The elevated serum urea:creatinine ratio in canine babesiosis in South Africa is not of renal origin

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ABSTRACT

Pigmented serum, usually due to free haemoglobin and/or bilirubin, is a common finding in dogs with babesiosis, resulting in interference with all biochemical tests that rely on photochemistry. This is particularly true of urea and creatinine determinations, complicating the diagnosis of acute renal failure, which is a serious complication of babesiosis. A disproportionately raised serum urea concentration of unknown origin occurs in severely anaemic canine babesiosis patients and gives rise to an increased serum urea:creatinine ratio. The assay for cystatin-C, an excellent measure of glomerular filtration rate, is unaffected by free serum haemoglobin, and due to its different intrinsic origins, is free of influence by the metabolic derangements and organ pathology, other than renal disease, encountered in canine babesiosis. Serum cystatin-C was used to compare the concentrations of serum urea and serum creatinine in dogs with the severely anaemic form of canine babesiosis as well as a canine babesiosis-free reference group. Mean serum urea and mean serum urea:creatinine ratio were significantly elevated in the babesia-infected group relative to the reference population in this study. Mean serum creatinine and mean serum cystatin-C were within the reference ranges. Therefore an elevated urea:creatinine ratio in canine babesiosis in the presence of a normal serum creatinine concentration is considered to be caused by an elevated serum urea concentration and is most likely of non-renal origin. Serum creatinine was therefore as specific a measure of renal function as serum cystatin-C in canine babesiosis in this study. The sensitivity of serum creatinine as a measure of renal function was not established by this study. Serum urea, however, proved to be of little use compared to serum cystatin-C and serum creatinine. Serum urea should therefore not be used to diagnose renal failure in canine babesiosis.

Key words: azotaemia, canine babesiosis, creatinine, cystatin-C, prerenal azotaemia, serum haemoglobin, urea.

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INTRODUCTION

The early diagnosis of acute renal failure in canine babesiosis is crucial. Azotaemia is an important biochemical finding in acute renal failure[12]. The serum concentrations of urea and/or creatinine are commonly used as indicators of azotaemia in canine babesiosis. Serum urea is commonly elevated in canine babesiosis in a manner disproportionate to serum creatinine[9]. In a recent retrospective study of approximately 400 *Babesia canis* cases, the mean and median serum urea concentrations were approximately double the normal laboratory serum concentrations, whilst mean and median serum creatinine concentrations fell within normal laboratory values[9]. Although it was not evaluated, this phenomenon would have caused an increased serum urea:creatinine ratio.

The normal serum urea:creatinine ratio reported in dogs is 10–15 (when calculated using mg/dℓ as the unit of measurement)[28]. In human medicine a serum urea:creatinine ratio in an azotaemic patient of ≥ 20 indicates prerenal azotaemia, whereas a serum urea:creatinine ratio of <20 in an azotaemic patient indicates intrinsic renal disease[6]. This would indicate that serum urea is more likely to increase due to prerenal factors than serum creatinine, whereas both parameters are equally likely to increase due to renal disease. It has been speculated that this phenomenon in canine babesiosis may be caused by a falsely raised serum urea, a form of laboratory error inherent in various test methods[34], or by prerenal azotaemia, or by serum substances interfering with the analysis of serum creatinine[4,5,9,15,39]. Some of this speculation may have its origins in the increased serum urea:creatinine ratios found in cases of intestinal haemorrhage, excessive protein loading or increased protein catabolism, where hyperureagenesis is common[32,37]. It is hypothesised that ammonia loading occurs in canine babesiosis as a result of haemolysis, blood transfusions and gastrointestinal haemorrhage. This could lead to a non-renal related elevation in serum urea concentrations *via* hyperureagenesis and could cause the increased serum urea: creatinine ratio apparent in canine babesiosis[9,30,32]. In humans reduced cardiac output has been associated with a raised urea:creatinine ratio and this could play a role in babesiosis as there is evidence of myocardial injury in this disease[3,10,11,23].

Although minimal intrinsic renal impairment without overt renal failure has been shown to be common in canine babesiosis[24], renal failure is a reported complication of canine babesiosis infection[16,40]. It occurs in less than 3% of South African canine babesiosis infections and appears to be more common in the haemoconcentrated form of the disease[16]. When it does occur it is usually fatal[24,40]. Typically canine babesiosis cases seen at the Onderstepoort Veterinary Academic Hospital (OVAH) with renal failure are severely azotaemic, with both serum urea and creatinine raised. In addition, cases are frequently oliguric or anuric, with any residual urine being darkly pigmented[16].

