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Capillary and venous *Babesia canis rossi* parasitaemias and their association with outcome of infection and circulatory compromise

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Abstract

This observational study of 100 dogs naturally infected with *Babesia canis rossi* determined whether severity of parasitaemia was associated with outcome of infection and documented the relative distribution of parasitised red blood cells (pRBC) in capillary and venous circulation. The association between increased parasitaemias and outcome with a clinically compromised circulation was also investigated. Outcome was defined as either hospitalisation with death, or hospitalisation with eventual recovery or treatment as an outpatient. Dogs were enrolled if large babesias were found on stained thin capillary blood smears made from an ear prick. Thin venous smears were prepared from jugular or cephalic blood. Parasitaemias were manually counted and expressed as the percent pRBC. Ten dogs died, 50 recovered after hospitalisation and 40 were treated as outpatients. Venous sampling site did not affect venous parasitaemia ($P = 0.6$). Both capillary and venous parasitaemias of dogs that died were significantly higher than those of dogs that recovered after hospitalisation ($P = 0.002$) and dogs that were treated as outpatients ($P < 0.0001$). When assessing the whole group, capillary parasitaemia (median 0.61%, range <0.05–71.6%, interquartile range (IQR) 0.22–3.75%) was significantly higher than venous parasitaemia (median 0.14%, range 0–30.6%, IQR 0.046–0.52%) with $P < 0.0001$. The 21 dogs with a clinically compromised circulation were more likely to die ($P < 0.0001$) and had significantly higher capillary (median 5.98%, range 0.09–71.6%, IQR 2.44–19.41%) and venous (median 2.81%, range <0.05–30.6%, IQR 0.17–9.03%) parasitaemias than the 79 dogs with a clinically normal circulation (capillary median parasitaemia 0.38%, range <0.05–12.87%, IQR 0.16–1.42%; venous median parasitaemia 0.096%, range 0–6.13%, IQR <0.05–0.33%; $P < 0.0001$). This study shows that high parasitaemia is significantly associated with death in *B c rossi* infected dogs. The previous clinical suspicion that capillary parasitaemias are usually higher than venous parasitaemias is confirmed. Thus capillary samples are the most appropriate diagnostic samples. Prior observations that a clinically compromised circulation

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is associated with death are confirmed. Despite the highly significant association between compromised circulation and higher parasitaemia, it is thought unlikely that parasite burden is the sole trigger for circulatory collapse.

Keywords: *Babesia canis rossi*; Capillary parasitaemia; Venous parasitaemia; Circulation; Outcome

1. Introduction

Canine babesiosis is an economically important disease in South Africa. Amongst dogs presented to the Onderstepoort Veterinary Academic Hospital's (OVAH) first opinion service it was diagnosed in an average of 1170 dogs annually between 1988 and 1993 (Shakespeare, 1995). Three large canine babesia (*Babesia canis rossi*, *Babesia canis canis* and *Babesia canis vogeli*) are well described (Uilenberg et al., 1989). It is suggested that they represent different species (Carret et al., 1999). All species may cause pyrexia, anorexia, splenomegaly and anaemia in susceptible dogs, but some distinct differences in pathophysiology (Schetters et al., 1997) and virulence have been described (Uilenberg et al., 1989).

B c rossi and *B c vogeli* have been identified in South Africa (Matjila et al., 2004). Prior to 2005, the babesia species infecting dogs presenting to the OVAH was not routinely determined. A total of six *B c vogeli* infections were detected amongst a convenience sample from approximately 400 dogs presenting to the OVAH with clinical signs of babesiosis during 2005 (P.T. Matjila, personal communication 2006). These included the dogs sampled for this study. Thus *B c rossi* appears to be the most prevalent species infecting dogs presenting to the OVAH with clinical signs of babesiosis. *B c rossi* is highly pathogenic (Uilenberg et al., 1989) and infected dogs require treatment to prevent mortality (Schetters et al., 1997). In two large studies of clinical cases admitted to the OVAH for treatment, mortality rate was between 12 and 15% (Reyers et al., 1998; Nel et al., 2004). The clinical signs of dogs infected with South African strains of *B c vogeli* have not been described, but this parasite usually causes mild or subclinical signs in adult dogs elsewhere in the world (Uilenberg et al., 1989).

