Research Articles

Urinary markers in *Babesia canis vogeli*-infected dogs

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Abstract

Canine babesiosis, a life-threatening tick-borne blood parasitic disease in dogs, caused by *Babesia canis vogeli*, is a health problem in companion animals. The disease causes febrile illness, hemolytic anemia, pre-hepatic jaundice, and thrombocytopenia. Moreover, renal dysfunction from babesiosis has been reported. The purpose of this study was to investigate urinary markers that might be sensitive and specific for the early detection of renal dysfunction in *B. c. vogeli*-infected dogs. Blood and urine samples were collected from 11 dogs. The blood and urine samples were divided into two groups. *B. c. vogeli*-infected dogs group including six infected dogs as confirmed by microscopic examination and multiplex polymerase chain reaction. Non-infected dogs group included five healthy dogs. Blood samples were subjected to hematology and biochemistry analysis while urine samples were stored at -80°C until analyzed. Three candidate urinary markers (urinary immunoglobulin G, uIgG; urinary C-reactive protein, uCRP; and urinary retinol-binding protein, uRBP) were examined using commercial enzyme-linked immunosorbent assays (ELISA); two additional candidate markers, aspartate aminotransferase to alanine aminotransferase ratio (AST/ALT) and urinary creatinine to serum creatinine ratio (UCr/SCr) were also studied. The results demonstrated that hemoglobin, red blood cell count, and hematocrit were significantly different between *B. c. vogeli*-infected dogs and non-infected dogs while the candidate markers were not. In conclusion, the selected candidate markers could not be used as urinary markers for renal dysfunction in *B. c. vogeli*-infected dogs. However, further study should investigate other urinary markers such as albumin, tubular enzymes, and tubular proteins as well as high-throughput technologies such as the proteomic approach.

Keywords: *Babesia canis vogeli*, babesiosis, dog, urinary marker, urine

ตัวบ่งชี้ในปัสสาวะของสุนัขที่ติดเชื้อ *Babesia canis vogeli*

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บทคัดย่อ

โรคบาบีซิโอสิสในสุนัข ซึ่งเป็นโรคที่นำโดยเห็บและทำให้สุนัขถึงแก่ความตาย อันมีสาเหตุมาจากเชื้อ *Babesia canis vogeli* ยังคงเป็นปัญหาด้านสุขภาพในสัตว์เลี้ยงเป็นเพื่อน อาการของโรคได้แก่ อาการไข้ ภาวะโลหิตจางจากการแตกทำลายของเม็ดเลือดแดง ภาวะดีซ่านที่มีสาเหตุก่อนตับ และเกล็ดเลือดต่ำ นอกจากนั้น มีรายงานว่าโรคบาบีซิโอสิสทำให้เกิดภาวะไตทำงานผิดปกติ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อสำรวจตัวบ่งชี้ในปัสสาวะ ซึ่งน่าจะมีความไวและความจำเพาะ สำหรับการตรวจหาภาวะไตทำงานผิดปกติในระยะเริ่มต้น ในสุนัขที่ติดเชื้อ *B. c. vogeli* ตัวอย่างเลือดและปัสสาวะถูกเก็บจากสุนัข 11 ตัว โดยแบ่งตัวอย่างออกเป็น 2 กลุ่ม คือ กลุ่มสุนัขที่ติดเชื้อ *B. c. vogeli* ประกอบด้วยสุนัข 6 ตัว ซึ่งยืนยันโดยการตรวจภายใต้กล้องจุลทรรศน์ และวิธีมัลติเพล็กซ์พีซีอาร์ กลุ่มสุนัขที่ไม่ติดเชื้อ ประกอบด้วยสุนัข 5 ตัว ที่มีสุขภาพดี ตัวอย่างเลือดได้ถูกนำไปวิเคราะห์หาค่าทางโลหิตวิทยาและชีวเคมี ในขณะที่ปัสสาวะถูกเก็บไว้ที่อุณหภูมิ -80°C ก่อนการวิเคราะห์ ตัวบ่งชี้ในปัสสาวะของสุนัข 3 ชนิด คือ อิมมูโนโกลบูลินจี ซี-รีแอคทีฟโปรตีน และเรตินอลบายด์ดิ้งโปรตีน ถูกตรวจโดยใช้วิธีเอนไซม์-ลิงค์ อิมมูโนแอสเซย์-อีไลซา รวมทั้งสารบ่งชี้อีก 2 ชนิด คือ ค่าสัดส่วนของแอสพาเตต อะมิโนทรานสเฟอเรส กับ อะลานีน อะมิโนทรานสเฟอเรส และ ค่าสัดส่วนของยูรินารี ครีเอตินีน กับ ซีรัม ครีเอตินีน ได้ถูกนำมาศึกษา ผลการศึกษาแสดงให้เห็นว่าค่าฮีโมโกลบิน จำนวนเม็ดเลือดแดง และปริมาตรเม็ดเลือดแดงอัดแน่น มีความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่มสุนัขที่ติดเชื้อ *B. c. vogeli* และกลุ่มสุนัขที่ไม่ติดเชื้อ ในขณะที่สารบ่งชี้ไม่มีความแตกต่าง โดยสรุป ตัวบ่งชี้ที่นำมาศึกษา ไม่สามารถใช้บ่งชี้ภาวะไตทำงานผิดปกติในสุนัขที่ติดเชื้อ *B. c. vogeli* อย่างไรก็ตาม ควรมีการตรวจสอบตัวบ่งชี้ตัวอื่น ได้แก่ อัลบูมิน ทูบูลาร์ เอนไซม์ และทูบูลาร์ โปรตีน รวมทั้งการใช้เทคโนโลยีที่ให้ผลผลิตสูง เช่น เทคนิคทางโปรตีโอมิกส์ เป็นต้น

