

**ECHOCARDIOGRAPHIC CHANGES OF LEFT  
VENTRICULAR SIZE AND FUNCTION IN A CANINE  
NORMOVOLAEMIC ANAEMIA MODEL**

**by**

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**Submitted in partial fulfilment of the requirements  
for the degree of M.Med.Vet (Diagnostic Imaging)  
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## **Dedication**

To Nina, Neve and Isla

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## Table of contents

<b>List of Figures and Tables</b> .....		<b>3</b>
<b>Glossary</b> .....		<b>5</b>
<b>Summary</b> .....		<b>7</b>
<b>1</b>	<b>Objectives and Benefits</b> .....	<b>8</b>
<b>1.1</b>	<b>Objective:</b> .....	8
<b>1.2</b>	<b>Benefit:</b> .....	8
<b>2</b>	<b>Hypotheses</b> .....	<b>9</b>
<b>3</b>	<b>Literature Review</b> .....	<b>10</b>
<b>3.1</b>	<b>Evaluation of cardiovascular function</b> .....	10
<b>3.2</b>	<b>Echocardiographic evaluation of cardiac function</b> .....	10
3.2.1	Ejection fraction.....	10
3.2.2	Fractional shortening .....	11
3.2.3	Left ventricular volumetrics.....	11
3.2.4	Cardiac output.....	15
3.2.5	Cardiac index and stroke index.....	15
3.2.6	End systolic volume index.....	15
3.2.7	Systolic time intervals.....	15
<b>3.3</b>	<b>The heart in anaemia</b> .....	18
3.3.1	Overview of anaemia .....	18
3.3.2	Cardiovascular studies in human anaemia.....	19
3.3.3	Overview of the haemodynamics during the acute anaemic state .....	20
3.3.4	Cardiovascular studies in canine anaemia .....	21
3.3.5	The role of decreased afterload in acute normovolaemic anaemia.....	22
3.3.6	Evaluation of cardiovascular function in anaemia using echocardiography .....	23
3.3.7	The heart in canine babesiosis .....	24
<b>4</b>	<b>Materials and Methods</b> .....	<b>25</b>
<b>4.1</b>	<b>Overview</b> .....	25
<b>4.2</b>	<b>Study design</b> .....	26
4.2.1	Sample group .....	26
4.2.2	Induction of normovolaemic anaemia .....	27
4.2.3	Echocardiography .....	28
<b>4.3</b>	<b>Statistical analysis</b> .....	29
<b>4.4</b>	<b>Ethical considerations</b> .....	30

<b>5</b>	<b>Results .....</b>	<b>31</b>
<b>5.1</b>	<b>Subjective 2D assessment .....</b>	<b>32</b>
<b>5.2</b>	<b>Heart rate.....</b>	<b>32</b>
<b>5.3</b>	<b>M-mode derived parameters.....</b>	<b>33</b>
<b>5.4</b>	<b>2-D derived parameters.....</b>	<b>38</b>
<b>5.5</b>	<b>Systolic time intervals .....</b>	<b>42</b>
<b>6</b>	<b>Discussion.....</b>	<b>45</b>
<b>7</b>	<b>References .....</b>	<b>50</b>
<b>8</b>	<b>Appendix.....</b>	<b>59</b>

## List of Figures and Tables

<b>Figure 1</b>	Bullet method .....	<b>13,14</b>
<b>Figure 2</b>	Systolic time intervals (M mode) .....	<b>17</b>
<b>Figure 3</b>	Systolic time intervals (Doppler mode) .....	<b>17</b>
<b>Figure 4</b>	Haematocrit .....	<b>31</b>
<b>Figure 5</b>	Heart rate .....	<b>32</b>
<b>Figure 6</b>	Fractional shortening .....	<b>34</b>
<b>Figure 7</b>	E point to septal separation .....	<b>34</b>
<b>Figure 8</b>	Interventricular septum in diastole .....	<b>35</b>
<b>Figure 9</b>	Left ventricular interior diameter in diastole .....	<b>35</b>
<b>Figure 10</b>	Left ventricular posterior wall in diastole .....	<b>36</b>
<b>Figure 11</b>	Interventricular septum in systole .....	<b>36</b>
<b>Figure 12</b>	Left ventricular interior diameter in systole .....	<b>37</b>
<b>Figure 13</b>	Left ventricular posterior wall in systole .....	<b>37</b>
<b>Figure 14</b>	End diastolic volume .....	<b>38</b>
<b>Figure 15</b>	End systolic volume .....	<b>39</b>
<b>Figure 16</b>	End systolic volume index .....	<b>39</b>
<b>Figure 17</b>	Ejection fraction .....	<b>40</b>
<b>Figure 18</b>	Stroke volume .....	<b>40</b>
<b>Figure 19</b>	Stroke index .....	<b>41</b>
<b>Figure 20</b>	Cardiac output .....	<b>41</b>
<b>Figure 21</b>	Left ventricular ejection time .....	<b>42</b>
<b>Figure 22</b>	Left ventricular pre-ejection period .....	<b>43</b>

<b>Figure 23</b>	Velocity of circumferential shortening .....	<b>43</b>
<b>Figure 24</b>	Left ventricular ejection time/pre-ejection period .....	<b>44</b>
<b>Figure 25</b>	Left ventricular ejection time index .....	<b>44</b>

**Tables**

<b>Table 1</b>	Systolic time intervals .....	<b>18</b>
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## Glossary

Abbreviations used in the text:

<b>ANH</b>	Acute normovolaemic haemodilution
<b>AO</b>	Aorta
<b>ASE</b>	American society of echocardiography
<b>BSA</b>	Body surface area
<b>CI</b>	Cardiac index
<b>CO</b>	Cardiac output
<b>ECG</b>	Electrocardiogram
<b>EDRF</b>	Endothelium-derived relaxing factor
<b>EDV</b>	End diastolic volume
<b>EF</b>	Ejection fraction
<b>EPSS</b>	E point to septal separation
<b>ESV</b>	End systolic volume
<b>ESVI</b>	End systolic volume index
<b>FS</b>	Fractional shortening
<b>Hct</b>	Haematocrit
<b>HR</b>	Heart rate
<b>IVSd</b>	Interventricular septum in diastole
<b>IVSs</b>	Interventricular septum in systole
<b>LA</b>	Left atrium
<b>Lax</b>	Long axis
<b>LV</b>	Left ventricle
<b>LVET</b>	Left ventricular ejection time
<b>LVEF</b>	Left ventricular ejection fraction
<b>LVETI</b>	Left ventricular ejection time index
<b>LVIDd</b>	Left ventricular internal diameter in diastole
<b>LVIDs</b>	Left ventricular internal diameter in systole
<b>LVPWd</b>	Left ventricular posterior wall diameter in diastole
<b>LVPWds</b>	Left ventricular posterior wall diameter in systole

<b>LVVd</b>	Left ventricular volume in diastole
<b>LVVs</b>	Left ventricular volume in systole
<b>MODS</b>	Multiple organ dysfunction syndrome
<b>NO</b>	Nitric oxide
<b>OVAH</b>	Onderstepoort Veterinary Academic Hospital
<b>OVARU</b>	Onderstepoort Veterinary Academic Research Unit
<b>PCV</b>	Packed cell volume
<b>PEP</b>	Left ventricular pre-ejection period
<b>RMANOVA</b>	Repeated measures analysis of variance
<b>Sax</b>	Short axis
<b>SD</b>	Standard deviation
<b>SI</b>	Stroke index
<b>SIRS</b>	Systemic inflammatory response syndrome
<b>STI</b>	Systolic time interval
<b>SV</b>	Stroke volume
<b>TVI</b>	Time velocity integral
<b>Vcf</b>	Velocity of circumferential shortening

## Summary

The objective of this study was to non-invasively document the changes in echocardiographic variables of left ventricular size and function during acute normovolaemic anaemia. This model was developed as a pilot study with the purpose of providing baseline information to investigate the pathophysiology, and more specifically the effect on the heart, of canine babesiosis-induced anaemia. The study group comprised of 11 mature healthy Beagle dogs that weighed between 9 and 15 kg. Severe normovolaemic anaemia was induced over a 3-4 day period by serial bleeding while maintaining normovolaemia by autotransfusing plasma and infusing crystalloids. The dogs were then allowed to recover. Pre-anaemic [mean haematocrit (Hct) 46.7%, standard deviation (SD) 2.4%] echocardiographic variables of left ventricular size and performance were statistically compared to those in the severely [mean Hct 15.3 %, SD 1.1%], moderately [mean Hct 24.7%, SD 1.5%] and mildly [mean Hct 33.5%, SD 2.5%] anaemic states, and between the anaemic states. The following variables were measured: left atrial size; left ventricular fractional shortening, ejection fraction, end-systolic and end-diastolic ventricular volumes and their derivatives [stroke volume, stroke index, cardiac output, cardiac index]; systolic time intervals [left ventricular ejection time (LVET), pre-ejection period (PEP), velocity of circumferential shortening, LVET/PEP and LVET index (LVETI)]; and heart rate. With the exception of end diastolic volume, left atrial size, LVET/PEP and LVETI, there was a statistically significant ( $p < 0.05$ ) change in all variables in the severely anaemic state versus the pre-anaemic and the mild and moderate anaemic states. In accordance with previous invasive models, this study demonstrates the hyperdynamic state of the left ventricle that develops in response to experimentally induced acute canine normovolaemic anaemia in the conscious dog, and shows promise as a non-invasive technique of evaluating the cardiac changes in dogs suffering from canine babesiosis.

# 1 Objectives and Benefits

## 1.1 Objective:

The objective of this study was to gather baseline echocardiographic data on left ventricular size and function in normovolaemic acutely anaemic dogs.

## 1.2 Benefit:

This project is a precursor to follow-up work that will evaluate LV size and performance in anaemic dogs with babesiosis. More specifically, this study is intended to provide reference data for comparing the echocardiographic changes to left ventricular function and size in anaemic dogs suffering from canine babesiosis.

This project was part of a larger PhD project entitled: “Abdominal splanchnic haemodynamics in a canine normovolaemic anaemia model and uncomplicated canine babesiosis: A comparative Doppler study.” The researcher was Dr LMPK Koma from the Section Diagnostic Imaging, Companion Animal Clinical Studies.

This project forms part of the research requirements for Dr Spotswood’s MMedVet (DiagIm) degree.

## 2 Hypotheses

Acute normovolaemic anaemia causes significantly altered left ventricular size and function as measured by echocardiography in otherwise healthy dogs.

1. Normovolaemic anaemia causes a significant change in fractional shortening
2. Normovolaemic anaemia causes a significant change in ejection fraction

The degree of change in left ventricular size and function is related to the severity of acute normovolaemic anaemia (as measured by Hct).

1. The degree of change in fractional shortening increases with decreasing Hct
2. The degree of change in ejection fraction increases with decreasing Hct

### 3 Literature Review

#### 3.1 Evaluation of cardiovascular function

Traditionally most studies of cardiac haemodynamics involved invasive methods using catheterisation, such as the Fick's technique, indicator dye or thermodilution<sup>1</sup>. The thermodilution method is considered the "gold standard" for determining cardiac output (CO)<sup>2</sup>. Radionuclide cardioscintigraphy has also been described in the dog for measuring cardiac function<sup>3</sup>. However, these methods are impractical for clinical use in the conscious animal<sup>4</sup>.

#### 3.2 Echocardiographic evaluation of cardiac function

Echocardiography is readily available, non-invasive and a clinically useful tool to assess cardiac size and function. The echocardiographic evaluation of size, function and haemodynamics of the canine heart has been thoroughly reviewed<sup>5</sup>.

There are several echocardiographically-derived variables available to the clinician when measuring left ventricular systolic performance, including ejection fraction (EF), fractional shortening (FS), CO, and systolic time intervals (STI)<sup>5</sup>.

##### 3.2.1 Ejection fraction

Ejection fraction is calculated as follows:

$$\text{LVEF} = \frac{\text{LVVd} - \text{LVVs}}{\text{LVVd}} \times 100$$

where LVVd is the diastolic and LVVs is the systolic left ventricular volume.

The LVEF has traditionally been used as an index of LV systolic function and is affected by contractility as well loading conditions (preload and afterload) and thus provides a measurement of overall LV function. Ejection fraction is considered a useful clinical variable for assessment of global systolic function<sup>4</sup>, and in people, has

been found to be useful in evaluating the extent of ventricular dysfunction in ischaemic heart disease<sup>6</sup>.

### 3.2.2 Fractional shortening

Fraction shortening is an easily measurable and rapidly acquired derivative calculated from a frozen M-mode cardiac image as follows:

$$FS = \frac{LVIDd - LVIDs}{LVIDd} \times 100$$

where LVIDd is the diastolic and LVIDs is the systolic left ventricular dimension.

The FS may be measured from short or long axis views<sup>7</sup>. Similar to EF (the FS is essentially the one dimensional equivalent thereof), FS is dependant on contractility and loading conditions.

### 3.2.3 Left ventricular volumetrics

A fair correlation (0.87 compared to the thermodilution technique) has been found when calculating cardiac output from LV volumes derived from using the Teicholz method in M-mode in the transverse (short –axis) plane in anaesthetised dogs<sup>3</sup>. This method relies on mathematical assumptions made from measuring the width of the left ventricle in one dimension. However this method is highly dependent on the exact location in the ventricle where the measurement is made, and small errors are compounded by the Teicholz formula<sup>8</sup>. This is especially true if the ventricle is asymmetrical. In humans The American Society of Echocardiography (ASE) recommends two-dimensional (2D) methods for LV volumetry using the modified Simpson's rule (or summated-disc method) for calculating LV volumes<sup>9</sup>. In dogs an alternative reportedly accurate method of volumetry is the bullet method<sup>3</sup>. The bullet method assumes a bullet-shaped LV chamber with a wider base that tapers to a rounded apex and is performed as follows:

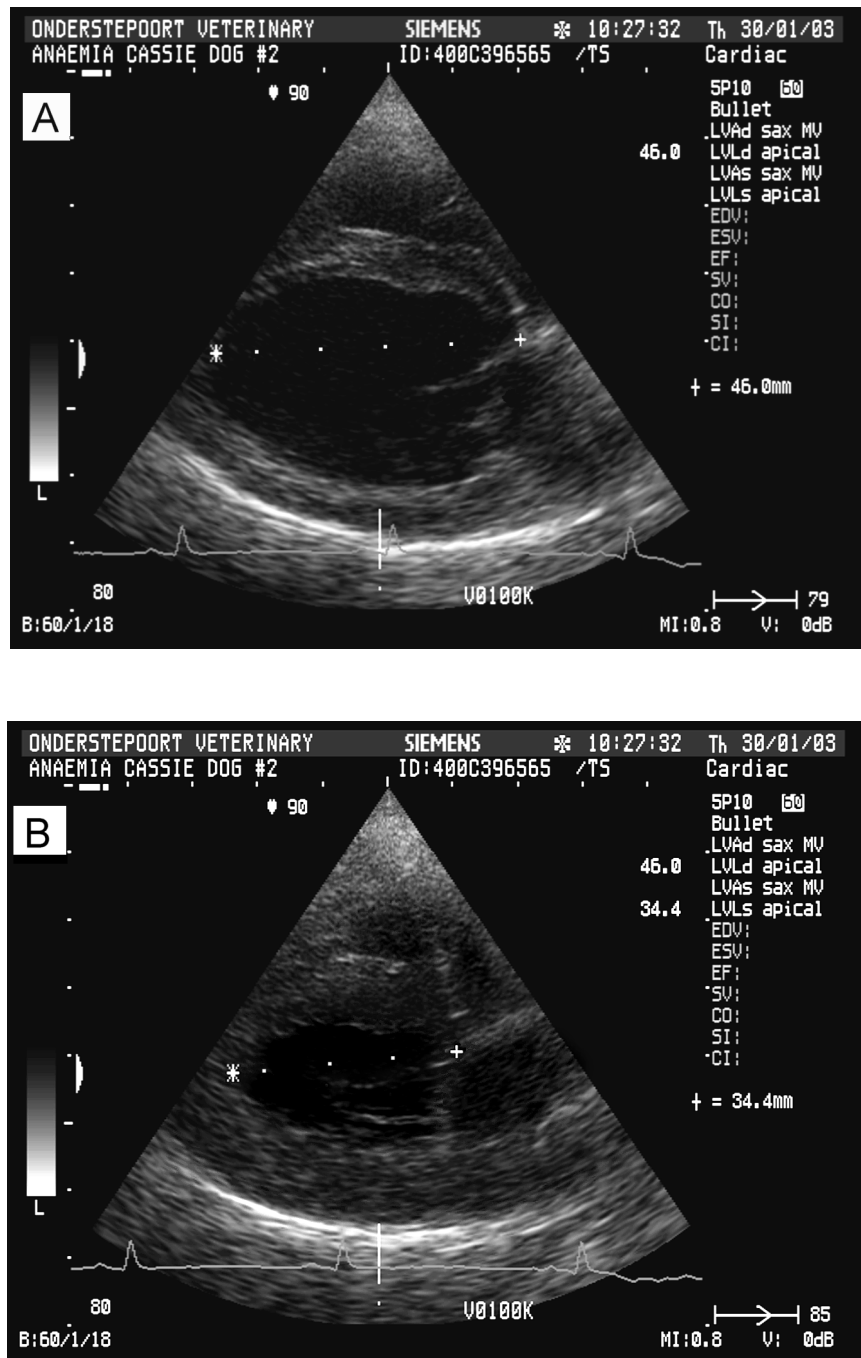
The right parasternal long axis view is used to measure the length of the LV chamber from the apex to the mitral-aortic junction in systole and diastole (Fig. 1 A and B) . Right parasternal short axis view at the level of the chordae tendinae is then used to trace the LV chamber circumference in systole and diastole (Fig. 1 B and C). The ultrasound machine then calculates the systolic and diastolic chamber volumes respectively by the following formula<sup>3</sup>:

$$\text{LV Volume} = 5/6 \times \text{Area} \times \text{Length} = 5/6AL$$

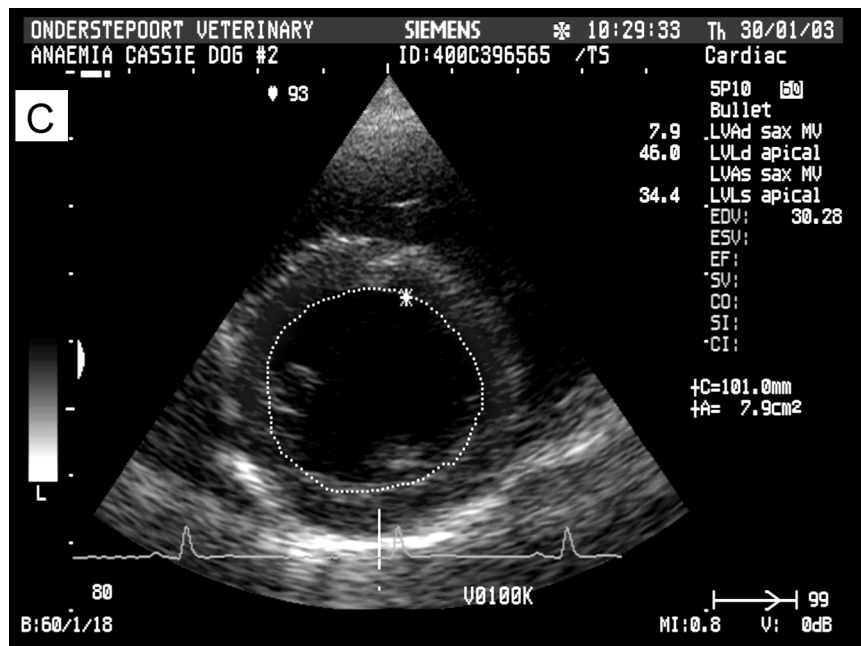
A study in healthy dogs using the bullet method for volume determination found a good correlation (0.87) between echocardiographically determined ejection fractions in anaesthetised dogs and gated equilibrium radionuclide ventriculography<sup>3</sup>. The same study found the same correlation (0.87) between the bullet and thermodilution methods in determining cardiac output<sup>3</sup>. Another study in healthy dogs compared bullet formula-derived echocardiographic volumes with post mortem values and found a very good correlation (0.97)<sup>10</sup>. Another study in healthy dogs compared three-dimensional (3D) ultrasonographic techniques with 2D techniques (i.e. the modified Simpson's method and bullet method) for measuring LV volumes<sup>11</sup>. The study concluded that 3D techniques were more accurate for determining LV volumes, SV and EF than the 2D techniques (when compared to more accurate invasive methods), especially when the ventricles were more asymmetrical. The accuracy of the bullet and modified Simpson's methods were fairly similar in this study (ejection fraction: error = 7.7% ± 8.5%, and 6.8% ± 12.3% by bullet and modified Simpson's methods respectively). From this limited study, it appears that in dogs, the variability of EF derived by the Bullet methods is less than the Simpson's rule derived EF values.

An alternative method of measuring SV by using spectral Doppler is described for dogs<sup>4</sup>. Here the angle-corrected time velocity integral (TVI) of the aortic systolic flow is multiplied by the cross sectional area of the aortic annulus. A good correlation (0.93) was found when comparing the Doppler method to thermodilution when measuring cardiac output.





**Figure 1.** Long axis lengths of the left ventricle in end diastole (A) and end systole (B) for the bullet method. The cursor on the ECG tracing shows the moment in the cardiac cycle where the image was frozen at the maximum (A) and minimum (B) ventricular areas.



**Figure 1 (continued).** Short axis areas of the left ventricle in end diastole (C) and end systole (D) for the bullet method. The cursor on the ECG tracing shows the moment in the cardiac cycle where the image was frozen at the maximum (A) and minimum (B) ventricular areas. Once all four dimensions are entered, the derivatives (EDV, ESV, EF, SV, CO, SI and CI) are automatically calculated.

### 3.2.4 Cardiac output

Cardiac output is calculated as follows:

$$\mathbf{CO = SV \times HR}$$

where SV is stroke volume and HR is heart rate. Stroke volume is calculated as follows:

$$\mathbf{SV = LVVd - LVVs}$$

The CO is dependent on afterload, preload and heart rate.

### 3.2.5 Cardiac index and stroke index

Cardiac index (CI) is CO divided by the animal's body surface area (BSA). The body surface area (in metres squared) may be calculated from the body mass (in kilograms) by the following formula<sup>12</sup>:

$$\mathbf{BSA = \frac{Mass^{2/3} \times 10.1}{100}}$$

The stroke index is calculated by dividing the SV by the BSA.

### 3.2.6 End systolic volume index

End systolic volume index (ESVI) is calculated by dividing LVVs by the animals BSA. The ESVI has been used to assess dogs with mitral insufficiency, and found it to be a useful index in grading the degree of myocardial failure<sup>13</sup>. According to this study, normal dogs have an ESVI of less than 30ml/m<sup>2</sup>.

### 3.2.7 Systolic time intervals

Systolic time intervals (STI) are measured from M-mode (Fig. 2) or spectral Doppler mode (Fig. 3) echocardiographic measurements of the aorta and left

ventricular chamber with a concurrent electrocardiogram (ECG)<sup>14</sup>. They are reportedly useful indicators of overall cardiac systolic function in humans<sup>15;16</sup> and dogs<sup>17-20</sup> and are not subject to geometrical assumptions of chamber size or irregularities in ventricular wall motion. The following STIs are used:

Left ventricular ejection time (**LVET**)

Left ventricular pre-ejection period (**PEP**)

Velocity of circumferential shortening (**Vcf**)

Left ventricular ejection time to pre-ejection period ratio (**LVET/PEP**)

The LVET is measured from the time the aortic valve opens to the time it closes. The PEP is measured from the start of the QRS complex (on the ECG) to the opening of the aortic valve, and corresponds with isovolumic contraction phase of the cardiac cycle. The Vcf incorporates the ejection time into the fractional shortening equation (see above) and is calculated as follows:

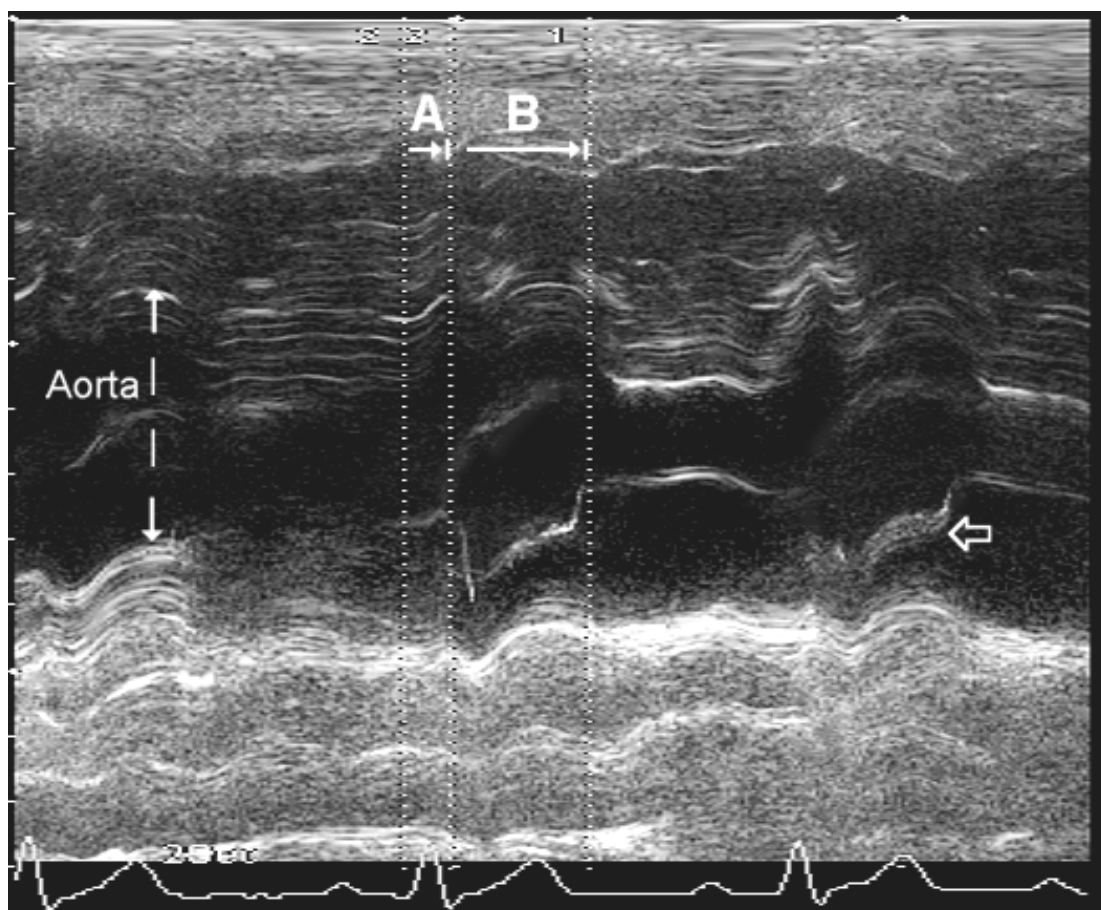
$$\mathbf{Vcf} = \frac{\mathbf{LVd} - \mathbf{LVs}}{\mathbf{LVd} \times \mathbf{LVET}}$$

The LVET is inversely related to heart rate. This effect may be minimised by normalising the interval. The heart-rate-corrected LVET (also called left ventricular ejection time index or LVETI) is calculated thus:

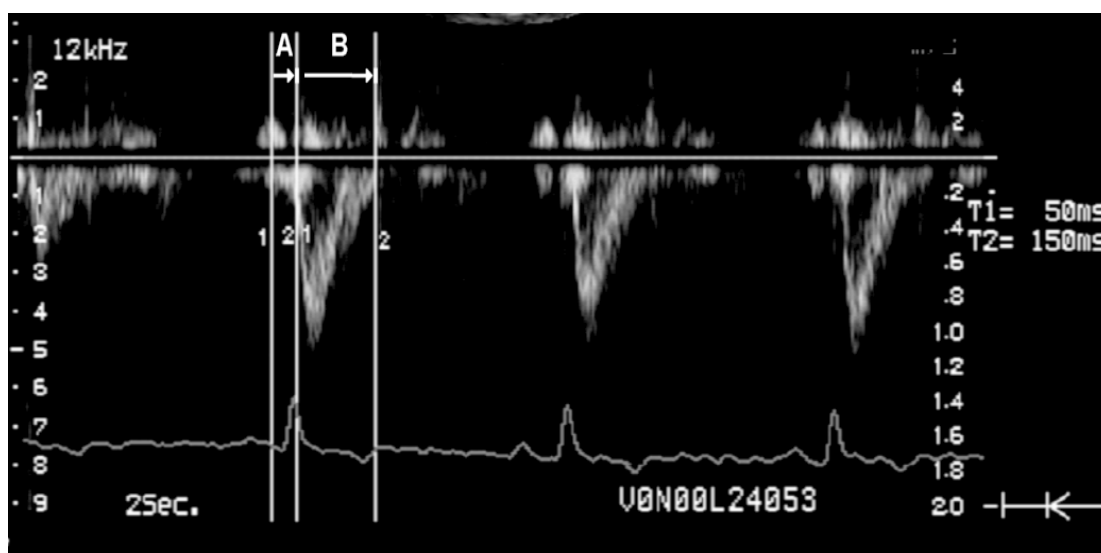
$$\mathbf{LVETI} = \mathbf{LVET} + (\mathbf{0.55} \times \mathbf{HR})$$

The LVET/PEP is also reportedly relatively independent to the effects of heart rate, and is a sensitive indicator of contractility<sup>17;19</sup>. The PEP is minimally affected by heart rate<sup>15;20;21</sup>

Systolic time intervals are affected by preload, afterload and contractility<sup>5</sup> (Table 1). In people, there is considered to be an excellent correlation between STIs and the peak rate of change of left ventricular pressure (or  $dp/dt_{\max}$ , which is considered the “gold standard” of measuring contractility by intracardiac catheterisation), in patients with cardiac disease, and correlated closely with ejection fraction<sup>15</sup>.



**Figure 2.** Pre-ejection period (A) and left ventricular ejection time (B) measurements made from an M-mode tracing through the aortic valve. The aortic width is between the vertical arrows. The open arrow denotes an open aortic valve leaflet during systole.



**Figure 3.** Pre-ejection period (A) and left ventricular ejection time (B) measurements made from a spectral Doppler tracing of aortic flow, with the sample gate placed just distal to the aortic valve.

Table 1: The effects of preload, afterload and heart rate on STI<sup>5</sup>.

	PEP	LVET	LVET / PEP	Vcf
↑ Preload	-	+	-	me
↓ Preload	+	-	+	me
↑ Afterload	+	+	me	-
↓ Afterload	-	+	-	+
↓ Heart rate	me	me	-	-
↑ Heart rate	me	-	+	+

Increase denoted by “+” ; decrease by “-”; “me” indicates minimal effect

### 3.3 The heart in anaemia

#### 3.3.1 Overview of anaemia

Anaemia is defined as a reduction in the number or volume of erythrocytes or a reduction in the haemoglobin in the blood<sup>22</sup>. Depending on the study, anaemia is defined by any one of these three in the literature, and the units will vary accordingly. For instance, red cell count is measured in number per litre and haemoglobin concentration is measured in grams per litre. Haematocrit (Hct) is the percentage of blood composed of erythrocytes, and may be calculated from the measured mean cell volume (MCV) and red cell count (RCC), the latter being the standard method used by automated cell counters. Alternatively Hct may be measured directly by microhaematocrit centrifuge, and is referred to as packed cell volume (PCV)<sup>22</sup>.

