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ORIGINAL ARTICLE

Measurements of cardiac troponin I and creatine kinase myocardium isoform in dogs with diabetic ketoacidosis

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Abstract Diabetic ketoacidosis (DKA) is a complication of diabetes mellitus (DM) which may cause cardiac injury. The study aimed to compare concentrations of cardiac troponin I (cTnI) and creatine kinase myocardium isoform (CK-MB) in DKA and normal dogs and determine the relationship between cTnI and CK-MB concentrations and blood profile values, complications, and the risk of death. Plasma samples from 20 normal and 24 DKA dogs were collected. The cTnI concentration of DKA dogs (0.053 [0.034–0.200] ng/ml) was higher than those of normal dogs (0.020 [0.015–0.043] ng/ml) ($p < 0.0001$). Dogs with higher cTnI concentration had a higher risk of death than those with lower cTnI concentrations (HR 12.34; 95 % CI 1.66, 91.97). DKA dogs (203.5 [142.8–290.0] U/l) had higher CK-MB concentrations than normal dogs (90 [78–134.5] ng/ml) ($p = 0.008$). In conclusion, cTnI and CK-MB concentrations increase in DKA dogs. With higher cTnI concentrations, dogs have a higher risk of death.

Keywords Canine · Cardiac injury · Cardiac marker · Diabetes

Introduction

Diabetic ketoacidosis (DKA) is a complication of diabetes mellitus (DM), one of the most common diseases in dogs. DKA has been reported in approximately 15 % of dogs diagnosed with DM (Hess et al. 2000). DKA commonly occurs in dogs not receiving appropriate treatments for DM. DKA can develop in diabetic dogs in different time periods after DM diagnosis. One study reported the development of DKA in 0–72 months after DM diagnosis (Hume et al. 2006). The clinical signs of dogs with DKA include lethargy, weakness, anorexia, vomiting, and diarrhea. On physical examination, dogs may show signs of dehydration, tachypnea, abdominal pain, and acetone-like breath odor. Laboratory abnormalities like high blood glucose, leukocytosis, pre-renal azotemia, electrolyte imbalance, glucosuria, ketonuria, and occasional urinary tract infection may be observed (Hess et al. 2000; Hume et al. 2006).

Also, DKA could induce myocardial infarction in humans (Geddes al. 2007; Eubanks et al. 2012). Some researchers suggested that myocardial injury might occur after an increase of acid in the circulation of human patients with DKA (Moller et al. 2005; Ammann et al. 2003). The cardiac injury secondary to DKA is usually clinically unrecognized; however, it is associated with increased morbidity and death in human patients (Ammann et al. 2003). Malfunction of cardiac muscles caused by an increased stiffness of myocardium due to glycoprotein accumulation has been reported in experimental DM dogs (Regan et al. 1974). However, the evidence of cardiac injury in dogs affected with DKA has not been reported yet.

In human patients, cardiac injury can be detected by an increased concentration of cardiac biomarkers, including cardiac troponin I (cTnI) and creatine kinase cardiac isoform (CK-MB) and the presence of ST segment depression or elevation on electrocardiography.

Creatine kinase (CK) is a non-specific biomarker releasing from several organs including brain, cardiac muscle, and skeletal muscle. Serum CK rises in case of injury to those organs or tissues. Nowadays, a CK test specific to cardiac muscle, creatine kinase myocardium isoform (CK-MB), has been developed. This test can detect cardiac injury more specifically than serum CK. CK-MB has been

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shown to be an adequate biomarker for the myocardial infarction in humans (Puleo et al. 1994). Also, in veterinary medicine, CK-MB has been reported to use as a biomarker for cardiac injury (Aktas et al. 1993).

