ht 31-37 %). A FBC was repeated in each Ht range.

Collected data were also applied retrospectively to the theoretical haemodilution equation, described by Bourke and Smith (Bourke & Smith 1974) to evaluate its accuracy in this group of dogs. The total volumes of the actual extracted blood were compared with the theoretically predicted volumes calculated from Bourke and Smith's (1974) equation for the same final Ht.

A.5   RESULTS

The body weight median, mean, standard deviation (SD) and range of the 11 Beagles was 11.3, 11.9 (1.8), 9.5-15.2 kg. On Day 0, 33 % of dogs were sedated, Day 1, 76 %, Day 2, 74 % and Day 3, 60 %. Dogs three and seven, which received a morphine diazepam combination, were judged to be nauseous and were treated effectively with metaclopramide (Clopamon, Pharmacare, Port Elizabeth, RSA). In Dog ten an apparent adverse reaction to the medetomidine occurred on the third day of bleeding. The dog collapsed towards the end of the phlebotomy, approximately 20 min after medetomidine injection. On immediate medetomidine reversal with atipamazole, the dog recovered uneventfully.

 The process of phlebotomy together with the laboratory work took about 40 min (15-20 min bleeding and 20 min plasma preparation). Ringer's infusion took about 20 min, which at times ran concurrently with the plasma. Plasma infusion took about 60 min, which was slow due to its high viscosity, and also to monitor for possible transfusion reactions in dogs not receiving their own plasma (Schneider 2000). Eight dogs were bled once, two dogs twice and one dog was bled three times on the first day. This variation was purely for convenience to get dogs into the system. On the second day all dogs were bled twice with the exception of one dog that was only bled once. On the third day eight dogs were bled twice and the remaining three dogs had attained the required Ht by the afternoon and were thus bled only in the morning. On the fourth

day, five dogs were bled once. The number of phlebotomies required to obtain the optimal Ht ranged from 4-7 with a mean (SD) of 5.3(0.9). The mean (SD) and range of removed blood volume (in $m\ell$) on Day 1 was 226 (32), 190-304; Day 2 was 225 (36.6), 190-304; Day 3 was 219 (43.1), 114-290; and Day 4 was 211 (43.7), 171-285. The low volume of 114 $m\ell$ removed in one dog on Day 3 was due to the sedation collapse described above. Complications of the procedure were minimal. Two dogs (Dog four once and Dog five twice) received donor plasma instead of their own plasma. On one occasion in each dog this was due to a power failure that would have caused a delay in re-infusion and in the other due to rupture of the blood collecting bag in the centrifuge. Dog four developed a mild facial oedema which was not treated, and which resolved spontaneously within 36 h.