Cystatin-C is a cysteine protease inhibitor[1,19]. It is constantly produced by all nucleated cells, it is freely filtered by the glomerulus, it is metabolised by the luminal tubular epithelium, and is without significant unmetabolised tubular reabsorption[33]. As in human medicine[13,20,33], cystatin-C has been proposed as a more sensitive marker of reduced glomerular

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Table 1: **Summary of serum chemistry data in canine babesiosis and human falciparum malaria cases.**

Variable	Group	*n*	Mean	Standard deviation	Median	Range (min–max)	*P*-value*
Urea (mmol/ℓ)	Babesiosis	25	12.8	6.7	11.8	3.9–32.8	<0.001
	Control	13	5.1	1.9	4.3	3.0–8.9	
Creatinine (μmol/ℓ)	Babesiosis	25	72.1	24.6	67.0	22–119	0.27
	Control	13	78.7	16.6	75.0	56–114	
Cystatin-C (mg/ℓ)	Babesiosis	25	0.71	0.39	0.7	0.0–1.4	0.45
	Control	13	0.67	0.085	0.7	0.48–0.77	
Urea:creatinine ratio calculated using mg/dℓ	Babesiosis	25	46.3	21.7	41.4	17.3–94.9	<0.001
	Control	13	15.9	4.2	15.4	11.2–25.0	
	Human Malaria group	363	20.6	9.5	18.8	5.4–64.0	
Serum free Hb (g/ℓ)	Babesiosis	25	1.72	1.06	1.3	0.7–5.1	0.002
	Control	13	0.88	0.48	0.8	0.0–1.6	

*P-value for the Wilcoxon-rank sum test for differences in medians between babesiosis and control groups.

filtration rate than creatinine in dogs, and thus an earlier indicator of renal disease[1,2,19]. Serum haemoglobin, bilirubin, and triglycerides do not interfere with the cystatin-C assay[22]. The human test for cystatin-C has been validated for canine use[1].

In a recent study in humans in which cystatin-C was compared with creatinine in falciparum malaria, where renal failure is common in both children and adults[7,14], it was shown that renal disease appears to have been underestimated when using creatinine as a screening test[13]. This may have been due to intrinsic sensitivity properties of the analyte or due to laboratory perturbations specific to the measurement of creatinine in the presence of haemolysis[12,34,39]. Serum creatinine usually only becomes elevated once two-thirds of renal function is lost. Furthermore the by-products of haemolysis, namely bilirubin and haemoglobin, have been reported to interfere with the spectrophotometric analysis of creatinine. Because there appear to be disease mechanisms in common between canine babesiosis and human falciparum malaria[8], it is possible that serum creatinine also underestimates renal disease in canine babesiosis.

The objective of this study was to report the presence of an increased serum urea:creatinine ratio in canine babesiosis and to determine whether the elevated ratio originates from elevated serum urea or depressed serum creatinine concentrations or both, using serum cystatin-C concentrations as a reference.

MATERIALS AND METHODS

This was a prospective study involving 25 dogs with babesiosis treated at the OVAH (Group 1), and 13 normal reference dogs (Group 2). Dogs were included in the babesia-infected group if they were positive for babesia parasites on thin blood smear and negative for macroscopic serum lipaemia and had a packed cell volume of ≤0.2 ℓ/ℓ. Group 2 consisted of clinically healthy dogs determined to be free of canine babesiosis by thin blood smear examination.

A serum sample was collected from the jugular vein of each dog into 3 mℓ serum vacuum tubes. All the dogs in the study also had a peripheral blood smear, PCV (by whole blood centrifugation) and urine specific gravity (collected by cystocentesis) performed. Samples were collected prior to any treatment. Serum chemistry was determined by the OVAH Clinical Pathology Laboratory using the following standard methods[4,19,38].

