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*See next page for additional authors*

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## Evaluation of Hematology Profiles and Measurement of Serum Cardiac Troponin Level in Canine Monocytic Ehrlichiosis

Rungrote Osathanon<sup>1\*</sup> Walasinee Moonarmart<sup>1</sup> Naiyana Suksantilap<sup>2</sup> Nattiya Krajangpit<sup>2</sup>  
Piyanart Lekcharoensook<sup>2</sup> Pruksa Julapanthong<sup>3</sup> Nutthakulporn Wongrerkgam<sup>3</sup>

### Abstract

Canine monocytic ehrlichiosis (CME) is an important multisystemic disease in dogs which is caused by *Ehrlichia canis* (*E. canis*). Systemic inflammatory response syndrome (SIRS) and severe anemia from *E. canis* might be the potential causes of myocardial damage. Cardiac troponin T (cTnT) is considered to be a sensitive and specific biomarker for myocardial damage in many mammalian species. The objectives of this study were to evaluate the effect of CME on serum cTnT levels in dogs and to determine the relationship between serum cTnT and values obtained from hematology. Fifty-two client-owned dogs were recruited into this study and clinical data were thoroughly recorded. Blood samples were collected from 52 dogs and tested for antigen of *E. canis* by multiplex PCR. The cTnT levels were measured by Elecsys®/cobas e™ cTnT fourth-generation assay. The dogs were divided into 4 groups. The control group consisted of 15 healthy dogs. The negative group included 20 dogs presented with at least 2 inclusion criteria but yielded negative results for Multiplex PCR. The *E. canis* infected group consisted of 10 dogs, which were positive for *E. canis* only. Lastly, the other blood parasite infected group included 7 dogs with multiplex PCR positive for *Babesia spp.* and/or *Hepatozoon canis*. Results showed that serum cTnT concentration was not different among the 4 groups ( $p = 0.70$ ) and did not significantly increase in dogs with natural *E. canis* infection. However, there was a negative correlation between cTnT and RBC, and a correlation of cTnT with age. These implied that anemia and aging might cause myocardial injury, consequently, increased serum cTnT level in both normal and *E. canis* infected dogs.

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**Keywords:** Cardiac troponin T, *Ehrlichia canis*, hematology profiles, myocardial damage

<sup>1</sup> Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Thailand

<sup>2</sup> Veterinary Student, Faculty of Veterinary Science, Mahidol University, Thailand

<sup>3</sup> Prasu-Arthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University, Thailand

\*Corresponding author E-mail: [rungrote.osa@mahidol.ac.th](mailto:rungrote.osa@mahidol.ac.th)

## บทคัดย่อ

### การประเมินค่าโลหิตวิทยาและการตรวจวัดระดับของซีรัม Cardiac Troponin T ในสุนัขที่ป่วยด้วยโรค Canine Monocytic Ehrlichiosis

รุ่งโรจน์ โอสถานนท์<sup>1\*</sup> วลาสินี มุลอามาตย์<sup>1</sup> นัยนา สุขสันติลาภ<sup>2</sup> ณัฐฐิญา กระจ่างพิศ<sup>2</sup> ปิยนาด เล็กเจริญสุข<sup>2</sup> พงกษา จุฬพันธ์ทอง<sup>3</sup> ณัฐกุลภรณ์ วงษ์ฤกษ์งาม<sup>3</sup>