Body temperature remained normal throughout the trial. Heart rate increased from a mean (SD) on Day 0 of 96(13.2) (n=9) to a peak of 132(14.7) on Day 2 and decreased to 128(13.6) (n=10) on Day 4. Habitus remained at Level 4 in seven dogs throughout the trial. Dog eight had a Level 3 the last day it was bled as well as the next day. The remaining dogs (Dogs two, four, six and ten) had a Level 3 the day after the final bleed. Two of these dogs had other problems that could have accounted for the mild depression; Dog four had the facial oedema and Dog ten had a lame hind leg of uncertain aetiology for that day. Appetite remained at Level 4 in eight dogs. Dogs six and nine had slightly reduced appetites on the last phlebotomy day with Dog 6 eating only a small amount the next day as well. Dog one ate nothing on the second and third days after bleeding but this was believed to be due to a change in food consistency.
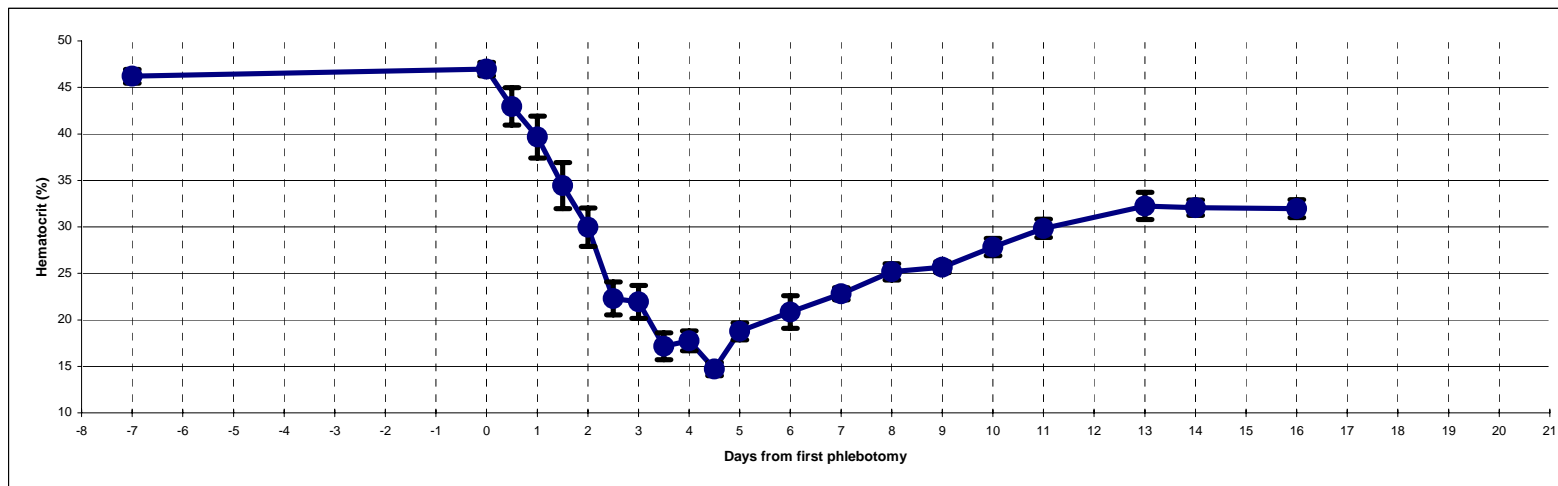
FIG. A.1 - The mean Ht value of all dogs measured on any particular day relative to the first phlebotomy being set as Day 0. During the first four days Ht readings were often taken twice daily

and the afternoon readings have been plotted as "half-days". The pre-trial "screening" data were all assigned to Day - 7 for convenience, although some were conducted more than one week

before the "active trial". The error bars represent 2SE on either side of the mean values.

