Renal involvement in dogs with babesiosis

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ABSTRACT
Proteinuria, and renal tubular casts and epithelial cells in urine sediment, are commonly observed in both complicated and uncomplicated babesiosis, but do not necessarily reflect or predict renal failure. This study investigated the presence and degree of renal damage in canine babesiosis. Renal function and integrity were evaluated using serum urea and creatinine, serum electrolytes (sodium and potassium), fractional clearance of sodium (FcNa) and potassium (FcK), urine enzyme activity of gamma-glutamyl transpeptidase and alkaline phosphatase, urine protein:creatinine ratio, and urinalysis. One control group (n = 10) and 3 groups of babesiosis cases were studied: mild uncomplicated (n = 10), severe uncomplicated (n = 11), and complicated (n = 9). All babesiosis groups showed well-concentrated urine. Mean serum urea was elevated in the severe and complicated groups, and was significantly different from the control group. There was no statistically significant difference between the groups for creatinine, although the complicated group had a mean value above the normal reference range. Hyperkalaemia was uncommon in all the groups. Hyperkalaemia was present in only 2 dogs in the complicated group. Marginal hyponatraemia was present in a minority of dogs in all groups. Serum electrolytes were not significantly different between groups. There was no overall elevation, nor any statistically significant difference in both the FcNa and FcK between the groups. Only 1 dog, in the complicated group, showed marked enzymuria. Proteinuria was a common finding and was significantly different between the severe and complicated groups and the control group. Some dogs in all groups had renal tubular epithelial cells in the urinary sediment, which increased in severity from the mild to the complicated groups and was significantly different from the control group. This study demonstrated that minimal renal damage occurs more often in canine babesiosis than significant damage or acute renal failure.

Key words: babesiosis, canine, kidney, renal function.


INTRODUCTION
Canine babesiosis is an important worldwide tick-borne disease caused by the intra-erythrocytic protozoal parasites Babesia canis or B. gibsoni¹. Although the disease primarily involves erythrocyte destruction, it may also result in multi-systemic involvement.¹⁻⁴ Acute renal failure (ARF) is an uncommon complication of babesiosis and typically presents as anuria or oliguria despite adequate rehydration.⁵ Evidence of renal damage, reflected on urinalysis by the presence of proteinuria, casts and renal tubular epithelial cells, is common in both complicated and uncomplicated cases, but does not necessarily reflect or predict renal failure.⁶ In babesiosis, elevated serum urea alone is an unreliable indicator of renal insufficiency, as a disproportionate rise in urea, compared with creatinine, occurs, possibly due to catabolism of lysed erythrocytes.⁶ Renal failure is diagnosed on the basis of ongoing evaluation of urine volume, urinalysis and degree of azotaemia. In humans, falciparum malaria, a disease clinically similar to canine babesiosis, can result in ARF, which resembles sepsis-related acute tubular necrosis.⁶ Glomerulonephritis may also be evident.⁶

Primary or intrinsic ARF is a syndrome characterised by the sudden onset of impaired renal function, resulting in azotaemia, fractional clearance of sodium (FcNa) that is greater than 1, the presence of renal tubular epithelial (RTE) cells and/or casts in the urine sediment, and characteristic histological changes.¹⁷⁻¹⁹ Any toxic or ischaemic renal insult may result in cellular degeneration and/or necrosis, with consequent RTE cell loss into the urine. In humans, overt necrosis is not evident in all cases but tubular dysfunction is a uniform hallmark of this form of ARF.¹⁷ Ischaemic injury occurs when renal blood flow is attenuated by decreased blood pressure or renal vasocostriction.¹⁸ Glomerular afferent arteriolar vasocostriction caused by the effects of angiotensin II and antidiuretic hormone (ADH), in response to increased renin release, is a proposed mechanism of decreased glomerular filtration rate (GFR) in ARF.²⁰ Decreased renal blood flow results in reduced amounts of oxygen and metabolic substrates presented to tubular cells, and this ‘cellular starvation’ initiates the development of acute tubular necrosis with consequent ARF.²¹ Acute renal failure associated with malaria has been attributed to hypo-volaemia and/or hypotension, intra-vascular haemolysis, hyperparasitaemia, cholestatic jaundice, catecholamines, and endotoxaemia.²²⁻²⁵