Canine monocytic ehrlichiosis (CME) เป็นโรคที่สำคัญที่ส่งผลกระทบต่อหลายระบบในสุนัขและมีสาเหตุมาจากเชื้อ *Ehrlichia canis* (*E. canis*) ซึ่งอาจทำให้เกิดการเสียหายของกล้ามเนื้อหัวใจอันเนื่องมาจากภาวะอักเสบทั่วร่างกายและภาวะโลหิตจางแบบรุนแรง ในสัตว์เลี้ยงลูกด้วยนม การตรวจ cardiac troponin T (cTnT) เป็นวิธีที่ไวและจำเพาะต่อการวินิจฉัยภาวะการเสียหายของกล้ามเนื้อหัวใจ การทดลองในครั้งนี้มีวัตถุประสงค์เพื่อประเมินผลของการติดเชื้อ CME และ หาความสัมพันธ์ระหว่างค่าโลหิตวิทยากับระดับของซีรัม cTnT ในสุนัข โดยทำการศึกษาในสุนัขที่มีเจ้าของจำนวน 52 ตัว บันทึกประวัติ ข้อมูลทางคลินิก และ เก็บตัวอย่างเลือดเพื่อนำไปตรวจหา antigen ของ *E. canis* ด้วยวิธี multiplex PCR และ วัดระดับ cTnT โดย Elecsys®/cobas e™ cTnT fourth-generation assay ทำการแบ่งกลุ่มสุนัขออกเป็น 4 กลุ่มได้แก่ กลุ่มควบคุมมีสุนัขจำนวนปกติ 15 ตัว กลุ่มที่ให้ผลลบมีสุนัขจำนวน 20 ตัวที่พบความผิดปกติอย่างน้อย 2 ชนิดจากข้อกำหนดของการทดลองและได้ผลลบต่อการตรวจ antigen ของ *E. canis* ด้วยวิธี multiplex PCR กลุ่มที่เป็น *E. canis* มีสุนัขจำนวน 10 ตัวที่แสดงผลบวกต่อการตรวจ *E. canis* ด้วยวิธี multiplex PCR เท่านั้น และ กลุ่มที่เป็นพยาธิในเม็ดเลือดชนิดอื่น ๆ มีสุนัขจำนวน 7 ตัว ที่ให้ผลบวกต่อการตรวจ *Babesia spp.* และ/หรือ *Hepatozoon canis* ด้วยวิธี multiplex PCR จากการตรวจพบวาระดับซีรัม cTnT ไม่มีความแตกต่างระหว่าง 4 กลุ่ม ( $p = 0.70$ ) และไม่เพิ่มสูงขึ้นอย่างมีนัยสำคัญในสุนัขที่ติดเชื้อ *E. canis* ตามธรรมชาติ นอกจากนี้ยังพบความสัมพันธ์ระหว่าง cTnT กับอายุ และความสัมพันธ์ผกผันกับจำนวนเม็ดเลือดแดง จึงเป็นไปได้ว่าภาวะเลือดจางและอายุที่มากขึ้นอาจส่งผลให้กล้ามเนื้อหัวใจเสียหายจนเป็นผลทำให้ระดับ cTnT ในกระแสเลือดเพิ่มขึ้น

**คำสำคัญ:** การเสียหายของกล้ามเนื้อหัวใจ คาร์ดิแอค โโทรโปนิน ที ค่าโลหิตวิทยา โรคพยาธิในเม็ดเลือด

<sup>1</sup> ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

<sup>2</sup> นักศึกษาคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

<sup>3</sup> โรงพยาบาลประจักษ์ศิลปาคม คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

\*ผู้รับผิดชอบบทความ E-mail: rungrrote.osa@mahidol.ac.th

## Introduction

Canine monocytic ehrlichiosis (CME) is firstly described by Donation and Lestoquard in Algeria in 1965 (Harrus et al., 1999). It is now recognized as an important canine disease that can be found throughout the world (Kelly, 2000; McQuiston et al., 2003; Harrus and Waner, 2011). CME is caused by gram-negative obligate intracellular cocci bacteria, named *Ehrlichia canis* (Cohn, 2003; Rikihisa, 2010). This bacterium is a tick-borne organism and transmitted by *Rhipicephalus sanguineus* (Groves et al., 1975). CME infected dogs is presented with asymptomatic or multisystemic clinical signs (Harrus and Waner, 2011). Examples of acute or chronic clinical signs are lethargy, obtundation, weight loss, fever, loss of appetite, lymphadenopathy, splenomegaly, and bleeding disorders (Harrus et al., 1999; Procaglio et al., 2011).

CME can be diagnosed using several techniques, for example, presentation of intracytoplasmic *E. canis*-morulae in blood smear, hematology, cytology, serology, isolation and molecular detection (Harrus and Waner, 2011). Molecular biology approach using polymerase chain reaction (PCR) to detect experimental *E. canis* infection was introduced (Iqbal et al., 1994). This molecular technique is commonly employed as a definitive diagnosis for *E. canis* infection (Harrus and Waner, 2011). The *E. canis* DNA detection using PCR technique illustrated a high sensitivity and specificity for the detection of concurrent ehrlichial infections (Baneth et al., 2009). The most common target genes are p30-based PCR and 16S rRNA. However, the p30-based PCR assay is more sensitive than the 16S rRNA-based PCR assay (Stich et al., 2002). A multiplex polymerase chain reaction (PCR) was developed for simultaneous detection of canine blood parasites, including *E. canis*, *Babesia spp* and *Hepatozoon canis*,

from blood samples in a single reaction. This multiplex PCR primers were specific to *E. canis* VirB9, *Babesia spp* 16S rRNA and *H. canis* 16S rRNA that yielded 100% identity to the sequences of these blood parasites (Kledmanee et al., 2009).