คำสำคัญ : *Babesia canis vogeli* Babesiosis ปัสสาวะ สารบ่งชี้ในปัสสาวะ สุนัข

Introduction

Canine babesiosis is a tick-borne blood parasitic disease caused by *Babesia* spp. which was first discovered in 1888. Babesia parasites in dog are divided into two types, large babesia (*Babesia canis canis, B. canis vogeli* and *B. canis rossi*) and small babesia (*Babesia gibsoni*), based on the size of merozoites located in the red blood cells (Schoeman, 2009). The clinical signs of infected dogs vary depending on the subtypes of babesia, host immunity, age, endemic area, and co-infection with other blood parasites such as *Ehrlichia canis, Anaplasma platys,* and *Hepatozoon canis* (de Caprariis et al., 2011; Al Izzi et al., 2013; Rojas et al., 2014). The dog infected with the virulent subtype *B. c. rossi* shows severe clinical signs such as hemolytic anemia and acute devastating inflammatory response (Reyers et al., 1998), whereas infection with *B. c. canis* causes lethargy, anorexia, fever, icterus, anemia and thrombocytopenia. On the other hand, *B. c. vogeli* infection causes mild disease and no clinical signs are often recorded (Caccio et al., 2002). However, Salem and Farag (2014) reported that infection with *B. c. vogeli* showed various clinical findings such as fever, anorexia, anemia, enlargement of lymph nodes and splenomegaly. Unfortunately, the supporting documents regarding the effects of *B. c. vogeli* on renal function and pathophysiology of the disease have been limited.

Previous studies have showed that renal hypoxia, met-hemoglobinuria and cytokine induced by *Babesia* spp. are related to renal damage. Decreased blood pressure (hypotension) and azotemia in babesiosis are the factors that might cause renal damage as well as acute tubular necrosis (Zygner and Gójska-Zygner, 2014). Intravascular hemolysis caused by *B. c. canis* produces the nephrotoxic substance met-hemoglobin, which can impair renal function (Harrison et al., 1947; Lobetti and Reyers, 1996; Lobetti et al., 1996). Increasing serum TNF-α concentration in *B. c. canis*-infected dogs leads to hypotension, pre-renal azotemia, and subsequently to renal failure (Zygner et al., 2014). In addition, a study using ultrasonography showing increasing echogenicity and corticomedullary ratios in canine babesiosis and glomerulonephritis has also been reported (Fraga et al., 2011).