Anaemia is a common presentation in humans and animals. In a joint statement issued by the World Health Organisation (WHO) and the United Nations Children’s Fund (UNICEF) on the WHO website in 2004, the number of anaemic people worldwide was estimated at 2 billion, with approximately 50% of these attributed to iron deficiency<sup>23</sup>. This is seen by the WHO as a major public health problem: not only does iron deficient anaemia have dramatic effects on human health , such as increased maternal and child mortality, but it also has more insidious consequences, such as

retardation in cognitive and physical development in children and reduced work productivity in adults. This document emphasised the multifactorial aetiology of anaemia: these vary from blood loss from trauma; helminth infestations (particularly hookworm and schistosomiasis); toxicosis (such as rodenticide and drug-induced); tuberculosis; HIV/AIDS; hereditary; chronic renal failure; immune mediated and blood parasites such as falciparum malaria in people. The last is very important with 300-500 million clinical cases of malaria occurring each year worldwide with approximately 2 million of these being fatal<sup>24</sup>.

In dogs, immune-mediated haemolytic anaemia, particularly the idiopathic form<sup>25</sup>, is the most common cause except in countries such as South Africa, where canine babesiosis is caused by a particularly virulent strain of parasite (*Babesia canis rossi*). (see 3.3.7) Some workers have highlighted the similar disease mechanisms of canine babesiosis and falciparum malaria, and have proposed canine babesiosis as a useful model for studying the pathophysiology of falciparal malaria<sup>26,27</sup>.

### 3.3.2 Cardiovascular studies in human anaemia

Cardiovascular studies in human anaemia have focused on chronic anaemic disease conditions, such as thalassaemia<sup>28-33</sup>, sickle-cell anaemia<sup>34-39</sup>, anaemia of chronic renal failure<sup>40-42</sup> and iron deficiency anaemia<sup>43;45</sup>. There has also been a large body of work focussing on acute normovolaemic haemodilution (ANH)<sup>45-49</sup>. In humans, ANH is a technique used to eliminate or decrease the need for homologous blood transfusions in surgical patients<sup>48</sup>. The advantages of ANH include avoiding disease transmission and transfusion reactions, and the acquisition of fresh autologous blood for transfusion<sup>48</sup>. Reduction in red blood cell loss during surgery is limited: for example, a patient with a Hct of 40 % who loses a litre of blood loses 400 ml of RBCs. By inducing ANH and reducing the Hct to 25 % preoperatively, the patient will only lose 250 ml of RBCs per litre of blood loss. In practice, ANH has been used extensively in elderly patients and small children, especially for cardiac surgery<sup>48</sup>. Briefly, the technique of ANH involves the removal of blood before surgery and its simultaneous replacement with a crystalloid or colloid.



### 3.3.3 Overview of the haemodynamics during the acute anaemic state

The compensatory mechanisms to avoid hypoxia when total body oxygen delivery is compromised during acute anaemia are: increase in CO; redistribution of blood flow to more vital tissues; and an increase in the whole body oxygen extraction ratio<sup>50</sup>. Increased tissue extraction of oxygen is achieved by shifting the oxyhaemoglobin-dissociation curve to the right (due to increased levels of 2,3-DPG)<sup>51</sup>.

The hyperdynamic cardiovascular response to the anaemic state has been well established. In severe human anaemia (less than 70g/l haemoglobin concentration where the normal haemoglobin concentration is around 140g/l), CO at rest is almost always elevated<sup>51</sup>: increased CO is regarded as the most important compensatory mechanism in maintaining adequate tissue oxygenation, particularly in the face of moderate to severe chronic anaemia where the other mechanisms are already saturated. This hyperkinetic state is exaggerated by exercise<sup>51</sup>. An exaggerated response to exercise is often seen in patients with haemoglobin levels of 100g/l who showed normal CO at rest<sup>51</sup>. Response to exercise is therefore considered a more sensitive indicator of altered blood haemodynamics in anaemia. This was also shown in an echocardiographic study of anaemic children where exercise was found to be useful in unmasking subtle left ventricular dysfunction that was not detected at rest<sup>52</sup>.

An increase in CO may be induced by an increased heart rate or by an increased stroke-volume, or both<sup>53</sup>. Several factors that contribute to the increase in cardiac output during acute anaemia have been studied, including: reduction in blood viscosity<sup>54;55</sup> (see below); sympathetic innervation to the heart<sup>56</sup>; increased myocardial contractility<sup>57</sup>; and reduced afterload (arteriolar dilation)<sup>58;59</sup>. The increased heart rate may occur as a result of a decrease in vagal efferent activity, or an increase in the sympathetic efferent activity, or both<sup>53</sup>. Varat *et al* showed that in human patients with chronic anaemia, the increased CO is mainly due to an increased stroke volume rather than increased heart rate as tachycardia is frequently absent in these patients<sup>51</sup>. Decreased afterload was considered to be the major determinant of increased cardiac output in chronic anaemia<sup>51</sup>.



### 3.3.4 Cardiovascular studies in canine anaemia

A large number of invasive cardiovascular studies of acute anaemia have involved canine models<sup>50;53;54;56;60-65</sup>. An experimental canine normovolaemic anaemia model reported that increased stroke volume was not prevented by beta-adrenergic blockers or cardiac denervation, implying that this mechanism is largely independent of sympathetic control<sup>61</sup>. Another study of experimentally induced, acutely normovolaemic-anaemic dogs however showed that the aortic chemoreceptors did contribute significantly to increased CO<sup>56</sup>. These findings are, at first glance, apparently contradictory.

This complexity was addressed in a study investigating the haemodynamic responses of anaesthetised dogs during acute normovolaemic haemodiluted (Dextran-exchange) anaemia<sup>53</sup>. Experiments were performed on dogs with intact autonomic innervation; dogs with  $\beta$ -adrenergic blockade; dogs with cholinergic blockade; dogs with bilateral vagotomy; and dogs with combined bilateral vagotomy and  $\beta$ -adrenergic blockade. Haemodilution induced an increased CO in all groups. However in dogs with low control heart rate (i.e. dogs with intact autonomic innervation and dogs with  $\beta$ -adrenergic blockade) the increase in CO was almost wholly due to increased heart rate. This is in contrast to the dogs with high control heart rate (i.e. the dogs with cholinergic blockade; dogs with bilateral vagotomy; and dogs with combined bilateral vagotomy and  $\beta$ -adrenergic blockade) where the increase in CO was almost wholly due to an increase in stroke volume. There was no significant difference in response to haemodilution between the dogs with intact autonomic innervation and the dogs with  $\beta$ -adrenergic blockade.

The study concluded that the cardioacceleration in these dogs was probably primarily mediated through the efferent vagus nerves, and that the efferent sympathetic nerves did not make significant contribution to the reflex. The study conceded that other mechanisms, including auto-regulation of the cardiovascular system, could be operative in increasing cardiac output during normovolaemic haemodilution. This is supported by other studies where cardiac output in dogs increased with exercise even after cardiac denervation<sup>66;67</sup>. In another study of circulatory response to acutely induced anaemia in non-anaesthetised dogs, a wide variation in the control heart rates was observed<sup>68</sup>. This was thought to be largely due

to the stress of the experimental procedures, resulting in increased sympathetic and decreased vagal tone<sup>53</sup>.

The effect of ANH on myocardial contractility has also been studied. Extrinsic loading factors (preload and afterload) have a major influence on cardiac contractility: in anaemia preload has been shown to increase as a result of decreased peripheral resistance<sup>69</sup> and afterload to decrease (see below). Previous studies relied on the maximum rate of left ventricular pressure increase ( $dp/dt_{max}$ ) to determine myocardial contractility<sup>70-74</sup> but this index is influenced by pre- and afterload<sup>75</sup>. Habler *et al* measured myocardial contractility during ANH in anaesthetised dogs using end-systolic elastance and preload recruitable stroke work indices (which are load insensitive): a true increase in myocardial contractility during ANH was documented in this study<sup>57</sup>.

### 3.3.5 The role of decreased afterload in acute normovolaemic anaemia

Decreased afterload reduces the impedance of left ventricle ejection, resulting in increased stroke volume. The role of decreased afterload accounting for increased left ventricular stroke volume has been well documented in ANH<sup>57</sup>. Two main mechanisms for the reduced afterload have been studied: decreased peripheral resistance and decreased blood viscosity.

Vascular resistance depends on the calibre of the peripheral vessels and the presence or absence of arteriovenous shunts. A human study found that increased basal levels of nitric oxide (endothelium-derived relaxing factor or EDRF) made an important contribution to lower systemic vascular resistance in chronic severe anaemia<sup>58</sup>. The nitric oxide (NO) molecule is a potent vasodilator. The basal levels of NO are raised in anaemic patients because haemoglobin is a potent inhibitor of NO<sup>58</sup>. A study in rats also evaluated the role of EDRF and neural reflexes in reducing peripheral vascular resistance<sup>59</sup>. The study found that by inhibiting nitric oxide synthase, the enzyme responsible for EDRF synthesis, with the NO synthase inhibitor L-nitroarginine, the vasodilator response to ANH was abolished. The study also found by “pithing” the rats, no change in peripheral vascular resistance was seen, and concluded that neural reflexes do not play a role in modulating peripheral resistance in ANH. Another study evaluated cerebral blood flow patterns in ANH induced

rats<sup>55</sup>. The study found an increased cerebral blood flow with proportional decreased vascular resistance with anaemia, but no concurrent dilation of the plial microvessels. It was concluded that the hyperaemia accompanying haemodilution was therefore largely viscosity mediated.

In a study evaluating the effects of viscosity on CO, significant alterations in blood viscosity were induced in four groups of anaesthetised dogs by exchange transfusions with different blood replacement fluids<sup>54</sup>: blood was exchanged with 70 000 molecular weight Dextran in group I (thus reducing blood viscosity and haematocrit to 18.1% mean), and 500 000 molecular weight Dextran in group II (thus maintaining blood viscosity and reducing Hct to 19.9% mean). Group III received clinical Dextran and red blood cell mixture (thus maintaining blood viscosity and Hct) while group IV received 500 000 Dextran and red blood cell mixture (thus increasing blood viscosity above normal while maintaining Hct). There was a greater significant increase in CO in group I compared to group II (as measured by invasive means). Group III showed no change in CO, while group IV (the hyperviscosity group) showed a significant fall in CO. The study concluded that a change in blood viscosity exerts a significant effect on cardiac output, especially during dextran-exchange anaemia, and is supported by other studies<sup>76;77</sup>.

### 3.3.6 Evaluation of cardiovascular function in anaemia using echocardiography

In humans with anaemia, echocardiography is used as a non-invasive means of evaluating cardiac function, especially left ventricular function<sup>33;52;78-81</sup>. An echocardiographic study of adolescents and young adults with sickle cell anaemia showed an increase in: chamber diameters; LV mass (eccentric hypertrophy); CI (due to increased SV); isovolumic contraction period; and ESVI<sup>81</sup>.

The investigator is not aware of any studies in the literature documenting echocardiographic changes in anaemic dogs, or echocardiographic changes in acute anaemia (less than 14 days).

### 3.3.7 The heart in canine babesiosis

In South Africa the single most important reason for anaemic animals to be presented to veterinarians is due to the haemoprotozoal disease, canine babesiosis and can account for up to 12% of all cases presented to veterinary practices<sup>82</sup>. Many of these patients suffer from severe anaemia necessitating hospitalization and may die if untreated. In a study performed at the Onderstepoort Academic Veterinary Hospital (OVAH), 11.7% of all sick dogs presented were positive for babesiosis, of which 31.4% were admitted for more intensive treatment<sup>83</sup>. Babesiosis cases commonly present in respiratory distress caused by anaemia, acidosis, pulmonary edema, or a combination thereof<sup>84;85</sup>. The pathophysiology of the various presentations of canine babesiosis is still poorly understood<sup>86;87</sup>. Reduced oxygen carrying capacity in anaemia or hypoperfusion (due to hypotension) leading to the development of tissue hypoxia occurs in canine babesiosis, and may result in the genesis of multiple organ dysfunction syndrome (MODS)<sup>85;87</sup>. The most popular view supports hypoperfusion-related hypoxia, with associated systemic inflammatory response syndrome (SIRS), as a possible cause of MODS<sup>86;87</sup>.

Little *in vivo* work has been done on the heart in the pathophysiology of canine babesiosis. One study documented increased cardiac troponin levels in the serum of dogs with severe complicated babesiosis, indicating probable myocardial damage<sup>88</sup>. Another study also showed that dogs with babesiosis had a high prevalence of arrhythmias and histopathological changes (inflammation, haemorrhage and necrosis) to the myocardium<sup>89</sup>. Cardiovascular size and function was not assessed in either of these studies. Recently the cardiac involvement in canine babesiosis was reviewed, with particular reference to the role of cardiac troponins and the histopathological changes to the myocardium, but any information relating to direct cardiac function and size was lacking or highly speculative<sup>90</sup>. The haemodynamic and cardiovascular changes, and their relationship with the concurrent anaemia, in the pathophysiology of babesiosis has not been elucidated.

## 4 Materials and Methods

### 4.1 Overview

This trial was a prospective, interventional, non-terminal, self-controlled experiment. The methodology of the induction of the normovolaemic anaemia is detailed in a recently published study<sup>91</sup> (see Appendix 1). Normovolaemic anaemia was induced in 11 Beagle dogs by removing about 20% of circulating blood volume from the jugular vein 1- 3 times per day over a 3-4 day period until a Hct of 13-17% was obtained. Normovolaemia was maintained by replacing the volume deficit of the red blood cells with Ringer's lactate whilst the blood bag was centrifuged for plasma separation. The separated plasma was then re-infused within an hour of collection. The dogs were allowed to recover from their anaemic state, while monitoring their haematocrits daily. Left ventricular size and performance variables were measured echocardiographically on 4 occasions: before the induction of anaemia (termed "Baseline"), at the lowest haematocrit ("Nadir") and then twice during the recovery phase ("Recovery 1" and "Recovery 2"). The changes in left ventricular variables were then evaluated statistically using repeated measures analysis of variance (RMANOVA) for significant differences between the 4 anaemic groups.

## 4.2 Study design

### 4.2.1 Sample group

One intact male, 3 neutered males and 7 non-pregnant female Beagle dogs in dioestrus, on loan from the Onderstepoort Veterinary Academic Research Unit (OVARU) were used. Nine dogs were 2.5 - 3 years old. The age of the other 2 dogs was uncertain, however, based on their vaccination records, they were less than 3 years.

#### 4.2.1.1 Selection of trial dogs

In order to ascertain their general health status, complete physical, faecal and urine examinations were performed 2-4 weeks before the trial on each dog prior to being included in the study. In addition, peripheral blood smear examinations (for blood parasites), full blood count (FBC), Hct 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, fasting ammonia and bile acids, alanine amino transferase and alkaline phosphatase. All dogs then underwent echocardiography, general abdominal ultrasonography and thoracic radiography. The echocardiographic variables of the sample group were compared to published reference ranges<sup>92</sup>. Any dogs with valvular abnormalities on Doppler examination were excluded. Only dogs in good physical condition and clinically healthy with suitable temperament with the above values and findings within normal limits were included in the trial.

#### 4.2.1.2 Management of trial dogs

All dogs were then dewormed and received a long acting ectoparasiticide. For the active trial period, the dogs were transferred from OVARU and housed at the OVAH in large kennels. They were fed a high protein and calorie commercial dog food. Prior to the first phlebotomy, and before the first (baseline) experimental ultrasonographic

examinations, the physical examination, peripheral blood smear, FBC and Hct were repeated. This point was termed “day 0”. The above biochemical profiles, excluding total calcium, phosphate, urea and bile acids were also repeated and checked for normality. Peripheral blood smears 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 1-4. 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. Once the active phase of the trial was completed, the dogs were returned to OVARU.