Troponins are myofibrillar proteins that are present in both skeletal and cardiac muscles (Babuín and Jaffe, 2005). It regulates the interaction between myosin and actin (DeFrancesco et al., 2002). Three types of troponins are reported, which are troponin T, troponin I, and troponin C (Babuín and Jaffe, 2005). Different types of troponins have different functions. It has been reported that troponin T is bound to tropomyosin. In addition, troponin C is bound to calcium, while, troponin I inhibits coupling of myosin and actin. Several cardiac troponin (cTn) isoforms have been identified. They are cardiac troponins C, I, and T (cTnC, cTnI, and cTnT) (Adams et al., 1993a). The cTnI and cTnT cardiac isoforms are specific to cardiac muscle and exhibit a high percentage of the conservation between humans and dogs (Adams et al., 1993a; O'Brien et al., 1997b). cTnT binds the troponin-tropomyosin complex to actin filament (Adams et al., 1993a). It has 260 amino acids with a molecular weight of 37 kDa. Increase in serum cTn levels in humans correlates with histopathological changes in cardiac muscle such as from ischemic injury and toxicity (O'Brien, 2006). Loss of membrane integrity of damaged cardiac myocytes causes release of cTn into blood circulation. After acute myocardial injury, initial increase in cTn levels in blood is caused by release of cytosolic pool. Consequently, the sustained blood cTn levels are due to the release of structurally bound troponin. Therefore, cTn is considered to be the myocardial leakage marker (Katus et al., 1991; Adams et al., 1994; Jaffe et al., 1996). cTnI was measured for diagnosis of cardiovascular diseases in dogs by an automated immunoassay method. A mouse monoclonal anti-troponin I antibody and a goat polyclonal anti-troponin I antibody were used for capture and detection of troponin I molecules directed against a unique 31 amino acid extension of the cTnI N-terminus, respectively (Spratt et al., 2005). This method has been validated using human serum samples which has been shown to be highly reliable and cardiospecific (Collinson et al., 2001). cTnI levels were significantly elevated in dogs with acquired mitral valve disease, dilated cardiomyopathy and pericardial effusion (Spratt et al., 2005). In cats with congestive heart failure from hypertrophic cardiomyopathy (HCM), the serum cTnI was significantly higher than in normal cats. This result may indicate that cats with HCM have ongoing myocardial damage (Herndon et al., 2002). Moreover, Brazilian dogs with acute *E. canis* infection had higher serum cTnI concentrations than healthy dogs. This suggested that acute *E. canis* infection might cause myocardial injury and systemic inflammatory response syndrome (Diniz et al., 2008).

cTnT has a high sensitivity and specificity for myocardial damage, leading to the potential utilization as a marker for cardiomyocyte injury (Adams et al., 1993b; Ohman et al., 1996; Fredericks et

al., 2001). In rat and canine myocardial infarction models, cTnT concentration was significantly increased and highly correlated with the size of infarction within 3 h of injury (O'Brien et al., 1997a). Taken together, these data imply the correlation of serum cTn levels and myocardial damage (Freda et al., 2002).

Recently, CME has been investigated extensively (Harrus et al., 1999). The main examination is focused on the pathogenesis clarification of the diseases (Harrus et al., 1999). Increasing evidences illustrate the involvement of immune mechanisms in the pathogenesis of acute CME associated with vasculitis (Cohn, 2003). These conditions include polymyositis (Shaw et al., 2001), glomerulonephritis (Avery and Avery, 2007), hepatitis (Mylonakis et al., 2010), uveitis (Komnenou et al., 2007), meningoencephalitis (Grindem et al., 2002) and polyarthritis (Weiss et al., 1999). The evidence illustrates that the naturally infected *E. canis* may cause myocardial damage in Brazilian dogs (Diniz et al., 2008). Moreover, the serum cTnI levels in dogs with non-myelosuppressive and myelosuppressive ehrlichiosis are significantly increased (Koutinas et al., 2012). Taken together, these results imply the relationship of CME, cardiac damage and serum troponin levels. Therefore, the aims of this study were to evaluate the effect of CME on serum cTnT levels in dogs and to determine the relationship between serum cTnT and values obtained from hematology.

## Materials and Methods

**Case Materials:** The study was performed in client-owned dogs that visited Prasu-Arthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University during a two-month period (April 2012 to May 2012). Thirty-seven dogs with at least two inclusion criteria were recruited into the potential CME group. The inclusion criteria were tick infestation, bleeding, nervous sign, inflammatory ocular disease, anemia (packed cell volume less than 35%), leucopenia (white blood cell less than 6000 cells/ $\mu$ l), hyperthermia (body temperature over than 102.9 $^{\circ}$ F), hyperproteinemia (total protein over than 7.8 g/dl), and thrombocytopenia (platelet less than 150,000 cells/ $\mu$ l). For the control group, fifteen healthy dogs were recruited. Dogs were excluded from the study if they had the evidences of trauma, antirickettsial drug administration (less than 30 days), and heart diseases. The protocol used in this study was approved by Faculty of Veterinary Science Animal Care and Use Committee (FVS-ACUC).

