

Comparison of effects of uncomplicated canine babesiosis and canine normovolaemic anaemia on abdominal splanchnic Doppler characteristics – A preliminary investigation

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ABSTRACT

A preliminary study was conducted to compare uncomplicated canine babesiosis (CB) and experimentally induced normovolaemic anaemia (EA) using Doppler ultrasonography of abdominal splanchnic vessels. Fourteen dogs with uncomplicated CB were investigated together with 11 healthy Beagles during severe EA, moderate EA and the physiological state as a control group. Canine babesiosis was compared with severe EA, moderate EA and the physiological state using Doppler variables of the abdominal aorta, cranial mesenteric artery (CMA), coeliac, left renal and interlobar, and hilar splenic arteries, and the main portal vein. Patterns of haemodynamic changes during CB and EA were broadly similar and were characterised by elevations in velocities and reductions in resistance indices in all vessels except the renal arteries when compared with the physiological state. Aortic and CMA peak systolic velocities and CMA end diastolic and time-averaged mean velocities in CB were significantly lower ($P < 0.023$) than those in severe EA. Patterns of renal haemodynamic changes during CB and EA were similar. However, the renal patterns differed from those of aortic and gastrointestinal arteries, having elevations in vascular resistance indices, a reduction in end diastolic velocity and unchanged time-averaged mean velocity. The left renal artery resistive index in CB was significantly higher ($P < 0.025$) than those in EA and the physiological state. Renal interlobar artery resistive and pulsatility indices in CB were significantly higher ($P < 0.016$) than those of moderate EA and the physiological state. The similar haemodynamic patterns in CB and EA are attributable to anaemia, while significant differences may additionally be attributed to pathophysiological factors peculiar to CB.

Key words: anaemia, Doppler ultrasonography, canine babesiosis, haemodynamics, renal artery, resistive index.

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INTRODUCTION

The virulent canine haemoprotezoan parasite, *Babesia canis rossi*, is widespread in South Africa²⁹. It has long been recognised that the disease caused by this parasite can involve multiple organs or systems and result in a wide variety of clinical manifestations^{27,28}. Canine babesiosis (CB) manifests as fever, anorexia

and anaemia, with parasites readily demonstrated in infected red blood cells of a peripheral blood smear²⁸. Atypical forms of CB may manifest as chronic disease; may involve the blood; one or several organs such as the spleen, kidney, liver, gastrointestinal tract, eyes, or body systems such as cardiovascular, respiratory, nervous and musculoskeletal^{3,28}. Parasites may be difficult to demonstrate in peripheral blood smears from atypical forms of the disease²⁸. More recently, the terms uncomplicated and complicated CB have been used in preference to typical and atypical CB, respectively^{17,19,28}. The involvement of many organs in an incident is referred to as multiple-organ damage/dysfunction syndrome^{17,19,41}. Despite years of dedicated research, disease mechanisms leading to multiple-organ

dysfunction syndrome remain subjects of investigation^{5,17,18,35,39}.

Systemic¹⁹ and microvascular (arterioles, capillaries and postcapillary venules)^{10,27,28} haemodynamic disturbances are thought to play significant roles in the pathophysiology and outcome of CB due to *B. c. rossi* infection. Hypotension is known to occur in some forms of the disease and reduction in mean systemic arterial pressure in dogs with severe and complicated disease provides evidence of disturbed systemic haemodynamics¹⁹. Haemoconcentration⁵ and the occurrence of ascites and oedema at various locations such as the subcutis or joints^{27,28} may also be associated with systemic haemodynamic alterations. Blockage of microvessels through sequestration of erythrocytes in various organs, for example the brain, liver, kidney, and spleen has been frequently reported at *post mortem*^{3,28}. Hypotension may favour sequestration within microvessels³⁵. The occurrence of sequestration led to a hypothesis of hypoperfusion in microvessels that is thought to contribute to the development of multiple-organ dysfunction syndrome in complicated CB^{3,28}. Many pathological processes and clinical manifestations of CB correlate with those of bovine babesiosis caused by *Babesia bovis*³⁵, or human malaria caused by *Plasmodium falciparum*^{6,27,28,35}. In human cerebral malaria, evidence of localised hypoperfusion in microvessels has been shown by single photon emission computed tomography²¹ and electron microscopy¹¹. Furthermore, cases of falciparum malaria presented with renal failure were reported to have compartmentalised reduction of renal cortical perfusion and reduced or normal total renal blood flow as determined by scintigraphy³⁷. In some malaria patients without renal failure, reduced mean systemic arterial pressure, renal vascular resistance and increased renin activity have been reported³⁶. In cerebral malaria, trans-cranial Doppler ultrasonography showed normal or hyperdynamic circulation of the middle cerebral artery^{7,21} despite the presence of local-

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ised hypoperfusion in cerebral microvessels²¹.

Little attention has been paid to the investigation of haemodynamic pathophysiology of CB, or its clinical evaluation in uncomplicated and complicated diseases. No report on microvascular or global haemodynamics of the commonly affected organs could be found. Doppler ultrasonographic changes in canine abdominal splanchnic haemodynamics during experimentally-induced normovolaemic anaemia have been reported elsewhere^{22,23}. The aim of this study was to investigate abdominal splanchnic haemodynamics in uncomplicated CB by use of Doppler ultrasonography and compare these with the haemodynamics of a control group of normal dogs in various states of experimentally-induced normovolaemic anaemia (EA). We hypothesised that there would be a significant increase in abdominal splanchnic haemodynamics during EA compared with the physiological state, and haemodynamic differences between uncomplicated CB and EA or the physiological state.