#### 4.2.2 Induction of normovolaemic anaemia

The bleeding procedure was a modification of a previously documented method<sup>93</sup> and has been described in detail elsewhere<sup>91</sup> (Appendix 1). Multiple jugular phlebotomies (2 to 3 per day) were performed at least 4 hours apart on a daily basis until an Hct of 13-17% was obtained. About 20% of the estimated blood volume was removed at each phlebotomy, which is considered a safe volume to be removed at one time without inducing shock<sup>94</sup>. The blood was centrifuged immediately after each blood collection to separate the plasma from the packed red cells. Normovolaemia was maintained after each bleed by reinfusing the harvested plasma plus a volume of Ringer’s lactate equal to the volume of packed cells (to account for the volume deficit of the removed packed cells). The exact amount of blood to be withdrawn each day was recalculated daily and depended on the individual animal’s response to the bleeding as monitored by the Hct readings prior to each bleed. Once the predetermined Hct range was reached, dogs were allowed to recover naturally from their anaemia with daily Hct monitoring.

### 4.2.3 Echocardiography

#### 4.2.3.1 Technique

The dogs were shaved with an Oster clipper and no. 40 blade over the heart just caudal to the elbows bilaterally, and scanned in lateral recumbency from the dependent side. Echocardiography was performed by the investigator on the Siemens Omnia Sonoline\* ultrasound machine using a multifrequency (5-6MHz) phased array transducer. A dedicated echocardiogram ECG 3-lead system was attached in the standard manner and a lead 2 electronic tracing was continuously recorded on the ultrasonogram for each scan. For the echocardiograms the dogs were restrained manually without sedation. To avoid excitement-induced tachycardia, care was taken to ensure the dogs were as calm and relaxed as possible. All echocardiographic variables were measured immediately before and after induction of severe, acute anaemia (Hct 14-17%). Measurements were repeated during recovery in moderate chronic (Hct 25-27%) and mild chronic anaemia (Hct 31-37%). These Hct-determined data acquisition ranges were termed “Baseline”, “Nadir”, “Recovery 1” and “Recovery 2” respectively. A full blood count (FBC) was repeated in each Hct range.

#### 4.2.3.2 Evaluation

The left ventricular wall and interventricular septal motion was evaluated subjectively in 2D. The ECG was evaluated continuously during the scan and for heart rate and rhythm. The heart rate (HR) for each variable was calculated by measuring the preceding R-R interval in milliseconds on the ECG tracing. As the R-R intervals varied considerably during the examination of each variable, a series of cardiac cycles was subjectively assessed over the length of the cine loop, and three similar ones were selected from approximately the middle of the R-R range for each variable.

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\* Omnia Sonoline, Siemens Medical Systems Inc, Ultrasound Group, Erlangen Germany



The following measurements were made during M-mode evaluation:

E-point to septal separation (EPSS), end-systolic (LVIDs) and end-diastolic (LVIDd) left ventricular interior chamber diameters; end-systolic (LVPWs) and end-diastolic (LVPWd) left ventricular posterior wall thicknesses; end-systolic (IVSs) and end-diastolic (IVSd) interventricular septum thicknesses; and aortic- to- left atrial (LA) widths and their ratio (Ao:LA). The fractional shortening (FS) was calculated. The M-mode measurements were made in short axis at the level of the aortic valve for the Ao:LA; at the level of the maximum mitral valve leaflet excursion for EPSS; and at the level of the chordae tendinae just below the maximum excursion of the mitral valve leaflets for the other measurements.

The following measurements were made during 2D evaluation:

Right parasternal short axis views at the level of the chordae tendinae were used to trace the LV chamber circumference (and automatically calculate the area) in systole and diastole. Right parasternal long axis views were used to measure LV length from apex to the mitral-aortic junction in systole and diastole. From these measurements the LVVs and LVVd were calculated by means of the bullet method<sup>3</sup> (see 3.2.3). Their derivatives SV, EF, CO and corresponding indices SI, ESVI and CI were calculated.

The systolic time intervals LVET and LVPEP were measured during spectral Doppler evaluation of the aortic flow profile, and their derivatives LVET/PEP, Vcf and LVETI were calculated (see 3.2.7).

All variables were averaged from the three measurements made from the middle of the R-R interval range. All data were saved digitally on the ultrasound machine's hard drive memory, printed on thermal printer, and then transferred onto an Excel®<sup>§</sup> spreadsheet. Each examination was also taped with a high resolution video cassette recorder.

### 4.3 Statistical analysis

After assessing the data for normality, each LV echocardiographic parameter was compared between the 4 Hct groups ("Baseline", "Nadir", "Recovery 1" and "Recovery 2") using repeated measures analysis of variance (RMANOVA). The

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<sup>§</sup> Microsoft Corporation, Redmond , USA

Bonferroni test was then used for multiple between-stage comparisons in order to keep the experimental-wise significance ( $\alpha$ ) level of 0.05. Analyses were done using a statistical software package<sup>†</sup>.

#### **4.4 Ethical considerations**

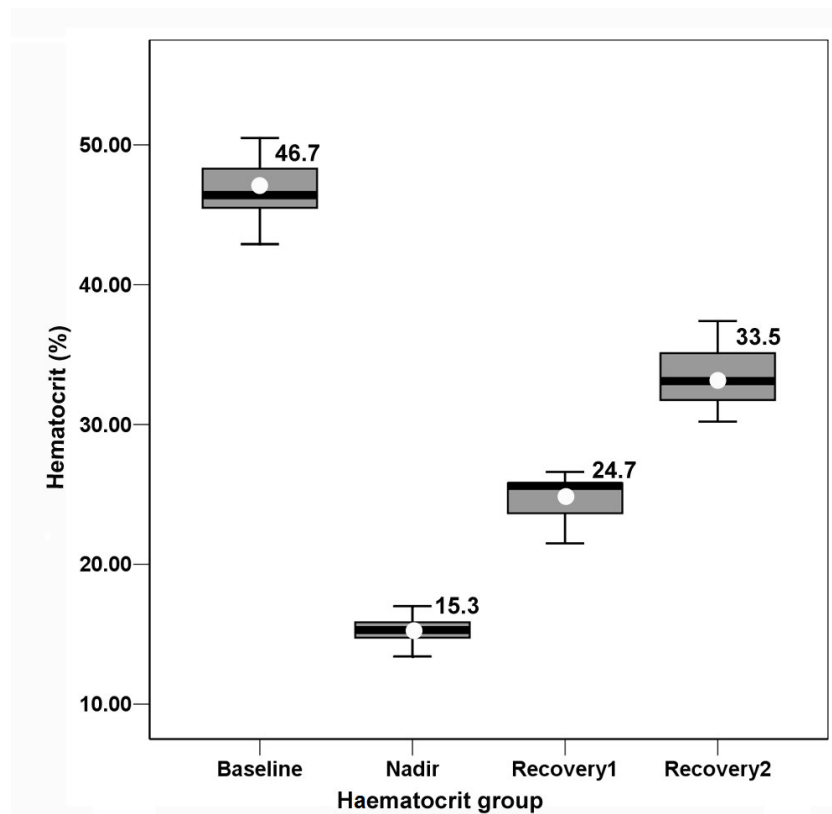
The Animal Use and Care Committee of the Faculty of Veterinary Science, University of Pretoria, approved this study (reference 36-5-562).

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<sup>†</sup> NCSS 2001, NCSS, Kaysville, Utah, U.S.A.

## 5 Results

The body weight mean of the 11 Beagles was 11.9 kg mean, 1.8 standard deviation (SD) with a range of 9.5-15.2. The Hct's were: 46.7% mean (2.4% SD) at Baseline; 15.3 % mean (1.1% SD) at Nadir; 24.7% mean (1.5% SD) at Recovery 1 and 33.5% mean (2.5% SD) at Recovery 2. There were highly statistically significant differences ( $p < 0.001$ ) in Hct between the four stages (Fig. 4). The mean time from Baseline to Nadir (induction of anaemia) was 3 (0.8 SD) days, 5.3 (1.7 SD) days from Nadir to Recovery 1, and 5.4 (SD 1.1) days from Recovery 1 and Recovery 2. The dogs maintained good habitus and appetite throughout the trial and showed minimal adverse effects, even in the profoundly anaemic state. All echocardiograms were completed within 45 minutes.



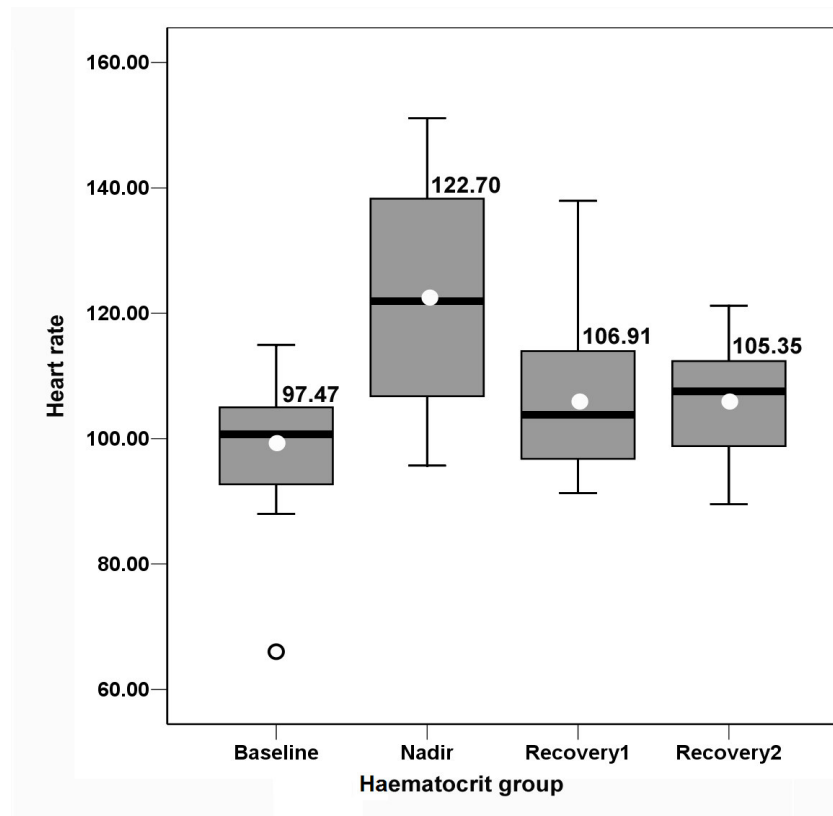
**Figure 4** Haematocrit (%) ranges of Beagles (n = 11) at the four anaemia levels. Box = interquartile range; whiskers = maximum observation beyond lower and upper fence ( $1.5 \times$  interquartile range); bold line = median; circle within box and numerical value = mean.

### 5.1 Subjective 2D assessment

Abnormal myocardial kinesis was not detected in any dogs, and no changes in the echogenicity of the endocardium or myocardium were seen. No arrhythmias were detected on the ECG at any stage of the study.

### 5.2 Heart rate

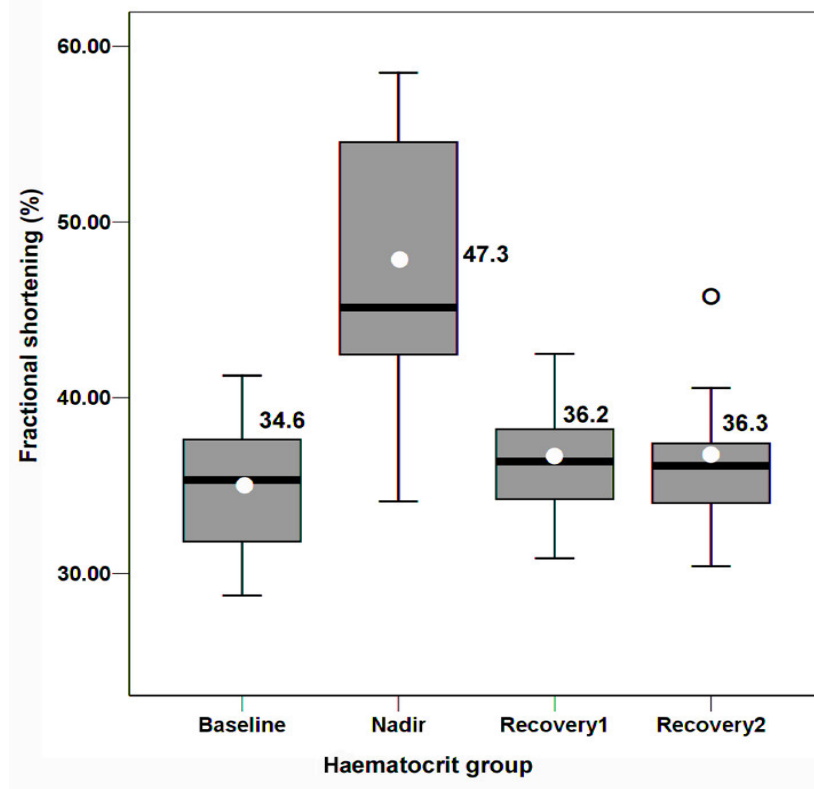
Heart rate varied considerably during the echocardiographic examination. The HR recorded during acquisition of the LV volumes are reported in Fig. 5. Heart rate increased during severe anaemia. There was a highly significant difference ( $p < 0.001$ ) in HR at Nadir vs. the other groups. There were no significant differences between the recorded HR of the Baseline, Recovery 1 and Recovery 2 groups.



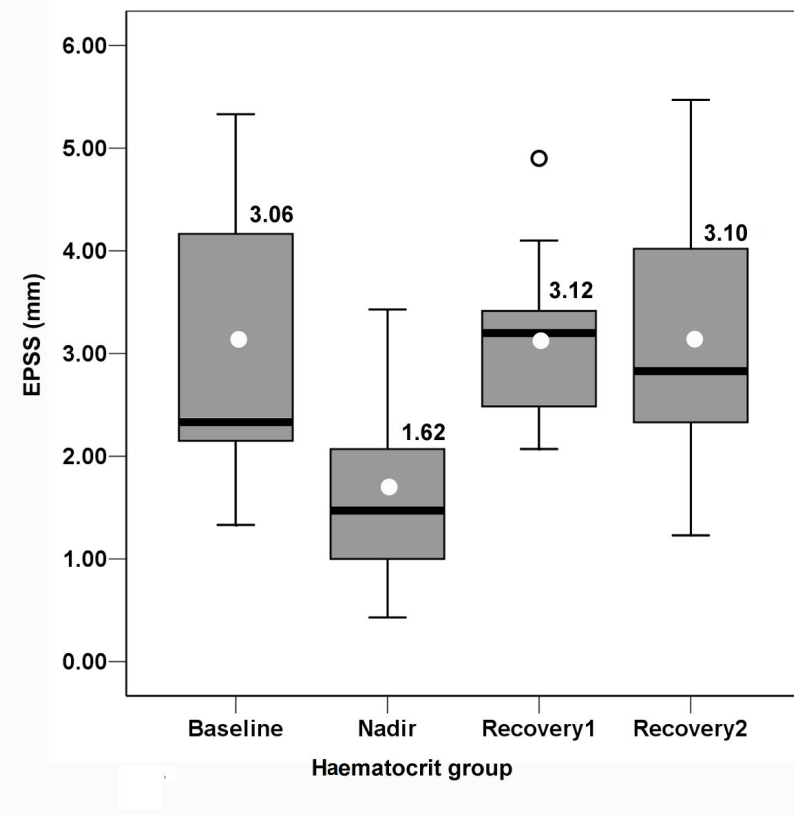
**Figure 5** Heart rate (beats per minute) ranges of Beagles (n = 11) at four anaemia levels. Circle outside box = outlier (observations exceeding the upper and lower fence). See Fig. 4 for key.