**Data collection:** Comprehensive data including sex, breed, age, body weight, body condition score, medical history, medication and external parasite infestation history of all dogs recruited into this study were completely recorded. Blood (7 ml) was taken from cephalic or saphenous veins and further collected into K3-EDTA-treated tube (Becton Dickinson) and plain tube (Becton Dickinson). A single drop of blood in K3-EDTA-treated tube was tested by WITNESS<sup>®</sup> EHRlichia IgG antibody test. A small amount of EDTA anti-coagulated blood was employed for hematology by a VetABC hematology

analyser (SCIL Animal Care Company, IL, USA). All blood samples in K3-EDTA-treated tube were stored at -80°C for subsequent analysis of DNA extraction and multiplex polymerase chain reaction (PCR) amplification. Blood samples in plain tubes were centrifuged at 4°C and 3000 rpm for 10 minutes and serum were collected and stored at -80°C for subsequent cTnT analysis.

**Multiplex PCR:** *E. canis* antigen in the specimens was evaluated using multiplex PCR that has been developed by Kledmanee et al. (2009). The multiplex PCR primers were specific to *E. canis* VirB9, *Babesia spp.* 16S rRNA and *Hepatozoon canis* 16S rRNA (Kledmanee et al., 2009). Parasite DNA was extracted by proteinase K digestion and phenol:chloroform : isoamyl (25 : 24 : 1). Primer designed by GenBank was used for multiple alignments using Bio Edit v 7.0.4 software. The multiplex PCR amplification was performed in thermocycler (PCT-200). The amplicons were further separated by electrophoresis in 2.5% agarose gel and visualized under ultraviolet light. All selected samples were detected at Laboratory Department of the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University. This method from multiplex PCR could 100% identify the sequences of *E. canis*, *Babesia spp.* and *Hepatozoon canis*, providing a highly specific tool for diagnosis of tick-borne parasitic co-infections.

**Serum cTnT:** Serum cTnT was measured by Elecsys®/cobas e™ cTnT fourth-generation assay (Roche Diagnostics) on Elecsys 2010/cobas e 411 and Modular® Analytix E170/cobase 601 immunoanalyzers (Roche Diagnostics), according to the manufacturer's instruction. All samples were measured at Clinical Pathology Unit, Siriraj Hospital, Mahidol University, Thailand. Principles and protocol of the assay were described (Giannitsis et al., 2010). Briefly, the assay uses fragment antigen-binding (FAB) fragments of 2 cTnT-specific mouse monoclonal antibodies in a sandwich format. The antibodies recognize epitopes located in the central part of the cTnT molecule (amino acid positions 125-131 and 135-147, respectively). Detection is based on an electrochemiluminescence immunoassay (ECLIA), using a Tris(bipyridyl)-ruthenium(II) complex as label. This assay was performed based on human cTnT that yielded 99% sensitivity and 98% specificity.

**Statistical Analyses:** All data were analyzed by computerized statistical software (SPSS 18.0 for Windows, Chicago, IL, USA). The data in each group were tested for normality by using Shapiro-Wilk test. Comparisons between groups were tested by Kruskal-Wallis test, followed by post hoc Mann-Whitney U test. Spearman's rank correlation coefficient was used to assess the correlation between dependent variables cTnT and independent variables: age and values obtained from hematology. *P* value less than 0.05 was considered statistically significant.

## Results

Fifty-two dogs were recruited into this study, consisting of 29 males (6 neutered, 22 entire, and 1 no data) and 23 females (12 spayed, 9 entire, and 2 no data). There were 16 breeds, including 15 mixed breeds, 9 Thai breeds, 6 Golden retrievers, 4 Siberian huskies, 3 Beagles, 3 Labrador retrievers, 3 Poodles, 1 Cocker spaniel; 1 Bangkaew, 1 German shepherd, 1 Pitbull, 1 Pug, 1 Shih Tzu, 1 St. Bernard, 1 Welsh Corgi and 1 unrecorded. The dogs were classified according to the positive results for *E. canis* or other parasites based on multiplex PCR results. They were divided into 4 groups (Table 1), which were control group, negative group, *E. canis* infected group, and other blood parasite infected group. The control group consisted of 15 healthy dogs. The negative group including 20 dogs presented with at least 2 inclusion criteria but negative results for Multiplex PCR. The *E. canis* infected group included 10 dogs which were positive for *E. canis* only. Lastly, the other blood parasite infected group included 7 dogs with multiplex PCR positive for *Babesia spp.* and/or *Hepatozoon canis*. Clinical variables obtained from the 52 dogs are illustrated in Table 2.