MATERIALS AND METHODS

Fourteen dogs of various breeds and ages, diagnosed with uncomplicated CB by microscopic detection of *B. canis* parasites within red blood cells of a thin capillary blood smear (from the tip of the pinna), and with no evidence of confirmed or suspected concurrent ehrlichiosis were examined. Presence of moderate to severe lymphadenopathy of the mandibular, parotid and popliteal lymph nodes; epistaxis; petechial haemorrhages of the oral and conjunctival mucosae; neutropenia seen in a peripheral thin blood smear or presence of morulae in monocytes were considered suspicious for concurrent ehrlichiosis, this being a common occurrence in South Africa (A L Leisewitz, Onderstepoort Veterinary Animal Hospital, pers. comm., 2003). All dogs were seen as outpatients at the Onderstepoort Veterinary Academic Hospital during one season from September 2003 to May 2004. Any dog that received treatment other than, or in addition to, diminazene aceturate (Dimisol, Virbac Animal Health, Halfway House, South Africa) was excluded from the study. Other exclusion criteria were clinical collapse (non-ambulatory), positive in-saline agglutination (indicating concurrent secondary immune-mediated haemolytic anaemia⁸), haematocrit (Hct) greater than 37 % or lower than 14 %, or Dobermans, Spaniels, Great Danes and Irish Wolfhounds that are predisposed to dilated cardiomyopathy. All dogs were first treated with diminazene aceturate and then admitted to the study for Doppler examinations.

Owner consent was obtained in all cases. The Animal Use and Care Committee of the University of Pretoria approved the study.

After admission, dogs were excluded if found with any disorder of the respiratory, cardiovascular or gastrointestinal system, or of the liver, spleen, kidneys or urogenital tract that was unrelated to CB (such as congenital renal hypoplasia, hydro-nephrosis, neoplasia or intussusception) as determined by thoracic radiography, echocardiography or complete abdominal ultrasound. Blood smear examination was repeated to confirm the presence of *Babesia* parasites. Full blood count was performed to screen haemograms for any abnormalities that were unrelated to CB. Total serum protein, creatinine and blood urea nitrogen levels were recorded in all dogs.

Doppler examinations of the abdominal aorta (AAo), cranial mesenteric artery (CMA), coeliac artery (CA), left renal artery (LRA), one of the renal interlobar arteries (ILA), one of the hilar splenic arteries (HSA) and main portal vein (MPV) were then performed on all selected dogs, commencing within 1–2 hours after treatment and completed within 4 hours. Dogs were discharged the next day.

One investigator (LMK) carried out all examinations without administering any anaesthesia or sedation. The ventral and left lateral abdomen and right lateral thorax from the 9th to 13th ribs were clipped, cleaned and covered with acoustic gel. For general abdominal imaging, dogs were placed in dorsal recumbency. The spleen was imaged in parasagittal, transverse, and oblique planes and the left kidney was imaged in dorsal, transverse and oblique planes slightly caudal in position to that of the spleen. Right lateral recumbency was used for imaging the AAo, CMA, CA, LRA, ILA and HSA in a parasagittal or dorsal plane. Dogs were then turned into left lateral recumbency to image the MPV in dorsal and transverse planes through the right 9–12th intercostal spaces caudal to the liver. All the examinations were carried out using an ultrasound-imaging machine (Sonoline Omnia, Siemens Medical Systems, Ultrasound, Erlangen, Germany) with a convex array, linear array or phased array transducer. B-mode examination of the general abdominal cavity, organs and AAo were done with a 5.0 MHz convex array transducer; identification and evaluation of the vessels was done with a 7.5 MHz linear array transducer or sometimes with a convex array transducer. The MPV was identified with a 6.0 MHz phased array transducer. All machine settings, including

overall gain, depth compensation gain, edge enhancement, focus and line density were adjusted during each examination to obtain optimal image quality.

Doppler examinations of the AAo and MPV were performed at 3.5 MHz Doppler frequency. Within the AAo the sample window was placed anywhere along its length cranial to the origin of the left renal artery. The sample window for the MPV was placed at the best location in the vessel, usually just caudal to the liver, where a long axis vessel-beam intercept angle of 60° or less could be obtained from the intercostal approach. Evaluation of the CMA, CA, LRA, ILA and HSA were performed at 5.2 MHz Doppler frequency, or sometimes at 3.5 MHz for the CMA and CA. The sample window in the CMA, CA or LRA was placed within the vessel about 5 mm away from the aortic origin. The sample window for the ILA was placed within the renal pelvis 5–10 mm from the hilus and for the HSA was placed about 5 mm from the splenic hilus, within or outside the parenchyma. Using a 7.5 MHz frequency on B-mode and colour Doppler facilitated the placement of these sample windows.

In the AAo and MPV, placing one cursor of the sample window close to each inner surface of the opposite vessel walls was done as a modification of the technique of uniform insonation¹⁴. This kept the size of the sample window within at least two-thirds of the vessel diameter and avoided wall artefacts or spectral contamination from adjacent vessels. In the CMA, CA and LRA cursors of the sample window were positioned transversely across each vessel diameter as it coursed towards the transducer. In all cases, except the ILA and HSA, the vessel-beam alignment was angle-corrected before recording the spectral tracing. Angle correction was assisted by manual adjustment of the transducer and by the electronic steering capacity of the linear array transducer. The vessel-beam angle achieved was recorded. Machine settings were optimised for high sensitivity imaging in the Doppler mode. Doppler variables recorded were resistive index and pulsatility index for all arteries. In addition, peak systolic velocity, end diastolic velocity and time-averaged mean velocity for the larger arteries were recorded. Ratios of peak systolic velocity and time-averaged mean velocity of a splanchnic artery to the corresponding variables of the AAo were calculated. In the MPV, peak velocity, time-averaged mean velocity, blood flow and congestion index were recorded. All Doppler examinations had a simultaneous electrocardiographic (ECG) tracing, from which heart rate was calculated.