The effect of phlebotomy on the Ht as well as during the dogs' recovery over the next ten days is illustrated (Fig. 1). The percentage differences between the actual and predicted volumes using Bourke & Smith's (1974) haemodilution equation to achieve the final haematocrit are summarized (Fig. 2). The standard deviation of the percentage difference between actual and predicted volumes in this group of dogs was 17.4 % volume.
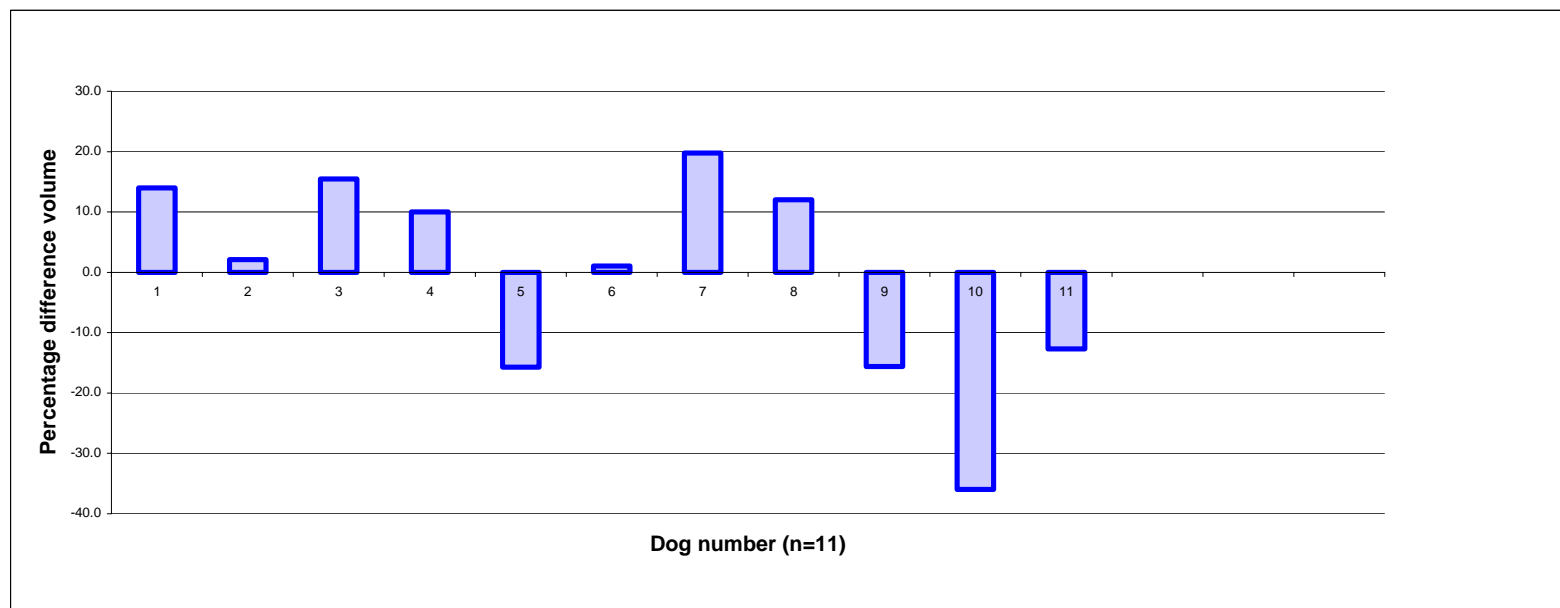
FIG. A.2 - The percentage difference between the actual and predicted volumes of blood by Bourke and Smith's haemodilution equation to achieve the final Ht for each dog. A percentage difference of 0 would indicate that the actual and predicted blood volumes were the same. A positive percentage means that the actual blood volume was in excess of the predicted blood volume, while a negative percentage means that the actual volume was less than the volume predicted.

A.6   DISCUSSION

This experimental anaemia model provides a technique to produce acute anaemia over a period of 3-4 four days in dogs with minimal side effects. Sedation was only required in a third of the dogs on Day 0, but was increased up to 75 % of the dogs on the remaining days as dogs became aware that they were going to be bled. The one dog that collapsed towards the end of the phlebotomy, after medetomidine injection, was judged to have acute cardiac output failure. This is likely to have been due to a combination of the sedative and anaemic state of the animal. On immediate reversal of the medetomidine, the dog recovered uneventfully. Medetomidine was used on this occasion in this dog as it was particularly uncooperative, and the other sedation protocols, having been used with previous phlebotomies, were judged to be inadequate. It is not recommended to use medetomidine as a sedative in this anaemia model, and uncooperative or fractious dogs should rather be excluded from trials

The phlebotomy and re-infusion process could be completed within 90 min if Ringer's infusion took place at the same time as extracting the plasma. Plasma infusion could be done while the next dog was being bled and thus with adequate labour, four to six dogs could be bled in a single day. In this study a maximum of two dogs were bled per day as the same workers had to do other experimental procedures during the acute anaemia and recovery stages. The induction of anaemia and procedures performed up to recovery to mild chronic anaemia thus took a month in this group of dogs and two weeks in individual dogs.

The blood volume was removed acutely and directly from the vasculature of the Beagles. Fluid replacement with Ringer's lactate, equal in volume to the calculated red blood cell volume removed, was administered in the first 30 min after phlebotomy. The plasma was transfused

after 30-60 min, often together with the Ringer's lactate as a colloid as well as for volume replacement. A minimum of four hours was allowed between phlebotomies to allow for normal trans-membrane fluid movements.