There was no statistical difference in age, WBCs, monocytes, lymphocytes, eosinophils, basophils, band neutrophils, RBCs, MCV, MCH, MCHC, RDW, and plasma protein among all 4 groups (Table 3). The neutrophils of dogs were significantly different among the 4 groups ( $p = 0.039$ ). The neutrophils of the negative group were significantly higher than those of the *E. canis* infected group ( $p = 0.025$ ) and the other blood parasite infected group ( $p = 0.027$ ). Medians of the neutrophil in the control and the negative groups were in normal range, whereas medians in the *E. canis* infected group and the other blood parasite infected group were neutropenia (Table 3).

RBCs of the dogs in all groups was significantly different ( $p = 0.002$ ). The RBC of the *E. canis* infected group and the other blood parasite infected group was significantly lower than the control group ( $p < 0.001$  and  $p = 0.001$ , respectively). Moreover, the RBC of the *E. canis* infected group was also significantly lower than the negative group ( $p = 0.016$ ) (Table 3).

**Table 1** Group distribution of 37 dogs according to multiplex PCR results and 15 healthy dogs

Dog groups	N	PCR positive Organism (s)	N	<i>E. canis</i> Seroreactivity <sup>a</sup> (N)
Control	15	-	-	0
Negative <sup>b</sup>	20	-	-	15
<i>E. canis</i> infected	10	<i>E. canis</i>	10	10
Other blood parasite infected	7	<i>Babesia spp.</i> <i>Hepatozoon canis</i> <i>Babesia spp.</i> + <i>Hepatozoon canis</i>	4 2 1	6

<sup>a</sup>WITNESS® EHRLICHIA

<sup>b</sup>Dogs presented with at least 2 inclusion criteria but negative results for Multiplex PCR

Hematocrit of the dogs in all groups was significantly different ( $p = 0.003$ ). The hematocrit of the *E. canis* infected group was significantly lower than the control group ( $p < 0.001$ ) and the negative group ( $p = 0.025$ ). Medians of the RBC in the control group and the negative group were in normal range. However, medians in the *E. canis* infected group and the other blood parasite infected group were

normocytic normochromic anemia (Table 3).

Hemoglobin of the dogs in all groups was significantly different ( $p = 0.002$ ). The hemoglobin of the *E. canis* infected group was significantly lower than the control group ( $p < 0.001$ ). In addition, the hemoglobin of the *E. canis* infected group was significantly lower than the negative group ( $p = 0.016$ ) (Table 3).

**Table 2** Descriptive statistics of clinical variables of 52 dogs

Variable	Quartiles			Range
	Low	Median	High	
Age (years)	2.0	4.08	7.0	0.25 - 12.42
WBC ( $10^3$ cells/ $\mu$ l)	8.3	10.5	13.0	0.9 - 23.4
Neutrophil ( $10^3$ cells/ $\mu$ l)	5.9	7.7	9.8	0 - 16.4
Band-neutrophil ( $10^3$ cells/ $\mu$ l)	0	0	0	0 - 0.3
Lymphocyte ( $10^3$ cells/ $\mu$ l)	1.4	2.0	3.2	0 - 7.0
Monocyte ( $10^3$ cells/ $\mu$ l)	0	0.12	0.28	0 - 13.3
Eosinophil ( $10^3$ cells/ $\mu$ l)	0	0.19	0.78	0 - 1.7
Basophil ( $10^3$ cells/ $\mu$ l)	0	0	0	0 - 0.2
RBC ( $\times 10^6$ cells/ $\mu$ l)	4.60	5.46	6.91	0.80 - 8.50
Hb (g/dL)	10.40	13.10	16.00	1.70 - 19.50
Hct (%)	32.0	38.6	48.0	5.4 - 57.1
MCV (fL)	67	69	71	62 - 74
MCH (pg)	22	23.10	24	19 - 25
MCHC (g/dl)	32.80	33.30	34.20	30 - 36
Plt ( $\times 10^3$ cells/ $\mu$ l)	68	126	232	7 - 386
RDW (%)	14.50	15.30	16.50	11.50 - 22.10
Plasma protein g/dl	8.60	9.20	10	6 - 12
cTnT (pg/ml)	3	4	5	0 - 370