Table 1: Haematocrit, heart rate, and total serum protein, creatinine and urea levels in uncomplicated canine babesiosis, experimentally induced normovolaemic anaemia, and the physiological state.

Group	Haematocrit (%)		Heart rate (b/m)		Total serum protein (g/l)		Creatinine (mol/l)		Blood urea nitrogen (mmol/l)	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Uncomplicated CB	14	21.3 ± 5.1	12	114.0 ± 23.8	14	61.9 ± 13.0	14	67.1 ± 20.7	14	6.1 ± 3.2
Severe EA	11	16.0 ± 0.8	11	134.0 ± 13.0	10	54.0 ± 3.2	–	–	–	–
Moderate EA	11	26.3 ± 0.7	11	105.4 ± 7.5	5	61.8 ± 3.0	–	–	–	–
Physiological state	11	47.5 ± 2.2	10	95.9 ± 16.3	11	59.8 ± 3.0	11	68.4 ± 8.1	3	4.5 ± 0.7
Normal reference range						53–75		40–133		3.6–8.9

CB = canine babesiosis; EA = experimentally-induced normovolaemic anaemia; n = sample size; SD = standard deviation.

Abdominal organs were evaluated on B-mode. Echogenicity of the spleen, liver and kidneys were subjectively compared with each other. Renal function was estimated by plasma creatinine level. Measurement of the thickest area of the spleen estimated its size. The diameter of the largest splenic vein branch in the splenic body region was measured at the hilus from a long axis view and its cross-sectional area was calculated. The largest short axis cross-sectional area of the MPV was obtained by using the cine loop function for up to about 120 frames, and utilising the elliptical program of the onboard computer. This minimised errors of measurement in cross-sectional area due to variation with time and respiration. Doppler spectra of individual vessels were evaluated for their flow patterns with the help of a simultaneous ECG tracing.

During each examination, an attempt was made to obtain measurements from spectral cycles whose preceding R–R intervals were approximately equal, excluding obvious sinus arrhythmias. The value recorded for heart rate and each Doppler variable was obtained by averaging 3 measurements or calculations from 3 ECG or Doppler spectral cycles. Doppler variables were measured with the onboard computer. Pulsatility index (PI) was calculated from the equation³²:

PI = (Peak systolic velocity – end diastolic velocity)/time-averaged mean velocity.

Resistive index (RI) was calculated from the equation³²:

RI = (Peak systolic velocity – end diastolic velocity)/Peak systolic velocity.

Blood flow was computed using the equation³⁰:

Blood flow = cross-sectional area × time-averaged mean velocity.

Congestion index was calculated using the equation³¹:

Congestion index = cross-sectional area/ time-averaged mean velocity.

Results of each examination were entered into a spreadsheet program (MS Excel, Microsoft Corporation, Redmond, USA) on a personal computer. All ultrasonographic procedures were recorded on videotape for possible re-evaluation and future reference. All data were tested with the Shapiro-Wilk test and were normally distributed. The data are presented as mean ± standard deviation (SD).

Data from uncomplicated CB were compared with similar data from 11 healthy Beagles with a mean ± SD age of 2.63 ± 0.05 years and weight 12.0 ± 1.8 kg obtained before withdrawal of blood (mean ± SD Hct 47.5 ± 2.2 %, physiological state); after repetitive blood withdrawal to produce acute severe normovolaemic anaemia (mean ± SD Hct 16.0 ± 0.77 %, severe EA); and during recovery (mean ± SD Hct 26.3 ± 0.74 %, moderate EA). Comparison was done using a 2-group *t*-test providing for unequal variances (Welch *t*-test) at the 0.05 level of significance. A statistical software program (Stata Release 8, Stata Press, College Station, Texas, USA) was used to perform the data analysis.

RESULTS

Eighteen dogs with uncomplicated CB were admitted to the study. Four of the 18 dogs were excluded because they were in-saline agglutination positive, had Hct higher than 37 % or had clinical complications a week later. Of the remaining 14, there were 8 males and 6 females comprising 7 large breeds, 4 small to medium breeds and 3 crossbreeds. Mean ± SD age was 17.8 ± 14.9 months (range 2–56 months), mean ± SD body weight was 17.7 ± 13.1 kg (range 5.4–30.6 kg), and mean ± SD Hct was 21.3 ± 5.1 % (14.6–32.4 %).

Table 1 gives normal reference values of mean ± SD total serum protein, creatinine and blood urea nitrogen levels and those in uncomplicated CB, severe EA, moderate EA and the physiological state. Comparisons of severe and moderate EA with the physiological state using Doppler vari-

ables have been reported elsewhere^{22,23}. Comparisons of uncomplicated CB with severe EA, moderate EA and the physiological state regarding haemodynamic patterns are shown in Figures 1–5. Figures 1–2 and Table 2 compare uncomplicated CB with severe EA, moderate EA and the physiological state regarding mean resistive, pulsatility and congestion indices; Figures 3–5 and Table 3 with regard to mean peak, end diastolic and time-averaged mean velocities; and Table 4 with regard to mean peak systolic velocity ratio, time-averaged mean velocity ratio and blood flow.