Under normal conditions, Starlings forces across the extra-cellular membranes cause approximately 80 % of transfused isotonic crystalloids to filter into the interstitium within 1 h (Mathews 1998). The value of replacing the lost red blood cell mass with crystalloids is thus debatable but it did give volume support in the immediate post-phlebotomy phase while the plasma was being prepared for re-infusion. Auto-plasma re-infusion appears to be preferable as one dog that received donor plasma developed mild facial oedema, indicating a mild Type 1 anaphylactic reaction against donor proteins (Hohenhaus 2000).

The true volume deficit created by red cell removal, assuming an initial Ht of 45 % and volume collection of 200 mℓ, was only 9 % and would probably have been compensated for by normal fluid redistribution between compartments during normal water intake and albumin production by the liver. As dogs became progressively more anaemic the effective red blood cell mass volume deficit would become even less significant due to the compounding effects of the iatrogenic anaemia.

The decision not to use colloids as post collection replacement fluids was based on the above premise as well as the fact that the oncotic effect would have been cumulative as the half-life of 6% hetastarch (Haes-Steril, Fresenius Kabi, Bad Homburg, Germany); the volume replacement fluid of choice at our institution is 25 hours (Rudloff & Kirby 1997). This would have had a marked effect when blood was being drawn daily and even twice daily and would have interfered with natural fluid homeostatic compensatory mechanisms causing a degree of

haemodilution (Rudloff & Kirby 1997). The ideal post collection transfusion would have been own plasma plus the red cell volume of a mixture of donor plasma and 0.9 % saline to a concentration of 30 g/ℓ. This would have been laborious and unnecessary considering the small volumes involved and the ability of the body to compensate for these changes. In this model, echocardiographic and Doppler parameters were measured several hours after re-infusion in unsedated dogs and thus blood volumes should have reinstated their fluid equilibrium.

The microhaematocrit technique to measure PCV was selected to monitor the anaemia on a day-to-day basis because of its ease of use, low cost, convenience (such as after hours monitoring), and the rapid acquisition of results. For data recording it was, however, rejected early in the study in favour of the analyser-calculated reading (calculated automatically from the mean cell volume and the RBC number) due to discrepancies of up to 2.4 % higher PCV when compared with the analyser-calculated Ht. Although the microhaematocrit method was very useful in monitoring the anaemia during the trial it is recommended that the automated cell counter values be used to verify the exact Ht when necessary. It is believed that the discrepancy between the values using the two methods was due to inter-equipment variation which needs to be investigated further.

 From the Ht data (Fig. 1) it can be seen that the pre-trial screening Ht values and the values obtained on the day that (but before) phlebotomy commenced, were essentially the same and well within the reference range of 37 to 55 % (Meinkoth & Clinkenbeard 2000). The anaemia model reproducibly produced a decline of Ht to its nadir (at approximately 15 %) by the 4th and 5th days which is similar to the anaemia experimental model described in the horse (Smith & Agar 1976).

By comparing the actual and predicted volumes of blood for the final Ht in this small group of dogs (Fig. 2), it would appear that the theoretical haemodilution equation described (Bourke & Smith 1974) is unsuitable for clinical application in dogs. More work would be needed to establish an equivalent predictive equation in this species. Until then, careful monitoring of the Ht during the bleeding phase is recommended.

Recovery, from Day 5, was progressive and essentially linear, for the next week (until a Ht of 30 % was achieved by Day 11). It would appear that after that level of recovery had been reached, the stimulus for regeneration abated somewhat and the recovery tailed off quite markedly. The original trial design did not include monitoring beyond two weeks, but one dog was fortuitously sampled on Day 43 and was found to have an Ht of 42 %. The recovery rate was within the range that had been anticipated but the plateau after day 11 was unexpected as it had been assumed that a plateau would be reached at a Ht in the 35 to 40 % range.