**Table 3** Comparisons of control group (n = 15), negative group (n = 20), *E. canis* infected group (n = 10), and other blood parasite infected group (n = 7). Statistical differences were evaluated by the Kruskal-Wallis test, followed by Mann-Whitney U post hoc analysis. Results are in frequencies or medians (interquartile range)

Variable	Control (n = 15)	Negative (n = 20)	<i>E. canis</i> infected (n = 10)	Other blood parasite infected (n = 7)	P
Age (years)	4.3 (1.7, 6.6)	4.0 (2.1, 7.7)	3.0 (1.0, 6.2)	6.0 (4.0, 9)	0.618
WBC ( $10^3$ cells/ $\mu$ l)	11.3 (8.9, 14.0)	12.0 (8.98, 13.72)	8.95 (7.18, 12.5)	8.3 (6.6, 12.2)	0.153
Neutrophil ( $10^3$ cells/ $\mu$ l)	8.18 (6.37, 10.14)	8.20 (7.08, 10.40) <sup>a,b</sup>	6.67 (3.33, 8.60) <sup>a</sup>	5.48 (4.54, 9.27) <sup>b</sup>	0.039*
Band-neutrophil ( $10^3$ cells/ $\mu$ l)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.514
Lymphocyte ( $10^3$ cells/ $\mu$ l)	2.00 (1.51, 2.27)	1.93 (1.49, 3.39)	2.98 (1.10, 3.64)	1.95 (1.12, 2.83)	0.855
Monocyte ( $10^3$ cells/ $\mu$ l)	0.25 (0, 0.28)	0 (0, 0.19)	0.22 (0, 0.50)	0.27 (0, 0.36)	0.099
Eosinophil ( $10^3$ cells/ $\mu$ l)	0.29 (0.09, 0.78)	0.28 (0.02, 0.93)	0.04 (0, 0.19)	0.57 (0.05, 0.82)	0.120
Basophil ( $10^3$ cells/ $\mu$ l)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.309
RBC ( $\times 10^6$ cells/ $\mu$ l)	6.50 (6.20, 7.40) <sup>c,d</sup>	6.27 (4.53, 7.15) <sup>e</sup>	3.95 (2.43, 5.15) <sup>c,e</sup>	5.20 (4.70, 7.00) <sup>d</sup>	0.002**
Hb (g/dl)	15.30 (14.10, 17.00) <sup>f</sup>	14.25 (10.78, 16.45) <sup>g</sup>	9.45 (5.93, 12.28) <sup>g</sup>	12.90 (10.40, 16.30)	0.002**
Hct (%)	45.8 (42.7, 51.1) <sup>h</sup>	43.2 (32.2, 49.8) <sup>i</sup>	27.8 (17.8, 36.6) <sup>h,i</sup>	37.4 (32.0, 48.3)	0.003**
MCV (fl)	69 (68, 72)	69 (67, 71)	68 (66, 71)	69 (65, 72)	0.825
MCH (pg)	23.4 (22.6, 24.1)	22.8 (22.0, 23.7)	23.2 (21.4, 24.1)	23.0 (21.5, 24.7)	0.749
MCHC (g/dl)	33.8 (32.7, 34.3)	33.1 (32.6, 33.8)	33.3 (32.8, 34.6)	33.3 (33.0, 34.4)	0.649
Plt ( $\times 10^3$ cells/ $\mu$ l)	270 (232, 334) <sup>k,l</sup>	99 (72, 133) <sup>j</sup>	54 (28, 114) <sup>k</sup>	127 (67, 172) <sup>l</sup>	< 0.001***
RDW (%)	15.1 (14.7, 15.6)	15.3 (14.1, 16.1)	17.2 (14.8, 17.5)	15.4 (14.3, 16.6)	0.208
Plasma protein (g/dl)	9.0 (8.2, 9.2)	9.4 (8.3, 10.4)	10.1 (8.8, 11.6)	9.6 (9.0, 10.6)	0.080
cTnT (pg/ml)	0.004 (0.003, 0.007)	0.004 (0.003, 0.005)	0.005 (0.004, 0.013)	0.003 (0.003, 0.005)	0.703

<sup>a</sup>Significant difference between negative group and *E. canis* infected group ( $p = 0.025$ )

<sup>b</sup>Significant difference between negative group and other blood parasite infected group ( $p = 0.027$ )

<sup>c</sup>Significant difference between control group and *E. canis* infected group ( $p < 0.001$ )

<sup>d</sup>Significant difference between control group and other blood parasite infected group ( $p = 0.001$ )

<sup>e</sup>Significant difference between negative group and *E. canis* infected group ( $p = 0.016$ )

<sup>f</sup>Significant difference between control group and *E. canis* infected group ( $p < 0.001$ )