For some variables fewer observations were recorded than the number of dogs investigated for various reasons. These were ECG malfunctioning or animal's intolerance to placement of ECG pads (heart rate), excessive vessel mobility (HSA), or obscurity by lung or gastrointestinal gas (MPV, CA) and time constraints.

DISCUSSION

Aortic, gastrointestinal and splenic haemodynamics

The similarity between uncomplicated CB and EA regarding patterns of change in Doppler variables is most likely attributable to a common mechanism. It is well established that moderate to severe acute normovolaemic haemodilution in animals^{13,34,40}, or chronic anaemia in human patients¹, induces a hyperdynamic cardiovascular response both systemically and regionally, characterised among others by a reduction in vascular resistance and an increase in blood flow. The lowered vascular resistance during anaemia is mainly due to a reduction in blood viscosity as a result of reduced Hct¹⁰. In uncomplicated CB, we anticipated mild or no hypovolaemia, hypotension or hyperviscosity – pathophysiological changes that may be seen in the complicated disease. This means any change in Doppler variables during uncomplicated CB would mainly be due to the concur-

rent anaemia and should be proportional to the Hct levels. This would explain the modest differences observed between uncomplicated CB and severe or moderate EA seen in the AAO, CMA, CA, HSA and MPV (Tables 1–4; Figs 1–5). Another possible reason for lack of significant differences between uncomplicated CB and any of the grades of EA may be due to a small sample of the cases investigated.

In severe human falciparum malaria, pathophysiological changes associated with systemic inflammatory response leading to hypovolaemia, hypotension, or an increase in plasma fibrinogen levels occur in addition to anaemia. Rheological changes involving red blood cell (RBC) cytoadherence (sequestration), reduced deformability and aggregation also occur¹⁰. Plasma hyperviscosity and the RBC changes would lead to an increase in peripheral vascular resistance¹⁰. All of the above changes, singly or in combination, would in turn lead to hypoperfusion. These changes are of particular significance in microvessels¹⁰. In the larger vessels, plasma hyperviscosity is often counterbalanced by hypoviscosity due to reduced Hct such that the net effect is often little or no change in whole blood viscosity³⁷. Transcranial Doppler in malaria patients that had evidence of hypoperfusion in the cerebral microvessels found normal or increased middle cerebral artery haemodynamics²¹, supporting the above argument.

Pathophysiological mechanisms similar to those of complicated malaria have been suggested for complicated CB that is known to cause hypoalbuminaemia²⁴. This is thought to be due to the leakage of protein through the endothelium as is characteristic of critical illness¹². Mild hyperglobinaemia is also a characteristic of CB, which may be due to antigenic stimulation²⁴. Our data show a normal mean total serum protein in the uncomplicated cases of CB (Table 1) although 2 of the 14 dogs had values above and 2 others had values below the normal range. Although hypoalbuminaemia or hyperglobinaemia alone may lower or raise blood viscosity, respectively, it is thought that mild changes in the levels of these serum proteins, such as during uncomplicated CB, have little significance for haemodynamics since their effects frequently cancel each other out. Hypotension has also been reported in complicated CB¹⁹. A reduction in arterial blood pressure or circulating blood volume would be expected to increase peripheral vascular resistance indices. We were unable to confirm such changes since we did not measure blood volume or blood pressure. However, we were able to

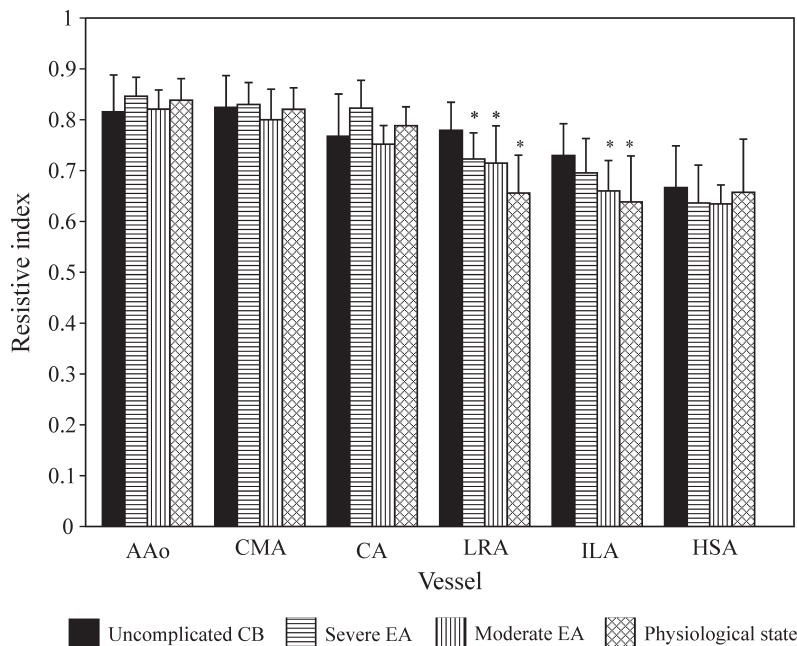


Fig. 1: Resistive index in dogs with uncomplicated CB, severe EA, moderate EA and the physiological state. Data are shown as mean $\pm$ SD. (*) Denotes a significant difference between uncomplicated CB and the marked group.

exclude profound hypotension (non-ambulatory) during physical examination of dogs with uncomplicated CB. Another cause of difference between the uncomplicated CB in our study and EA would be due to a possible effect of diminazene on blood pressure of the CB dogs. However, Joubert *et al.*²⁰ found no influence of diminazene aceturate on systemic blood pressure of healthy adult dogs.