### 5.3 M-mode derived parameters

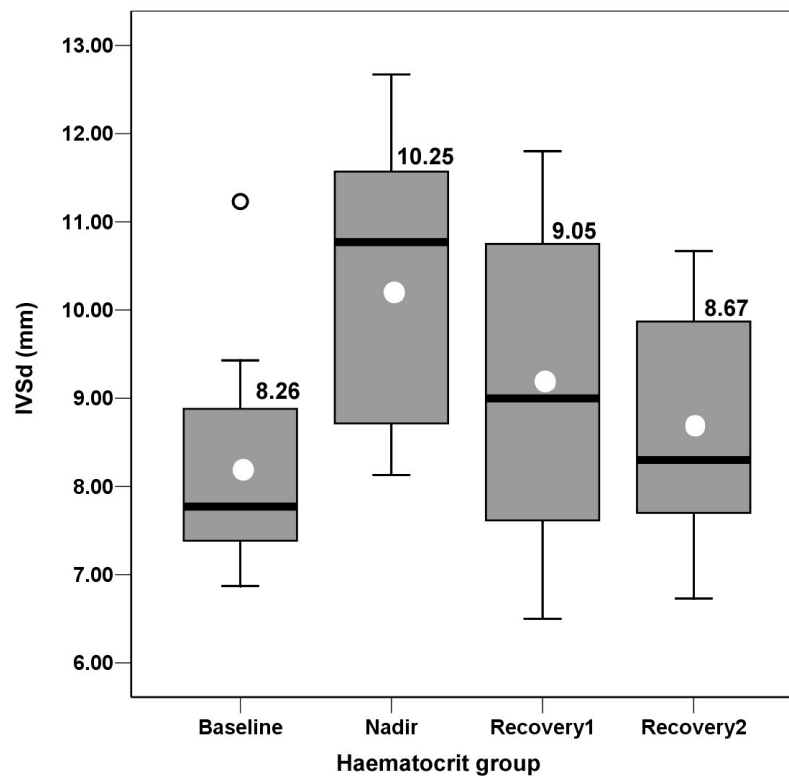
The FS increased and EPSS decreased in severe anaemia (Figs. 6 and 7). There was a highly statistically significant difference ( $p < 0.001$ ) between the FS and EPSS at Nadir vs. the other groups. There were no statistically significant differences between the FS and EPSS of the Baseline, Recovery 1 and Recovery 2 groups. There was no significant difference in the LA width or Ao:LA ratio between groups. The IVSd and IVSs increased in severe anaemia (Figs. 8 and 11), with significant difference ( $p < 0.05$ ) between the IVSd of the Baseline and Nadir groups only, and between the IVSs at Nadir vs. the other groups. There was no significant difference in the LVIDd between groups (Fig. 9). The LVIDs however decreased with severe anaemia (Fig. 12), with significant difference ( $p < 0.05$ ) between LVIDs at Nadir vs. other groups. The LVPWd and LVPWs increased with anaemia (Figs. 10 and 13) with statistically significant difference ( $p < 0.05$ ) between LVPWd of the Baseline group vs. the other groups, and between LVPWs at Nadir vs. the other groups.



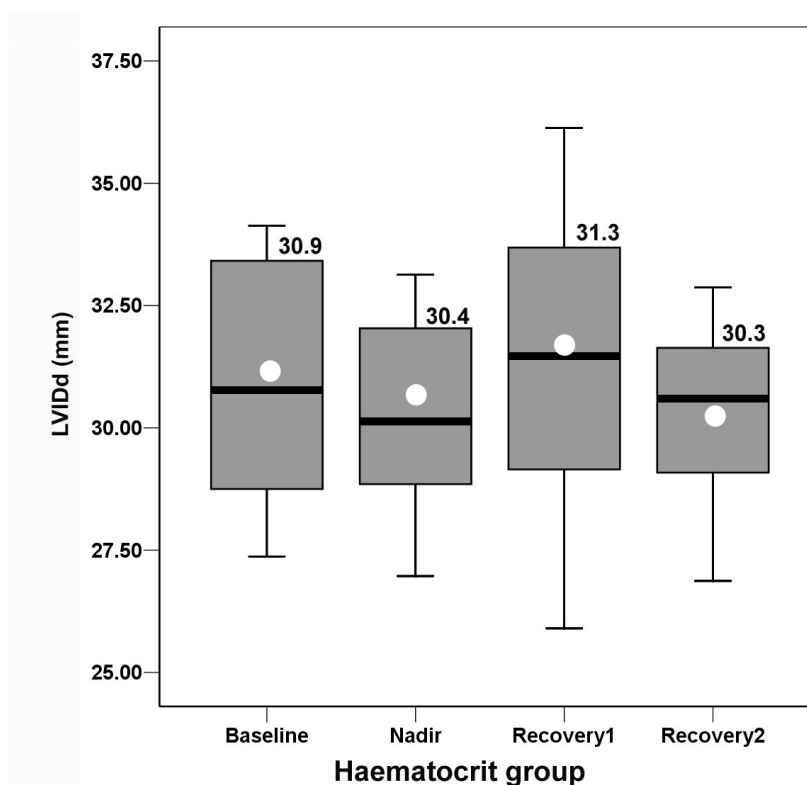
**Figure 6** Fractional shortening (%) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



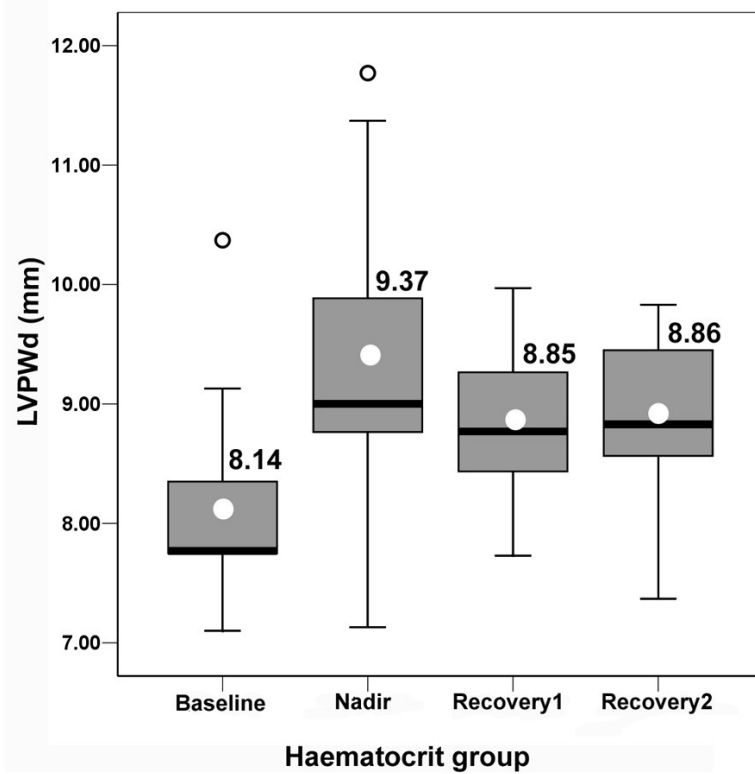
**Figure 7** E-point to septal separation (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



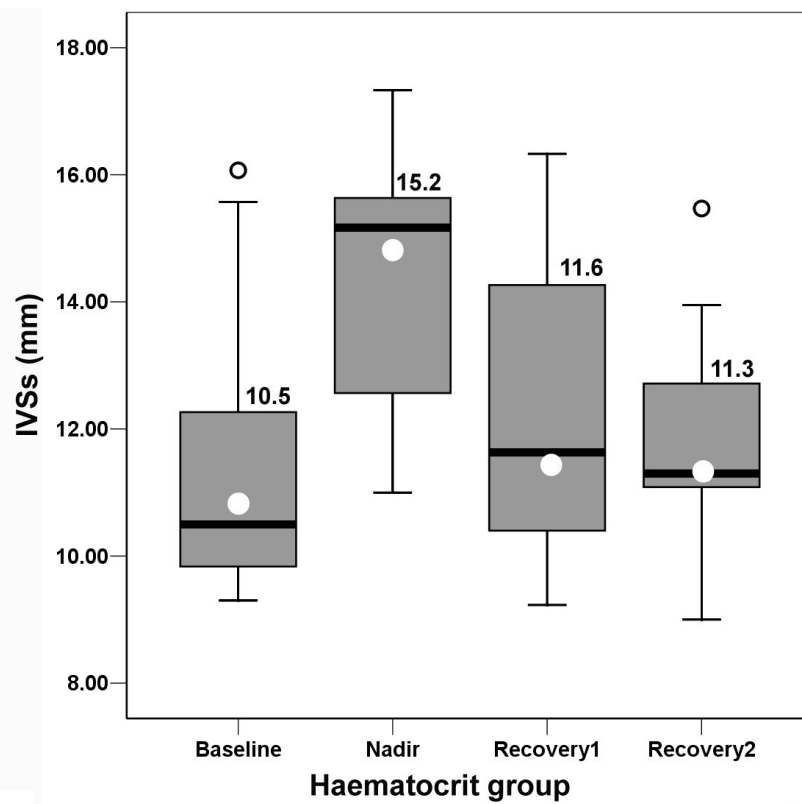
**Figure 8** Interventricular septum in diastole (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



**Figure 9** Left ventricular interior diameter in diastole (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.

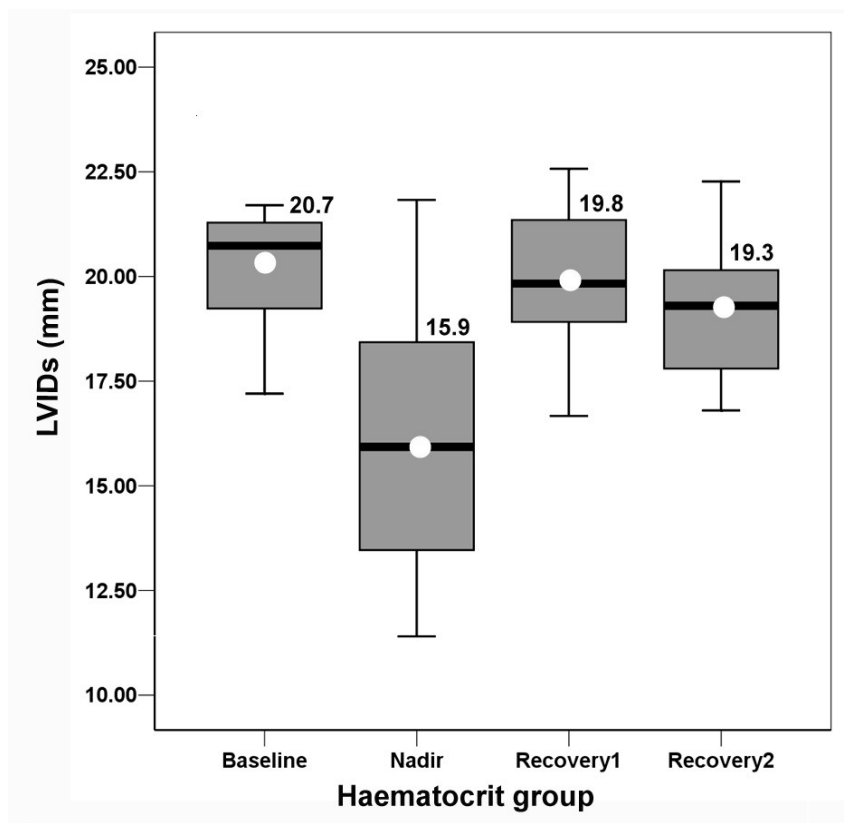


**Figure 10** Left ventricular posterior wall in diastole (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.

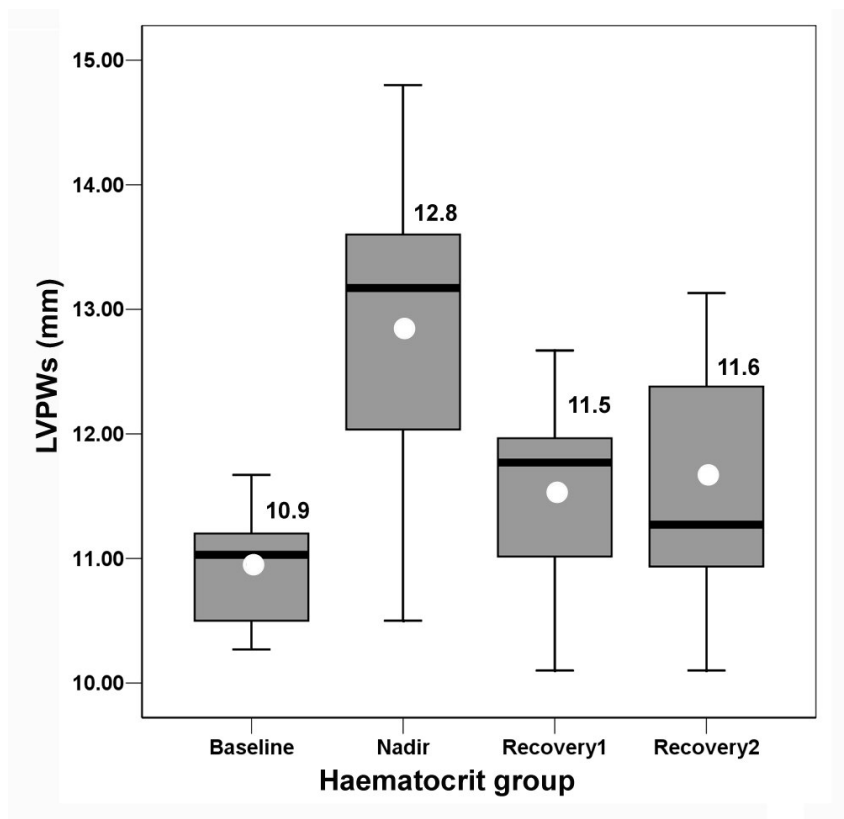


**Figure 11** Interventricular septum in systole (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.





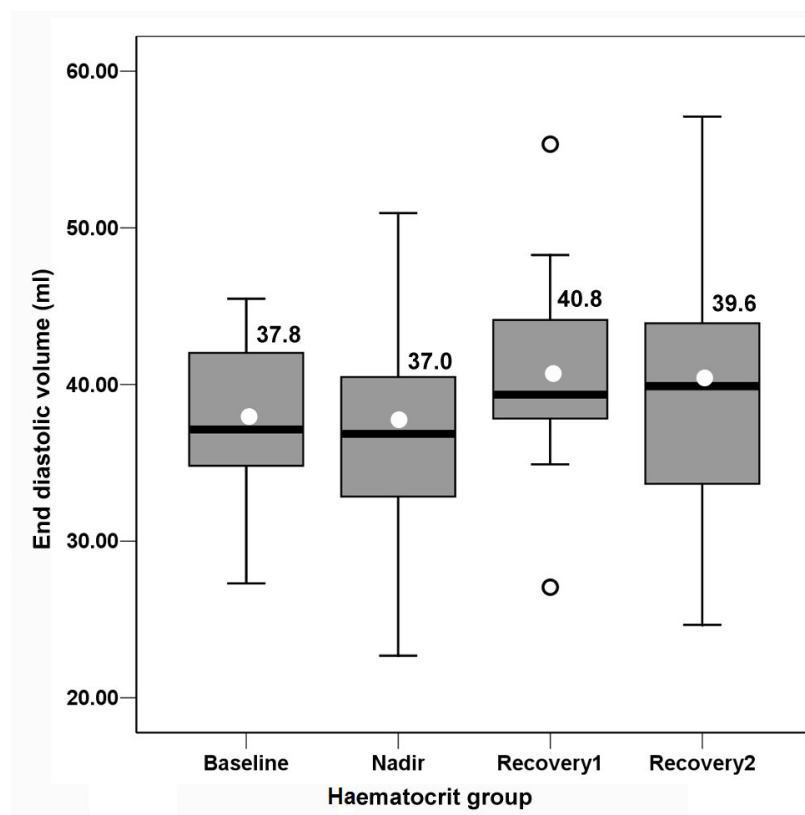
**Figure 12** Left ventricular interior diameter in systole (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