<sup>g</sup>Significant difference between negative group and *E. canis* infected group ( $p = 0.016$ )

<sup>h</sup>Significant difference between control group and *E. canis* infected group ( $p < 0.001$ )

<sup>i</sup>Significant difference between negative group and *E. canis* infected group ( $p = 0.025$ )

<sup>j</sup>Significant difference between control group and negative group ( $p < 0.001$ )

<sup>k</sup>Significant difference between control group and *E. canis* infected group ( $p < 0.001$ )

<sup>l</sup>Significant difference between control group and other blood parasite infected group ( $p = 0.001$ )

Platelets of the dogs in all groups were significantly different ( $p < 0.001$ ). The platelets of the negative group ( $p < 0.001$ ), *E. canis* infected group ( $p < 0.001$ ) and the other blood parasite infected group ( $p = 0.001$ ) were significantly lower than the control group. Median of the platelets in the control group were in normal range. In contrast, medians of the platelets in the negative, *E. canis* infected group and the other blood parasite infected group were thrombocytopenia (Table 3).

There was no difference in serum cTnT concentrations among the control group, negative group, *E. canis* infected group, and other blood parasite infected group ( $p = 0.70$ ). Median and interquartiles of serum cTnT concentrations in each group are shown in Table 3.

Correlation between cTnT and age and hematology values are displayed in Table 4. Serum cTnT was correlated with age in 52 dogs (Spearman's correlation coefficients  $r_s = 0.42$ ,  $p = 0.002$ ) and the correlation was stronger in the control dogs exclusively (Spearman's correlation coefficients  $r_s = 0.75$ ,  $p = 0.001$ ).

Serum cTnT was weakly negatively correlated with lymphocytes in all dogs (Spearman's correlation coefficients  $r_s = -0.32$ ,  $p = 0.02$ ). This negative correlation was stronger in the control dogs exclusively (Spearman's correlation coefficients  $r_s = -0.77$ ,  $p = 0.001$ ) and was weaker in the dogs with *E. canis* exclusively (Spearman's correlation coefficients  $r_s = -0.65$ ,  $p = 0.04$ ) (Table 4). Serum cTnT was weakly negatively correlated with RBC in all dogs (Spearman's correlation coefficients  $r_s = -0.29$ ,  $p = 0.03$ ).

## Discussion

Troponin T is detected in the blood at 4 to 12 hours after acute myocardial infarction. The mean hours to peak is between 10 to 24 hours, and return to baseline at 5 to 10 days (Goldmann et al., 2001). The serum half-life of cTnT is around 4 hours. Thus, the

prolong detection of serum cTnT may suggest a continuing release due to myocardial damage (Herman et al., 1999). In human, cTnT has been employed as a biomarker for myocardial injury due to its sensitivity and specificity (Babuin and Jaffe, 2005). In veterinary medicine, increase in serum troponin level can be detected in patients with various cardiac and noncardiac diseases such as gastric dilatation and volvulus (Schober et al., 2002), pyometra (Hagman et al., 2007), cardiac contusion (Schober et al., 1999), babesiosis (Lobetti et al., 2002), acquired and congenital cardiac diseases (Oyama and Sisson, 2004; Spratt et al., 2005), arrhythmogenic right ventricular cardiomyopathy in Boxer (Baumwart et al., 2007), experimental infarction (Ricchiuti et al., 1998), and pericardial effusion (Shaw et al., 2004; Spratt et al., 2005; Linde et al., 2006).

In human medicine, the high-sensitivity cardiac troponin T (hs-cTnT) has been introduced as a gold standard to diagnose myocardial infarction (Santalo et al., 2013). In addition, the high value of hs-cTnT is related with increasing risk of cardiovascular problems in patients with hypertrophic cardiomyopathy (Kubo et al., 2013). In acute ischemic stroke patients, the serum cTnT levels were increased above the normal limit about 36.4% of the patients in the study group (Kral et al., 2013). Furthermore, both cTnT and cTnI are effectively used for identifying doxorubicin-induced myocardial injury (Reagan et al., 2013). Unfortunately, the hs-cTnT cannot improve the diagnostic performance of acute coronary syndrome, yet the acute myocardial infarction detection sensitivity is considerably good (Borna et al., 2013). Together, these informations demonstrated the appropriate utilization of cTnT for the diagnosis of myocardial problem in human.

