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Arterial blood pressure values in dogs naturally infected with *Babesia canis*

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ABSTRACT

Babesiosis is a significant disease of domestic and wild animals caused by haemoprotozooan parasites of the *Babesia* genus. The aim of the present study was to compare arterial blood pressure values in healthy dogs and dogs naturally infected with *Babesia canis* (*B. canis*), and to compare arterial blood pressure values in dogs with complicated and uncomplicated babesiosis. An additional aim was to determine if hypotension is an important contributing factor in development of complicated babesiosis and whether it affects the outcome of the disease. Fifty dogs were included in this study, and were divided into two groups: healthy dogs (20 dogs) and dogs with babesiosis (30 dogs). The group of dogs with babesiosis was subdivided into a subgroup of dogs with complicated (12 dogs) and a subgroup of dogs with uncomplicated babesiosis (18 dogs). Babesiosis was diagnosed by demonstrating the presence of *B. canis* in Romanowsky stained blood smears. The study showed that dogs with babesiosis had lower blood pressure (systolic arterial pressure = 120 ± 96.76, diastolic arterial pressure = 68 ± 59.1, mean arterial pressure = 83 ± 65.08) compared to healthy dogs (systolic arterial pressure = 145 ± 44.02; diastolic arterial pressure = 92 ± 32.96; mean arterial pressure = 106 ± 22.38). Furthermore, blood pressure was higher in dogs with uncomplicated babesiosis (systolic arterial pressure = 162 ± 51.96, diastolic arterial pressure = 95 ± 48.38, mean arterial pressure = 116 ± 41.33) compared to healthy dogs (systolic arterial pressure = 145 ± 44.02; diastolic arterial pressure = 92 ± 32.96; mean arterial pressure = 106 ± 22.38), and lower in dogs with complicated babesiosis (systolic arterial pressure = 90 ± 69.38, diastolic arterial pressure = 48 ± 15.86, mean arterial pressure = 60 ± 22.38) compared to dogs with uncomplicated babesiosis (systolic arterial pressure = 162 ± 51.96, diastolic arterial pressure = 95 ± 48.38, mean arterial pressure = 116 ± 41.33). The results of this study confirm that hypotension is present in complicated forms of canine babesiosis caused by *B. canis*. Serial blood pressure measurements should be carried out in order to estimate the influence of blood pressure changes on the course and outcome of the disease.

Key words: arterial blood pressure, systolic blood pressure, diastolic blood pressure, mean arterial pressure, dog, babesiosis

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Introduction

Babesiosis is a significant disease of domestic and wild animals transmitted by ticks, which is caused by haemoprotozooan parasites of the *Babesia* genus (TABOADA and MERCHANT, 1991). In dogs, babesiosis is caused by three different species of large babesia: *Babesia canis* (*B. canis*), *Babesia* spp. (*Coco*) and *Babesia caballi* (*B. caballi*) (BIRKENHEUER et al., 2004; LEHTINEN et al., 2008). Apart from the large babesia, the following small babesia can also cause canine babesiosis: *Babesia gibsoni* (*B. gibsoni*), *Babesia conradae* (*B. conradae*), *Theileria annae* (*T. annae*) (ZAHLER et al., 2000) and *Theileria equi* (*T. equi*) (CRIADO-FORNELIO et al., 2003). Based on biological and antigenic differences it has been suggested that *B. canis* can be divided into three distinct subspecies: *B. canis canis*, *B. canis vogeli* and *B. canis rossi* (UILENBERG, 1989). Since additional molecular biological studies have shown that these three groups of parasites do not cluster in a single clade, which therefore suggest they are not subspecies (CARRET et al., 1999; UILENBERG, 2006), classical nomenclature (*B. canis*, *B. rossi* and *B. vogeli*) will be used, as suggested by SCHETTERS (1997), CARRET et al. (1999), PASSOS et al. (2005) and SCHETTERS (2005). Canine babesiosis caused by *B. canis* has been reported to be a significant cause of morbidity and mortality in Croatia, especially in the area of the City of Zagreb (CACCIO et al., 2002; MATIJATKO et al., 2007; BRKLJAČIĆ et al., 2010).