Currently, to diagnose renal damage in canine babesiosis as rapidly as possible in order to provide effective treatment, specific markers of renal damage are being studied. The presence of proteinuria, renal tubular casts, and epithelium cells, which represent renal damage, were detected in both complicated and uncomplicated babesiosis (Lobetti and Jacobson, 2001). Unfortunately, serum urea and creatinine could not predict azotemia caused by babesiosis (de Scally et al., 2004). The previous report showed that *B. c. rossi* affected renal function in the infected dog. The urinary markers of *B. c. rossi*-infected dogs showed statistically significant difference of urinary immunoglobulin G (uIgG) and urinary C-reactive protein (uCRP), which are markers for glomerular dysfunction, and urinary retinol-binding protein (uRBP), which is a marker for tubular dysfunction, compared with healthy dogs (Defauw et al., 2012). In addition, the previous study demonstrated that increased aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio or AST/ALT, in azotemic compared with non-azotemic *B. c. canis* infection combined with elevated serum urea and creatinine may be useful for the early detection of renal dysfunction caused by babesiosis (Zygner et al., 2012). Urinary creatinine to serum creatinine ratio (UCr/SCr)

and renal failure index (RFI) were also studied. In azotemic dogs infected with *B. c. canis*, the UCr/SCr was significantly lower and the RFI was significantly higher than in non-azotemic dogs infected with *B. c. canis* (Zygner et al., 2013). Recently, serum Cystatin-C, which is responsible for renal glomerular function, was used as a novel renal marker for the early detection of renal dysfunction of *B. c. vogeli* infection (Didem et al., 2015).

The aim of the current study was to assess the renal dysfunction in infected dogs caused by *B. c. vogeli*, which is the major *B. canis* subtype in Thailand, using AST/ALT, UCr/SCr, uIgG, uCRP, and uRBP as biomarkers.

Materials and Methods

Animals

The present study was approved by The Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC), Mahidol University, protocol No. MUVS-2014-22, protocol title "A study of urinary marker in dog infected with *B. c. vogeli* at Prasu Arthorn Animal Hospital"

A total of 11 dogs were enrolled in the study and divided into two groups. Group 1 (babesia-positive dogs) included six dogs that were prospectively sampled after they presented with babesiosis to the Prasu-Arthorn Animal Hospital, faculty of Veterinary Science, Mahidol University, Nakhon pathom, Thailand. Group 2 (non-infected dogs) included five clinically healthy control dogs of comparable age and body weight, that also presented to the animal hospital during the same period. The control dogs were considered healthy based on a thorough anamnesis, physical examination, complete blood count, basic biochemistry profile, urinalysis, and urinary protein to creatinine (UPC) ratio < 0.5. The identification of the *Babesia* spp. responsible for the infection, in both babesia-infected and healthy samples, was confirmed by Modified Wright-Giemsa staining, morphology examined under a light microscope, and multiplex polymerase chain reaction (multiplex PCR).

Sampling regimen

Blood and urine samples were collected at admission in both groups. All urine samples were collected by catheterization or voiding. Urinalysis consisted of a dipstick, urine specific gravity (USG), and urine protein-to-creatinine (UPC) ratio. For the study of urinary markers, urine samples were performed by quick centrifugation (3 min) and then supernatant was divided into aliquots of 1 mL and stored at -80°C until analysis. All analyses of urinary markers using enzyme-linked immunosorbent assay (ELISA) kits were performed within 1 year of sample collection. Smets and his colleagues (Smets et al., 2010) reported that the stability of uRBP concentration during storage was not significantly different after storage for 12 months at -80°C compared with fresh samples. However, storage information about uCRP and uIgG are lacking in veterinary medicine.

A thorough anamnesis, physical examination, complete blood count, basic biochemistry profile [including serum urea, serum creatinine (sCr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total serum protein, serum albumin (sAlb), glucose, and electrolytes (potassium and calcium)], and a urinalysis were performed to exclude the presence of concurrent

diseases. AST/ALT and UCr/SCr were calculated using the following formulae:

AST/ALT = aspartate aminotransferase ÷ alanine aminotransferase,

UCr/SCr = urinary creatinine concentration ÷ serum creatinine concentration.