The current model was adapted from one used to determine the effect of hypoxia in the development of nephropathy in the dog (Lobetti *et al* 1996). The acute anaemia model can be used in many other studies such as the effect of haematinics or cardiovascular drugs, acute and subacute blood loss (e.g. gastric ulceration), evaluating the effects of various degrees of anaemia on splanchnic Doppler blood flow, the usefulness of cell-counter-derived erythrocyte parameters (red cell distribution width and machine-generated reticulocyte counts) in haemorrhagic and immune-mediated anaemias as well as studies on canine babesiosis as a model for falciparum malaria in humans.

A.7   ACKNOWLEDGEMENTS

A.8   REFERENCES

ARTAVANIS-TSAKONAS, K., TONGREN, J.E. & RILEY, E.M. 2003. The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. *Clinical Experimental Immunology,* 133:145-152.

BHARGAVE, M. & GABBE, E.E. 1984. Chronic experimental iron losses in rats not leading to overt Iron deficiency: a model for the regulation of the whole-body iron balance in an iron-replete condition. *Journal of Nutrition,* 114:1060-1069.

BOURKE, D.L. & SMITH, T.G. 1974. Estimating allowable haemodilution. *Anaesthesia.* 41:609-612.

CONTAMIN, H., BEHR, C., MERCEREAU-PUIJALON, O., MICHEL, J. 2000. Plasmodium falciparum in the squirrel monkey (*Saimiri sciureus*): infection of non-splenectomized animals as a model for exploring clinical manifestations of anaemia. *Microbes and Infection.* 2:945-954

DONALD, D.E., FERGUSON, D.A. & MILBURN, S.E. 1968. Effect of beta-adrenergic receptor blockade on racing performance of greyhounds with normal and with denervated hearts. *Circulation Research.* 22:127-134.

FAHIM, M. & SINGH, M. 1992. Haemodynamic responses during acute normovolemic haemodilution in anaesthetized dogs. *Japanese Journal of Physiology* 42:753-763.

FOWLER, N.O., FRANCH, R.H. & BLOOM, W.L. 1956. Haemodynamic effects of anaemia with and without plasma volume expansion. *Circulation Research.* 4:319-324.

FOWLER, N.O. & HOLMES, J.C. 1971. Ventricular function in anaemia. *Journal of Applied Physiology.* 31:260-265.

FOWLER, N.O. & HOLMES, J.C. 1975. Blood viscosity and cardiac output in acute experimental anaemia. *Journal of Applied Physiology.* 39:453-456.

GAMBLING, L., CHARANIA, Z & HANNAH, L., ANTIPATIS, C., LEA, R.G. & McARDLE, H.J. 2002. Effect of iron deficiency on placental cytokine expression and foetal growth in the pregnant rat. *Biology and Reproduction*. 66:516-523.

GEHA, A.H. 1976.Coronary and cardiovascular dynamics and oxygen availability during acute normovolemic anaemia. *Surgery.* 80:4-53.

GROSS, J.B. 1983. Estimating allowable blood loss: corrected for dilution. *Anaesthesia.* 58:277-280.

HABLER, O.P., KLEEN, M.S., PODTSCHASKE, A.H., HUTTER, J.W., TIEDE, M., KEMMING, G.I., WELTE, M.V., CORSO, C.O. & MESSMER, K.F. 1996. The effect of acute normovolaemic haemodilution (ANH) on myocardial contractility in anesthetised dogs. *Anaesthesia & Analgesia*. 83:451-458.

HOHENHAUS, A.E. 2000. Transfusion reactions. in *Schalm's Veterinary Haematology*, 5th edn., Edited by B.F. Feldman, J.G. Zinkl, & N.C. Jain Philadelphia: Lippincot, Williams & Wilkins, 864-868.

KAWAI, S., AIKAWA, M & KANO, S. 1993. A primate model for severe human malaria with cerebral   involvement: *Plasmodium coatneyi*-infected *Macaca fuscata*. *American Journal of Tropical Medicine and Hygiene,* 48:630-636.