On the basis of the clinical picture, canine babesiosis can be described as uncomplicated and complicated (LOBETTI, 1998; JACOBSON, 2006). Complications of canine babesiosis can develop singularly or in combination, as part of the multiple organ dysfunction syndrome (MODS), which is the consequence of a generalized and uncontrolled systemic inflammatory response (JACOBSON and CLARK, 1994). Complicated babesiosis can be classified as "protozoal sepsis", and it is similar to other septic conditions characterized by systemic inflammatory response syndrome (SIRS) and MODS (BONE et al., 1992; JACOBSON et al., 2002).

One of the key factors that affects tissue perfusion is arterial blood pressure, which is maintained within the physiological values by various regulatory mechanisms (EGNER, 2002). Peripheral baroreceptors are the first mechanism of blood pressure regulation. They act via cardiovascular reflex and arterial tonus. If changes implemented by the action of the peripheral baroreceptors do not restore blood pressure to physiological value, the renin-angiotensin-aldosterone system is activated, together with prostaglandins, atrial natriuretic peptide and antidiuretic hormones. Arterial blood pressure values differ between dog breeds, and small breeds have higher blood pressure values compared to large breeds. The average values in dogs are 133 mmHg for systolic, and 75 mmHg for diastolic arterial blood pressure (BODEY and MICHELL, 1996). It is known that physiological oscillations (from 10 to 15 mmHg) in arterial blood pressure occur between measurements, and therefore it is generally accepted that a mean value of 3 to 5

measurements should be calculated to improve the accuracy (ERHARD and HENKE, 2002; STEPIEN, 2005). Pathological changes in arterial blood pressure values are: increased blood pressure (hypertension) or decreased blood pressure (hypotension), and as such they can significantly influence the functioning of various organs and promote the progression of the disease, which in the end can increase mortality.

Hypotension in dogs is defined as a systolic arterial blood pressure value lower than 90 mmHg, or a mean arterial blood pressure value lower than 80 mmHg (BRADY and OTTO, 2001). There are many causes of hypotension, and it develops because of the interplay of various factors on peripheral arterial resistance, heart frequency and/or cardiac output. Primary goals of treatment are patient stabilization and correction of hypotension (ERHARD and HENKE, 2002; WADDEL, 2005). Hypotension is commonly reported in septic dogs (DE LAFORCADE and ROZANSKI, 2001), and has been reported in canine babesiosis caused by *B. rossi* (JACOBSON et al., 2000), an experimental study of canine babesiosis caused by *B. canis* (SCHETTERS et al., 2009), and a study of septic shock in canine babesiosis caused by *B. canis* (MATIJATKO et al., 2009).

The aim of this study was to compare arterial blood pressure values in healthy dogs and dogs naturally infected with *B. canis*, and also to compare arterial blood pressure values in dogs with complicated and uncomplicated babesiosis, in order to determine if hypotension is an important contributing factor in the development of complicated babesiosis and whether it affects the outcome of the disease.

Materials and methods

Fifty dogs were included in this study. They were divided into two groups: group 1 consisted of 30 dogs naturally infected with *B. canis* and group 2 of 20 healthy dogs. Group 1 was further divided into groups with complicated and uncomplicated babesiosis. All the dogs were clinically examined, and the blood samples for haematological and biochemical parameters were taken from the cephalic vein. In each dog blood pressure was measured at admission.

Babesiosis was diagnosed by demonstrating *B. canis* in Romanowsky stained blood smears. Group 2 was used as healthy controls.