In canine babesiosis, the morphological lesions in the kidney have been attributed to anaemic hypoxia resulting from erythrocyte destruction.¹⁷ However, recent unpublished data, from 84 dogs with complicated babesiosis, have shown that the mean haematocrit of dogs with elevated creatinine was significantly higher (mean 36.5 %, SD 20.19) than of those with normal creatinine (mean 22 %, SD 16.38), making hypovolaemia a more likely cause than anaemia for the renal failure described in this disease. Babesiosis can result in a kidney that is swollen and dark in colour, with red-brown urine in the bladder. Microscopically the RTE cells are swollen and contain haemoglobin (Hb) droplets and small vacuoles. In severe cases, necrosis of the RTE cells is evident. The lumen of the nephron contains multiple Hb casts.¹⁷ The net effect of babesiosis on the kidney can be ARF, which has been attributed to haemoglobinuric nephropathy.²⁶ However, ARF is uncommon in babesiosis,²⁶ and recent work has demonstrated that severe haemoglobinuria, of the magnitude seen in canine babesiosis, did not induce significant nephropathy, regardless of whether or not concomitant anaemia was present.²⁶

The true pathogenesis of renal lesions in
babesiosis is still obscure. Maegraith25 noted the development of oliguria or anuria in dogs without concomitant haemoglobinuria. Several authors have reported that anoxia, reduction in renal blood flow, and possibly hypotension with intra-renal vasoconstriction and renal ischaemia must be considered of major importance in the pathogenesis, as opposed to mechanical obstruction of tubules by Hb and the toxic effects of Hb7,26,46. It has also been demonstrated that hypoxia results in more injury to renal tubules than haemoglobinuria and that the nephrotoxic effect of Hb appears to be highly individual27. Malherbe28 also suggested that the renal damage in babesiosis is usually reversible.

The purpose of this study was to investigate the presence and degree of renal damage in naturally occurring canine babesiosis. Renal function and integrity were evaluated using urinalysis, serum urea and creatinine, serum electrolytes (sodium and potassium), fractional clearance of sodium (FcNa) and potassium (FcK), urine enzyme activity of gammaglutamyl transpeptidase (GGT) and alkaline phosphatase (ALP), and quantification of proteinuria.

MATERIALS AND METHODS

Study design

The Ethics and Research Committees of the Faculty of Veterinary Science, University of Pretoria, approved this study and written consent by the dogs’ owners was obtained. Thirty dogs with babesiosis, presented to the Onderstepoort Veterinary Academic Hospital (OVAH), were sequentially enrolled. The diagnosis of babesiosis was based on finding B. canis parasites on a thin capillary blood smear, stained with Cams Quick stain (CA Milsch). These dogs were categorised into 3 groups: mild uncomplicated (Group 1), severe uncomplicated (Group 2) and complicated (Group 3). Mild cases had mild-to-moderate anaemia (haematocrit 20-30 %) with no clinical or biochemical signs of complicated disease. Severe cases had severe anaemia (haematocrit <15 %) with no clinical or biochemical signs of complicated disease. Complicated cases had one or more of the following complications: cerebral signs, ARF, acute respiratory distress syndrome, hypotensive shock, or haemoconcentration. Clinically healthy, aparastaeic, dogs, presented to the OVAH for routine ovario-hysterectomy, were used as controls (Group 4). Groups 1 and 4 comprised 10 dogs each and groups 2 and 3, 11 and 9 dogs respectively.

Data collection

Blood was collected from the cephalic vein, using a 22G venoject needle, a holder and a serum vacuum tube (Vacutainer System, Becton Dickinson). A cytospin’s urine sample was collected aseptically using a 23G needle and a 10-mL syringe. All samples were collected before any treatment.

Analytical methods

Urea and creatinine were determined on a Technicon RA 1000 system (Technicon Instruments Corporation) using the Technicon modification of the kinetic method for urea29 and the alkaline picrate reaction for creatinine, modified as a first order rate reaction30. Urine and serum sodium and potassium were determined using an ion selective analyser (Nova 1, Nova Biomedical). Urine ALP and GGT were determined on a Technicon RA 1000 system using the Technicon modification of the p-nitrophenyl phosphate substrate method in AMP buffer for ALP31 and glutamyl-p-nitroanilide substrate with glycylglycine peptide acceptor for GGT32. Urine Hb was determined on a Technicon RA 1000 system using the Drabkins method (CA Milsch). Total urine protein was determined using a spectrophotometer (Lange L6P Photometer) based on the Richterich technique using perchloric acid and biuret reagent33. Urine Hb was subtracted from the total urine protein and the corrected protein value was expressed as a ratio with the urine creatinine.