Researchers have long remarked on the similarities between the diseases caused by virulent *B canis* strains (Malherbe, 1956; Maegraith et al., 1957; Clark and Jacobson, 1998); *Babesia bovis* (*syn. argentina*) (Wright et al., 1988; Schetters and Eling, 1999; Allred and Al-Khedery, 2004), the most pathogenic babesia infecting cattle; and *Plasmodium falciparum*, the most pathogenic of the plasmodium species infecting humans. All three protozoa cause acute disease that may include severe haemolysis and haemoglobinuria, icterus, circulatory collapse, multiple organ failure and neurological signs (Jacobson and Clark, 1994). On post mortem capillaries, particularly cerebral capillaries, may be packed with parasitised red blood cells (pRBC) (Graham-Smith, 1905; Maegraith et al., 1957; Wright, 1972; Macpherson et al., 1985; Pardini, 2000). This phenomenon has not been observed during infection with less pathogenic babesia (de Vos and Potgieter, 1994) or plasmodium species (Berendt et al., 1990; Schetters and Eling, 1999).

Electron-microscopic studies have demonstrated that *P falciparum* (Berendt et al., 1990; Cooke et al., 2005) and *B bovis* pRBC (Potgieter and Els, 1979; Aikawa et al., 1985; Allred and Al-Khedery, 2004; Cooke et al., 2005) as well as *B canis* infected red cells of dogs presented to the OVAH (Pardini, 2000) adhere to capillary endothelium. Sequestration, the interaction between parasite derived antigens on pRBC surfaces and endothelial receptors, is an obligatory part of *P falciparum*'s lifecycle (Newbold et al., 1999). It has been studied extensively in falciparum malaria as it is thought to be an important determinant of this parasite's virulence (Newbold et al., 1999; Allred and Al-Khedery, 2004). The study of specific antigen–receptor interactions is in its infancy in babesiosis (Cooke et al., 2005). The prevalence of sequestration and its association with relative parasite distribution and outcome have not been studied in canine babesiosis.

In South Africa, the diagnosis of canine babesiosis is made on examination of a capillary smear taken from the ear pinna (Malherbe and Parkin, 1951; Keller et al., 2004; Jacobson and Lobetti, 2005). Blood smears are typically used to quantify parasitaemia in babesia research (Van Heerden et al., 1983; Schetters et al., 1994, 1996, 1997; Lewis et al., 1995). Despite

widespread use of smears, there is limited information on the association between parasitaemia and outcome in *B c rossi* infections. Early workers' impressions (Purchase, 1947; Maegraith et al., 1957) and a preliminary study (Reyers and van Zyl, 1995) could show no association between virulent *B canis* sp. parasitaemia and outcome. However parasitaemia was associated with the severity of clinical signs in experimental *B c rossi* infections (Schetters et al., 1997).

Although many workers have remarked that canine babesiosis is best diagnosed on capillary smears (Paine, 1934; Malherbe and Parkin, 1951; Malherbe, 1956; Maegraith et al., 1957), the relative distribution of pRBC in *B c rossi* infections has not been formally determined by any means. In *B bovis* infections, capillary parasitaemia is significantly greater than venous parasitaemia (Callow and Pepper, 1974). The converse has been shown for a North American large babesia assumed to be *B c vogeli* (Ewing, 1966; Birkenheuer et al., 2005). Capillary parasitaemias should exceed venous parasitaemias if sequestration is prevalent.

The association between parasitaemia and circulatory compromise has not been studied in canine babesiosis. Circulatory collapse has been offered as a common cause of death (Maegraith et al., 1957) and appeared to be associated with a poor prognosis in this disease (Malherbe et al., 1976; Abdullahi et al., 1990). Clinical collapse has been associated with hypotension (Jacobson et al., 2000), hyperlactaemia (Malherbe et al., 1976; Leisewitz et al., 2001; Jacobson and Lobetti, 2005), hypoglycaemia (Keller et al., 2004) and acid–base disturbances (Leisewitz et al., 2001). Thus circulatory compromise appears to be associated with severely affected individuals.

The aims of this study were to determine whether parasitaemia was associated with outcome and to determine the relative distribution of pRBC in capillary and venous circulation of dogs naturally infected with *B c rossi*. Additional aims were to confirm prior studies' conclusions that circulatory compromise was associated with outcome and to determine whether circulatory compromise was also associated with increased parasitaemias. The confirmation of relative *B c rossi* parasite distribution is long overdue. If an association between capillary (rather than venous) parasitaemia and outcome or circulatory compromise could be shown, this would provide clinical evidence to support the theory that sequestration may be an important part of *B c rossi*'s pathomechanism.

2. Materials and methods

2.1. Study population

One hundred and seventeen naturally infected dogs that had large babesias identified on a stained thin capillary blood smear were enrolled in this observational study. Dogs were not sampled if their condition was complicated by any of the following: treatment for babesiosis in the preceding 3 weeks, prior splenectomy, suspected or confirmed severe concurrent disease, or detection of *Ehrlichia canis* morula on capillary smear. Dogs were excluded after sampling if their slides were damaged, there was inadequate follow-up, elective euthanasia was performed, severe concurrent disease became evident or *B c vogeli* or *Ehrlichia canis* were detected by polymerase chain reaction (PCR) and reverse line blot (RLB) (Matjila et al., 2004). The study was approved by the University of Pretoria's Animal Use and Care Committee.

