Assessment of renal dysfunction using urinary markers in canine babesiosis caused by *Babesia rossi*

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Abstract

Renal damage is deemed a common, yet poorly documented, complication in canine babesiosis. Serum urea and creatinine are insensitive and non-specific markers of early renal dysfunction and

their measurements are influenced by hemolysis caused by babesiosis. Therefore, the aim of this study was to use urinary markers to assess the localization and degree of renal dysfunction in dogs with *Babesia rossi* infection. Urinary immunoglobulin G (uIgG) and urinary C-reactive protein (uCRP) were measured as markers for glomerular dysfunction, while urinary retinol-binding protein (uRBP) was used as a marker for tubular dysfunction. Eighteen dogs presenting with uncomplicated babesiosis were included and compared with eight clinically healthy dogs. Previously validated commercial ELISA kits were used for the measurement of uIgG, uCRP, and uRBP. Results were related to urinary creatinine concentrations (c). Dogs with babesiosis had significantly higher concentrations of all three measured urinary markers compared to healthy dogs. Except for urinary protein/c ratio (UPC), routine urinary and serum markers for renal function (urine specific gravity (USG), serum urea and creatinine (sCr)) were not significantly different between dogs with babesiosis and healthy dogs. All three urinary markers were positively correlated with each other and with UPC. The data supports the presence of both glomerular and tubular dysfunction in dogs suffering from uncomplicated *B. rossi* infection. Urinary markers were superior to USG, serum urea and creatinine concentrations for the early detection of renal dysfunction in dogs with babesiosis.

Keywords: Babesiosis; Dog; Renal dysfunction; C-reactive protein; Immunoglobulin G; Retinol-binding protein

1. Introduction

Canine babesiosis is a tick-borne disease with worldwide significance, caused by an intra-erythrocytic protozoan. *Babesia* species have historically been divided into large (*Babesia canis*) and small (*Babesia gibsoni*) piroplasms (Taboada and Merchant, 1991). Three distinct species of large piroplasms can be identified in canine babesiosis: *B. canis, B. rossi*, *and B. vogeli* (Carret et al., 1999). Canine babesiosis in South Africa is most frequently caused by the more virulent *B. rossi*, although *B. vogeli* has also been identified recently (Matjila et al., 2004). In one study, 12% of all sick dogs

presented to the Onderstepoort Veterinary Academic Hospital in South Africa were diagnosed with babesiosis (Shakespeare, 1995), making it an important and frequently diagnosed disease in this country. Moreover, canine babesiosis is considered an important emerging disease in Europe, chiefly due to increased transport of pets and climate change (Beugnet and Marié, 2009).

The clinical severity of canine babesiosis is variable, and is determined by the *Babesia* species and the immune response of the host. *Babesia rossi* is the most virulent form of the disease and infections are frequently fatal, while *B. vogeli* often only causes a mild to moderate clinical disease (Uilenberg et al., 1989). Two main pathophysiologic mechanisms are thought to be responsible for the clinical signs: a hemolytic anemia, primarily of immune-mediated origin, and a severe systemic inflammatory response (Reyers et al., 1998). The presence of a systemic inflammatory response syndrome or multiple-organ dysfunction syndrome did not significantly affect the outcome in cases of complicated canine babesiosis (Welzl et al., 2001). However, the involvement of specific organ systems was found to influence outcome. The study further concluded that renal involvement, defined as an increase of admission serum creatinine (sCr) (>150 µmol/l) in the absence of clinical dehydration, resulted in a five times increased risk of death.

True acute renal failure is a severe complication of infection by several species of *Babesia*, with a prevalence varying from 2.2% to 36% (Jacobson and Clark, 1994; Camacho et al., 2004; Garcia, 2006; Máthé et al., 2006). However, pre-renal azotemia could not be excluded in some of these cases (Garcia, 2006). Minimal renal damage is identified more often than overt acute renal failure, making 'renal involvement' a more suitable description for this complication (Lobetti and Jacobson, 2001).

Many potential causes of renal damage in babesiosis have been proposed. Hemoglobinemia was proposed to be the main cause of renal damage in babesiosis, however a study on healthy dogs, experimentally infused with hemoglobin, showed that a significant nephropathy was not induced (Lobetti et al., 1996). Moreover, histological changes typical for hemoglobinuric nephropathy were rarely observed in canine babesiosis (Máthé et al., 2007). Instead, the histopathological lesions were most consistent with hypoxic or toxic damage. Renal hypoxia, caused by anemia and systemic

hypotension, seems to be the primary cause of renal damage as opposed to hemoglobinuria (Lobetti et al., 1996; Máthé et al., 2007). It has also been suggested that met-hemoglobinuria, reported in canine babesiosis, may be another cause of renal damage (Lobetti and Reyers, 1996). Moreover, cytokine-induced renal damage due to a systemic inflammatory response is another likely mechanism (Jacobson and Clark, 1994; Reyers et al., 1998).