Extraction of parasite DNA

Total DNA was prepared from blood specimens using a QIAamp Mini Blood Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's instruction. The extracted blood DNA was eluted in 50 μL elution buffer and kept at -20°C for long-term storage.

PCR amplification and sequencing

To exclude co-infection with *Ehrlichia canis, Anaplasma platys*, and *Hepatozoon canis*, only dogs that presented with uncomplicated babesiosis caused by *B. c. vogeli* were included in this study. Multiplex PCR for the detection of *B. c. vogeli*, *E. canis*, and *H. canis* (Kledmanee et al., 2009) was carried out using 5 μL of the total DNA as a template and 20 μL of 0.4 pmol of each primer, 300 μM of each dNTP, 4 units of HotStar *Taq* DNA Polymerase (QIAGEN®, Germany), 1x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, and RNase-free water. Amplification was performed in a T100™ Thermal cycler (BIO-RAD, Hercules, CA, USA) and thermocycling consisted of one step of 15 min at 95°C followed by 35 cycles of 45 s at 94°C, 45 s at 65°C, and 90 s at 72°C with a final extension step of 10 min at 72°C. Aliquots of the amplicons were detected on 2.5% agarose gel electrophoresis.

Conventional PCR for the detection of *A. platys* was carried out using 5 μL of the total DNA as a template and 20 μL of 0.4 pmol of each primer [(Ana45F; 5'GTCGAACGGATTTTTGTCGT3' and Ana671R; 5'GCCACTGGTGTTCCTCCTAA3'): Sungpradit et al., unpublished data], 300 μM of each dNTP, 4 units of iTaq™ DNA Polymerase (iNtRON Biotechnology, Kyungki-Do, South Korea), 1x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, and RNase-free water. Amplification was performed in a T100™ Thermal cycler (BIO-RAD, Hercules, CA, USA) and thermocycling consisted of one step of 10 min at 95°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C with a final extension step of 10 min at 72°C. Aliquots of the amplicons were detected on 2.0% agarose gel electrophoresis.

Six babesia-positive PCR products were procured for DNA sequencing by First BASE Sdn Bhd (Selangor, Malaysia). DNA sequences were analyzed using a BigDye Terminator V3.1 cycle sequencing kit chemistry. DNA sequences were performed using Ba103F and Ba721R primers (Kledmanee et al., 2009). The 18s ribosomal RNA (18s rRNA) gene sequence results were analyzed using Chromas version 2.4.3. All 18s rRNA sequence results were compared with the available sequences in the GenBank database using the Basic Local Alignment Search Tool, BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

ELISA

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 uIgG, uCRP, and uRBP. For each immunoassay, the absorbance was measured at a wavelength of 450 nm,

using an ELISA plate reader, and the KC junior program (Biotek®, ELX808, Winooski, VT, USA) was used to generate the standard curve and calculate the concentrations of uIgG, uCRP, and uRBP. Finally, results were indexed to urinary creatinine concentration (c) and expressed as ratios (Defauw et al., 2012).

Statistical analysis

The commercial software SPSS version 18 was used for data analysis. A normal distribution Shapiro-Wilk test and the non-parametric Mann-Whitney U-test were used to compare *B. c. vogeli*-infected group with non-infected group. Differences were considered statistically significant at $P < 0.05$.

Results

All babesia-positive dogs were diagnosed with uncomplicated babesiosis caused by *B. c. vogeli* by screening with multiplex PCR to exclude co-infection with *E. canis, A. platys,* and *H. canis*. The sequencing results of the 18s rRNA gene showed 100% identity with the *B. c. vogeli* 18s rRNA gene deposited in the GenBank database. Dogs with pyometra, chronic renal disease (CRD) and Cushing's syndrome were excluded. The median age in this group was 2.3 years, which was not significantly different from the median age of non -infected group, 3.5 years ($P = 0.09$). In *B. c. vogeli*-infected group, five dogs were female and one male, whereas in non-infected group, one was female and four were male, which was significantly different from *B. c. vogeli*-infected group ($P = 0.04$). Hematology and biochemistry results of both groups are shown in Table 1.