Renal haemodynamics

Acute normovolaemic haemodilution in the dog is known to cause an increase in cardiac output and a reduction in systemic vascular resistance¹³. Renal

blood flow is increased during severe acute haemodilution^{15,34} and severe chronic anaemia⁴⁰. However, no change in renal blood flow was observed during moderate chronic anaemia in the dog⁴⁰ or moderate acute anaemia in rabbits⁴. We did not measure renal artery blood flow although we found an increased renal artery peak systolic velocity²². However, the lack of an increase in renal artery time-averaged mean velocity could not support presence of an increased blood flow²². Regarding renal vascular resistance, Vatner *et al.*⁴⁰ reported a reduction during severe chronic anaemia in the dog although we found an increased renal

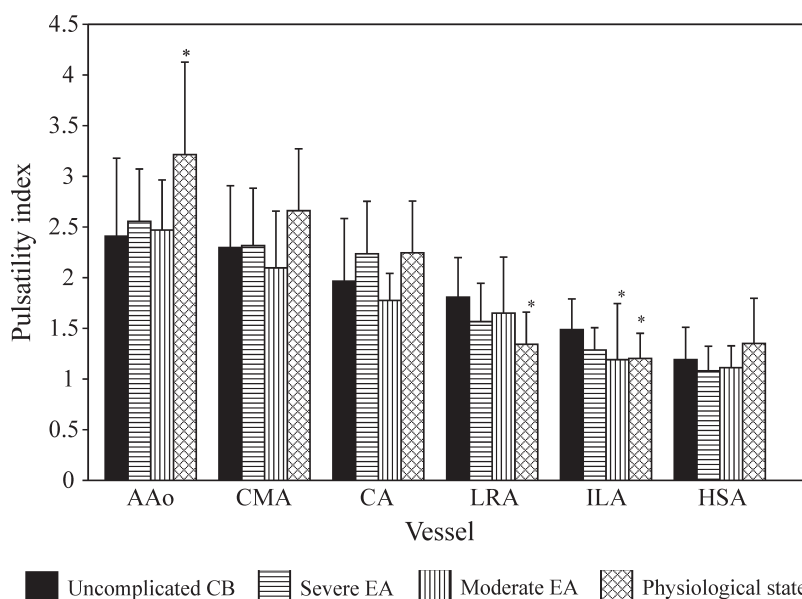


Fig. 2: Pulsatility index in dogs with uncomplicated CB, severe EA, moderate EA and the physiological state. Data are shown as mean $\pm$ SD. (*) Denotes a significant difference between uncomplicated CB and the marked group.

artery resistive index during severe acute and moderate chronic anaemia²². The apparent contradiction between our results and those of other investigators may be due to differences in anaemia grades or the staging and methods of measurement. Depending on the stage and grade of anaemia, an increased renal vascular resistance may co-exist with a modest increase in renal blood flow. Available evidence suggests that the increase in renal blood flow during anaemia is proportionately lower than that of the cardiac output determined under similar conditions^{15,33,34}. Thus one investigator found decreased effective renal blood flow with increasing severity of anaemia³³ while others asserted that the renal flow fraction of the cardiac output fell significantly during anaemia¹⁵. This is possible since it has been shown that renal artery resistance increases in response to increasing flow rate over an arterial pressure range of 75–200 mm Hg¹⁶. The increase in blood flow during anaemia attempts to compensate for reduced oxygen carrying capacity and maintain optimum tissue oxygen delivery³⁸. Blood with normal oxygen carrying capacity surpasses the oxygen requirements of the kidney, which rarely experiences an oxygen deficit except during profound anaemia³³. Therefore, in an attempt to maintain a constant blood flow, renal auto-regulation occurs during an elevated flow rate, probably mediated by vasoconstriction and increased renal vascular resistance¹⁶. The mechanism of auto-regulation is unclear but it may be activated by the increased peak systolic velocity during haemodilution or anaemia. This would explain the different patterns of haemodynamic changes between the LRA on one hand and the AAo, CMA, CA and HSA on the other as observed in our study (Figs 1, 2, 4).

In spite of having similar renal haemodynamic change patterns, our data show that the uncomplicated CB group differed significantly from severe EA, moderate EA and the physiological state with respect to LRA resistive index (Table 2). The higher LRA resistive index in the uncomplicated CB could not be explained on the basis of Hct levels. Also, significant differences between uncomplicated CB and the physiological state, based on resistive and pulsatility indices of the ILA (Table 2), without a similar difference between severe EA and the physiological state²² could not be explained by severity of anaemia. The observed differences between uncomplicated CB and severe EA may be explained by the presence of additional factors (pathology) peculiar to the disease. An increase in renal vascular