In a previous study, cTnI was employed as a myocardial damage indicator in Brazilian dogs with an acute *E. canis* infection (Diniz et al., 2008). The results suggested that *E. canis* infection was a risk factor for cardiac injury associated with severe anemia

**Table 4** Spearman's correlation coefficients ( $r_s$ ) of cTnT and age, hematological values. Correlation coefficients were illustrated for all dogs, control dogs, negative dogs, *E. canis* infected dogs and other blood parasite infected dogs

Variable	All dogs		Control		Negative		<i>E. canis</i> infected		Other blood parasite infected	
	$r_s$	$P$	$r_s$	$P$	$r_s$	$P$	$r_s$	$P$	$r_s$	$P$
Age	0.42	0.002*	0.75	0.001*	0.25	0.28	0.49	0.22	0.78	0.40
WBC	-0.19	0.16	-0.334	0.22	0.13	0.58	-0.52	0.12	-0.12	0.80
Neutrophil	-0.54	0.69	0.03	0.92	0.20	0.40	-0.48	0.16	-0.48	0.28
Band-neutrophil	0.21	0.13	-	-	0.35	0.13	-0.18	0.62	-	-
Lymphocyte	-0.32	0.02*	-0.77	0.001*	0.20	0.40	-0.65	0.04*	-0.34	0.45
Monocyte	-0.24	0.08	-0.05	0.87	-0.33	0.16	-0.14	0.70	0.12	0.80
Eosinophil	-0.15	0.28	-0.10	0.72	-0.01	0.98	-0.30	0.41	-0.30	0.52
Basophil	-0.001	0.99	0.36	0.19	-	-	-	-	-0.34	0.45
RBC	-0.29	0.03*	-0.21	0.45	-0.33	0.15	0.03	0.93	-0.36	0.42
Hb	-0.26	0.06	-0.21	0.46	-0.35	0.13	0.17	0.63	-0.18	0.70
Hct	-0.25	0.06	-0.23	0.41	-0.32	0.17	0.12	0.74	-0.18	0.70
MCV	0.05	0.70	-0.03	0.92	0.21	0.38	0.30	0.41	-0.24	0.60
MCH	0.09	0.52	0.05	0.92	0.16	0.49	0.54	0.10	-0.42	0.34
MCHC	.010	0.45	0.15	0.59	-0.01	0.98	0.38	0.27	-0.24	0.61
Platelet	-0.13	0.35	-0.08	0.79	-0.11	0.64	-0.38	0.28	0.00	1.00
RDW	0.08	0.56	-0.19	0.49	-0.10	0.67	-0.07	0.83	-0.18	0.70
Plasma protein	0.20	0.15	0.40	0.14	0.27	0.24	-0.34	0.34	0.48	0.28



and systemic inflammatory response syndrome (SIRS) (Diniz et al., 2008). Together, it was hypothesized that the pathogenesis of a canine cardiac injury with ehrlichiosis involved the vasculitis, myocardial hemorrhages, tecidual hypoperfusion, and intense inflammation (Diniz et al., 2008). However, there is no evidence of *E. canis* infected cardiomyocytes, thus, the mechanism of cardiac cell injury and the troponin release into the bloodstream is still unknown (Diniz et al., 2008).

In the present study, the serum cTnT levels in CME were determined. The results showed that serum cTnT concentrations were not different among the 4 groups ( $p = 0.70$ ), implying that there is no evidence of severe myocardial injury in dogs with natural occurring *E. canis* infection. However, the serum cTnT was correlated with age of the dogs, similar to one study in humans (Chapelle et al., 2002). This suggested that patients in advanced age might develop subclinical myocardial lesions and continuous release of the cTnT. This evidence may be applied to that occurred in dogs.

Interestingly, serum cTnT was negatively correlated with lymphocytes and RBCs. Indeed, previous studies showed that serum cTnT was increased in anemic dogs because myocardial ischemia induced myocardial injury (Ohman et al., 1996; O'Brien et al., 1997<sup>a</sup>). However, the relationship between lymphocyte count and serum cTnT level remains unclear and needs further investigation.

We hypothesized that the severity of myocardial damage might be related to the phase of CME because dogs infected with *E. canis* in acute phase and anemic dogs had a higher risk of myocardial injury (Diniz et al., 2008). The lack of circulating cTnT suggests that although some myocardial disease is present, it may not be severe enough to cause an increase in serum cTnT concentrations (DeFrancesco et al., 2002) or it may not cause ongoing cardiac injury (Serra et al., 2010). Furthermore, due to the serum half-life of cTnT, the length of time after myocardial injury may be involved (Alpert et al., 2000). Further studies are indeed required to confirm the association of *E. canis* infection and myocardial injury in dogs.

### Conclusion

In conclusion, serum cTnT levels were not increased in dogs naturally infected with *E. canis*. However, it was positively correlated with age and negatively correlated with RBC. This suggests that anemia may cause an increase in serum cTnT level, and it should be treated with caution in older dogs infected with CME.

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