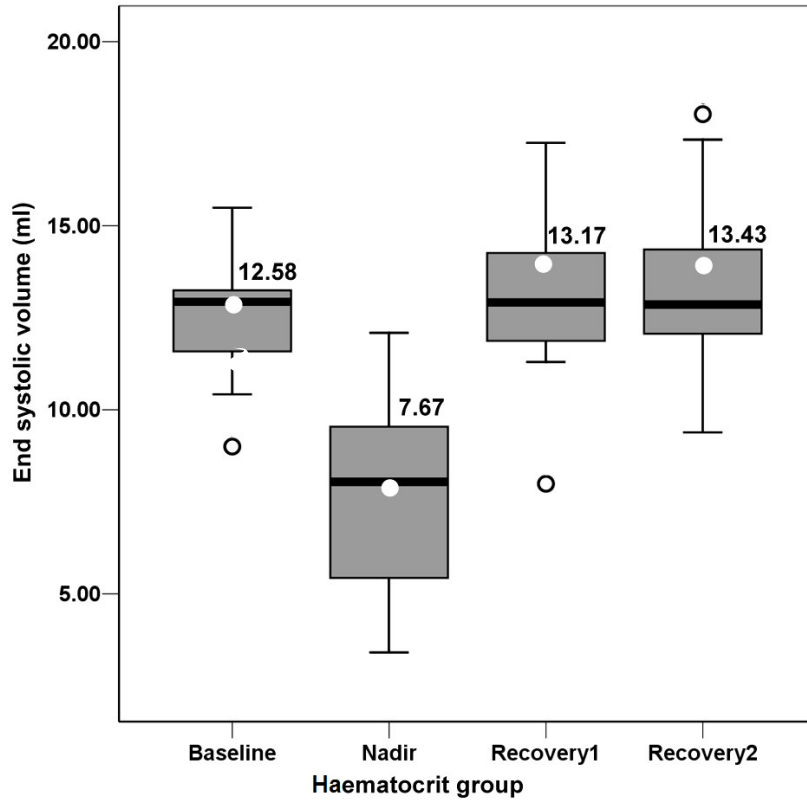
**Figure 13** Left ventricular posterior wall in systole (millimetres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.

#### 5.4 2-D derived parameters

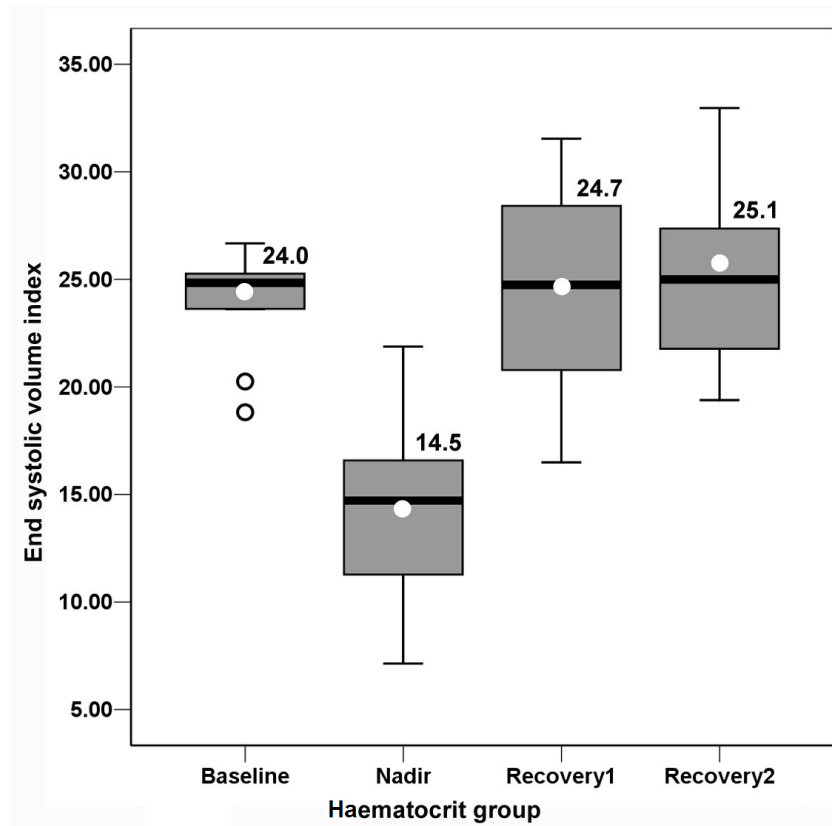
The LV EDV changed minimally in the anaemic state (Fig. 14) with insignificant differences between the four groups. The LV ESV and ESVI decreased while the EF increased with severe anaemia (Figs. 15, 16 and 17). There was a highly significant difference ( $p < 0.001$ ) between the ESV, ESVI and EF at Nadir vs. the other groups. There were no significant differences between the ESV, ESVI and EF of the Baseline, Recovery 1 and Recovery 2 groups. The SV and SI (Fig. 19) increased with anaemia. There was a significant difference ( $p < 0.05$ ) between the SV and SI at Nadir vs. Baseline and Recovery 2, and no significant difference between Nadir and Recovery 1. There were no statistically significant differences between the SV and SI of the Baseline, Recovery 1 and Recovery 2 groups. The CO (Fig. 20) and CI increased with anaemia. There was a highly significant difference ( $p < 0.001$ ) between the CO and CI at Nadir vs. the other groups. The CO and CI at Recovery 1 differed significantly ( $p < 0.05$ ) from CO and CI at Baseline and Nadir. The CO and CI at Baseline did not differ significantly from CO and CI at Recovery 2.



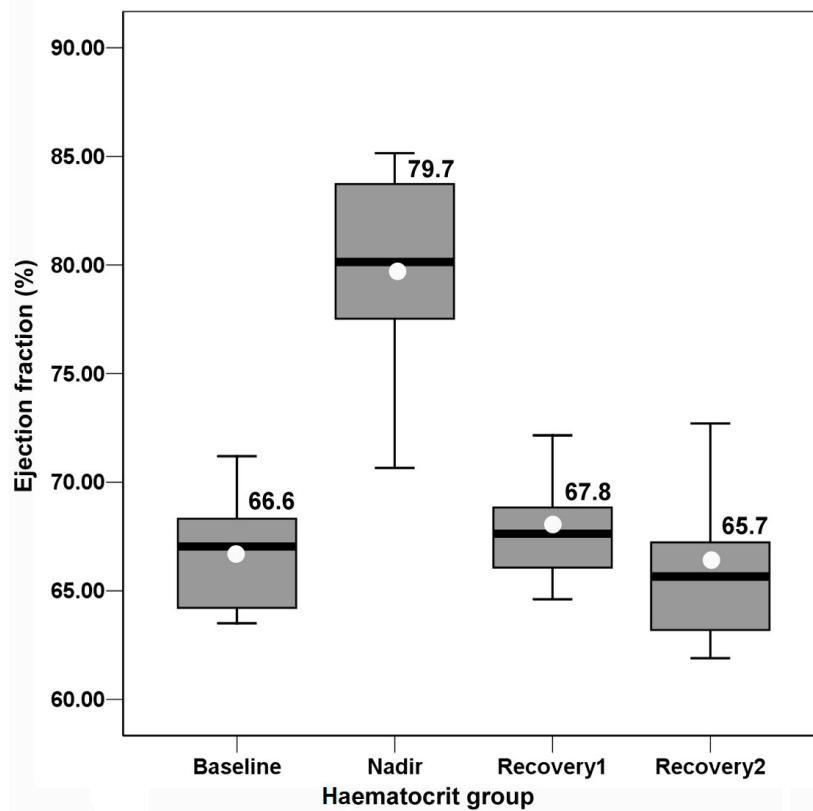
**Figure 14** End diastolic LV volume (millilitres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



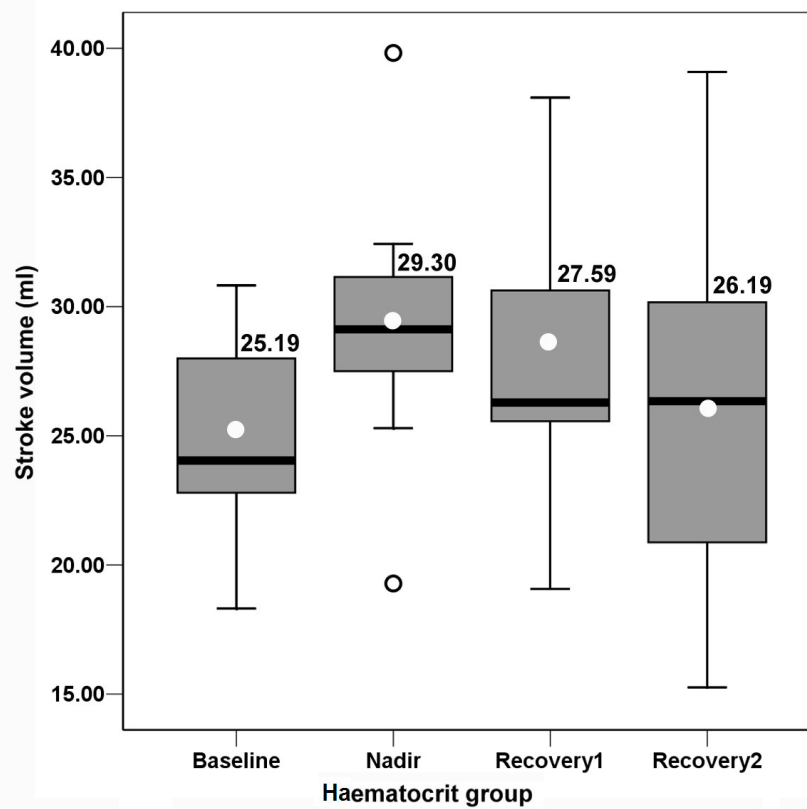
**Figure 15** End systolic LV volume (millilitres) ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



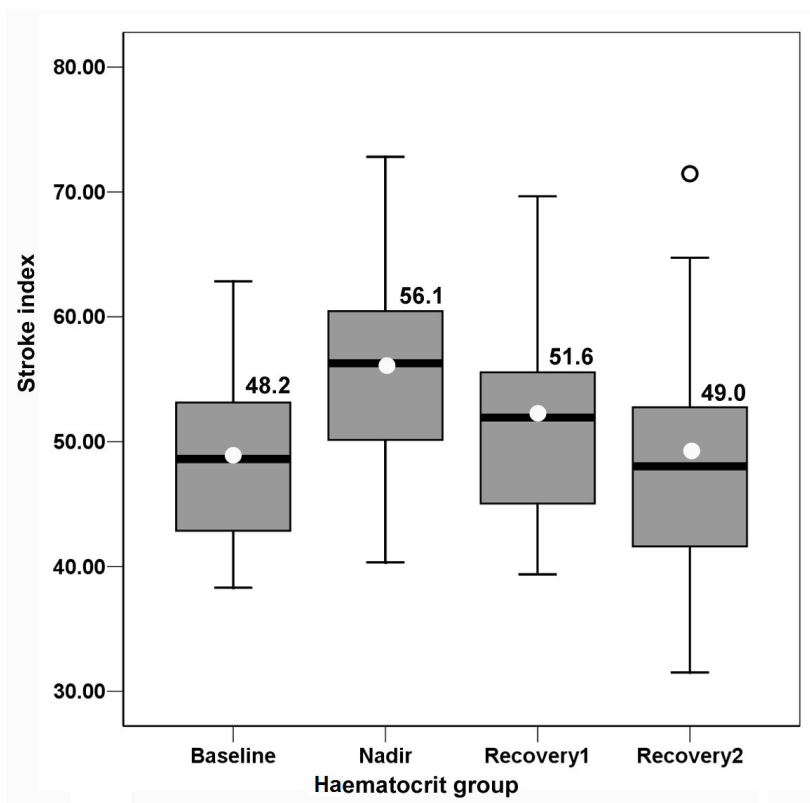
**Figure 16** End systolic volume index ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



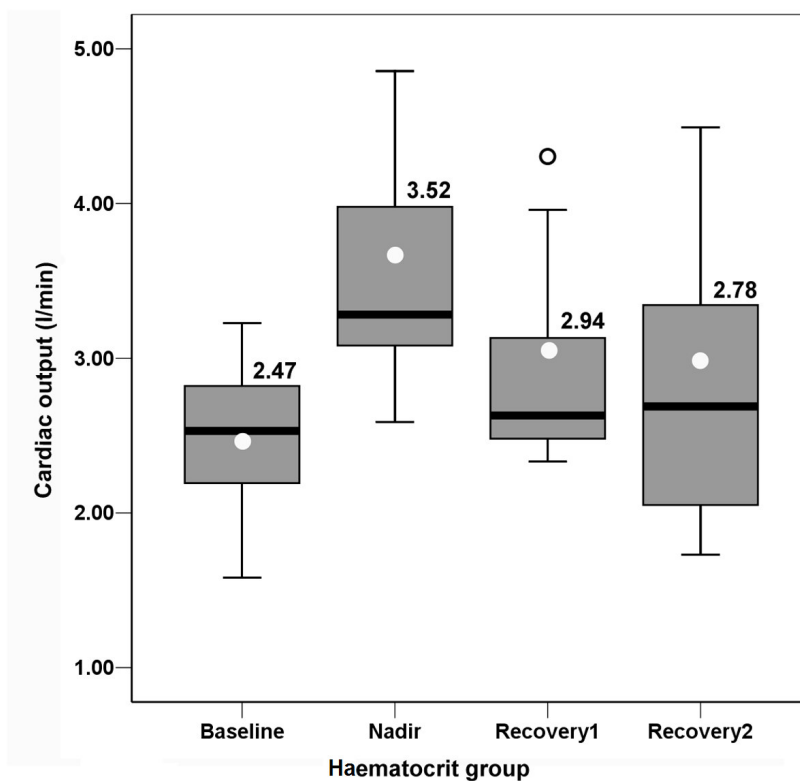
**Figure 17** Left ventricular ejection fraction (%) ranges of Beagles (n = 11) at four anaemia levels. See Fig. 4 for key.



**Figure 18** Left ventricular stroke volume (millilitres) ranges of Beagles (n = 11) at four anaemia levels. See Fig. 4 for key.