Serum urea and creatinine will only increase when more than 75% of renal functional mass is lost, making them insensitive markers for early detection of renal dysfunction. In addition, serum urea concentration can be elevated due to extra-renal causes such as hemolysis and rhabdomyolysis, both of which occur in babesiosis. Thus, serum urea should not be used in the diagnosis of renal failure caused by babesiosis (Jacobson and Lobetti, 1996; de Scally et al., 2004; de Scally et al., 2006). Furthermore, serum hemoglobin and bilirubin can interfere with the chemical analysis of sCr, leading to an underestimation of sCr concentration in several methods of sCr measurement (de Scally et al., 2004). Proteinuria, caused by hemolysis in babesiosis, induces a false increase in specific gravity (Chadha et al., 2001), which makes the measurement of urine specific gravity (USG) a less reliable diagnostic method for renal function in babesiosis.

There is enough evidence to suggest that renal dysfunction is a serious complication associated with canine babesiosis that has been shown to influence outcome (Lobetti and Jacobson, 2001; Welzl et al., 2001). Therefore, sensitive markers are necessary for the early diagnosis of renal dysfunction in canine babesiosis. Urinary markers may allow this and additionally have the capacity to quantify and localize the site of renal injury (Price, 2002). Immunoglobulin G (IgG), involved in the humoral immune response, and C-reactive protein (CRP), a major acute phase protein in dogs, are two high molecular weight (HMW) proteins, to which an intact glomerular barrier is impermeable (D'Amico and Bazzi, 2003). Glomerular damage consequently leads to the urinary presence of these HMW proteins. Urinary IgG (uIgG) and/or urinary CRP (uCRP) detected glomerular dysfunction in dogs with *Escherichia coli* pyometra, chronic kidney disease (CKD), and Cushing's syndrome (Maddens et al., 2010a; Smets et al., 2010b; Smets et al., 2011). Retinol binding protein (RBP) is filtered through the

renal glomeruli because of its low molecular weight and is reabsorbed in the proximal tubules under physiological circumstances (Christensen et al., 1999). Therefore, increased urinary RBP (uRBP) indicates tubular dysfunction, as previously reported in dogs with *E. coli* pyometra, CKD, and Cushing's syndrome (Maddens et al., 2010a; Smets et al., 2010b; Smets et al., 2011).

The aim of the current study was to assess renal dysfunction in dogs with babesiosis, caused by *B. rossi*, using urinary markers for both glomerular (uIgG and uCRP) and proximal tubular dysfunction (uRBP). The potential interference of hemoglobinuria with these urinary marker analyses was additionally considered.

2. Material and methods

2.1. Animals

The study was approved by the Animal Use and Care Committee of the Faculty of Veterinary Science, University of Pretoria. A total of 26 dogs were enrolled in the study and divided into two groups.

Group 1 (B) included 18 dogs that were prospectively sampled after they presented with babesiosis to the Onderstepoort Veterinary Academic Hospital (OVAH), University of Pretoria in South Africa during 2010. The identity of the *Babesia spp.* responsible for the infection was confirmed by polymerase chain reaction (PCR) and reverse line blot (RLB). PCR and RLB was also used to exclude co-infection with *Ehrlichia canis*. Only dogs that presented with uncomplicated babesiosis caused by *B. rossi* were included in this study. Uncomplicated babesiosis was defined as a clinical presentation attributable to hemolytic anemia only (Jacobson and Clark, 1994). A thorough anamnesis, physical examination, complete blood count, basic biochemistry profile [incl. serum urea, creatinine (sCr), glucose (Glu), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total serum protein (TSP), albumin (sAlb), globulin (Glob), and electrolytes (sodium, potassium, total and ionized calcium)], and a urinalysis, including bacterial culture, was performed to exclude presence of concurrent diseases.

Group 2 (H) included eight clinically healthy control dogs of comparable age and body weight, that also presented to the OVAH during the same period. The control dogs were considered healthy based on a thorough anamnesis, physical examination, complete blood count, basic biochemistry profile, and a urinalysis, including bacterial culture (negative culture and a urinary protein to creatinine ratio (UPC)<0.5). PCR and RLB was performed to exclude infection with babesiosis or ehrlichiosis.

An additional group included four clinically healthy dogs of which a urine sample, collected by cystocentesis, was used for hemoglobin interference testing. These dogs were considered healthy based on a thorough anamnesis, physical examination, and a urinalysis, including bacterial culture (negative culture, no hemoglobinuria, and a UPC<0.5).