Hemoglobin, red blood cell count, and packed cell volume were significantly different between *B. c. vogeli*-infected group and non-infected group ($P < 0.05$), but all of the parameters in both groups were in the reference range. Platelet count was significantly different between both groups ($P < 0.05$). Three of six dogs in *B. c. vogeli*-infected group (50%) had thrombocytopenia while one of five dogs (20%) in non-infected group had thrombocytopenia.

ALT was significantly different between *B. c. vogeli*-infected group and non-infected group ($P = 0.02$) but ALT in both groups was in the reference range. Two of six dogs in babesia-positive group (33%) had hypoalbuminemia while all dogs in babesia-positive group had normal total serum protein, but albumin and total serum protein were not significantly different between both groups ($P = 0.11$ and $P = 0.31$, respectively). Urine color was yellow in both groups. Urine appearance varied from clear to turbid in *B. c. vogeli*-infected group and from clear to slightly turbid in non-infected group. The urinary pH ranged from 6 to 8 in *B. c. vogeli*-infected group and from 5 to 8 in non-infected group. Bilirubinuria ranged from negative to 1+ in *B. c. vogeli*-infected group and ranged from negative to 2+ in non-infected group.

Results of routine parameters for urinalysis for *B. c. vogeli*-infected group and non-infected group are presented in Table 2. Serum urea, serum creatinine, and urine specific gravity did not differ significantly between both groups ($P = 0.34$, $P = 0.85$, and $P = 0.86$, respectively), while urinary protein-to-creatinine (UPC) ratio was not significantly different between both groups ($P = 0.58$).

Results of the selected urinary marker analysis compared between *B. c. vogeli*-infected group and non-infected group are shown in Table 3. AST/ALT and UCr/SCr were not differ significantly between both

groups (P = 0.54 and P = 0.43, respectively). Urinary immunoglobulin G-to-creatinine ratio (uIgG/c), urinary C-reactive protein-to-creatinine ratio (uCRP/c), and urinary retinol binding protein-to-creatinine ratio (uRBP/c) did not differ significantly between both groups (P = 0.41, P = 0.20, and P = 0.72, respectively).

Discussion

In this study, we excluded other tick-borne blood parasitic samples, such as *E. canis, H. canis,* and *A. platys* which might be co-infections with *B. c. vogeli*-infected samples, using the multiplex PCR technique. Acute canine monocytotropic ehrlichiosis (CME) caused by *E. canis* can be the cause of chronic renal insufficiency (Morar et al., 2015), protein-losing nephropathy (Codner and Maslin, 1992; Codner et al., 1992), and minimal change glomerulopathy (Codner et al., 1992).

The AST/ALT ratio may not act as a useful indicator for the early detection of renal dysfunction in *B. c. vogeli* infection. The previous study revealed that kidney injury caused by *B. c. canis* was correlated with AST/ALT (Zygner et al., 2012). In contrast, the present study showed that AST/ALT was not significantly different between both groups. Anemia followed by intra- and extravascular hemolysis, which may cause elevation of AST, was not observed in this work, and the biochemical profile of *B. c. vogeli* and healthy samples also confirmed that there was no azotemia dogs in both groups.

This study did not reveal significant difference between the uCr/sCr in *B. c. vogeli*-infected group and non-infected group. This result is in disagreement with observations on uCr/sCr in *B. c. canis*-infected dogs showing acute tubular necrosis may occur (Zygner et al., 2013).