2.2. Clinical examination to determine circulatory score

The primary investigator examined all the dogs and recorded rectal temperature, heart rate, pulse quality, capillary refill time (CRT), habitus and hydration status. A circulatory score was assigned based on clinical information (Tables 1 and 2). The owner was questioned about previous episodes of babesiosis in all cases and about prior surgery if the spleen was not clearly palpable.

Shock has been defined as ''acute circulatory failure with inadequate or inappropriately distributed tissue perfusion resulting in generalised cellular hypoxia'' (Graham and Parke, 2005). The clinical criteria used to define the circulatory score were selected by consulting a standard text on shock (Day, 2000) and omitting parameters that would usually be abnormal in babesia cases as a consequence of anaemia (Table 3). Clinically detectable dehydration was added to the parameters as this would not be the direct result of babesiosis and could contribute towards circulatory failure. Dehydration was recorded

Table 1
Circulatory score

Score	Description
1	Presence of 2 or more of the following • Pulse weak • Bradycardia • CRT <1 s or >2 s • Collapse, i.e. unable to stand • Clinically detectable dehydration • Hypothermia <37.5 °C
0	Presence of 1 or none of the above

Table 2
Criteria for diagnosis of bradycardia (Miller et al., 1999; Spotswood et al., 2005)

	<10 kg	>10–25 kg	>25 kg
PCV <15%	<120 bpm	<100 bpm	<80 bpm
PCV 15 to <25%	<110 bpm	<90 bpm	<70 bpm
PCV 25% or more	<100 bpm	<80 bpm	<60 bpm

if the eyes were sunken, skin fold turgidity was decreased and/or the oral mucosae were dry. Blood glucose was determined and hypoglycaemia treated before habitus was assessed. Mental depression, stupor and coma were included under the criterion 'collapse, unable to stand' as the latter was more easily assessed objectively (Table 1). Bradycardia would be very unusual in babesiosis. It has been defined as a heart rate below 100 for small dogs and below 60 for large breeds (Miller et al., 1999). In a normovolaemic anaemia model in conscious beagles weighing 9.5–15.2 kg, heart rate increased by a mean of 25 bpm as the haematocrit fell from a mean of 0.47 to 0.13–0.17 l/l (Spotswood et al., 2005). This information was used to define bradycardia for the study population (Table 2).

2.3. Sampling

Samples were collected prior to treatment. A thin capillary blood smear was made from the first drop of blood that formed after the clipped inner surface of an ear was pricked with a 21G needle 5–15 mm away from the ear margin and away from the marginal ear vein.

Venous blood (1/2 to 4 ml) was collected into EDTA within 10 min of making the capillary smear and a thin smear was made from this blood. Jugular samples were usually collected. Cephalic samples were collected if the jugular vein was not accessible for some reason, if the dog was fractious or if a cephalic catheter needed to be placed for therapeutic reasons. In the latter case, a

Table 3
Selection of criteria included in the circulatory score

	Sign of shock (Day, 2000)	Sign expected as result of anaemia	Included in circulatory score
Compensatory stage of shock	CRT < 1 s	No	Included
	Tachycardia	Yes	No
	Injected mucosae	Difficult to show when anaemic	No
Early decompensatory stage of shock	CRT > 2 s	No	Included
	Tachycardia	Yes	No
	Pale mucosae	Yes	No
	Mental depression	No	Included, after hypoglycaemia ruled out (Keller et al., 2004)
	Hypothermia	No	Included
Terminal shock	Bradycardia	No	Included
	Pale mucosae	Yes	No
	Cyanotic mucosae	No: need haemoglobin to become cyanotic	No
	Hypothermia	No	Included
	Weak pulses	No	Included
	Stupor/coma	No	Included, after hypoglycaemia ruled out (Keller et al., 2004)

sample was withdrawn immediately after the catheter was placed. The remaining EDTA blood was stored at 4 °C until it was submitted for PCR and RLB. The primary investigator made all the smears.

2.4. PCR and RLB

The PCR and RLB were performed as previously described (Matjila et al., 2004). PCR was conducted with a set of primers that amplified a 460–540 base pair fragment of the 18S SSU rRNA spanning the V4 region, a region conserved for *Babesia* and *Theileria*. The *Ehrlichia* PCR amplified the V1 hypervariable region of the 16S SSU rRNA (Schouls et al., 1999; Bekker et al., 2002). The membrane used for RLB included probes for *B c vogeli*, *B c rossi*, *B c canis* and *Ehrlichia canis*.