2.2. Sampling regimen

Blood and urine samples were collected at admission in all B and H dogs. All urine samples were collected by cystocentesis. Urinalysis consisted of a dipstick analysis (Combur 9 Test®, Roche Diagnostics, Germany), microscopic sediment analysis, USG, UPC, and a bacteriological culture. After urine collection, quick centrifugation (three minutes at 447 x g) was performed and the supernatant was divided into aliquots of 0.5 ml and stored at -80°C. Frozen urine samples were transported for analysis to Belgium on dry ice. Upon arrival, samples were stored at -80°C until analysis. All analyses of urinary markers were performed within six months of sample collection. To the author's knowledge, only one report on stability of urinary markers during storage is published in veterinary medicine (Smets et al., 2010a). In that study, uRBP concentration was not different after storage for 12 months at -80°C compared to fresh samples. Storage information about uCRP and uIgG are lacking in veterinary medicine.

2.3. Laboratory methods

All urine samples were analyzed with commercially available canine- or human-specific sandwich enzyme-linked immunosorbent assays (ELISA) (Immunology Consultants Laboratory, Newberg, USA) to determine the concentrations of either uIgG, uCRP, or uRBP, respectively. All assays were previously validated for use with canine urine in our laboratory (Maddens et al., 2010b). For each immunoassay, the absorbance was measured at a wavelength of 450 nm, using an ELISA plate reader (Multiskan MS®, Labsystems Thermo Fisher Scientific, Waltham, USA). A 4-parameter logistic curve fitting program (Deltasoft JV®, Biometallics Incorporated, Princeton, USA) was used to generate the standard curve and calculate the concentrations of uIgG, uCRP, and uRBP. Finally, results were indexed to urinary creatinine concentrations (c) and expressed as ratios.

2.4. Hemoglobin interference testing

To determine whether the presence of hemoglobinuria affects the urinary marker analyses, urinary markers were measured on urine samples of four healthy dogs, to which different concentrations (0 g/L, +1 g/L, +4 g/L, +12 g/L, +24 g/L) of a human hemoglobin standard (Sigma-Aldrich, St. Louis, MO, USA) were added. These concentrations were based on previously published urinary hemoglobin concentrations in dogs with babesiosis (Lobetti and Reyers, 1996; Lobetti and Jacobson, 2001).

2.5. Statistical analysis

Two commercial software programs were used for data analysis (SPSS 16, SPSS Inc., Chicago, USA; GraphPad Prism 5, GraphPad Software Inc., CA, USA). The non-parametric Mann-Whitney *U*-test was used to compare group B with group H dogs. The non-parametric Wilcoxon signed-rank test was used as a paired difference test for hemoglobin interference testing. Correlations between the different

urinary markers and between these markers and other variables (serum urea and sCr, USG, UPC, sAlb) were calculated using the non-parametric Spearman's correlation coefficient. Differences were considered statistically significant at $P<0.05$.

3. Results

3.1. Study population

All dogs in group B were diagnosed with uncomplicated babesiosis, caused by *B. rossi*. Median age in group B was 3.25 years (range: 0.6-11 years), which was not significantly different from the median age of group H (3 years; range: 0.5-8 years) ($P=0.484$). There was no significant difference between the median body weight in group B (18.5 kg; range: 3.2-60 kg) and group H (22.5 kg; range: 6-40 kg) ($P=0.868$). In group B, three dogs were mixed breed, while pure-bred dogs included three Boerboels, two Rottweilers, two Dachshunds, and one Cocker Spaniel, Pekingese, Great Dane, Staffordshire Bull Terrier, Toy Pomeranian, Chow Chow, Fox Terrier, and Jack Russell Terrier. There were seven neutered and four intact females, and three neutered and two intact male dogs in group B, while one dog in both sexes was of unknown neuter status. Group H consisted of two mixed breed dogs, two Boerboels, two Bouviers des Flandres, one German Shepherd dog, and one Beagle. In group H, there were five intact and two neutered females, and one intact male dog.

Hematology and biochemistry results of groups B and H are shown in Table 1. Anemia ranged from mild to severe in group B. None of the dogs in group B had a leucocytosis, while all were moderately to severely thrombocytopenic. Sixteen of 18 dogs in group B (89%) had hypoalbuminemia, while eight (44%) were hypoproteinemic. Serum albumin was significantly lower in group B than in group H ($P=0.001$), while TSP and Glob were not significantly different between both groups ($P=0.075$ and $P=0.374$, respectively).

Table 1. Clinicopathologic findings (hematology and biochemistry results) in dogs with uncomplicated babesiosis (B) and healthy dogs (H) (expressed as median and range).