The blood samples were placed in tubes with ethylenediaminetetraacetic acid (EDTA) for haematological analysis and in tubes with no anticoagulant, which were centrifuged at 1200 x g prior to biochemical analysis. White blood cell count (WBC), platelet count and haematocrit (HCT) were determined using an automatic haematology analyser (System 9120; Serono Baker Diagnostic) while serum creatinine, alanine aminotranspherase (ALT), alkaline phosphatase (AP), creatine phosphokinase (CPK), glucose and serum bilirubin were determined using a biochemical autoanalyser, Olympus AU 600 (Olympus Diagnostica, Hamburg). Analyses were performed using standard methods and the

original reagents of the manufacturer (Olympus Diagnostica, Hamburg). The animal was classified as having the complicated form of the babesiosis if one of the following criteria were fulfilled: renal dysfunction (serum creatinine more than 180 μmol/L - reference values 44-140 μmol/L), liver dysfunction (alanine aminotranspherase, ALT, more than 176 U/L - reference value less than 88 U/L, and alkaline phosphatase, AP, more than 360 U/L - reference value less than 156 U/L), central nervous system dysfunction (a modified Glasgow coma scale score less than 9) (SHORES, 1989; WELZL et al., 2001), respiratory system dysfunction (radiographic signs of pulmonary oedema, dyspnoea or blood/tinged frothy nasal discharge), muscular involvement (creatine phosphokinase, CPK, more than 600 U/L - reference value less than 160 U/L). We included bilirubin values greater than 100 μmol/L (reference value 1.7-8.6 μmol/L) as an additional criterion for liver dysfunction (WEISER, 1992). If two or more of the aforementioned criteria were fulfilled the animal was classified as MODS positive and included in the complicated babesiosis group.

One dose (6 mg/kg) of imidocarb dipropionat (Imizol®, Schering-Plough) was administered to all the dogs subcutaneously on the day of admission. Additional treatment consisted of various fluids (hydroxyethyl starch and crystalloids), whole blood transfusion, and in dogs that were dyspnoeic or had radiographic signs of pulmonary oedema, oxygen supplementation was administered, via intranasal tubes or oxygen cage.

Blood pressure was determined non-invasively by an oscillometric technique: we used a PM-9000Vet Veterinary Portable Multi-Parameter Patient Monitor (Grady Medical Systems, Inc., Temecula, CA, USA) and standard dog cuffs. Each dog was placed in right lateral recumbency, and the cuff was placed on the distal left forelimb, immediately proximal to the carpus, over the radial artery. The cuff size was selected so that its width was approximately 40% of the limb circumference. Five measurements of systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were recorded (JACOBSON et al., 2000). Hypotension was defined as MAP lower than 80 mmHg, and refractory hypotension was defined as hypotension (SAP less than 90 mmHg) that persisted despite adequate volume repletion (BRADY and OTTO, 2001).

Arterial blood pressure values in healthy dogs and dogs with babesiosis, as well as in dogs with uncomplicated and complicated babesiosis, were compared using the Mann-Whitney U test, and P values of <0.05 were considered to be significant.

Results

Out of 30 dogs with confirmed babesiosis, 12 developed a complicated form and 18 an uncomplicated form of babesiosis. In two dogs with complicated babesiosis, single organ dysfunction was present (acute hepatic failure), while in rest of the dogs (10) MODS developed.

In dogs that developed MODS, one dog had four organ system involvement, five dogs had three organ system involvement, while in four dogs two organ system involvement was present. Within MODS, the most common feature noted was acute renal dysfunction (10 dogs), while central nervous system dysfunction was the rarest complication (1 dog). The most frequent combination of organ system dysfunction within MODS was acute renal dysfunction and muscle involvement (7 dogs), followed by acute renal dysfunction and liver dysfunction (5 dogs), acute renal dysfunction and ARDS (3 dogs), liver dysfunction and muscle involvement (2 dogs), ARDS and muscle involvement (2 dogs), and finally liver dysfunction and ARDS (1 dog) (Table 1). All the dogs with uncomplicated forms of babesiosis survived, while nine out of twelve dogs with complicated forms of babesiosis died. Mortality was significantly higher ($P<0.05$) in cases of complicated babesiosis compared to uncomplicated babesiosis.

Arterial blood pressure values in healthy dogs and dogs with babesiosis are shown in Table 2. In 11/12 dogs with complicated babesiosis hypotension was present. None of the 18 dogs with uncomplicated babesiosis were hypotensive. A statistically significant difference ($P<0.05$) was noted in blood pressure values of healthy dogs and dogs with babesiosis. Arterial blood pressure in dogs with uncomplicated babesiosis was higher when compared to healthy dogs, as well as dogs with complicated babesiosis.