2.5. Groups of dogs

Animals were grouped according to the following outcomes: treated with an antibabesial during the consultation and sent home immediately (H), admitted for treatment and survived until discharge (A), and death despite treatment or euthanasia owing to poor prognosis (D). Owners of dogs in the H group were contacted on completion of the study to confirm that their pet had recovered completely. Dogs that were represented to the OVAH with complications of their babesiosis after being treated as an outpatient were transferred to group A.

The duty clinician decided whether the dog should be admitted to the hospital or treated as an outpatient. All diagnostic and therapeutic decisions were made by the attending clinician, not the primary investigator. Because this was a clinical study of client owned dogs, therapy was not standardised. Nevertheless babesia cases are treated in a similar manner by all clinicians in the OVAH.

2.6. Analysis of parasitaemia

Blood smears were stained with Kyro-quick (Kyron Laboratories, Benrose, South Africa), a Romanowski stain, and scored at 1000× magnification with the aid of a digital image analysis program (Optimas 6 for Win 95/NT 4.0, Media Cybernetics, distributed by Carl Zeiss Ltd., Randburg, South Africa). A digital photograph of a section of the smear was transferred to a computer screen and magnified. The computer program allowed markers to be placed over counted red blood cells (RBC), which ensured accurate counting.

A semi-quantitative non-volumetric method (Jacobson, 1994) that was a combination of those used by Vaughan-Scott (2001) and Pardini (2000) was used to quantify parasitaemias. Free parasites were ignored. Unparasitised RBC and pRBC were counted separately in each field. The dogs' PCVs ranged between 5 and 60%, thus counting a specified number of fields would have resulted in less reliable results as anaemic samples would have had significantly fewer RBC examined. Instead, approximately 650 RBC were examined in the red cell area and the same number along the feather edge and along the sides of the smears. Thus at least 1950 RBC were examined per smear. Complete oil immersion fields were scored, as scoring only proportions of fields could artificially increase parasitaemias. Identification markings on the slides were covered to blind analysis. The primary investigator scored all the slides.

Results were added and expressed as a percent pRBC. A score of $<0.05\%$ was given if no pRBC were detected in the designated areas but were observed somewhere else on the smear. A score of 0% was assigned if no pRBC were detected on the smear after 15 min of scanning the slide without counting.

2.7. Data analysis

Multiple regression analysis was used to compare parasitaemias from cephalic and jugular samples, adjusting for outcome group. This was performed to demonstrate that venous blood sampling site was not responsible for any bias in the results. The significance level was set at $\alpha = 0.05$. Medians, ranges and interquartile ranges (IQR) were used to describe the capillary and venous parasitaemias as these were not normally distributed and log-transformation did not achieve a near-normal distribution. Kruskal–Wallis one-way ANOVA on ranks was used to determine whether capillary and venous parasitaemia differed between outcome groups. The Wilcoxon signed-rank test was used to compare capillary and venous parasitaemias for all the dogs together as well as within each of the three outcome groups. The Fisher exact test was used to confirm reports by previous

investigators that dogs with a collapsed circulation were significantly more likely to die. The Wilcoxon rank-sum test was performed to compare the capillary and venous parasitaemias for dogs with and without circulatory compromise. Statistical analyses were performed using NCSS 2004 (Kaysville, UT, USA).

3. Results

3.1. Study population

Of the 117 dogs sampled, 17 dogs were excluded for the following reasons: 8 dogs were infected with *Ehrlichia*. One dog had concurrent pneumonia. One had demodecosis and its babesia parasitaemia, anaemia and splenomegaly persisted for 3 months despite repeated treatments. Two dogs were euthanased electively. One dog died unexpectedly 2 days after diagnosis, having made an uneventful recovery from its babesiosis. Another died a week after being treated as an outpatient. The owner had observed this dog's condition deteriorate but felt he could not afford further treatment. Neither of the last two dogs was available for a post mortem examination. One dog treated as an outpatient (group H) was excluded because no follow-up information could be obtained. Two dogs were excluded because their slides were damaged. This left 100 dogs in the study group.

3.2. Circulatory score

There were 21 dogs with a clinically compromised circulation and 79 dogs with normal circulation. A CRT below 1 s was the most prevalent circulatory abnormality ($n = 75$). This was followed by collapse ($n = 17$), hypothermia ($n = 6$), dehydration ($n = 4$), bradycardia ($n = 1$) and weak pulse ($n = 1$). The CRT was not determined in 4 dogs as they were muzzled. The temperature was not recorded for 1 dog. These 5 dogs had no other abnormal criterion, thus the missing information could not have changed their classification (Table 1).