Variable	B	H	Reference range
Hematology			
Hemoglobin (g/L)	89 (37-145)	171 (125-198)	120-180
Red blood cell count (x 10^e12/L)	3.8 (1.6-6.3)	7.3 (5.3-8.3)	5.5-8.5
Packed cell volume (L/L)	0.27 (0.12-0.45)	0.51 (0.38-0.60)	0.37-0.55
Leukocyte count (x 10^e9/L)	6 (2-14)	8 (7-20)	6-15
Platelet count (x 10^e9/L)	35 (8-64)	302 (32-401)	200-500
Biochemistry			
Total serum protein (g/L)	53 (41-81)	60 (50-68)	53-75
Serum albumin (g/L)	25 (17-29)	35 (24-39)	27-35
Serum globulin (g/L)	30 (16-62)	24 (18-39)	20-37
Serum urea (mmol/L)	6.0 (2.6-47.4)	5.2 (4.0-7.7)	3.6-8.9
Serum creatinine (µmol/L)	68 (39-215)	92 (48-103)	40-133
Glucose (mmol/L)	4.6 (1.7-5.4)	4.5 (3.1-5.4)	3.3-5.5
Alanine aminotransferase (U/L)	25 (17-49)	28 (2-51)	9-73
Alkaline phosphatase (U/L)	98 (43-370)	38 (15-110)	65-311
Sodium (mmol/L)	142 (137-147)	145 (142-148)	140-155
Potassium (mmol/L)	3.9 (3.4-4.7)	4.7 (4.0-5.2)	3.6-5.1
Calcium (total) (mmol/L)	2.2 (2.0-2.5)	2.6 (2.5-2.9)	2.2-2.9

Urine color varied from yellow to dark brown in group B, and was yellow in group H. Urine appearance varied from clear to turbid in both groups. The urinary pH ranged from 6 to 7 in group B, and from 5 to 9 in group H. Urinary glucose, ketone and urobilinogen measurements were negative in both groups. Bilirubinuria ranged from negative to 3+ in group B and was negative in group H. Hemoglobinuria was severe (4+) (range: 0-4+) in 17 of 18 dogs in group B, and was mild (1+) in one dog. In group H, hemoglobinuria varied from negative to 3+. Bilirubin crystalluria was present in seven of 18 dogs in group B (39%). Micro-organisms were not observed during microscopic examination, and bacterial urine cultures were negative in all dogs.

3.2. Routine serum and urinary parameters for renal dysfunction

Results of routine parameters for renal function are presented in Table 2, for both groups. Serum urea, sCr, and USG did not differ significantly between group B and group H (P=0.331, P=0.221, and P=0.373, respectively), while UPC was significantly higher in group B (P<0.001). Only one of 18 dogs

Table 2. Results of routine serum and urinary parameters for renal function and of urinary markers in dogs with uncomplicated babesiosis (B) and healthy dogs (H) (expressed as median and range).

Variable	**B**	**H**	***P*-value**
Serum urea (mmol/L)	6.0 (2.6-47.4)	5.2 (4.0-7.7)	0.331
Serum creatinine (µmol/L)	68 (39-215)	92 (48-103)	0.221
USG	1.036 (1.012-1.065)	1.031 (1.014-1.048)	0.373
UPC	1.6 (0.23-5.53)	0.1 (0.05-0.35)	<0.001
uIgG/c (mg/g)	226.71 (11.32-2296.35)	1.27 (0.52-3.23)	<0.001
uCRP/c (mg/g)	0.02 (0.01-0.81) BDL (n=6); BQL (=2)	BDL (n=8)	0.011
uRBP/c (mg/g)	10.84 (0.91-58.23)	0.05 (0.04-0.16) BDL (n=2); BQL (n=1)	<0.001

USG: urine specific gravity; UPC: urinary protein to creatinine ratio; uIgG/c: urinary immunoglobulin G-to-creatinine ratio; uCRP/c: urinary C-reactive protein-to-creatinine ratio; uRBP/c: urinary retinol binding protein-to-creatinine ratio; BDL: below detection limit; BQL: below quantification limit

in group B had a UPC<0.5. The USG of one of the dogs in group B was not included, because fluid therapy was initiated before sample collection.

Upon sediment evaluation, granular casts were present in five dogs of group B (28%) and renal tubular epithelial (RTE) cells were present in four dogs of group B (22%). In contrast, urinary casts and RTE cells were absent in the urine sediment of all the dogs in group H.

3.3. Hemoglobin interference testing

Table 3. Results of urinary markers in healthy dogs with different concentrations of added human hemoglobin (expressed as median and range).