Our results show concentrations of the three urinary markers (uIgG, uCRP, and uRBP) were not significantly different between babesia-positive dogs (*B. c. vogeli*-infected group) and healthy dogs (non-infected group), indicating that the glomerular and tubular functions were not affected in uncomplicated canine babesiosis, caused by *B. c. vogeli*. However, *B. c. vogeli*-infected group had slightly increased of uRBP/c, uIgG/c, and uCRP/c when compared with non-infected group. The previous study demonstrated that uRBP/c and uIgG/c concentration were increased (about 200 times) in canine babesiosis, caused by *B. c. rossi* (Defauw et al., 2012). The severity of clinical presentation of the dog infected with *B. c. rossi* is higher than that of *B. c. vogeli* and the mortality rate of *B. c. rossi* is about 12% and approximately 1% for *B. c. vogeli* (Schoeman, 2009). *B. c. vogeli* causes severe babesiosis in puppies, but the clinical signs are often mild in adult dogs (Irwin, 2010). In addition, our result showed the median age in *B. c. vogeli*-infected group was 2.3 years (range: 1-3.17) and median age in non-infected group was 3.5 years (range: 1.92-4.42). Moreover, two of six babesia-infected dogs showed negative results for the microscopic examination because of low parasitemia. The previous study has showed that *B. c. vogeli* causes elevation of Cystatin-C, indicating that renal glomerular was affected by the disease (Didem et al., 2015). These factors may lead to normal concentrations of the three urinary markers.

We found that hemoglobin, red blood cell count, packed cell volume, and platelet count were significantly different between *B. c. vogeli*-infected group and non-infected group ($P < 0.05$). This association is consistent with knowledge of the hematology changes in canine babesiosis (Salem and Farag, 2014). Babesiosis can

Table 1. Clinicopathologic findings (hematology and biochemistry results) in dogs with uncomplicated babesiosis and non-infected dogs (expressed as median and range)

Parameter	*B. c. vogeli*-infected group Median (range)	Non-infected group Median (range)	Reference range
Hematology			
Hemoglobin (g/dL)	15.30[a] (12.3-16.9)	18.80[b] (15.9-19.9)	10-18
Red blood cell count x 10^6 (cell/mm^3)	6.80[a] (5.7-7.2)	8.05[b] (6.94-8.24)	5-9
Packed cell volume (%)	43[a] (35.1-47.6)	52.30[b] (43.9-56)	35-55
Leukocyte count (cell/mm^3)	11000 (5400-18400)	11800 (7040-16100)	6000-17000
Platelet count x 10^3 (cell/μL)	203[a] (71-273)	305[b] (97-318)	200-500
Mean cell volume (fL)	64.8 (57.8-69.8)	65.3 (55.7-67.9)	60-77
Mean corpuscular hemoglobin (pg)	22.8 (21.4-24.7)	23.3 (20.5-24.1)	20-25
Mean corpuscular hemoglobin concentration (g/dL)	35.3 (34.4-39.4)	35.7 (34.5-37.9)	32-36
Red cell distribution width (%)	16.85 (15.1-19.9)	17 (16.8-24.7)	12-15
Plasma protein (g/L)	9.2 (7.4-9.8)	9 (8.4-9.6)	6-7.5
Biochemistry			
Serum albumin (g/dL)	2.9 (2.3-3.4)	3.3 (2.7-3.7)	2.7-3.8
Alkaline phosphatase (U/L)	45 (29-87)	38 (8-65)	23-212
Aspartate aminotransferase (U/L)	33.5 (20-49)	40 (30-60)	0-50
Alanine aminotransferase (U/L)	28.5[a] (25-45)	49[b] (34-53)	10-100
Blood urea nitrogen (mg/dL)	12.5 (10-33)	12 (8-13)	7-27
Creatinine (mg/dL)	1.1 (0.9-1.8)	1.1 (1-1.2)	0.5-1.8
Glucose (mg/dL)	91.5 (83-107)	52 (44-115)	71-125
Calcium (mg/dL)	9.45 (9-11.6)	9.1 (6.9-10.2)	7.9-12
Potassium (mmol/L)	4.75 (3.7-5.3)	4.7 (4.43-5.24)	3.5-5.8
Total serum protein (g/dL)	7.05 (5.8-8.4)	7.4 (6.5-8.7)	5.2-8.2

[a,b] differ superscript were statistically significant (Mann-Whitney U test, P-value < 0.05)

Table 2. Clinicopathologic findings (urinalysis results) in dogs with uncomplicated babesiosis and non-infected dogs (expressed as median and range)