The fractional clearance of sodium and potassium was calculated, using the following formula:

\[ \text{FcNa} = \frac{\text{serum sodium} \times \text{urine volume} \times \text{urine specific gravity}}{\text{urine sodium}} \]

\[ \text{FcK} = \frac{\text{serum potassium} \times \text{urine volume} \times \text{urine specific gravity}}{\text{urine potassium}} \]

The physicochemical evaluation of the urine was performed using a urine dipstick (Lenstrip 8 Dipsticks, Benmore Diagnostics) and an AO veterinary refractometer (American Optical, Scientific Instrument division). Microscopic evaluation was done on urine sediment stained with Sternheimer-Malbin stain (Kyon Laboratories), which enabled the differentiation of RTE cells from other urinary epithelial cells. The presence of RTE cells in the urine was subjectively scored on a scale of 1-4 as follows: 1, represented 1 RTE cell per 2-3 high power fields (HPF); 2, represented 1-2 RTE cells per HPF; 3, represented 2-4 RTE cells per HPF; and 4, represented more than 5 RTE cells per HPF.

Data analysis

Statistical analysis of the data was performed using a commercial statistical software package (Sigma Stat, Jandel Scientific Software). Parameters that were statistically analysed were urine specific gravity (SG), serum urea and creatinine, serum sodium and potassium, FcNa, FcK, urineALP and GGT activity (expressed as a ratio to urine creatinine), proteinuria (expressed as urine protein: creatinine ratio), and urinalysis findings (haemoglobinuria and RTE cells in sediment). Data were compared between the groups using analysis of variance (ANOVA). The urinalysis findings were compared using Friedman repeated measures ANOVA on ranks. The Tukey correction was used for group comparisons. The Pearson test was used to check for correlation between haemoglobinuria and serum creatinine concentrations and urine RTE cell score. In all analyses, a value of \( P < 0.05 \) was considered significant.

RESULTS

Clinicopathological findings are summarised in Table 1.

Most dogs in the severe and complicated groups (8/11 and 8/9 respectively) had elevated serum urea, compared with only 1/10 in the mild group (Fig. 1). The serum creatinine did not mirror the serum urea in that 1/10 in the mild group, 0/10 in the severe group, and 3/10 in the complicated group had elevated serum creatinine levels (Fig. 2). There was a significant positive correlation between serum urea and creatinine in the mild group (\( r = 0.89, P < 0.05 \)) and complicated (\( r = 0.83, P < 0.05 \)) groups, but not in the severe group.

One dog in each of the babesiosis groups showed mild hypokalaemia. Severe hypokalaemia was present in 1 dog in the severe and 1 in the complicated group. Hyperkalaemia was present in 1 dog in the complicated group. There was no statistically significant difference between any of the groups for either hypo- or hyperkalaemia. Marginal hyponatraemia was present in 2/10 in the mild and severe groups, and 3/10 in the complicated group. There was no statistically significant difference between any of the groups. None of the dogs showed hypernatraemia. There was no elevation in the FcNa indicative of acute tubular dysfunction, but there was a statistically significant difference between all 3 groups and the control group in that the babesiosis groups had a much lower FcNa than the control group. There was neither elevation of, nor any statistically significant differences for, FcK.