Variable	**No added Hb**	**+ 1 g/L Hb**	**+ 4 g/L Hb**	**+ 12 g/L Hb**	**+ 24 g/L Hb**
uIgG/c (mg/g)	0.54 (0.22-1.06)	0.52 (0.19-0.95) (*P*=0.125)	0.45 (0.16-0.94) (*P*=0.125)	0.52 (0.17-0.93) (*P*=0.125)	0.53 (0.19-0.98) (*P*=0.125)
uCRP/c (mg/g)	BDL (n=4)	BDL (n=4)	BDL (n=4)	BDL (n=4)	BDL (n=4)
uRBP/c (mg/g)	0.06 (0.04-0.08)	0.06 (0.04-0.09) (*P*=0.625)	0.05 (0.03-0.08) (*P*=0.625)	0.07 (0.05-0.11) (*P*=0.25)	0.08 (0.07-0.13) (*P*=0.125)

uIgG/c: urinary immunoglobulin G-to-creatinine ratio; uCRP/c: urinary C-reactive protein-to-creatinine ratio; uRBP/c: urinary retinol binding protein-to-creatinine ratio; BDL: below detection limit

Results of urinary marker analysis with different concentrations of added hemoglobin are shown in Table 3. No significant differences were found when the samples without added hemoglobin were compared to the samples with different hemoglobin concentrations (*P* ranging from 0.125 to 0.625).

3.4. Urinary markers for renal dysfunction

Table 4. Correlations between urinary markers, routine parameters for renal dysfunction and other variables.

	uIgG/c	**uCRP/c**	**uRBP/c**
uIgG/c	1	0.82 (*P*<0.001)	0.91 (*P*<0.001)
uCRP/c	0.82 (*P*<0.001)	1	0.75 (*P*<0.001)
uRBP/c	0.91 (*P*<0.001)	0.75 (*P*<0.001)	1
sCr	-0.37 (*P*=0.060)	-0.20 (*P*=0.340)	-0.37 (*P*=0.065)
Urea	0.17 (*P*=0.405)	0.40 (*P*=0.042)	0.24 (*P*=0.234)
USG	0.19 (*P*=0.354)	0.25 (*P*=0.214)	0.28 (*P*=0.159)
UPC	0.93 (*P*<0.001)	0.84 (*P*<0.001)	0.89 (*P*<0.001)
sAlb	-0.74 (*P*<0.001)	-0.66 (*P*<0.001)	-0.73 (*P*<0.001)

uIgG/c: urinary immunoglobulin G-to-creatinine ratio; uCRP/c: urinary C-reactive protein-to-creatinine ratio; uRBP/c: urinary retinol binding protein-to-creatinine ratio; sCr: serum creatinine; Urea: serum urea; USG: urine specific gravity; UPC: urinary protein to creatinine ratio; sAlb: serum albumine

Results of urinary marker analysis and comparison between groups B and H are shown in Table 2. Significantly higher values were found in group B compared to group H for all three urinary markers (uIgG, uCRP, and uRBP; *P*<0.001, *P*=0.011, and *P*<0.001, respectively).

Correlations between urinary markers, routine parameters for renal dysfunction and other variables are listed in Table 4. All urinary markers were positively correlated with each other (*P*<0.001). A positive correlation was also found between UPC and each of the three urinary markers (*P*<0.001), while a negative correlation was present between sAlb and all urinary markers (*P*<0.001). Serum urea was positively correlated with uCRP/c (*P*=0.042), while none of the urinary markers were correlated with sCr nor USG.

3.5. Outcome

Sixteen of 18 dogs in group B (89%) made a full recovery. One dog presented with uncomplicated babesiosis, but developed acute renal failure and died two days after presentation. Another dog developed a mesenteric thrombus two weeks after presentation and died of septic peritonitis.

4. Discussion

Concentrations of the three urinary markers (uIgG, uCRP, and uRBP) were significantly higher in group B compared to group H, indicating the presence of both glomerular and tubular dysfunction in naturally occurring uncomplicated canine babesiosis, caused by *B. rossi*. The magnitude of increase in the median uRBP/c and uIgG/c in group B was similar (about 200 fold) for the proximal tubular and glomerular marker, suggesting a similar decrease in function at both levels of the nephron in dogs affected with uncomplicated babesiosis.

The potential interference of hemoglobin with the urinary marker analysis was excluded by performing interference testing. Previously published urinary hemoglobin concentrations in dogs with babesiosis ranged from 0.2 to 11.6 g/L (Lobetti and Reyers, 1996; Lobetti and Jacobson, 2001). No significant interferences were found on these, and even higher (24 g/L) concentrations.

Routine serum and urinary markers for renal dysfunction (with the exception of UPC) were not significantly different between the two groups, demonstrating the earlier detection of renal dysfunction when using urinary markers. Granular casts and RTE cells, possibly indicative of renal tubular injury (Lobetti and Jacobson, 2001), were only present in the urine of a minority of dogs in group B. Based on the pathogenesis of babesiosis, the observed proteinuria could be either of pre-renal (hemoglobinemia, myoglobinemia) or renal origin (Lees et al., 2005). Severe hemoglobinuria was present in almost all the dogs in group B, demonstrating a major pre-renal component in the observed proteinuria. Therefore, differentiation between glomerular and tubular proteinuria, which

is primarily based on the magnitude of proteinuria, is difficult to make based on the UPC in babesiosis. Nevertheless, the results of this study strongly suggest that both glomerular and tubular dysfunction occur in dogs with uncomplicated babesiosis.