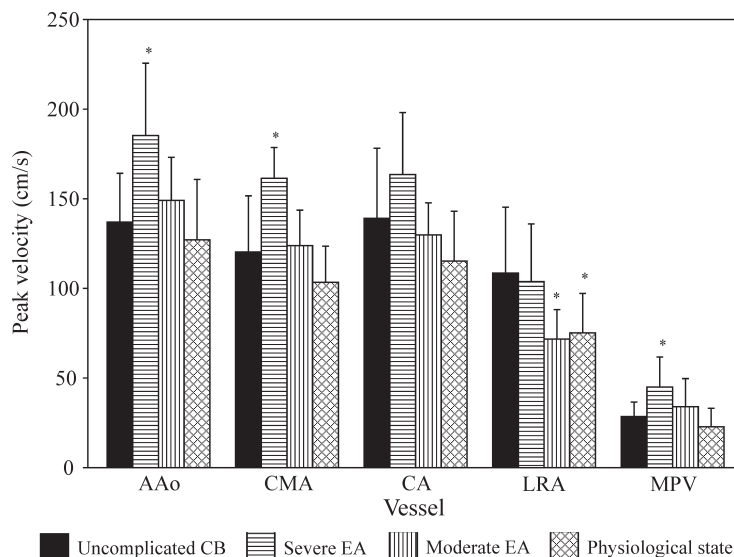


Fig. 3: Peak velocities in dogs with uncomplicated CB, severe EA, moderate EA and the physiological state. Data are shown as mean $\pm$ SD. (*) Denotes a significant difference between uncomplicated CB and the marked group.

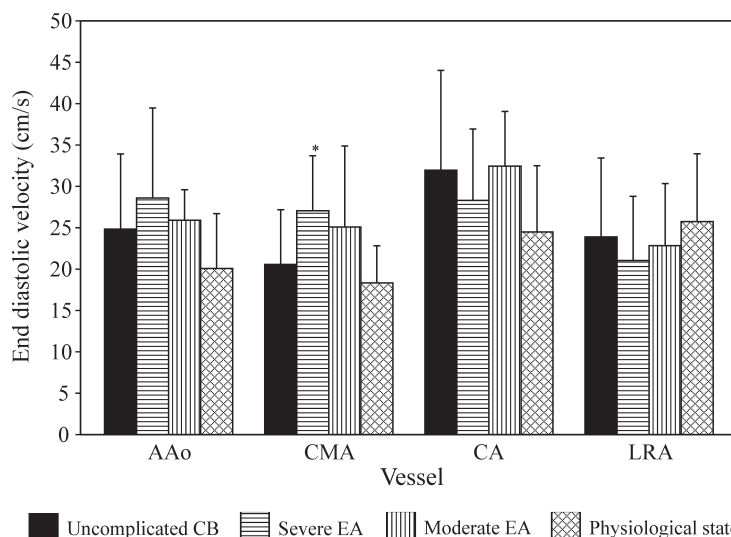


Fig. 4: End diastolic velocity in dogs with uncomplicated CB, severe EA, moderate EA and the physiological state. Data are shown as mean $\pm$ SD. (*) Denotes a significant difference between uncomplicated CB and the marked group.

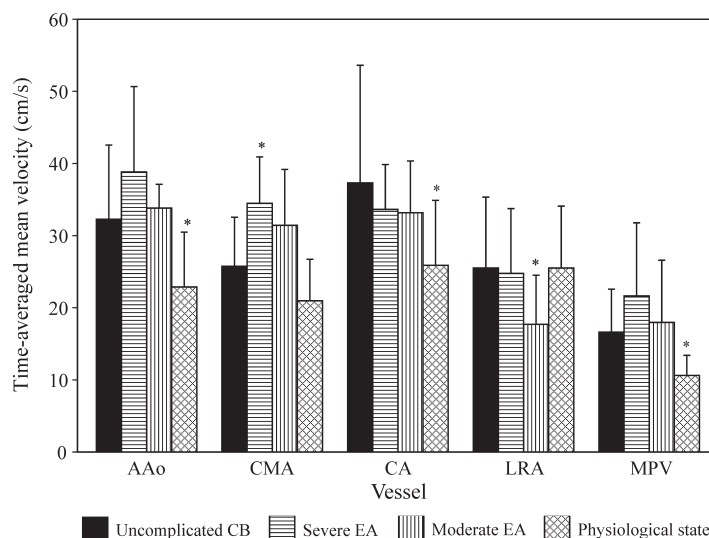


Fig. 5: Time-averaged mean velocity in dogs with uncomplicated CB, severe EA, moderate EA and the physiological state. Data are shown as mean $\pm$ SD. (*) Denotes a significant difference between uncomplicated CB and the marked group.

Table 2: Resistive index, pulsatility index and congestion index during babesiosis, experimental anaemia and the physiological state.

Variable	Vessel	Uncomplicated CB		Severe EA		Moderate EA		Physiological state	
		<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD
Resistive index	AAo	14	0.816 $\pm$ 0.072	11	0.846 $\pm$ 0.037	11	0.821 $\pm$ 0.038	11	0.839 $\pm$ 0.042
	CMA	14	0.824 $\pm$ 0.063	11	0.830 $\pm$ 0.043	11	0.800 $\pm$ 0.060	11	0.821 $\pm$ 0.042
	CA	13	0.768 $\pm$ 0.083	11	0.823 $\pm$ 0.055	10	0.752 $\pm$ 0.037	11	0.788 $\pm$ 0.037
	LRA	14	0.779 $\pm$ 0.055	11	0.723 $\pm$ 0.051	11	0.715 $\pm$ 0.073	10	0.656 $\pm$ 0.074
	ILA	14	0.730 $\pm$ 0.062	11	0.695 $\pm$ 0.068	11	0.660 $\pm$ 0.060	11	0.638 $\pm$ 0.090
	HSA	14	0.667 $\pm$ 0.082	11	0.636 $\pm$ 0.074	11	0.635 $\pm$ 0.037	10	0.657 $\pm$ 0.105
Pulsatility index	AAo	14	2.409 $\pm$ 0.770	11	2.555 $\pm$ 0.517	11	2.470 $\pm$ 0.494	11	3.214 $\pm$ 0.912
	CMA	14	2.296 $\pm$ 0.611	11	2.316 $\pm$ 0.566	11	2.096 $\pm$ 0.561	11	2.661 $\pm$ 0.632
	CA	13	1.965 $\pm$ 0.619	11	2.235 $\pm$ 0.518	10	1.776 $\pm$ 0.267	11	2.246 $\pm$ 0.510
	LRA	14	1.807 $\pm$ 0.391	11	1.566 $\pm$ 0.378	11	1.650 $\pm$ 0.554	10	1.343 $\pm$ 0.317
	ILA	14	1.487 $\pm$ 0.303	11	1.285 $\pm$ 0.221	11	1.191 $\pm$ 0.258	11	1.202 $\pm$ 0.249
	HSA	14	1.191 $\pm$ 0.319	11	1.081 $\pm$ 0.242	11	1.112 $\pm$ 0.216	10	1.351 $\pm$ 0.445
Congestion index	MPV	11	0.043 $\pm$ 0.022	9	0.030 $\pm$ 0.013	10	0.045 $\pm$ 0.020	9	0.066 $\pm$ 0.019