3.3. Venous sampling sites

Venous samples were collected from the jugular in 77 dogs and the cephalic vein in 22 dogs. The collection site was not recorded for 1 dog. Venous sampling site did not affect the magnitude of the venous parasitaemia ($P = 0.6$).

3.4. PCR and RLB

Five of the 8 dogs infected with *Ehrlichia* sp. were co-infected with *B c vogeli* and 3 with *B c rossi*. No dog was infected with more than one *Babesia* sp. No dog was infected with *B c vogeli* only. The five dogs infected with both *E canis* and *B c vogeli* in this study had capillary parasitaemias between <0.05 and 0.38% and venous parasitaemias below 0.05%. One venous smear was too damaged to score.

3.5. Groups of dogs

Of the 100 dogs included for further analysis, 60 dogs were admitted for further treatment and 40 discharged immediately after the consultation (group H). Ten of the admitted dogs died (group D), and 50 recovered (group A).

3.6. Parasitaemia and outcome

Both capillary and venous parasitaemias of the dogs that died (group D) were significantly higher than those of the dogs that were treated as outpatients (group H) ($P < 0.0001$ for both sampling sites) and also significantly higher than those of the dogs that were admitted for treatment and survived (group A) ($P = 0.002$ for both sampling sites). There was no significant difference in the capillary and venous parasitaemias of groups A and H ($P = 0.1$ and 0.2, respectively) (Fig. 1a and b).

3.6.1. Capillary compared with venous parasitaemia

All capillary smears contained parasites. One venous smear was negative for parasites after 15 min of continuous searching without scoring fields. Fourteen dogs had capillary parasitaemias above 10% (range 10.2–71.6%) and 5 dogs had venous parasitaemias above 10% (range 11.56–30.6%).

Capillary parasitaemias of the group of 100 dogs (median 0.61%, range <0.05–71.6%, IQR 0.22–3.75%) were significantly higher than venous parasitaemia (median 0.14%, range 0–30.6%, IQR 0.046–

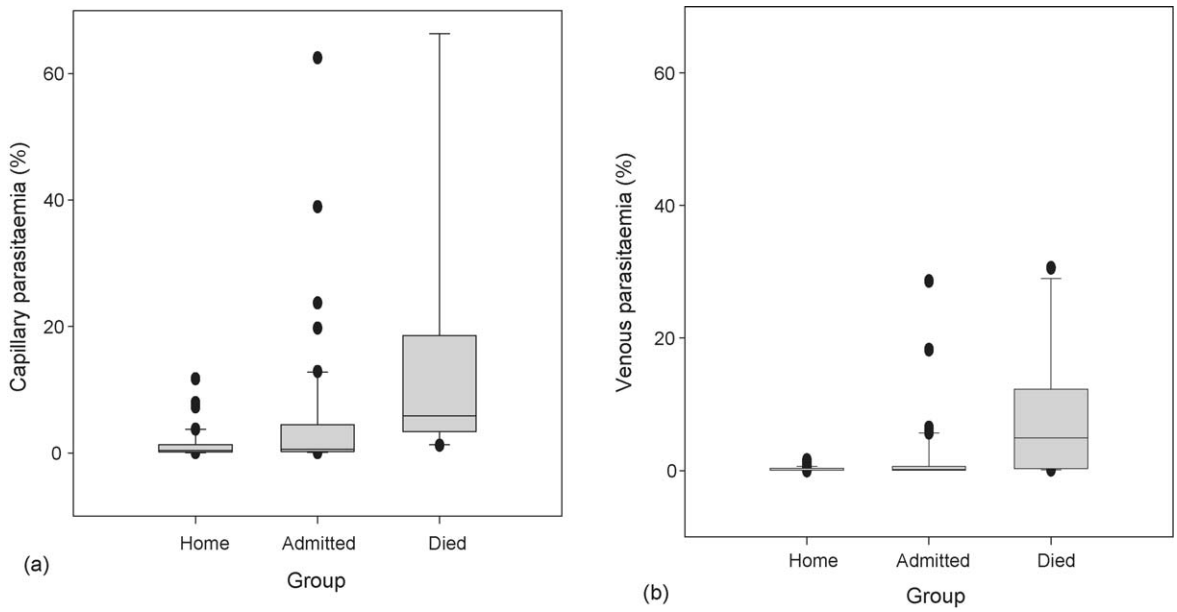

Fig. 1. Horizontal lines represent the median, boxes the interquartile range, whiskers the 10th and 90th percentiles, and circles the outside values. (a) Capillary parasitaemias of the three outcome groups. The difference was significant when the 10 dead dogs were compared with the 40 dogs that went home ($P = 0.0001$) and the 50 admitted dogs ($P = 0.002$). The difference between 'home' and the 'admitted' groups was not significant ($P = 0.1$). (b) Venous parasitaemias of the three outcome groups. The difference was significant when the 10 dead dogs were compared with the 40 dogs that went home ($P = 0.0001$) and the 50 admitted dogs ($P = 0.002$). The difference between 'home' and the 'admitted' groups was not significant ($P = 0.2$).