KNOTTENBELT.C. & MACKIN, A.J. 1998. Blood transfusions in the dog and cat part 1: Blood collection techniques, *In Practice* 20:110-117.

LOBETTI, R.G., REYERS, F & NESBIT, J.W. 1996. The comparative role of haemoglobinaemia and hypoxia in the development of canine babesial nephropathy in the dog. *Journal of the South African Veterinary Association,* 67:188-198.

MATHEWS, K.A. 1998. The various types of parenteral fluids and their indications. *Veterinary Clinics of North America. Small Animal Practice,* 28:483-513.

MEINKOTH, J.H. & CLINKENBEARD, K.D. 2000. Normal haematology of the dog. in *Schalm's*

Veterinary Haematology, 5th edn., Edited by B.F. Feldman, J.G. Zinkl, & N.C. Jain Philadelphia: Lippincot, Williams & Wilkins, 1057-1058.

REIMER, M.E, TROY, G.C. & WARNICK, L.D. 1999. Immune-mediated haemolytic anaemia: 70 cases (1988-1996). *Journal of the American Animal Hospital Association,* 35:384-391.

REYERS, F., LEISEWITZ, A.L., LOBETTI, R.G., MILNER, R.J., JACOBSON, L.S. & van ZYL, M.1998. Canine babesiosis in South Africa: more than one disease. Does this serve as a model for falciparum malaria? *Annals of Tropical Medicine and Parasitology*, 92:503-511.

RUDLOFF, E. & KIRBY, R. 1997. The critical need for colloids: Selecting the right colloid. *The Compendium of Continuing Education*, 19;811-825.

SCHNEIDER, A. 2000. Principles of blood collection and processing in *Schalm's Veterinary Haematology*, 5th edn., Edited by B.F. Feldman, J.G. Zinkl, & N.C. Jain Philadelphia: Lippincot, Williams & Wilkins, 827-832.

SCHOU, H., PEREZ DE SA, V., SIGURDARDOTTIR, M., ROSCHER, R., JONMARKER, C. & WERNER, O. 1996.Circulatory effects of hypoxia, acute normovolaemic haemodilution, and their combination in anaesthetised pigs. *Anaesthesia*. 84:1443-1454.

SMITH, J. & AGAR, N.S. 1976. Studies on erythrocyte metabolism following acute blood loss in the horse. *Equine Veterinary Journal*, 8:34-37.

STEHLING, L. & ZAUDER, H.L. 1991. Acute normovolaemic haemodilution. *Transfusion,* 31:857-868.

SZLYK, P.C., KING, C., JENNINGS, D.B., CAIN, S.M. & CHAPLER, C.K. 1984. The role of aortic chemoreceptors during acute anaemia. *Canadian Journal of Physiology and Pharmacology*. 62:519-523.

VATNER, S.F., HIGGINS, C.B. & FRANKLIN, D. 1972. Regional circulatory adjustments to moderate and severe chronic anaemia in conscious dogs at rest and during exercise. *Circulation Research 30*:731-740.

APPENDIX B

SELECTION OF UNCOMPLICATED CANINE BABESIOSIS CASES

B.1   Notice to Outpatient Clinicians

Dear Outpatient Clinician,

This is to inform you that Doppler ultrasonographic investigations cases of canine babesiosis (CB) reported to this Hospital, began with effect from 10th September 2003 and will continue until further notice.

The study requires that cases of CB be included / excluded according to the following criteria:

Inclusion Criteria for outpatients

- Blood smear is positive for *B. canis*

- Haematocrit is below 36%

- A freshly presented case

- Owner agrees to participate in the trial

Exclusion Criteria for outpatients

- Dog is Doberman, Spaniel, Great Dane or Irish Wolfhound

- Blood smear is positive for *E. canis* morulae

- Presence of epistaxis, petechiation or lymphadenopathy

- Haematocrit is above 36%

- Dog is clinically dehydrated

- Dog requires immediate blood transfusion

Exclusion criteria after admission of patient

- Radiographic evidence of abnormality of the chest, lungs or heart, or echocardiographic evidence of the heart that is not related to CB.