The urine ALP: creatinine ratio was elevated in 1 dog in the mild group and 1 in the severe group, and 2 in the complicated group. The urine GGT: creatinine
Table 1: Clinicopathological parameters in dogs with mild, severe, and complicated babesiosis and normal control dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild (n = 10)</th>
<th>Median</th>
<th>Range</th>
<th>Severe (n = 11)</th>
<th>Median</th>
<th>Range</th>
<th>Complicated (n = 9)</th>
<th>Median</th>
<th>Range</th>
<th>Control (n = 10)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea (mmol/l)</td>
<td></td>
<td>5.9</td>
<td>3.3–15.3</td>
<td>12.8</td>
<td>7.3–29</td>
<td></td>
<td>19.3</td>
<td>5.5–25.1</td>
<td></td>
<td>6.8</td>
<td>3.7–12.6</td>
<td></td>
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<tr>
<td>Serum creatinine (µmol/l)</td>
<td></td>
<td>98</td>
<td>74–168</td>
<td>93</td>
<td>38–130</td>
<td></td>
<td>125</td>
<td>59–281</td>
<td></td>
<td>119.5</td>
<td>105–155</td>
<td></td>
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<tr>
<td>Serum sodium(mmol/l)</td>
<td></td>
<td>141.5</td>
<td>138–145</td>
<td>142</td>
<td>139–150</td>
<td></td>
<td>142</td>
<td>133–149</td>
<td></td>
<td>17.5</td>
<td>141–152</td>
<td></td>
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<tr>
<td>Serum potassium (mmol/l)</td>
<td></td>
<td>3.85</td>
<td>3.6–4.0</td>
<td>3.9</td>
<td>2.8–5.0</td>
<td></td>
<td>4.3</td>
<td>2.4–6.6</td>
<td></td>
<td>4.4</td>
<td>4.2–4.7</td>
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<tr>
<td>Urine haemoglobin (g/l)</td>
<td></td>
<td>1.25</td>
<td>0.2–2.5</td>
<td>1.65</td>
<td>0.3–11.6</td>
<td></td>
<td>1.95</td>
<td>0.92–3.80</td>
<td></td>
<td>1.25</td>
<td>0.3–3.4</td>
<td></td>
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<tr>
<td>Serum urea (mmol/l)</td>
<td></td>
<td>1.17</td>
<td>0.17–2.5</td>
<td>1.5</td>
<td>0.3–11.6</td>
<td></td>
<td>1.95</td>
<td>0.92–3.80</td>
<td></td>
<td>1.25</td>
<td>0.3–3.4</td>
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</tr>
<tr>
<td>Serum sodium(mmol/l)</td>
<td></td>
<td>1.49</td>
<td>0.1–0.67</td>
<td>1.5</td>
<td>0.3–11.6</td>
<td></td>
<td>1.95</td>
<td>0.92–3.80</td>
<td></td>
<td>1.25</td>
<td>0.3–3.4</td>
<td></td>
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<tr>
<td>Serum potassium (mmol/l)</td>
<td></td>
<td>1.05</td>
<td>0.01–0.98</td>
<td>1.05</td>
<td>0.01–1.06</td>
<td></td>
<td>1.05</td>
<td>1.035–1.06</td>
<td></td>
<td>1.043</td>
<td>1.03–1.06</td>
<td></td>
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<tr>
<td>Specific gravity</td>
<td></td>
<td>3.85</td>
<td>3.6–4.0</td>
<td>3.9</td>
<td>2.8–5.0</td>
<td></td>
<td>4.3</td>
<td>2.4–6.6</td>
<td></td>
<td>4.4</td>
<td>4.2–4.7</td>
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</table>

DISCUSSION

In this study, the most consistent finding indicative of renal damage was proteinuria, and its severity was related to the severity of the babesiosis. Renal and renal-related disorders due to human falciparum malaria, a disease similar to canine babesiosis, include both extra-renal and renal manifestations. The former consist of fluid and electrolyte disorders. The latter vary widely, ranging from mild proteinuria and glomerulonephritis to ARF. In humans the reported incidence of ARF associated with falciparum malaria ranges from 1–4%. Jacobson and Clark reported that ARF is an uncommon complication in canine babesiosis, being diagnosed in only 3 of 134 cases reviewed, giving an incidence of 2.2%.

The elevated serum urea in the severe uncomplicated group in this study, without a corresponding increase in the serum creatinine, confirms previous findings. The phenomenon has been attributed to catabolism of lysed erythrocytes, resulting in an increased ammonia load on the liver and consequent increased urea production, but could also be associated with more generalised protein catabolism resulting from a febrile, inflammatory illness. Other possible causes would include gastrointestinal haemorrhage and ingestion of a high protein meal. These latter causes are unlikely in canine babesiosis. Thus elevated serum urea alone is an unreliable indicator of renal insufficiency in babesiosis. Elevated serum urea in the complicated group in this study was associated with a concomitant increase in the serum creatinine, hyperkalaemia and hyponatraemia, and 2 had moderate proteinuria and enzymeuria. None of the dogs showed elevated FcNa. All 4 dogs responded well to standard therapy for ARF.
which could reflect decreased renal blood flow, possibly as a result of decreased blood pressure and/or hypovolaemia. A recent study showed that hypotension occurred frequently in canine babesiosis and the presence and severity of hypotension increased with increased disease severity. As elevated creatinine, evident in some of the cases, was not correlated with any of the other parameters of renal function or integrity, it may have been pre-renal in origin. Another study has shown that elevated creatinine is associated with increased risk of death in canine babesiosis, indicating that it might nonetheless be a useful measure of renal insufficiency. In babesiosis, a higher cut-off value and/or serial creatinine determinations would assist in distinguishing pre-renal from renal causes.

Urine enzyme activity is both an early and persistent indicator of renal tubular damage. Both GGT and ALP are brush border enzymes present in the proximal convoluted tubule of the kidney. Although 24-hour urine enzyme activity is more accurate, evaluation of the ratio of urine enzyme: creatinine in a spot urine sample is technically simpler and has been shown to correlate well with a 24-hour sample. Urine ALP and GGT activity > 10 U/L and a ratio >2 (calculated using SI units) can be considered to be elevated. Only 1 dog in the complicated group revealed severe changes in the urine enzyme activity; however, this was not accompanied by abnormal urine SG or FcNa.