0.52%) with $P < 0.0001$. Within each individual outcome group, this difference was also significant: amongst the dogs that died, capillary parasitaemia (median 5.89%, range 1.21–71.6%) was significantly higher than venous parasitaemia (median 4.91%, range 0.1–30.6%) ($P = 0.025$). This was also true for the dogs surviving after hospitalisation (capillary parasitaemia median 0.56%, range <0.05–62.5%; venous parasitaemia median 0.17%, range <0.05–28.6%; $P < 0.0001$) and the dogs treated as outpatients (capillary parasitaemia median 0.37%, range <0.05–11.7%; venous parasitaemia median 0.09%, range 0–1.56%; $P < 0.0001$).

Seven of the 100 dogs had higher venous than capillary parasitaemias. One dog had both capillary and venous parasitaemias of 0.33%. Five dogs had both capillary and venous parasitaemias below 0.05%, making it difficult to compare relative parasite density in the two sampling sites. The difference between capillary and venous parasitaemias in individual dogs varied. The largest absolute differences were capillary and venous parasitaemias of 62.5 and 2.51%, and 71.6 and 30.6% in two dogs. The smallest percentage difference in the two sample sites were in two dogs with parasitaemias of 5.81 and 5.4%, and 0.095 and 0.09%, respectively. Thus even dogs with relatively high parasitaemias could have small differences in parasitaemias of the two sample sites. It is difficult to analyse this data further in the absence of information on the reliability of individual measurements and of an objective measure of whole body parasitaemia.

3.7. Circulatory score and outcome

Nine of the 21 dogs with a clinically compromised circulation and 1 of the 79 dogs with a clinically normal circulation died. Dogs with a compromised circulation were therefore significantly more likely to die ($P < 0.0001$).

3.7.1. Circulatory score and parasitaemia

Dogs with a clinically compromised circulation had a significantly higher capillary (median 5.98%, range 0.09–71.6%, IQR 2.44–19.4%) and venous

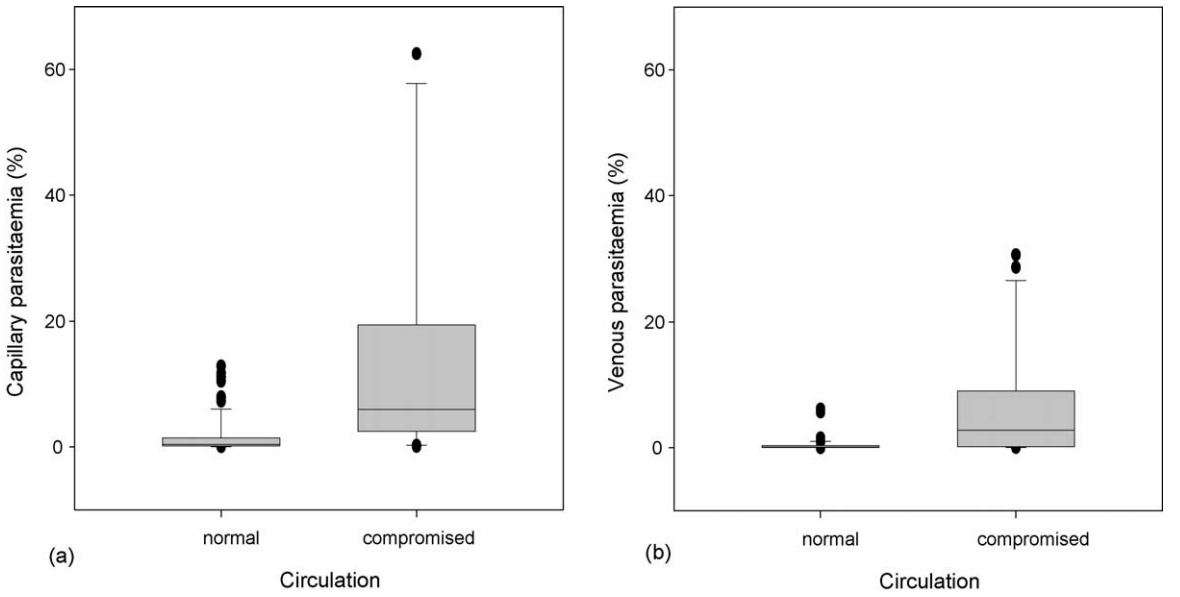

Fig. 2. (a) Capillary parasitaemia of dogs with normal ($n = 21$) and compromised circulation ($n = 79$). Horizontal lines represent the median, boxes the interquartile range, whiskers the 10th and 90th percentiles, and circles the outside values. The difference between the two groups was significant ($P < 0.0001$). (b) Venous parasitaemia of dogs with normal and compromised circulation. Horizontal lines represent the median, boxes the interquartile range, whiskers the 10th and 90th percentiles, and circles the outside values. The difference between the two groups was significant ($P < 0.0001$).