- Ultrasonographic evidence of abnormality of the abdomen, especially the liver, spleen and kidneys that is not related to CB.

The study will evaluate the heart and abdominal splanchnic blood flow, and will require three hours for completion. The selected dog will be admitted for 24 hours.

If a patient fulfils the criteria listed under I and II, and the owner is willing to participate in the study, please call Dr. Lee Koma / admit the patient, and contact any of the following:

1. Dr. Lee Koma (Tel. 012 529 8221 / Cell phone 072 611 5755), Room 4-64.

2. Dr. Tim Spotswood (Tel. 012 529 8005 / Cell phone: 082 511 5787), Room 4-52.

3. Prof. Robert Kirberger (Tel. 012 529 8270 / Cell phone 083 440 8211), Room 4-69.

Thank you for your co-operation and assistance.

Dr. Lee Koma

Section of Diagnostic Imaging

CACS Department

B.2   Client information sheet

University of Pretoria

Your dog has been diagnosed with canine babesiosis (biliary fever); a disease that sometimes becomes complicated and may cause death. The aim of this study is to investigate if any potential complication can be identified before it occurs, using a new ultrasound technique known as Doppler. No complication has been detected in your dog at this stage, which makes him/her suitable for the study. It is hoped that this study will improve our understanding, and subsequently the care for dogs suffering from biliary fever.

Your dog will have free advanced medical check-up that may give more information on the disease not detected by the usual tests. The additional tests will examine the function of the heart, and blood flow of the liver, spleen, kidneys, stomach and intestines. These procedures are non-invasive, completely safe and free from pain. However, the hair over the lower parts of the belly, chest and paws of the dog will be shaved, and about 5 ml of blood will be removed for further tests. Chest x-rays will also be made.

This investigation requires the dog to be admitted overnight. During this time, closer observation of the progress of its health, as well as its welfare will be provided without any additional cost.

If, however, we pick up any potential complication or additional disease, you will then be phoned, and informed of this. Your consent will be sought for any further treatment that may be required and, whose cost you are willing to meet.

If no further problem is detected, we will need to re-admit your dog overnight for a review three weeks after the initial discharge from the hospital. No fees will be charged for this review, and your dog will get a free vaccination or a small bag of dog feed during this visit.

To follow the progress of your dog, please, feel free to contact:

1. Dr Lee Koma (Tel. 012 529 8221 / Cell phone 072 611 5755), Room 4-64 OVAH

2. Dr Tim Spotswood (Tel. 012 529 8005 / Cell phone: 082 511 5787), Room 4-52 OVAH

3. Prof Robert Kirberger (Tel. 012 529 8270), Room 4-69 OVAH

Signed: --- Date: ----------------------

OWNER

B.3   Client consent form

University of Pretoria

I, …………………………...................………. (Full names), the undersigned owner /

authorised representative (delete not applicable), hereby permit this dog under my care,

identified as:

Name: ……………………............…………….. Breed: ………………………………….

Age: …………………………...............………. Gender: ………………………………...

Colour: …………………………………............

to participate in the ultrasonographic study for uncomplicated biliary fever.

I have received the information sheet on the study, and fully understand its contents. I am

aware that my pet will be admitted to the Outpatients Clinic of Onderstepoort Veterinary

Academic Hospital overnight (24 hours) on each of two occasions: the first being to-day, and

the second three weeks later. I understand that during each occasion the following procedures,

all of which are completely safe, will be carried out on my pet at no additional cost:

1.   Hair clipped over areas of the belly, chest and paws

2.   Small amount (5ml) of blood removed

3.   Chest x-rays made

4.   Ultrasonography of the heart and abdomen performed

I am also aware that if any complication or other disease is found, I will be informed, and be

given opportunity to decide on any recommended further treatment, the cost of which I will

210

meet. I am further aware that I may remove my pet from this study at any time, at my request, and this will in no way jeopardise the proper care for my dog.