Table 1. Overview of complications in dogs with developed complicated babesiosis and their number

	Renal failure	Hepatic failure	CNS dysfunction	Muscle damage	Respiratory dysfunction	Total number
1.	+	-	-	+	-	2
2.	-	+	-	-	-	1
3.	+	-	-	+	+	3
4.	+	+	-	-	-	2
5.	+	-	-	+	+	3
6.	-	+	-	-	-	1
7.	+	-	-	+	-	2
8.	+	+	-	-	-	2
9.	+	+	-	+	-	3
10.	+	+	-	+	-	3
11.	+	+	+	-	+	4
12.	+	-	-	+	-	2
Total number	10	7	1	7	3	

Table 2. Arterial blood pressure values in healthy dogs and dogs with babesiosis.

	SAP/DAP/MAP (min-max)	(SAP) (mean ± 2 SD)	(DAP) (mean ± 2 SD)	(MAP) (mean ± 2 SD)
Healthy dogs	123-193/ 59-117/ 70-134	145 ± 44.02*	92 ± 32.96*	106 ± 22.38*
Dogs with babesiosis	60-202/ 34-129/ 42-143	120 ± 96.76*	68 ± 59.1*	83 ± 65.08*
Uncomplicated babesiosis	146-202/ 55-124/ 75-143	162 ± 51.96*	95 ± 48.38*	116 ± 41.33*
Complicated babesiosis	60-193/ 34-59/ 42-73	90 ± 69.38*	48 ± 15.86*	60 ± 22.38*
Reference values	-	133	75	104

(SAP) Systolic arterial blood pressure. (DAP) Diastolic arterial blood pressure. (MAP) Mean arterial pressure. (*) Significant difference ($P<0.05$) between healty dogs and dogs with babesiosis. Reference values for SAP and DAP according to Bodey and Michell (1996).

Discussion

Arterial blood pressure is present in the arterial part of circulation, and is often defined as the product of cardiac output and peripheral resistance (EGNER, 2002). In sepsis, hypotension commonly occurs, and is one of the criteria used to define the progression of inflammatory response, from severe sepsis to septic shock (BONE et al., 1992). The release of pro-inflammatory mediators and vasodilators plays a crucial role in the genesis of hypotension in sepsis (PARRATT, 1997).

Babesiosis is a widespread disease of various species of animals, with the severity of the disease ranging from mild to fatal. Complications of babesiosis can occur individually or in combination, and although they are different, the mechanism which promotes them is uniform (JACOBSON and CLARK, 1994; JACOBSON and LOBETTI, 1996; LOBETTI et al., 1996; WELZL et al., 2001; JACOBSON, 2006). This mechanism is the consequence of poorly regulated inflammatory response and is similar in various conditions, such as bacterial sepsis, babesiosis, malaria, multiple trauma and burns (JACOBSON and CLARK, 1994). All these conditions are similar in clinical presentation, and are characterized by multi-system signs, caused by dysfunction of different organ systems (JACOBSON, 2006).

In our study, 10/12 dogs with a complicated form of babesiosis (83%) developed MODS. MODS as a complication of babesiosis was first described in the study by WELZL et al. (2001), in canine babesiosis caused by *B. rossi*. Later, the development of MODS was also reported in canine babesiosis caused by *B. canis* (MÁTHÉ et al., 2006; MATIJATKO et al., 2009; MATIJATKO et al., 2010). Within MODS, the most frequent complication was acute renal dysfunction (83%), followed by liver dysfunction (58%), muscle involvement

(58%), ARDS (33%) and central nervous system dysfunction (8%). These results are similar to the results of other studies of MODS in canine babesiosis (WELZL et al., 2001; MATIJATKO et al., 2010).