(median 2.81%, range <0.05–30.6%, IQR 0.17–9.03%) parasitaemia than dogs with a clinically normal circulation ($P < 0.0001$ for both). The latter had a median capillary parasitaemia of 0.38% (range <0.05–12.9%, IQR 0.16–1.42%) and a median venous parasitaemia of 0.096% (range 0–6.13%, IQR <0.05–0.33%) (Fig. 2a and b). Thus median capillary parasitaemia was 15 times and median venous parasitaemia was 29 times higher in dogs with a clinically compromised circulation.

4. Discussion

The results presented here show that *B c rossi* infected dogs that died had significantly higher capillary and venous parasitaemias than dogs that survived. A similar association has been shown in falciparum malaria (World Health Organisation, 2000). The only previous study that evaluated this aspect in canine babesiosis could not show a significant difference between the parasitaemias of survivors and non-survivors (Reyers and van Zyl, 1995). The study was designed to assess the prognostic significance of haematological parameters in babesia-infected dogs. The venous samples used in the study were drawn in the first 24 h after admission and treatment with an antibabesial. It was shown subsequently that parasitaemia will, on average, increase slightly during the first 2 h after treatment with diminazene aceturate or trypan blue and then fall dramatically by 6 h post treatment (Jacobson et al., 1996). Sampling animals at variable times post treatment probably obscured significant findings in the earlier study.

Despite the highly significant association between dogs that died and high parasitaemias, there is so much overlap between the groups (Fig. 1a and b) that this finding is not clinically helpful. The reason parasitaemia does not correlate more closely with outcome may lie in the varied host response to infection. For most dogs, parasite-induced anaemia is the most serious and potentially life-threatening problem. A proportion develop complications, some of which (hepatopathy, immune-mediated haemolysis) typically extend hospital stay but do not affect mortality if appropriately treated (van Zyl, 1995; Miller, 1999; Jacobson and Clark, 1994), while others (haemoconcentration, neurological signs not associated with hypoglycaemia,

acute renal failure, pulmonary oedema) require early, aggressive and intensive therapy and carry a poor prognosis (Jacobson and Clark, 1994; Reyers and van Zyl, 1995; Welzl et al., 2001; Keller et al., 2004). Individual dogs may develop several different complications to varying degrees (Welzl et al., 2001), thus disease manifestations form a continuum and affected dogs cannot be separated perfectly into uniform groups. An additional reason for the lack of a significant difference in parasitaemias between the two surviving groups is that the decision to hospitalise a dog was made by different people, as discussed in Section 2.5. Some dogs were hospitalised overnight but were discharged without further treatment when, for example, urine production was maintained or the PCV did not drop precipitously. Others were treated as outpatients at the owner's request owing to cost constraints although hospitalisation was indicated. Lastly the varied times after infection that the dogs were presented for treatment may have affected parasitaemia (Lewis et al., 1995).

This study shows that that capillary parasitaemia is greater than venous parasitaemia in most *B c rossi* infected dogs. Thus parasites of the most pathogenic large canine babesia species accumulate in capillaries while the large babesia studied in the USA (assumed to be *B c vogeli*) is more prevalent in venous samples (Ewing, 1966; Birkenheuer et al., 2005). This mirrors the observations in human malaria that the highly pathogenic *P falciparum* results in higher capillary parasitaemias while the less pathogenic *P vivax* appear with equal frequency in capillary and venous samples (Singh et al., 2003). This observation is consistent with the theory that *B c rossi* sequesters. The fact that capillary parasitaemias were occasionally similar to or lower than venous parasitaemias suggests that sequestration, if present, occurs only in a proportion of *B c rossi* infected dogs, or only during certain stages of the evolution of the disease.

An alternative explanation for the higher capillary parasitaemias could be that parasite-induced increases in RBC membrane rigidity may slow the transit of pRBC through capillaries and result in more pRBC being found there at any one time. Such changes in RBC membrane deformability have been demonstrated in falciparum malaria (Suwanarusk et al., 2004) and septic shock (Hinshaw, 1996), a condition that bears some resemblance to complicated canine babesiosis and falciparum malaria (Clark and Jacobson, 1998; Jacobson et al., 2000). Lastly, direct observations of capillary blood flow in 600 humans affected by a variety of diseases showed that RBC invariably lost their laminar flow in people sufficiently ill to seek medical care (Knisely et al., 1947). RBC travelled more slowly, aggregated at the dependent edge of vessels and RBC conglomerates hampered capillary flow. It has been postulated that parasites within aggregated RBC could find themselves in a microenvironment more favourable to multiplication (Malherbe, 1956; Schetters et al., 1998) and indirect evidence of local proliferation of *B c canis* parasites has been presented (Schetters et al., 1998). Thus any mechanism that retards capillary flow could favour localized parasite proliferation and consequently higher capillary parasitaemias.