In the unlikely event of my dog dying, I give permission for a full post-mortem to be performed.

Signed at Onderstepoort on this …… day of ………......................… in the year ………

....

      (*Signature*)

B.4   Data Sheets

B.4.1   B-mode appearance of organs:

CASE ID: Date ------------------   Dog's Name / No. -------------------------   Ht. --------------------   Exam No. -----------

B.4.1.1   Spleen

Max thickness (mm)	------------------	Diameter of HSV --------------		
Shape	1. Normal	2. Deformed		
Contour	1. Smooth	2. Irregular		
Spleen Vs Renal cortex	1. – 2	2. – 1	3. 0	4. +1
Echotexture	1. Normal	2. Finer	3. Coarser	

LESIONS:

B.4.1.2   Left Kidney

Length (mm) ----------------   Width (mm) ----------- Height (S/A)(mm) --------------

Shape	1. Normal	2. Deformed		
Contour	1. Smooth	2. Irregular		
Corticomedullary distinction	1. Distinct	2. Indistinct	3. Absent	
Corticomedullary rim sign	1. Absent	2. Indistinct	3. Distinct	
R. Cortex Vs Spleen	1. + 2	2. + 1	3. 0	4. – 1	5. - 2
R. Medulla Vs R. Cortex	1. + 2	2. + 1	3. 0	4. – 1	5. - 2

LESIONS:

212

Table B.1 - Arterial numerical data

Vessel	Angle θ	HR (B/min)	ECG-RR interval (mS)	Spectral Doppler Measurements																				
				Peak Systolic Velocity (Cm/s)				End Diastolic Velocity (Cm/s)				Resistivity Index (RI)				Pulsatility Index (PI)				Time-averaged Mean Velocity (Cm/s)			
				1	2	3	A	1	2	3	A	1	2	3	A	1	2	3	A	1	2	3	A
AAo																							
CA																							
CMA																							
LRA																							
ILA																							
HSA																							

AAo = abdominal aorta; CA = coeliac artery; CMA = cranial mesenteric artery; LRA = left renal artery; ILA = interlobar artery; HSA = hilar splenic artery.

Table B.2 - Venous numerical data

Vessel	Angle θ	HR (B/min)	ECG-RR	2D Diameter (mm)	2D CSA (mm²)	Peak Velocity (Cm/s) 1	2	3	A	Minimum Forward Velocity (Cm/s) 1	2	3	A	Time-averaged Mean Velocity (Cm/s) 1	2	3	A	Peak Reverse Velocity (Cm/s) 1	2	3	A
LRV				■	■													■	■	■	■
ILV				■	■													■	■	■	■
HSV																		■	■	■	■
MPV																					
CVC																					

LRV = left renal vein; MPV = main portal vein; CVC = caudal vena cava; HSV = hilar splenic vein; ILV = interlobar vein

214

Table B.3 - Arterial descriptive data

Vessel	Doppler Spectra Analysis						
	Velocity Profile	Status of Spectral Window	Margination of Spectral Tracing	No of Peaks		No of Notches	
				Systole	Diastole	Systole	Diastole
AAo							
CA							
CMA							
LRA							
ILA							
HSA							

AAo = abdominal aorta; CA = coeliac artery; CMA = cranial mesenteric artery; LRA = left renal artery; ILA = interlobar artery; HSA = hilar splenic artery.

Table B.4 - Venous descriptive data

Vessel	Doppler Spectra Analysis				
	Velocity Profile	Status of Spectral Window	Margination of Spectral Tracing	No. of Peaks in a Respiratory Cycle	No. of Notches in a Respiratory Cycle
LRV					
ILV					
HSV					
MPV					
CVC					

LRV = left renal vein; MPV = main portal vein; CVC = caudal vena cava; HSV = hilar splenic vein; ILV = interlobar vein