Parameter	*B. c. vogeli*-infected group Median (range)	Non-infected group Median (range)	Reference range
Urine specific gravity	1.017 (1.015-1.051)	1.032 (1.014-1.046)	1.015-1.050
Urine pH	6.75 (6-8)	6.5 (5-8)	5.5-7.0
Urine protein (mg/dL)	16 (5-138)	13 (5-93)	10-50
Urine creatinine (mg/dL)	89.7 (50.2-417)	173.7 (31.7-223.5)	100-500
Urinary protein-to-creatinine ratio	0.202 (0.091 - 0.331)	0.158 (0.044 - 0.416)	≤0.5

Table 3. Results of the selected urinary parameters for urinary markers in dogs with uncomplicated babesiosis and non-infected dogs (expressed as median and range)

Parameter	*B. c. vogeli*-infected group Median (range)	Non-infected group Median (range)	*P*-value
AST/ALT	1.06 (0.67-1.53)	1.02 (0.61-1.18)	0.54
UCr/SCr	78.30 (27.89-379.09)	144.75 (31.70-186.25)	0.43
uIgG/c (mg/g)	0.077 (0.019 - 0.191)	0.041 (0.031 - 0.217)	0.41
uCRP/c (mg/g)	0.001 (0.000 - 0.010)	0.000 (0.000 - 0.001)	0.20
uRBP/c (mg/g)	0.047 (0.007 - 0.180)	0.027 (0.002 - 0.720)	0.72

AST/ALT: aspartate aminotransferase-to-alanine aminotransferase; UCr/SCr: urinary creatinine-to-serum creatinine; 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

induce red blood cell damage, increase osmotic fragility of the infected cells, and cause oxidative and secondary immune-mediated injury of the erythrocyte membrane resulting in a combination of intra- and extravascular hemolysis (Irwin, 2009), followed by acute renal failure due to decreased blood flow through the kidney (Lobetti and Jacobson, 2001). This finding differs from the former research because all of the samples that we collected were adult dogs presenting mild clinical signs of babesiosis, mainly anorexia and thrombocytopenia. Moreover, *B. c. vogeli* caused less disease severity than *B. c. rossi* (Defauw et al., 2012; Schetters et al., 2009). Therefore, the level of renal injury in dogs infected with *B. c. vogeli* should be less than that cause by *B. c. rossi* infection.

Renal damage is one of the most common complications of canine babesiosis (Ma'the' et al., 2006). Blood urea nitrogen and serum creatinine are commonly used as a renal screening test, but the former studies showed that an increase in blood urea nitrogen and serum creatinine can be caused by non-renal factors (Zygner et al., 2012). A previous study suggested that uCRP/c, uIgG/c, and uRBP/c could be used as urinary markers for the diagnosis of kidney injury caused by *B. c. rossi* (Defauw et al., 2012). In contrast, the present study revealed that all three urinary markers were not related to canine babesiosis caused by *B. c. vogeli*. To our knowledge, *B. c. vogeli* infection may not cause azothemia and renal glomerular and tubular dysfunction.

There are also many types of the urinary biomarkers such as tubular enzymes (N-acetyl-β-D-glucosaminidase, γ-glutamyl transferase, and lactate dehydrogenase) for acute kidney injury diagnosis (De Loor et al., 2013), and albumin and tubular protein (Tamm-Horsfall or uromodulin protein) for the diagnosis of chronic kidney disease (Raila et al., 2003). Moreover, the proteomic approach to identifying novel biomarkers in serum of canine babesiosis have been reported (Adaszek et al., 2014) and urinary markers for renal dysfunction caused by babesiosis might also be detected as rapidly as possible by this technique. In addition, Didem et al. (2015) proposed serum canine Cystatin-C (Cys-C) concentration as a novel endogenous marker for the early detection of renal dysfunction in *B. c. vogeli* infection.

In conclusion, the findings of the current study revealed that AST/ALT, UCr/SCr, uIgG, uCRP, and uRBP were not significantly different between *B. c. vogeli*-infected dogs and non-infected dogs. The other biomarkers should be investigated for their potential in the early detection of renal dysfunction in *B. c. vogeli* infection.

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