The parasitaemias reported in this study are higher than previously reported ones: maximum parasitaemias of 10% were recorded in capillary or venous samples from experimentally infected dogs (Stewart, 1983; Lewis et al., 1995; Schetters et al., 1997) and maximal venous parasitaemias around 2.43% in a group of 30 naturally infected dogs (Vaughan-Scott, 2001; Jacobson et al., 2002; Jacobson and Lobetti, 2005). The main reason for this discrepancy is probably that two-thirds of the RBC were scored on the feather edge and the edges of the smear, areas where pRBC are known to accumulate (Pardini, 2000). Despite widespread use of blood smears to determine parasitaemia in babesia research, no standard methodology is described. Only 3 of 20 papers discussing babesia parasitaemia described which area of the smear was scored (Van Heerden et al., 1983; Jacobson et al., 1996, 2002). Thus parasitaemias should not be directly compared between studies. Treatment of experimentally infected dogs before higher parasitaemias developed could have contributed to the observed discrepancy (Stewart, 1983; Lewis et al., 1995; Schetters et al., 1997). Although the babesia species infecting dogs in the above studies were not confirmed by PCR, all studies with the exception of Schetters et al. (1997) were performed on dogs living around the OVAH or experimentally infected at Onderstepoort. It is thus likely that most if not all were infected with *B c rossi*. Schetters et al. (1997) studied the same South African strain that Uilenberg et al. (1989) used when they first proposed the taxonomic system used here.

There was a highly significant association between a higher circulatory score and outcome. This confirms prior work that clinical collapse and shock are poor prognostic signs in dogs with babesiosis (Maegraith et al., 1957; Abdullahi et al., 1990).

In the group of dogs with a clinically collapsed circulation, both capillary and venous parasitaemias were significantly higher than those of dogs with clinically normal circulation. Capillary parasitaemia would be expected to be increased markedly and out of proportion to venous parasitaemia if parasite sequestration is more prevalent among animals with a collapsed circulation. This was not shown. Further studies will be necessary to determine the importance of parasite sequestration in *B c rossi* infections. Before this can be attempted, accurate means of determining the presence or absence of sequestration in individual dogs and of determining total parasite burden are required. These are not available at present.

The association between higher parasitaemias and circulatory compromise prompts the question whether the two are causally related. Although canine babesiosis has been described as a form of sepsis (Jacobson et al., 2000), high parasitaemias are unlikely to be the sole trigger of circulatory collapse in babesia infected dogs as some dogs with low parasitaemias developed signs of circulatory collapse. The type of immune response or inflammatory response mounted by babesia-infected dogs is likely to play a significant role (Jacobson and Clark, 1994; Reyers et al., 1998). In individual animals hypovolaemia (Jacobson et al., 2000) and decreased myocardial function (Lobetti, 2005) could contribute toward the development of shock. Although these abnormalities are recognised as independent triggers of circulatory collapse, all could be related to the type of host inflammatory or immune response in babesiosis. The study of inflammatory mediators in babesia-infected dogs has not revealed any strong correlations with parasitaemia, outcome or circulatory compromise yet (Vaughan-Scott, 2001; Jacobson et al., 2002; Ulutas et al., 2005). The small number of dogs studied and the global measures used to study inflammatory mediators that often have a short half-life and a local effect probably hampered detection of significant correlations. This aspect of canine babesiosis deserves further study.

5. Conclusion

In summary, this study shows that dogs infected with *B c rossi* that die have higher capillary and venous parasitaemias than those that survive. It confirms previous clinical suspicion that capillary parasitaemias are usually higher than venous parasitaemias. Thus capillary samples are the most appropriate diagnostic samples. The study confirms prior observations that a clinically compromised circulation is associated with death. It shows that both capillary and venous parasitaemias are significantly higher in animals with a higher circulatory score, providing a practical means of identifying dogs likely to harbour a high parasite load. The higher capillary parasitaemias found in most dogs are consistent with the theory that *B c rossi* pRBC sequester. The higher venous parasitaemias documented in 7 dogs suggest that sequestration, if present, does not occur in all *B c rossi* infected dogs at all stages of infection. The effects of host immune and inflammatory responses on parasite distribution, outcome and circulatory status warrant further study.

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