For each variable, the *P*-value represents a comparison of babesiosis with the group in the preceding column. Significance was set at $P \leq 0.05$.

EA = experimentally-induced normovolaemic anaemia; AAo = abdominal aorta; CMA = cranial mesenteric artery; CA = coeliac artery; LRA = left renal artery; HSA = hilar splenic artery; MPV = main portal vein.

Table 3: Blood velocities during uncomplicated canine babesiosis, experimental anaemia and the physiological state.

Variable	Vessel	Uncomplicated CB		Severe EA		Moderate EA		Physiological state	
		<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD
Peak systolic velocity (cm/s)	AAo	14	137.0 $\pm$ 27.4	11	185.3 $\pm$ 40.4	11	149.1 $\pm$ 024.0	11	127.1 $\pm$ 033.7
	CMA	14	120.3 $\pm$ 31.3	11	161.4 $\pm$ 17.2	11	123.9 $\pm$ 19.8	11	103.4 $\pm$ 20.2
	CA	13	139.1 $\pm$ 39.1	11	163.5 $\pm$ 34.6	10	129.9 $\pm$ 17.9	11	115.2 $\pm$ 27.8
	LRA	14	108.6 $\pm$ 36.7	11	103.8 $\pm$ 32.1	11	71.7 $\pm$ 16.5	10	75.2 $\pm$ 22.0
Peak velocity (cm/s)	MPV	12	28.5 $\pm$ 8.0	10	45.0 $\pm$ 16.8	10	34.0 $\pm$ 15.7	9	22.8 $\pm$ 10.3
End diastolic velocity (cm/s)	AAo	14	24.8 $\pm$ 9.1	11	28.6 $\pm$ 10.9	11	25.9 $\pm$ 3.7	11	20.1 $\pm$ 6.6
	CMA	14	20.5 $\pm$ 6.6	11	27.1 $\pm$ 6.6	11	25.1 $\pm$ 9.8	11	18.3 $\pm$ 4.5
	CA	13	32.0 $\pm$ 12.1	11	28.3 $\pm$ 8.6	10	32.4 $\pm$ 6.6	11	24.5 $\pm$ 8.0
	LRA	14	23.9 $\pm$ 9.5	10	21.0 $\pm$ 7.8	10	22.8 $\pm$ 7.5	10	25.7 $\pm$ 8.2
Time-averaged mean velocity (cm/s)	AAo	14	32.3 $\pm$ 10.3	11	38.8 $\pm$ 11.8	11	33.8 $\pm$ 3.3	11	22.9 $\pm$ 7.6
	CMA	14	25.8 $\pm$ 6.8	11	34.5 $\pm$ 6.4	11	31.4 $\pm$ 7.8	11	21.0 $\pm$ 5.8
	CA	13	37.3 $\pm$ 16.3	11	33.6 $\pm$ 6.2	10	33.2 $\pm$ 7.2	11	25.9 $\pm$ 9.0
	LRA	14	25.5 $\pm$ 9.8	11	24.8 $\pm$ 9.0	10	17.7 $\pm$ 6.8	8	24.1 $\pm$ 8.6
	MPV	11	16.6 $\pm$ 6.0	9	21.6 $\pm$ 10.1	10	18.0 $\pm$ 8.6	9	10.6 $\pm$ 2.8

For each variable, the *P*-value represents a comparison of babesiosis with the group in the preceding column. Significance was set at $P \leq 0.05$.

EA = experimentally-induced normovolaemic anaemia; AAo = abdominal aorta; CMA = cranial mesenteric artery; CA = coeliac artery; LRA = left renal artery; MPV = main portal vein.

Table 4: Velocity ratios and blood flow during uncomplicated canine babesiosis, experimental anaemia and the physiological state.