The high occurrence of renal dysfunction in our study (83%) can be explained by the fact that the patients which developed renal dysfunction had significantly lower arterial blood pressure ($P<0.05$), compared to uncomplicated babesiosis. It is a well-known fact that hypotension is one of the main causes of poor tissue perfusion, which is especially detrimental to renal tissue (BONE et al., 1992; BAGSHAW and BELLOMO, 2006; ALDRICH, 2007). Another possible cause of renal damage is rhabdomiolysis. Renal dysfunction caused by myoglobin release due to rhabdomiolysis has been documented in canine babesiosis (JACOBSON and LOBETTI; 1996, MATIJATKO et al., 2009), and has been confirmed in our study as well, because all dogs with muscle damage also had renal dysfunction (Table 1).

Hypoxia can be the single cause of diffuse hepatocyte swelling and resultant hepatic dysfunction in dogs with babesiosis (LOBETTI, 2000). Uncomplicated babesiosis has been suggested to be a consequence of haemolysis (JACOBSON and CLARK, 1994), but recent publications showed that both uncomplicated and complicated babesiosis appear to be the result of host inflammatory responses (MATIJATKO et al., 2007; SCHETTERS et al., 2009). Although haemolysis and consequent anaemia occur in canine babesiosis, it is unlikely that they are the main features of babesiosis that lead to tissue hypoxia. Therefore, hypotension could not be excluded as a contributing factor of hepatic tissue hypoxia that could result in hepatic dysfunction.

Regarding blood pressure (Table 2), this study showed that dogs with babesiosis have lower blood pressure (SAP = 120 ± 96.76, DAP = 68 ± 59.1, MAP = 83 ± 65.08) compared to healthy dogs (SAP = 145 ± 44.02; DAP = 92 ± 32.96; MAP = 106 ± 22.38). Furthermore, blood pressure was higher in dogs with uncomplicated babesiosis (SAP = 162 ± 51.96, DAP = 95 ± 48.38, MAP = 116 ± 41.33) compared to healthy dogs (SAP = 145 ± 44.02; DAP = 92 ± 32.96; MAP = 106 ± 22.38), and lower in dogs with complicated babesiosis (SAP = 90 ± 69.38, DAP = 48 ± 15.86, MAP = 60 ± 22.38) compared to dogs with uncomplicated babesiosis (SAP = 162 ± 51.96, DAP = 95 ± 48.38, MAP = 116 ± 41.33).

High blood pressure in dogs with babesiosis has already been documented, namely by JACOBSON et al. (2000) and LOBETTI et al. (2012). In the study by JACOBSON et al. (2000) one animal (out of 10) had high blood pressure, while in the study by LOBETTI et al. (2012) five individual animals were hypertensive. The higher blood pressure values in dogs with uncomplicated babesiosis can be explained by activation of the compensatory anti-inflammatory response, because of which fluids are translocated from the interstitial to vascular space, with the resultant increase in arterial blood pressure (JACOBSON et al.,

2000; SCHETTERS et al., 2009). It can be hypothesized that activation of compensatory mechanisms (primarily of the sympathetic nervous system and renin-angiotensin-aldosterone system) results in increased cardiac output and stroke volume, and, at least in the beginning, increased vascular tone. Contrary to these findings, SCHETTERS et al. (2009) reported hypotension despite haemodilution.

Hypotension has been documented in canine babesiosis caused by *B. rossi* (JACOBSON et al., 2000) and *B. canis* (MATIJATKO et al. 2009, SCHETTERS et al. 2009). In the study of canine babesiosis caused by *B. rossi* by JACOBSON et al. (2000) it was established that blood pressure values were lower in dogs that died compared to the values of dogs that survived, but that the differences were not statistically significant. In complicated forms of canine babesiosis, due to the overwhelming and uncontrolled inflammatory response and the absence of a compensatory anti-inflammatory response, blood pressure values are lower and hypotension develops.

Hypotension in canine babesiosis is most probably the result of vasodilatation, the decrease in intravascular volume caused by increased vascular permeability and decreased myocardial function. The degree of changes in the aforementioned mechanisms influences the severity of the disease (JACOBSON et al., 1999; LOBETTI, 2000).