The FcNa can be used as an indicator of acute tubular dysfunction, with an increase in the FcNa over 1 reportedly indicating acute tubular dysfunction. Two of the control dogs had FcNa greater than 1, raising the possibility that a FcNa greater than 1 is not always indicative of acute tubular dysfunction. In this study an unexpected finding was that the mean FcNa in the dogs with babesiosis was lower than that of the control dogs. Most sodium is actively re-absorbed from the proximal convoluted tubules of the kidneys, resulting in passive water re-absorption. Further sodium reabsorption occurs in the distal convoluted tubules (secondary to the active re-absorption of chloride ions) and collecting ducts (controlled by aldosterone). In this study, the lower FcNa can be interpreted as either renal retention of sodium secondary to aldosterone secretion or inhibition of prostaglandins, or as a result of activation of the renin-angiotensin-aldosterone system, in response to renal arterial hypotension. The well-concentrated urine in all dogs with babesiosis can also be attributed to sodium and water.
Hypokalaemia is an uncommon finding in malaria patients with either ARF or intravascular haemolysis. In this study, 2 dogs showed hyperkalaemia, of which 1 had ARF. In a study in children, plasma potassium was significantly higher and the Fc a significantly lower during the acute illness than after recovery. As canine red blood cells are much lower in potassium than human red blood cells, hyperkalaemia is unlikely to occur as a result of haemolysis in the dog.

In this study, a number of dogs had RTE cells in the urine sediment. In malaria, abnormal urinary sediment, consisting of red and white blood cells and occasional granular casts, commonly occurs in patients without renal failure. Other urinary sediment abnormalities, such as the presence of RTE cells, are not commonly reported. The presence of RTE cells in the urine sediment can indicate renal damage due to hypoxia, hypoperfusion, or toxic damage. In this study, the number of RTE cells in the urine sediment increased with increased disease severity. Renal hypoxia results in rounding and retraction of RTE cells with a disruption of actin microfilaments, as a result of which a large number of viable RTE cells are sloughed into the urine. Acute renal hypoxia has also been shown to induce apoptotic changes. This may explain the lack of correlation between urine enzyme activity and the presence of RTE cells.

Proteinuria was a common finding in this study. Proteinuria was demonstrated by Maegraith in experimental babesiosis, and occurred within 24 hours of the first appearance of parasites in the blood. In later stages of the infection, there was evidence of tubular damage in the form of granular and hyaline casts. Cast formation was not evident in this study. Mild proteinuria occurred in 40% of children with malaria during the acute illness but was not related to creatinine clearance, body temperature at presentation, or peripheral parasite density. Proteinuria was also absent after recovery. Babesia rodhaini-infected mice developed immune-complex-induced mesangiopathic glomerulonephropathy and moderate renal tubular necrosis. These mice had elevated serum urea and proteinuria. Babesia microti-infected mice showed relatively mild immune-complex-induced mesangiopathic glomerulonephropathy and mild renal tubular necrosis, with no increase in serum urea and no proteinuria.

The degree of proteinuria in this study was consistent with tubulo-interstitial disease. Canine babesiosis has not been associated with glomerulonephropathy.

Acute renal failure in malaria is commonly associated with blackwater fever, characterised by fever, massive intravascular haemolysis and haemoglobinuria. The condition is associated with quinine administration in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. The presence or absence of G6PD deficiency in dogs with babesiosis has never been evaluated, but severe haemoglobinuria is unlikely to mimic the human situation, since the common antibabesial drugs are dissimilar to quinine and haemoglobinuria generally occurs before treatment. In our experience, dogs with babesiosis, that die as a result of ARF show severe haemoglobinuria and oliguria, and frequently produce a small volume of urine that is almost black in colour. In this study there was no correlation between the haemoglobinuria and serum creatinine and RTE cells in the urine. This supports a previous study that showed that haemoglobinuria does not induce renal failure.

CONCLUSION
This study demonstrated RTE celluria, variable enzymuria, proteinuria, and variable azotaemia in dogs with babesiosis. However, these were all minimal changes and all could be consistent with hypoxia and/or hypovolaemia. To fully elucidate the effect of babesiosis on the kidney, further studies involving histological assessment are needed.

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Fig. 5: Renal tubular epithelial cells in urine sediment for the 4 groups. Data are shown as median (horizontal line within box), 25th and 75th percentiles (horizontal ends of boxes), and 10th and 90th percentiles (T-bars). An asterisk indicates significant differences between the babesiosis groups and the control group. Black dots represent outliers.

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REFERENCES