Variable	Vessel	Uncomplicated CB			Severe EA			Moderate EA			Physiological state		
		n	Mean $\pm$ SD	P	n	Mean $\pm$ SD	P	n	Mean $\pm$ SD	P	n	Mean $\pm$ SD	P
Peak systolic velocity ratio	CMA	14	0.888 $\pm$ 0.220		11	0.902 $\pm$ 0.189	0.853	11	0.849 $\pm$ 0.186	0.650	11	0.858 $\pm$ 0.243	0.762
	CA	13	1.024 $\pm$ 0.262		11	0.906 $\pm$ 0.238	0.261	10	0.859 $\pm$ 0.083	0.049	11	0.950 $\pm$ 0.255	0.492
	LRA	14	0.813 $\pm$ 0.305		11	0.570 $\pm$ 0.158	0.018	11	0.488 $\pm$ 0.119	0.002	10	0.587 $\pm$ 0.127	0.022
Time-averaged mean velocity ratio	CMA	14	0.889 $\pm$ 0.412		11	0.939 $\pm$ 0.244	0.706	11	0.935 $\pm$ 0.236	0.729	11	0.993 $\pm$ 0.370	0.511
	CA	13	1.198 $\pm$ 0.475		11	0.923 $\pm$ 0.275	0.092	10	0.984 $\pm$ 0.225	0.170	11	1.186 $\pm$ 0.358	0.944
	LRA	13	0.755 $\pm$ 0.298		11	0.668 $\pm$ 0.235	0.435	10	0.515 $\pm$ 0.193	0.029	8	1.004 $\pm$ 0.299	0.081
Blood flow (ml/kg/min)	MPV	11	73.9 $\pm$ 25.4		9	66.2 $\pm$ 38.8	0.616	10	61.5 $\pm$ 34.1	0.361	9	36.8 $\pm$ 13.0	0.001

For each variable P value represents comparison of uncomplicated CB with the group represented in the preceding column. Significance was set at $P \leq 0.05$. EA = experimentally-induced normovolaemic anaemia. AAO = abdominal aorta. CMA = cranial mesenteric artery. CA = coeliac artery. LRA = left renal artery. MPV = main portal vein.

resistance index supports reports of renal microvascular damage in uncomplicated CB, or acute renal failure in complicated CB^{25,41}.

In human falciparum malaria with renal failure, reduced renal microvascular blood flow leading to ischemia has been attributed to multiple pathophysiological changes including hypovolaemia, hyperviscosity of blood, catecholamine release and renin-angiotensin activation³⁷, all promoting an increase in renal vascular resistance. High plasma catecholamine levels and renin activity have also been demonstrated in malaria patients without renal failure³⁶. In Rhesus-alloimmunised human fetuses, an increase in splenic resistive index was attributed to blockage of splenic microvessels with damaged red blood cells². Observations on haemodynamics of human falciparum malaria^{11,21,36} have led to the suspicion that in CB renal microvascular haemodynamics may also be disrupted.

Mild renal damage is seen more frequently in clinically severe CB than a significant damage, or acute renal failure²⁵. We did not quantify urine protein, however, previous studies have shown that proteinuria is frequently encountered in CB and is not a good indicator of disturbed renal function²⁶. Serum urea may be elevated in complicated or uncomplicated CB²⁵. There is a disproportionate rise in urea compared to creatinine probably due to hyperureagenesis⁹. On the other hand, elevated creatinine levels are seen in cases of complicated CB with impaired renal function²⁵. Mean values for both urea and creatinine levels were normal in our sample of dogs with uncomplicated CB, although the standard deviations were relatively high (Table 1). We can therefore assume that no dog in this trial had significant renal disease. This is not surprising considering the selection criteria used. Although elevated creatinine is commonly seen in the complicated form of CB and is a risk factor for death⁴¹, we specifically selected dogs that had no evidence of complicated disease. The fact that renal vascular resistance indices were significantly increased in dogs that had no biochemical evidence of disturbed renal function was particularly interesting as this may suggest a higher sensitivity of resistive index compared with biochemical tests for the detection of early renal damage in CB. It is possible that the presence of a localised pathophysiological change such as increased renin activity, or a renal pathology in uncomplicated CB similar to that of human falciparum malaria may have contributed to the significantly higher renal resistive and pulsatility indices. In

human falciparum malaria with evidence of reduced cerebral microcirculation, Doppler ultrasound could not detect evidence of hypoperfusion in the middle cerebral artery. The resistance index of the middle cerebral artery was, however, not reported. Our observation raises hope that Doppler ultrasound may be useful in an early detection of renal involvement in CB.

The low likelihood of hypovolaemia, hypotension or hyperviscosity in our sample of uncomplicated CB may partly explain differences observed between renal and aortic or gastrointestinal haemodynamics. Greater vulnerability of the kidneys to CB and the fact that renal failure is a more frequent cause of morbidity and mortality when compared with the liver, spleen or gastrointestinal tract²⁵ may be another reason for the observed difference in renal haemodynamics. The haemodynamic changes described in this study are those of uncomplicated CB in which we do not see multiple-organ dysfunction syndrome. A similar study of complicated forms of the disease with multiple-organ dysfunction syndrome is envisaged and we hypothesise that far more haemodynamic disturbance will be observed. Further investigations of both uncomplicated and complicated forms of CB will improve our understanding of the haemodynamic changes in the disease, and possibly other similar diseases such as human malaria.

In conclusion, this study revealed a similarity between uncomplicated CB and EA regarding patterns of haemodynamic changes in Doppler variables of the abdominal aorta and splanchnic vessels. These were characteristic of a hyperdynamic circulatory state. While the similarity in patterns of haemodynamic changes during uncomplicated CB and EA may be attributable to anaemia, significantly higher renal vascular resistance in CB may have been suggestive of additional renal pathology or localised pathophysiological change peculiar to CB. Knowledge of organ haemodynamic changes in CB will improve our understanding of the disease pathophysiology. Clinical evaluation of haemodynamics may permit early identification of potential complications or improve management of complicated cases and monitor the disease response to therapy.

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