Increased amounts of serum CRP, together with other acute phase proteins, have been demonstrated in canine babesiosis, confirming the presence of an acute inflammatory response (Ulutas et al., 2005; Matijatko et al., 2007; Köster et al., 2009). The latter study failed to find an association between serum CRP concentration and outcome in canine babesiosis (Köster et al., 2009). Urinary CRP was previously measured in dogs with *E. coli* pyometra, Leptospirosis, and CKD (Maddens et al., 2010a; Oliveira et al., 2010; Smets et al., 2010b). The glomerular barrier must be damaged before HMW proteins such as CRP, irrespective of their circulating levels, can be detected in urine (D'Amico and Bazzi, 2003). The extent to which variations in serum CRP concentrations influence uCRP concentrations in canine babesiosis, remains to be determined.

Urinary IgG concentrations were also significantly higher in group B, demonstrating the trans-glomerular passage of yet another HMW protein. Theoretically, uIgG is a better and more selective marker of the severity of damage to the glomerular capillary wall than the overall degree of proteinuria, because increasing amounts of this HMW protein cross the glomerular barrier with an increasing severity of glomerular lesions (D'Amico and Bazzi, 2003).

Tubular dysfunction was assessed by measurement of uRBP, a LMW protein. The unbound fraction of this negative acute phase protein is freely filtered through the glomeruli. Under physiological circumstances, this filtered RBP is reabsorbed by the proximal tubular cells, but tubular dysfunction increases uRBP (Raila et al., 2003a; Raila et al., 2003b). Comparison of uRBP results from the dogs in group B with the reported results from dogs with different stages of CKD (Smets et al., 2010b) showed that the median uRBP/c of dogs with CKD was five times higher than in dogs with babesiosis, suggesting more severe tubular dysfunction in dogs with CKD. In another study (Raila et al., 2010), uRBP could not detect a reduced plasma creatinine clearance in apparently healthy dogs, leading to the conclusion that uRBP had no diagnostic value in detecting mildly decreased GFR in non-azotemic

dogs, however the GFR measurement is questioned, because a limited sampling technique was used. Further investigations regarding this finding are warranted.

The studied urinary protein markers were positively correlated even though they represent dysfunction at different levels of the nephron. Babesiosis might indeed cause direct damage at both the glomerular and tubular level. Another explanation could be a close interaction of these proteins in the nephron, resulting in an overload of filtered proteins that is harmful to tubular cells as described in humans (D'Amico and Bazzi, 2003; Vinge et al., 2010). In the process of reabsorption by the proximal tubular cells, HMW proteins compete not only amongst each other, but also with LMW proteins. If this reabsorption mechanism becomes saturated due to large amounts of filtered HMW proteins, filtered proteins of all sizes appear in the urine. An overload of filtered proteins, due to increasing severity of glomerular disease, induces progressive damage to the epithelial cells of the proximal tubules as well as activation of cytokines and growth factors that induces interstitial infiltration and fibrosis. Therefore, urinary LMW proteins, which escaped tubular reabsorption, are theoretically superior markers of the severity of tubulo-interstitial damage than the overall amount of proteinuria (D'Amico and Bazzi, 2003; Vinge et al., 2010).

A recent histological study, examining kidneys from eight dogs that suffered from a fatal *B. canis* infection, demonstrated mainly degenerative changes in the proximal renal tubules, with occasional complete necrosis of the proximal tubules, while no significant glomerular changes were reported (Máthé et al., 2007). This absence of glomerular pathology is in apparent contradiction with the evidence of glomerular dysfunction in the current study. One possible explanation for this might be the difference in virulence between the different *Babesia* species. Another argument is that the severe clinical presentation in the histological study may have resulted in death or euthanasia before significant glomerular damage could have occurred. Even more importantly, urinary markers represent functional processes, while histology shows structural findings. Therefore, although an association between the same urinary markers and renal structural lesions has recently been

suggested for canine *E. coli* pyometra (Maddens et al., 2011), the specificity of urinary markers for localization of renal injury needs further investigation.

5. Conclusion

The results of this study demonstrate the presence of both glomerular and tubular dysfunction in naturally occurring uncomplicated canine babesiosis, caused by *B. rossi*. Earlier detection of renal dysfunction was possible using the urinary markers uIgG, uRBP, and uCRP compared to USG, serum urea and sCr. Further studies are needed to assess the reversibility of renal dysfunction induced by *B. rossi* after treatment, to assess the ability of these urinary markers to predict the risk for renal azotemia, and to evaluate the correlation between changes in renal histology and urinary markers in canine babesiosis.