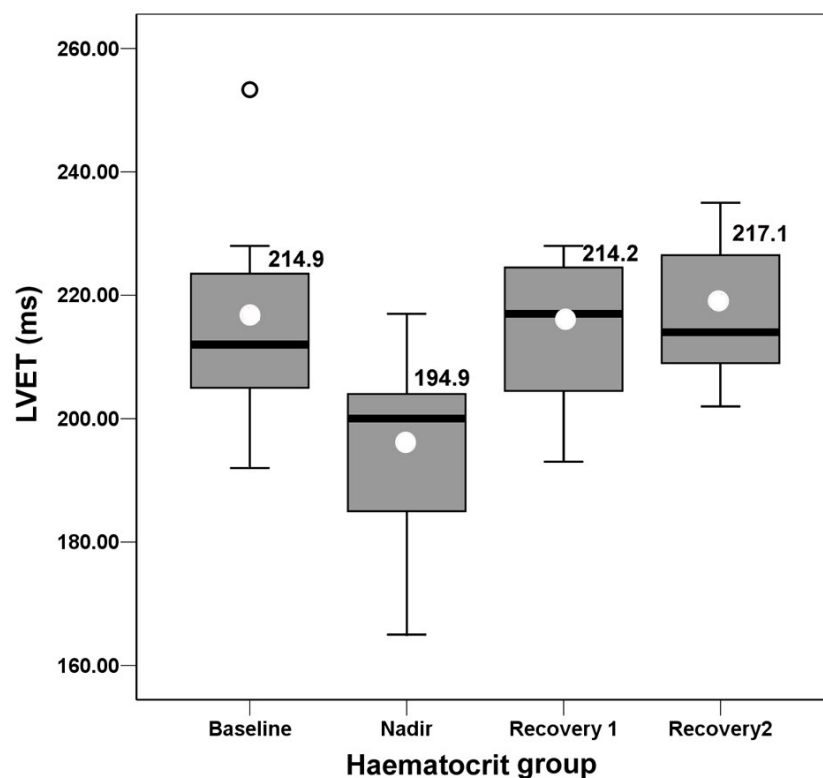
**Figure 19** Stroke index ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



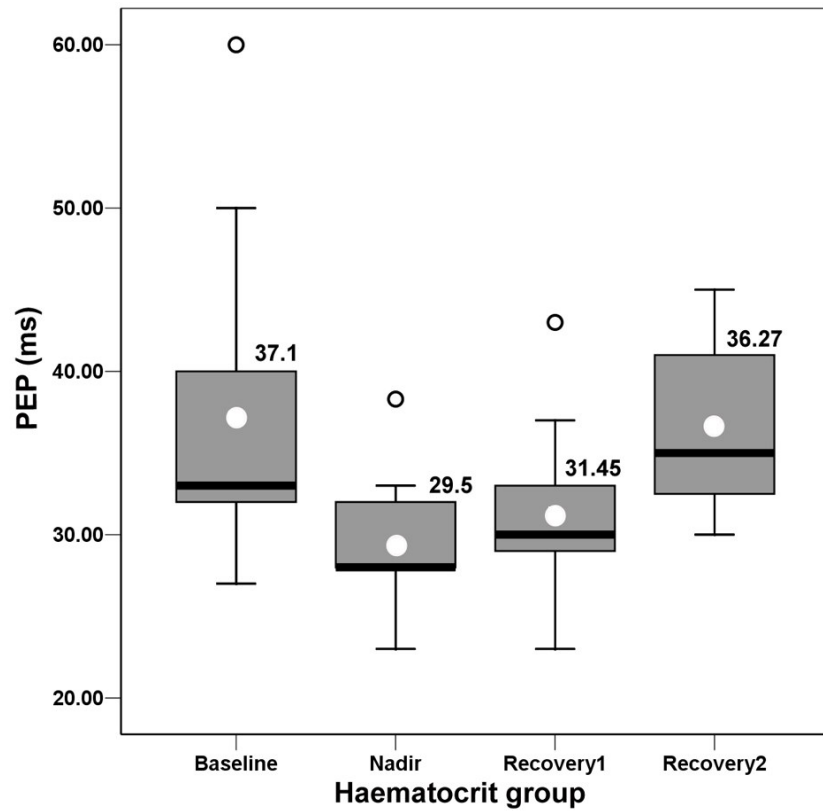
**Figure 20** Cardiac output ranges (litres per minute) of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.

### 5.5 Systolic time intervals

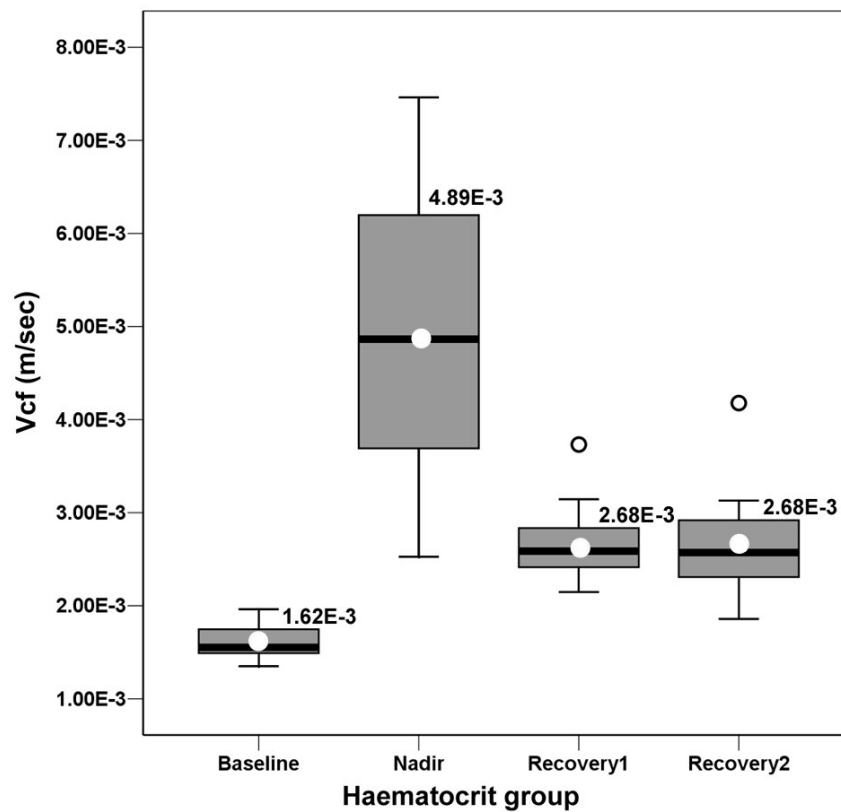
The LVET and PEP decreased in severe anaemia (Figs. 21 and 22). There was a statistically significant difference ( $p < 0.05$ ) between the LVET at Nadir *vs.* the other groups, and between the PEP at Nadir *vs.* the Baseline and Recovery 2 groups. There were no statistically significant differences between the LVET and PEP of the Baseline, Recovery 1 and Recovery 2 groups, or between the PEP of the Nadir or Recovery 1 groups. The Vcf increased in severe anaemia (Fig. 23). There was a highly statistically significant difference ( $p < 0.001$ ) between the Vcf at Nadir *vs.* the other groups. There were no statistically significant differences between the Vcf of the Baseline, Recovery 1 and Recovery 2 groups. There were no statistically significant differences in LVET/PEP or LVETI between the groups (Figs. 24 and 25).



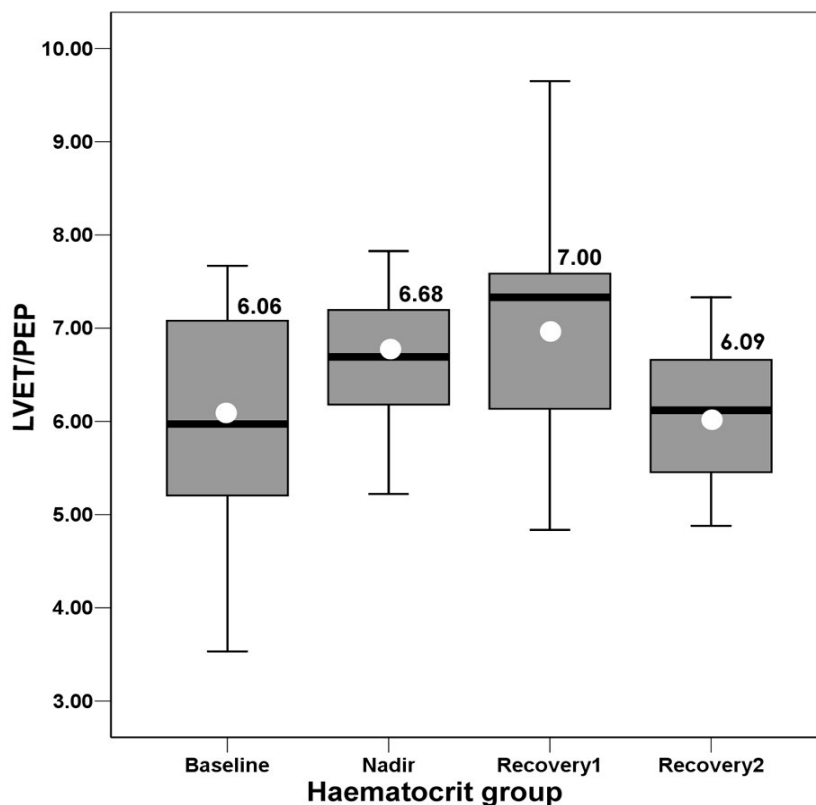
**Figure 21** Left ventricular ejection time ranges (milliseconds) of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



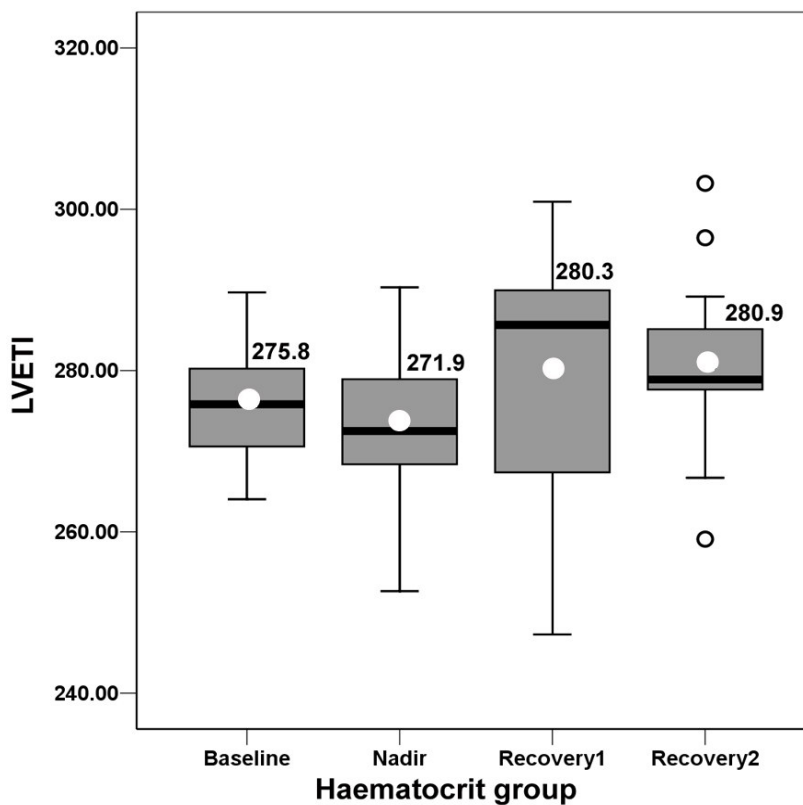
**Figure 22** Left ventricular pre-ejection period ranges (milliseconds) of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



**Figure 23** Velocity of circumferential shortening ranges (meters per second  $\times 10^{-3}$ ) of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



**Figure 24** Left ventricular ejection time/pre-ejection period ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



**Figure 25** Left ventricular ejection time index ranges of Beagles (n = 11) at four anaemia levels. See Figs. 4 and 5 for key.



## 6 Discussion

This study presents original echocardiographic evidence of the hyperdynamic response of the left ventricle to a severe normovolaemic anaemic state in experimental Beagle dogs, and that these changes tend to become less dramatic as the dogs' anaemic state improves. A parallel study evaluating the effect of anaemia on duplex Doppler parameters of abdominal aortic and splanchnic blood flows was conducted on the same animals, and revealed the hyperdynamic circulation in the abdominal aorta and splanchnic vessels in these dogs<sup>95</sup>. The findings in our non-invasive study are in accordance with numerous canine studies of acute normovolaemic anaemia in the human literature, where invasive techniques were used to document increased cardiac performance in anaesthetised dogs<sup>52;54;56;57;60;61;63;65;74;96;97</sup>.

In our study of conscious dogs, where no attempt was made to control heart rate, increased CO resulted from a combination of increased stroke volume and heart rate. The mean heart rates however varied little between dogs within each anaemia level group; this may have been due to the acclimatisation period that familiarised the animals with their surroundings, the non-invasive research procedures and the researchers. Preload has been shown to increase in anaemia as a result of decreased peripheral resistance<sup>69</sup>. No attempt was made to directly measure preload in this study. The increased stroke volume documented in the anaemic state was almost wholly as a result of decreased ESV rather than increased EDV, which remained essentially unchanged throughout the study. The LA size also remained unchanged between groups however the M-mode method of measuring LA size employed in this study has been shown to be less sensitive in detecting LA enlargement compared to other methods<sup>98</sup>, and more subtle changes in LA size may have been missed. Although increased dimensions of the LV and LA are seen in conditions of chronically increased preload, it is doubtful whether any changes in preload in the acute anaemic state in this experiment would have resulted in appreciable enlargement of these chambers. The decreased ESV could be as a result of decreased afterload, increased myocardial contractility or a combination thereof.

Decreased afterload is considered the major determinant of increased cardiac output in chronic anaemia<sup>51;57</sup>. Although blood viscosity was not measured in our study, the plasma-replacement method of inducing normovolaemic haemodilution

anaemia would also have significantly lowered the blood viscosity, thereby reducing the afterload. The presumed decreased afterload also may account for the decreased mean PEP as this would result in more rapid opening of the aortic valve against a lower aortic pressure, shortening the isovolumic contraction time. Decreased afterload should result in an increased mean LVET<sup>14</sup>, but in this study a significantly shortened LVET was documented in the severe anaemic state. This could be due to the increased heart rate which would have shortened LVET, overriding the effect of the decreased afterload. It is interesting to note that the LVET/PEP did not differ significantly between groups: this index is reportedly relatively unaffected by heart rate<sup>14;19</sup>, and suggests that the overriding influence on PEP and LVET in this study was probably heart rate. Similarly, the LVETI which compensates for changes in heart rate, was not significantly different between groups. The Vcf was markedly increased in the severe anaemic state, most likely due to the combined effect of decreased afterload and increased heart rate, both of which increase the velocity of contraction. Although myocardial contractility was not directly measured (as this involves more invasive methods<sup>57</sup>), increased contractility independent of loading factors has been documented in ANH<sup>57</sup>, and would also most likely account at least in part for the increased Vcf, FS, EF and their derivatives documented in this study.

There are several limitations of this study. Owing to manpower constraints a single observer (the author) acquired all data. This observer was not blinded to the stage of anaemia of the dogs during data acquisition, so observer bias was not accounted for. One study has shown that there is significant intraobserver and interobserver measurement variability in echocardiographic parameters with serial scans of dogs<sup>99</sup>. That study showed that coefficients of variation (CV) for all parameters measured (including Doppler, 2D volumetric, and M-mode parameters) ranged from 5.03 to 46.43%, but most were less than 20%. In general, least variation was found for the intraobserver category and the best reproducibility was for M-mode and left ventricular volumetric data (Simpson's method). That study suggested that differences greater than 20% for serial scans must be achieved to document genuine change (although the specific data should be consulted); and that variability and reproducibility are improved if a single experienced operator/observer acquires and measures serial scans. When compared to specific data in the above paper, most parameters in this study displayed changes exceeded the CV reported in that study.

For instance, in this study the percentage mean difference in the FS between the Baseline and Nadir was 26.6% versus 17.3% CV in the intraobserver category in that study<sup>99</sup>. This study utilised one observer for all echocardiograms, thereby eliminating the possibility of greater variation between observers, but at the expense of single observer bias,

Owing to financial constraints there was no control group and it was elected to use each dog as its own historical control. Although a separate control group would have been ideal, care was taken to maintain a constant environment and minimize the effects of the experimental intervention. For instance, the dogs were habituated to the scanning process and were housed and maintained in their familiar environment during the duration of the study. Nonetheless, by not implementing a control group, the impact of the actual echocardiographic procedure on the dogs cannot be assessed and accounted for. For instance, the stress of having been subjected to serial phlebotomies in the days preceding the Nadir echocardiogram could have resulted in a sympathetic response to being handled again for the echocardiographic procedure, most easily appreciated by an increased heart rate. However when the heart rates recorded during the echocardiograms were compared to the resting heart rates (monitored routinely every morning before the scans as part of their general health assessment), there was little appreciable difference in the average rates at these times.