Serum cystatin-C concentration was measured using the particle-enhanced turbidimetric immunoassay (Diagnostech, Honeydew Dako Cytomation, Cystatin-C PET kit, Denmark), designed for the determination of human cystatin-C[19]. Normal values were set at <1.7 mg/ℓ according to the group of reference dogs (group 2, n =13) used in this study. Serum urea concentrations were measured using the Technicon Method[38] which is a modification of the enzymatic method of Talke and Schubert for the RA-1000 analyser (Bayer (Pty) Ltd Isando, SA). Normal values were 3.6–8.9 mmol/ℓ as established in the OVAH Clinical Pathology Laboratory. Serum creatinine concentrations were measured using the Technicon Method[35] which is a kinetic modification of the Jaffé alkaline picrate reaction for the RA-1000 analyser (Bayer (Pty) Ltd Isando, SA). Normal values were <133 μmol/ℓ as established in the OVAH Clinical pathology Laboratory.

Serum urea, creatinine, cystatin-C and serum urea:creatinine ratios were measured or calculated for each dog. Urea and creatinine were converted to mg/dℓ for the calculation of the serum urea: creatinine ratio. Serum urea, creatinine, cystatin-C and serum urea:creatinine ratios were compared between the groups using the Wilcoxon rank-sum test for difference in medians. Two-tailed tests were used and the significance level was set at $\alpha = 0.05$. A statistical software package was used for data analysis (NCSS 2004, NCSS, Kaysville, Utah, USA).

RESULTS

A summary of the descriptive statistics of the serum chemistry data is provided in Table 1. Serum urea was above the reference range in 17/25 anaemic dogs. Serum urea was significantly elevated in the anaemic babesiosis group compared with the reference group ($P < 0.001$). Serum creatinine was not elevated in any of the 25 babesiosis dogs. Serum creatinine did not differ significantly between the reference group and the babesiosis group ($P = 0.27$). Serum cystatin-C was not elevated (>1.7 mg/ℓ) in any of the babesiosis dogs. Serum cystatin-C levels did not differ significantly between the reference group and the babesiosis group ($P = 0.45$). The serum urea:creatinine ratio was elevated in 23/25 babesiosis and 3/13 reference dogs. The urea:creatinine ratio was significantly elevated in the babesiosis group compared with the reference group ($P < 0.001$). Free serum haemoglobin was >1.6 g/ℓ in 8/25 babesiosis dogs. Free serum haemoglobin was significantly elevated in the anaemic babesiosis group compared with the reference group ($P = 0.002$).

DISCUSSION

This study confirmed that serum urea is often disproportionately increased relative to serum creatinine and serum cystatin-C in anaemic babesiosis dogs. It is therefore not surprising that the serum urea:creatinine ratio was found to be significantly

elevated in these dogs, with a number of dogs showing markedly increased ratios. Mean serum urea concentrations were significantly elevated in the babesiosis group in the presence of normal mean serum creatinine and serum cystatin-C concentrations. With cystatin-C considered free of the perturbations that serum urea and serum creatinine are subject to we can conclude that an elevated serum urea concentration in canine babesiosis in the absence of an elevated serum creatinine concentration does not reflect significant renal dysfunction. Serum creatinine thus proved to be a more specific indicator of significant renal dysfunction in canine babesiosis than serum urea. In fact, serum urea was so commonly elevated in the presence of a normal serum cystatin-C concentration that it can be said that urea should never be used alone (or even at all) to assess renal function in canine babesiosis.

The elevated serum urea concentrations encountered therefore represent either a form of laboratory error in the measurement of serum urea or serum creatinine, a form of pre-renal azotaemia, or a form of hyperureagenesis from substrate loading. The most likely cause of laboratory error would be photometric interference by free serum haemoglobin causing a positive bias on the measurement of serum urea concentrations and a negative bias on serum creatinine concentrations[9,15,34,38]. Free serum haemoglobin was significantly elevated in the anaemic babesiosis group compared with the control group in this study. Serum urea was measured by an enzymatic UV method[38]. This method has its absorbance spectra at wavelength 340 nm. This falls within the 300–500 nm range of high intrinsic absorbance that free serum haemoglobin displays[4,5]. Therefore it may be that a proportion of the elevated concentrations of serum urea encountered in the anaemic babesiosis group is a result of laboratory error in which free serum haemoglobin causes a positive bias on its measurement. Retrospectively calculated urea:creatinine ratios (unpublished data) in a study where comparative concentrations of free serum haemoglobin to those encountered in canine babesiosis were infused into dogs to measure the effect on the kidney, were however normal[25]. This indicates that free serum haemoglobin alone is not likely to be the cause of the elevated serum urea encountered in this study. Other potential causes of prerenal azotaemia in humans and dogs include dehydration, hypotensive shock, cardiac disease and rhabdomyolysis[3,11,17,28].