References

Beugnet, F., Marié, J.L., 2009. Emerging arthropod-borne diseases of companion animals in Europe. Vet. Parasitol. 163, 298-305.

Camacho, A.T., Guitian, E.J., Pallas, E., Gestal, J.J., Olmeda, A.S., Goethert, H.K., Telford, S.R., 3rd, Spielman, A., 2004. Azotemia and mortality among *Babesia microti*-like infected dogs. J. Vet. Intern. Med. 18, 141-146.

Carret, C., Walas, F., Carcy, B., Grande, N., Précigout, E., Moubri, K., Schetters, T.P., Gorenflot, A., 1999. *Babesia canis canis, Babesia canis vogeli, Babesia canis rossi*: differentiation of the three subspecies by a restriction fragment length polymorphism analysis on amplified small subunit ribosomal RNA genes. J. Eukaryot. Microbiol. 46, 298-303.

Chadha, V., Garg, U., Alon, U.S., 2001. Measurement of urinary concentration: a critical appraisal of methodologies. Pediatr. Nephrol. 16, 374-382.

Christensen, E.I., Moskaug, J.O., Vorum, H., Jacobsen, C., Gundersen, T.E., Nykjaer, A., Blomhoff, R., Willnow, T.E., Moestrup, S.K., 1999. Evidence for an essential role of megalin in transepithelial transport of retinol. J. Am. Soc. Nephrol. 10, 685-695.

D'Amico, G., Bazzi, C., 2003. Pathophysiology of proteinuria. Kidney Int. 63, 809-825.

de Scally, M.P., Lobetti, R.G., Reyers, F., Humphris, D., 2004. Are urea and creatinine values reliable indicators of azotaemia in canine babesiosis? J. S. Afr. Vet. Assoc. 75, 121-124.

de Scally, M.P., Leisewitz, A.L., Lobetti, R.G., Thompson, P.N., 2006. The elevated serum urea:creatinine ratio in canine babesiosis in South Africa is not of renal origin. J. S. Afr. Vet. Assoc. 77, 175-178.

Garcia, A.T., 2006. Piroplasma infection in dogs in northern Spain. Vet. Parasitol. 138, 97-102.

Jacobson, L.S., Clark, I.A., 1994. The pathophysiology of canine babesiosis: new approaches to an old puzzle. J. S. Afr. Vet. Assoc. 65, 134-145.

Jacobson, L.S., Lobetti, R.G., 1996. Rhabdomyolysis as a complication of canine babesiosis. J. Small Anim. Pract. 37, 286-291.

Köster, L.S., Van Schoor, M., Goddard, A., Thompson, P.N., Matjila, P.T., Kjelgaard-Hansen, M., 2009. C-reactive protein in canine babesiosis caused by *Babesia rossi* and its association with outcome. J. S. Afr. Vet. Assoc. 80, 87-91.

Lees, G.E., Brown, S.A., Elliott, J., Grauer, G.E., Vaden, S.L., 2005. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). J. Vet. Intern. Med. 19, 377-385.

Lobetti, R.G., Reyers, F., 1996. Met-haemoglobinuria in naturally occurring *Babesia canis* infection. J. S. Afr. Vet. Assoc. 67, 88-90.

Lobetti, R.G., Jacobson, L.S., 2001. Renal involvement in dogs with babesiosis. J. S. Afr. Vet. Assoc. 72, 23-28.

Lobetti, R.G., Reyers, F., Nesbit, J.W., 1996. The comparative role of haemoglobinaemia and hypoxia in the development of canine babesial nephropathy. J. S. Afr. Vet. Assoc. 67, 188-198.

Maddens, B., Daminet, S., Smets, P., Meyer, E., 2010a. *Escherichia coli* pyometra induces transient glomerular and tubular dysfunction in dogs. J. Vet. Intern. Med. 24, 1263-1270.

Maddens, B.E., Daminet, S., Demeyere, K., Demon, D., Smets, P., Meyer, E., 2010b. Validation of immunoassays for the candidate renal markers C-reactive protein, immunoglobulin G, thromboxane B2 and retinol binding protein in canine urine. Vet. Immunol. Immunopathol. 134, 259-264.

Maddens, B., Heiene, R., Smets, P., Svensson, M., Aresu, L., van der Lugt, J., Daminet, S., Meyer, E., 2011. Evaluation of kidney injury in dogs with pyometra based on proteinuria, renal histomorphology, and urinary biomarkers. J. Vet. Intern. Med. 25, 1075-1083.

Máthé, A., Vörös, K., Papp, L., Reiczigel, J., 2006. Clinical manifestations of canine babesiosis in Hungary (63 cases). Acta Vet. Hung. 54, 367-385.