Hypotension is well documented in dogs with complicated forms of babesiosis, as was established in our study as well, and is considered relevant when associated with poor tissue perfusion (LITTMAN and DROBATZ, 1995). Also, it is questionable if a single blood pressure measurement is a reliable predictor of the progression of the disease. In human medicine, the study of septic shock has shown that MAP at admission did not differ between survivors and non-survivors, but blood pressure values taken 24 hours after admission had prognostic value (BERNARDIN et al., 1996).

Finally, the results of this study confirm that hypotension is present in complicated forms of canine babesiosis caused by *B. canis*. However, serial blood pressure measurements should be carried out, in order to estimate the influence of blood pressure changes on the course and outcome of the disease. Early recognition of hypotension, coupled with the timely start of treatment, may influence disease progression, and possibly prevent development of MODS and septic shock.

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TORTI, M., M. ČERLEK, V. MATIJATKO, M. BRKLJAČIĆ, I. KIŠ, I. MAYER, D. POTOČNJAK, V. MRLJAK: Vrijednosti arterijskog krvnog tlaka u pasa prirodno invadiranih vrstom *Babesia canis*. Vet. arhiv 84, 563-574, 2014.

SAŽETAK

Babezioza predstavlja značajnu bolest domaćih i divljih životinja uzrokovanu protozoama roda *Babesia*. Cilj je istraživanja bio usporediti vrijednosti arterijskog krvnog tlaka u zdravih pasa, pasa oboljelih od nekompliciranog i pasa oboljelih od kompliciranog oblika babezioze uzrokovane vrstom *Babesia canis*. Dodatni je cilj bio utvrditi doprinosi li hipotenzija razvoju kompliciranog oblika babezioze i utječe li na ishod bolesti. Istraživanje je obuhvatilo 50 pasa koji su bili podijeljeni u dvije skupine: zdrave pse (20 pasa) i pse prirodno zaražene vrstom *B. canis* (30 pasa). Skupina prirodno inficiranih pasa dodatno je bila podijeljena na dvije podskupine: skupinu pasa oboljelu od kompliciranog oblika (12 pasa) i skupinu pasa oboljelu od nekompliciranog oblika (18 pasa). Babezioza je dokazana nalazom parazita u razmascima periferne krvi obojenima po Romanowskom. Istraživanje je pokazalo da psi oboljeli od babezioze imaju niži arterijski krvni tlak (sistolički arterijski tlak = 120 ± 96,76, dijastolički arterijski tlak = 68 ± 59,1, srednji arterijski tlak = 83 ± 65,08) u odnosu na zdrave pse (sistolički arterijski tlak = 145 ± 44,02; dijastolički arterijski tlak = 92 ± 32,96; srednji arterijski tlak = 106 ± 22,38). Arterijski krvni tlak bio je viši u pasa oboljelih od nekompliciranog oblika babezioze (sistolički arterijski tlak = 162 ± 51,96, dijastolički arterijski tlak = 95 ± 48,38, srednji arterijski tlak = 116 ± 41,33) u odnosu na zdrave pse (sistolički arterijski tlak = 145 ± 44,02; dijastolički arterijski tlak = 92 ± 32,96; srednji arterijski tlak = 106 ± 22,38), i nizak u pasa oboljelih od kompliciranog oblika babezioze (sistolički arterijski tlak = 90 ± 69,38, dijastolički arterijski tlak = 48 ± 15,86, srednji arterijski tlak = 60 ± 22,38) u odnosu na pse oboljele od nekompliciranog oblika babezioze (sistolički arterijski tlak = 162 ± 51,96, dijastolički arterijski tlak = 95 ± 48,38, srednji arterijski tlak = 116 ± 41,33). Rezultati istraživanja potvrđuju da je hipotenzija prisutna u pasa oboljelih od kompliciranog oblika babezioze uzrokovanog vrstom *Babesia canis*. Serijska mjerenja arterijskog krvnog tlaka potrebna su kako bi se mogao procijeniti utjecaj promjena arterijskog krvnog tlaka na tijek i ishod bolesti.

Ključne riječi: arterijski krvni tlak, sistolički krvni tlak, dijastolički krvni tlak, srednji arterijski krvni tlak, pas, babezioza