There are several possible pathophysiological explanations for the disproportionate increase in urea in canine babesia infections. Malaria studies have shown that total body water may be depleted in this disease[27]. It has been postulated that a similar event may occur in canine babesiosis[36]. Serum urea may increase before serum creatinine in an acute hypovolaemic state[6]. Hypotensive shock is thought to be a possible cause of renal failure in severe human falciparum malaria patients[21,31,41]. Hypotension has been reported in both complicated and severe uncomplicated canine babesiosis dogs[18]. In a human study on causes of an increased urea:creatinine ratio, chronic congestive cardiac disease was found to be major contributor[3]. There is some evidence for cardiac pathology in canine babesiosis[10,23]. The reporting of two cases of rhabdomyolysis and a significant increase in creatine kinase activity in South African canine babesiosis has led to speculation that increased muscle catabolism may account for the disproportionate increase in serum urea concentrations compared to serum creatinine concentrations observed[17,40]. Other possible sources of substrate for hyperureagenesis may include erythrocyte components released during haemolysis and blood from gastric ulceration[30,32]. A gastrointestinal (GIT) form of canine babesiosis has been described in South Africa[28] Pancreatitis has been shown to be a complication following babesiosis in a few dogs[30]. According to Maegraith[26], the erythrocyte count may fall by $1 \times 10^{12}/\ell$ of blood/day of babesia infection. This represents a daily loss of approximately 12.5 % of the dog's RBC. The normal percentage loss of RBC per day is 0.9 %. This means that during severe haemolysis 14 times the normal daily haemoglobin load on the body can occur.

Concentrations of both plasma ammonia and serum urea may also increase during a severe catabolic state due to the deamination process involved in using tissue proteins as an energy source. A catabolic state is likely in anaemic canine babesiosis dogs because of systemic inflammation, decreased appetite, tissue hypoxia, metabolic acidosis, pyrexia and the increased work of breathing. Substantial catabolism of endogenous proteins was found in critically ill dogs in a study by Michel[29]. Hyperureagenesis therefore remains a potential cause of the elevated serum urea:creatinine ratio seen in canine babesiosis.

Although the study was not designed to compare babesia-induced renal failure with babesia without renal failure, over the time during which material was collected for this study, 3 cases of the haemoconcentrating form of babesia infection were seen. These dogs had a mean haematocrit of 0.54 ℓ/ℓ. One of these dogs had a mildly elevated serum urea (13 mmol/ℓ) in the presence of a normal serum creatinine (86 μmol/ℓ) and serum cystatin-C (0.6 mg/ℓ) concentrations. The urea:creatinine ratio for this case was 37.43 and the dog made a full recovery. The second dog also had a mildly elevated serum urea (14.4 mmol/ℓ) but in the presence of mildly elevated serum creatinine (171 μmol/ℓ) and serum cystatin-C (2.6 mg/ℓ) concentrations. The urea:creatinine ratio for this case was 20.85 and this dog also made a full recovery. The third dog had acute renal failure proven on *post mortem* examination and histopathology. The serum urea in this dog was markedly elevated (41.8 mmol/ℓ) in the presence of markedly elevated serum creatinine (608 μmol/ℓ) and serum cystatin-C (5.7 mg/ℓ). The urea:creatinine ratio in this dog was 17.02. This small group of dogs reinforces the findings of the larger group namely that urea is not useful in the detection of acute renal failure and that cystatin-C and creatinine are as useful as one another for making this diagnosis. It is also interesting to note that in the case with proven acute renal failure, cystatin-C concentration tracked the change in creatinine concentration.

We conclude that serum urea is often elevated due to non-renal factors in canine babesiosis dogs, which causes an elevated serum urea:creatinine ratio. The cause of the elevated ratio remains undetermined, but may be as a result of laboratory error, pancreatitis, gastric ulceration, hypovolaemia, hypotensive shock, cardiac disease or hyperureagenesis. Therefore, serum urea concentration should not be used as an indicator of reduced glomerular filtration in canine babesiosis. The authors advocate the measurement of serum creatinine and hourly urine production to detect significant renal disease in canine babesiosis dogs. There appears to be no added benefit to be derived from the measurement of serum cystatin-C concentrations in canine babesiosis.

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