Máthé, A., Dobos-Kovács, M., Vörös, K., 2007. Histological and ultrastructural studies of renal lesions in *Babesia canis* infected dogs treated with imidocarb. Acta Vet. Hung. 55, 511-523.

Matijatko, V., Mrljak, V., Kis, I., Kucer, N., Forsek, J., Zivicnjak, T., Romić, Z., Simec, Z., Ceron, J.J., 2007. Evidence of an acute phase response in dogs naturally infected with *Babesia canis*. Vet. Parasitol. 144, 242-250.

Matjila, P.T., Penzhorn, B.L., Bekker, C.P., Nijhof, A.M., Jongejan, F., 2004. Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa. Vet. Parasitol. 122, 119-125.

Oliveira, S.T., Messick, J.B., Biondo, A.W., Santos, A.P., Guimarães, A.M.D., Mohamed, A.S., Neto, J., Dalmolin, M.L., González, F.H.D., 2010. Serum and Urinary C-Reactive Protein Concentrations in Dogs with Leptospirosis. Acta Scient. Vet. 38, 245-249.

Price, R.G., 2002. Early markers of nephrotoxicity. Comp. Clin. Pathol. 11, 2-7.

Raila, J., Forterre, S., Kohn, B., Brunnberg, L., Schweigert, F.J., 2003a. Effects of chronic renal disease on the transport of vitamin A in plasma and urine of dogs. Am. J. Vet. Res. 64, 874-879.

Raila, J., Neumann, U., Schweigert, F.J., 2003b. Immunochemical localization of megalin, retinol-binding protein and Tamm-Horsfall glycoprotein in the kidneys of dogs. Vet. Res. Commun. 27, 125-135.

Raila, J., Brunnberg, L., Schweigert, F.J., Kohn, B., 2010. Influence of kidney function on urinary excretion of albumin and retinol-binding protein in dogs with naturally occurring renal disease. Am. J. Vet. Res. 71, 1387-1394.

Reyers, F., Leisewitz, A.L., Lobetti, R.G., Milner, R.J., Jacobson, L.S., van Zyl, M., 1998. Canine babesiosis in South Africa: more than one disease. Does this serve as a model for falciparum malaria? Ann. Trop. Med. Parasitol. 92, 503-511.

Shakespeare, A.S., 1995. The incidence of canine babesiosis amongst sick dogs presented to the Onderstepoort Veterinary Academic Hospital. J. S. Afr. Vet. Assoc. 66, 247-250.

Smets, P.M., Meyer, E., Maddens, B., Duchateau, L., Daminet, S., 2010a. Effect of sampling method and storage conditions on albumin, retinol-binding protein, and N-acetyl-beta-D-glucosaminidase concentrations in canine urine samples. J. Vet. Diagn. Invest. 22, 896-902.

Smets, P.M., Meyer, E., Maddens, B.E., Duchateau, L., Daminet, S., 2010b. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. J. Vet. Intern. Med. 24, 65-72.

Smets, P.M., Lefebvre, H.P., Kooistra, H.S., Meyer, E., Croubels, S., Maddens, B.E., Vandenabeele, S., Saunders, J.H., Daminet, S., 2011. Hypercortisolism affects glomerular and tubular function in dogs. Vet. J. (in press).

Taboada, J., Merchant, S.R., 1991. Babesiosis of companion animals and man. Vet. Clin. North Am. Small Anim. Pract. 21, 103-123.

Uilenberg, G., Franssen, F.F., Perié, N.M., Spanjer, A.A., 1989. Three groups of *Babesia canis* distinguished and a proposal for nomenclature. Vet. Q. 11, 33-40.

Ulutas, B., Bayramli, G., Ulutas, P.A., Karagenc, T., 2005. Serum concentration of some acute phase proteins in naturally occurring canine babesiosis: a preliminary study. Vet. Clin. Pathol. 34, 144-147.

Vinge, L., Lees, G.E., Nielsen, R., Kashtan, C.E., Bahr, A., Christensen, E.I., 2010. The effect of progressive glomerular disease on megalin-mediated endocytosis in the kidney. Nephrol. Dial. Transplant. 25, 2458-2467.

Welzl, C., Leisewitz, A.L., Jacobson, L.S., Vaughan-Scott, T., Myburgh, E., 2001. Systemic inflammatory response syndrome and multiple-organ damage/dysfunction in complicated canine babesiosis. J. S. Afr. Vet. Assoc. 72, 158-162.

Wozniak, E.J., Barr, B.C., Thomford, J.W., Yamane, I., McDonough, S.P., Moore, P.F., Naydan, D., Robinson, T.W., Conrad, P.A., 1997. Clinical, anatomic, and immunopathologic characterization of *Babesia gibsoni* infection in the domestic dog (*Canis familiaris*). J. Parasitol. 83, 692-699.