Heart rate is a particularly tricky complex variable to assess, and has a profound effect on all other cardiac variables and derivatives: not only does it vary in the normal resting animal with respiration (sinus arrhythmia) from one beat to the next, but any change in sympathetic tone results in immediate and often marked fluctuations. Accounting for fluctuation in heart rate was a major challenge in this study. In an attempt to diminish the effects of moment-by-moment heart rate fluctuations, it was elected to take three measurements of each parameter from the middle range of R-R intervals selected from a run of beats on the ECG while keeping the dog as calm as possible, and then average them. We considered three sample points per parameter as the minimum number, and although more sample points may have provided a more homogenous R-R interval sample group, time constraints precluded this.

Continuous recording of heart rate by a Holter device or telemetric ECG device would have allowed detailed analysis of the effect of anaemia on the cardiac rate and

rhythm over a 24 hour period. A greater fluctuation (probably a relative increase) in heart rate during the data sampling period (echocardiograms) would then have been clearly appreciated. Several Holter monitors would have been needed for this study at any one time, and these were not available when the study was performed.

Blood pressure was not measured in this study. Blood pressure is most commonly measured non-invasively by oscillometric devices: such a device (Dinamap) is available at the OVAH, and has been used in a study that documented hypotension in dogs suffering from severe babesiosis<sup>100</sup>. However the accuracy of oscillometric devices has been questioned. One human study showed a variance of +40% to -29% compared to direct arterial measurements and concluded that oscillometric devices should not be used when a high degree of accuracy is needed to measure blood pressure<sup>101</sup>. Another study found that only 2 out of 5 oscillometric devices registered for hospital use passed the British Hypertension Society guidelines, and only one of which received an A/A rating<sup>102</sup>. Few devices have been tested in veterinary medicine: one study showed that only 59% of dogs' systolic blood pressure readings measured by oscillometry were within 20mmHg of arterial pressure when measured by direct arterial puncture<sup>103</sup>. Another study found that a commercially available oscillometric device to be reasonably accurate at lower blood pressures, but the error increased with increasing blood pressures<sup>104</sup>. Errors in oscillometric blood pressure readings may be due to a number of factors, such as cuff size and position, movement of the animal during measurements and restraint methods, and the algorithm of detection. Based on the questionable accuracy of oscillometric measurements, as well as the time constraints imposed on by the study design, we elected not to perform blood pressure measurements for this study. At the time that this study was performed, Doppler blood pressure measuring equipment was not available at the OVAH.

Despite there being evidence of cardiovascular disease in canine babesiosis<sup>7,8</sup>, any cardiovascular size and functional changes in canine babesiosis are yet to be investigated. In a recent review article of the cardiac involvement in canine babesiosis, data relating to cardiac performance was totally lacking<sup>88</sup>. Echocardiography has been accepted as a rapid, widely available, inexpensive, non-invasive and reasonably accurate technique of assessing cardiac function in people<sup>105-108</sup> and dogs<sup>3;5;18;109-115</sup> but until now any prospective echocardiographic studies in

anaemic dogs have been lacking. Echocardiography is particularly suitable in the clinical setting, especially in compromised patients where more invasive techniques may not be safe or practical. This study demonstrates that rapid, non-invasive evaluation of the left ventricular size and function in the conscious normovolaemic anaemic dog is practical. This is important in our local setting, particularly applicable in cardiac evaluation of canine babesiosis. It is envisioned that this study will provide baseline information for comparison with echocardiographic studies in babesiosis-induced anaemia.

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## **8. Appendix**



## A canine model of normovolaemic acute anaemia

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### ABSTRACT

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The objective was to develop a non-terminal, acute normovolaemic anaemia model in dogs that has minimal effects on patient well-being. Eleven normal Beagle dogs were used. About 20 % of the 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. Normovolaemia was maintained by replacing the volume deficit of the red blood cells with Ringer's lactate and re-infusing the plasma. 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 useful in the evaluation of the pathophysiology of anaemia in dogs or as a model for anaemia in humans.

**Keywords:** Anaemia model, dog, normovolaemia

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### INTRODUCTION

Anaemia is a common presentation in humans and animals. In a joint statement issued by the World Health Organisation (WHO) and the United Nations Children's Fund (UNICEF) on the WHO website in 2004, the number of anaemic people worldwide was estimated at 2 billion, with approximately 50 % of these attributed to iron deficiency (WHO/UNICEF 2004) This is seen by the WHO as a major public health problem: not only does iron deficient anaemia have dramatic effects on human health , such as

increased maternal and child mortality, but it also has more insidious consequences, such as retardation in cognitive and physical development in children and reduced work productivity in adults. This document emphasised the multifactorial aetiology of anaemia: these vary from blood loss; helminth infections (particularly hookworm and schistosomiasis); toxicosis (such as rodenticide and drug-induced); tuberculosis; HIV/AIDS; hereditary; chronic renal failure; immune mediated and blood parasites such as *falciparum* malaria in people. The last is very important with 300–500 million clinical cases of malaria occurring worldwide each year with approximately 2 million of them 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 such as like South Africa where canine babesiosis is caused by a particularly severe strain of parasite (*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 hospitalization and may die if untreated. The role of anaemia and haemodynamic changes in the pathophysiology of diseases such as babesiosis has not been clearly elucidated and is being investigated in our department. The research programme prompted the *inter alia* development of this model in an attempt to create a normotensive, normovolaemic anaemic state. 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.

Numerous anaemia models have been developed in a variety of species. 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 the animals being fed iron deficient diets (Gambling, Charania, Hannah, Antipatis, Lea & McArdle 2002) or undergoing 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 catheterization 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 euthanased at the end of the trial (Habler, Kleen, Podtschaske, Hutter, Tiede, Kemming, Welte, Corso & Messmer 1996) but in others nothing was stated on experimental animal survival. (Fowler *et al.* 1956; Fowler & Holmes 1971, 1975). 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 *et al.* 1956; Fowler & Holmes 1975; Szlyk *et al.* 1984; 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 euthanased 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 of blood loss. In practice, ANH has been used extensively in elderly patients and small children, especially for cardiac surgery (Stehling & Zauder 1991). Briefly, the tech-

nique of ANH involves the removal of blood before surgery and its 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 humans have been developed (Bourke & Smith 1974; Gross 1983), including the following one (Bourke & Smith 1974):

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

Where Lt = blood removed  
V = circulating blood volume  
Ln = natural logarithm  
Ho = initial Ht  
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.

The objectives of this experiment were 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.

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

## MATERIALS AND METHODS

### 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 between 2 and 3 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 and 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 findings within normal limits were included. All dogs were then dewormed and received a long acting ectoparasiticide. 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 biochemical profiles listed above but excluding total calcium, phosphate and urea were also repeated. Peripheral blood smear examinations were conducted weekly to ensure that haematocoon 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 and ranged to 4 for a good appetite. Every morning during the trial the rectal 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 pre-trial 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: a diazepam (Pax, Bayer Isando, RSA) and morphine sulphate [Bodene (Pty) Ltd., Port Elizabeth, RSA] combination; an 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.

### 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:

$$\text{Blood volume to be removed (mℓ)} = 20 \% \times \text{mass (kg)} \times 90$$

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 3 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 Academic Hospital 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).

### 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 solution, 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 3 500 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 yet finished. 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.

### 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 & 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.

## RESULTS

The body mass median, mean, standard deviation (SD) and range of the 11 Beagles were 11.3, 11.9 (1.8) and 9.5–15.2 kg, respectively. On day 0, 33 % of dogs were sedated, on day 1, 76 %, on day 2, 74 % and on day 3, 60 %. Dogs 3 and 7 that received a morphine diazepam combination were judged to be nauseous and were treated effectively with metaclopramide (Clopamon, Pharmicare, Port Elizabeth, RSA). In dog 10 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). The infusion of Ringer's lactate solution 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 during this period dogs not receiving their own plasma were monitored for possible transfusion reactions in (Schneider 2000). Eight dogs were bled once, two dogs twice and one dog three times on the first day. This variation was purely for convenience to get the dogs into the system. On the second day all of them were bled twice with the exception of one 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 mL) on day 1 was 226 (32) and 190–304; day 2 was 225 (36.6) and 90–304; day 3 was 219 (43.1) and 114–290; and day 4 was 211 (43.7) and 171–285. The low volume of 114 mL removed in one of the dogs on day 3 was due to the sedation collapse described above. Complications of the procedure were minimal. Two dogs (dog 4

once and dog 5 twice) received donor plasma instead of their own plasma. On one occasion in each dog this was due to an electrical power failure that caused a delay in re-infusion and in the other to rupture of the blood collecting bag in the centrifuge. Dog 4 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 8 had a level 3 on the last day it was bled as well as on the next day. The remaining dogs (dogs 2, 4, 6 and 10) had a level 3 on the day after the final bleed. Two of these dogs had other problems that could have accounted for the mild depression; dog 4 developed facial oedema and dog 10 a lame hind leg of uncertain aetiology on that day. Appetite remained at level 4 in eight of the dogs. Dogs 6 and 9 had slightly reduced appetites on the last day of phlebotomy. Dog 6 ate only a small amount on the following day. Dog 1 ate nothing on the second and third days after bleeding but this was believed to be due to a change in food consistency.

The effect of phlebotomy on the Ht as well as during the dogs' recovery over the next 10 days is illustrated in 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.

## DISCUSSION

This experimental anaemia model provides a technique to produce acute anaemia over a period of 3–4 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 the dogs became aware that they were going to be bled. One of the dogs 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



## Canine model of normovolaemic acute anaemia

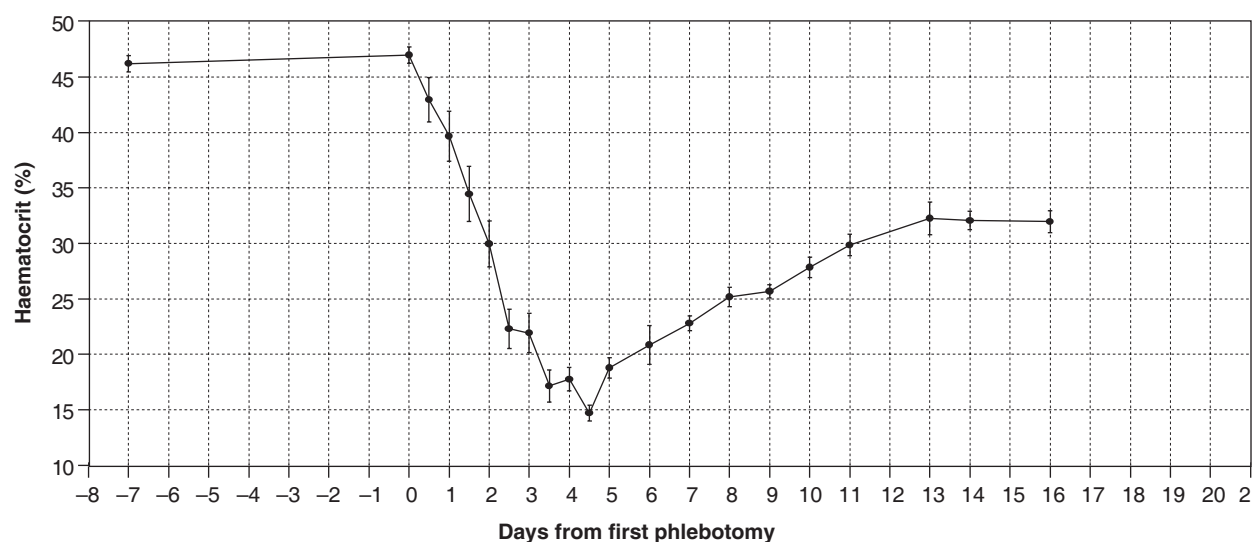


FIG. 1 The mean Ht value of all dogs measured on any particular day relative to the first phlebotomy being set as day 0. During the first 4 days Ht readings were often taken twice daily and the afternoon readings have been plotted as "half-days". The pre-trial "screening" data were all assigned to day 7 for convenience, although some were conducted more than one week before the "active trial". The error bars represent 2 SE on either side of the mean values

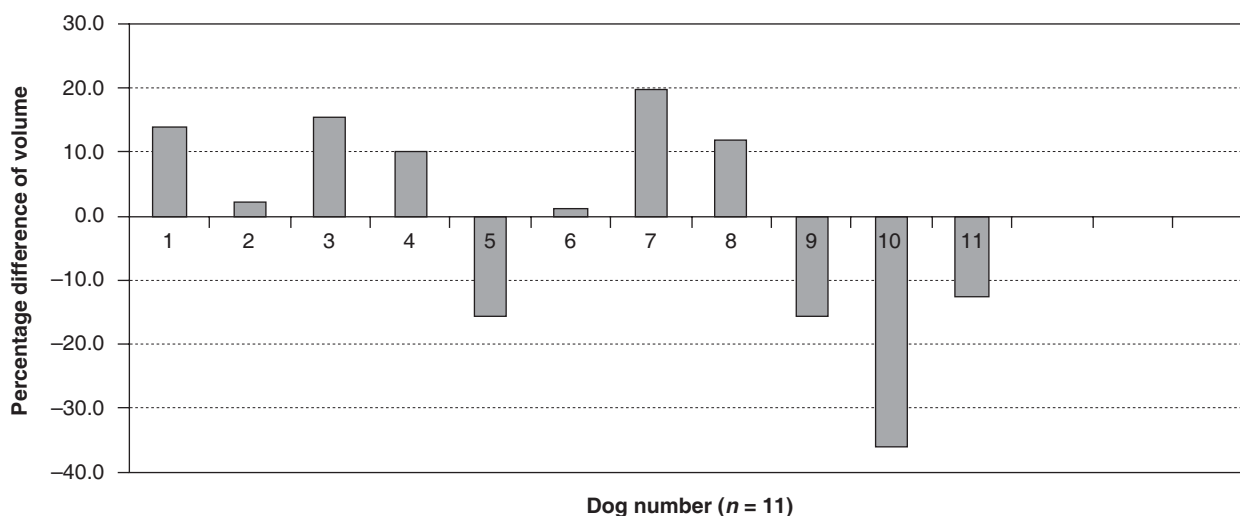


FIG. 2 The percentage difference between the actual and predicted volumes of blood by the haemodilution equation of Bourke and Smith (1974) to achieve the final Ht for each dog. A percentage difference of 0 would indicate that the actual and predicted blood volumes were the same. A positive percentage 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

it was particularly uncooperative, and the other sedation protocols, having been used with previous phlebotomies, were judged to be inadequate. Medetomidine is not recommended for use 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 the Ringer's infusion took place at the same time as the separating of the

plasma. Plasma infusion could be done while the next dog was being bled and thus with adequate labour, 4–6 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 perform 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 for the group (2 weeks for each dog).

The blood volume was removed acutely and directly from the central compartment 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 4 h 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 the one dog that received donor plasma developed mild facial oedema, indicating a mild Type 1 hypersensitivity 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 progressively 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 % heta-starch (Haes-Steril, Fresenius Kabi, Bad Homburg, Germany), the volume replacement fluid of choice at our institution, is 25 h (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-infu-

sion 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 37–55 % (Meinkoth & Clinkenbeard 2000). The anaemia model reproducibly produced a decline of Ht to its nadir (at approximately 15 %) by the 4<sup>th</sup> and 5<sup>th</sup> days, which are 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 an 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 2 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 an Ht in the 35–40 % 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). This 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.

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