Cardiac TnI is a biomarker for the diagnosis of cardiac damage secondary to myocardial ischemia in human patients (Braunwald et al. 2000). Cardiac TnI is uniquely expressed in the myocardium. Thus, it is a highly sensitive and specific marker for the diagnosis of cardiac injury. An increase of cTnI concentrations can be detected in the serum of human patients with ongoing cardiac damage (Auner et al. 2001). In veterinary medicine, an increase of cTnI has been found in several kinds of cardiac and extracardiac abnormalities such as pericardial effusion, cardiac contrusion, cardiomyopathy, degenerative mitral valve disease, babesiosis, ehrlichiosis, and gastric dilatation volvulus (Oyama and Sisson 2004). It has been suggested that cTnI is a suitable biomarker of myocardial damage in dogs (Fredericks et al. 2001). After the occurrence of cardiac injury, cTnI concentrations increase 3–8 h, peak in 12–24 h, and return to baseline in 7–10 days in dogs (Undhad et al. 2012).

A previous study showed an increase of cTnI concentrations in human patients with DKA (Atabek et al. 2004). A rise of cTnI concentrations returned to the normal limit within 24 h. Also, plasma cTnI concentrations were correlated negatively with pH and bicarbonate (Atabek et al. 2004). Another study recommended monitoring the concentration of cTnI and CK-MB in every human patient with DKA (Eubanks et al. 2012). It has been reported that DKA patients with high cTnI and CK-MB concentrations had a higher risk for a longer period of hospital admission and the development of cardiovascular complications (Eubank et al. 2012).

Cardiac damage secondary to DKA has not been studied in dogs. Therefore, this study was performed to test whether cTnI and CK-MB concentrations are different in dogs with DKA from normal dogs. The study aimed to compare cardiac biomarkers, cTnI and CK-MB concentrations, in dogs affected with DKA to normal dogs and determine the relationship between cTnI and CK-MB concentrations to hematology and blood chemistry values, electrolyte concentrations, complications, and the risk of death from DKA.

Materials and methods

Dogs

The study population consisted of normal dogs and dogs diagnosed with DKA at the Small Animal Veterinary Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand, from 2014 to 2015. The study protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University, Thailand (Animal Use Protocol No. 1431087). Informed consents were obtained from all owners. Normal dogs were determined healthy through a physical examination and the obtainment of complete blood count and blood chemistry profile values including serum alanine aminotransferase (ALT), alkaline phosphatase (AP), creatinine, blood urea nitrogen (BUN), and fasting blood glucose levels. Dogs with cardiovascular abnormalities, assessed by thoracic radiography, electrocardiography, and echocardiography, were not included in the normal group. Dogs in the DKA group were diagnosed with DKA on the basis of the presence of high fasting blood glucose, ketonemia, glucosuria, ketonuria, and metabolic acidosis (bicarbonate <15 mmol/l and pH <7.35) (Feldman and Nelson 2004; English and William 2004). Dogs diagnosed with cardiac diseases such as degenerative mitral valve disease, dilated cardiomyopathy, cardiac arrhythmia, heartworm infestation, and congenital heart diseases were excluded from the study. Dogs receiving drugs affecting the heart such as doxorubicin were not included in the study. All DKA dogs received a full cardiac examination including thoracic radiography, electrocardiography, and echocardiography when they were in a condition to undergo those tests. Echocardiography was performed by SS with an ultrasound machine (Eko7, Samsung Medison) with 2–4 and 4–10 multifrequency MHz-phased array transducers. Two-dimensional echocardiography was done on right parasternal long and short axis views to evaluate the cardiac structure. M-mode echocardiography was performed on right parasternal long axis view to measure the cardiac chamber size and wall thickness and evaluate the systolic function. Spectral Doppler echocardiography was performed on left apical and right parasternal transverse views to evaluate the diastolic function (Boon 2011). An echocardiographic examination was done following the recommendation of the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine (Thomas et al. 1993). Dogs which showed evidence of heart disease were further excluded. Pancreatitis was identified by the presence of abdominal discomfort, an increased level of canine pancreatic lipase by a canine pancreatic lipase test kit (IDEXX, Maine, USA), and the abnormality of the pancreas revealed by abdominal ultrasonography. Signalment including age, gender, breed, and weight were recorded as baseline data for all dogs. Blood gas, electrolyte, hematology, blood chemistry values, and urinalysis results were recorded for DKA dogs.

Biomarker measurements

Two milliliters of venous blood samples were collected from each dog. Blood collection was performed within 24 h after dogs had been diagnosed with DKA. Plasma was obtained by centrifuging at 1000 g for 15 min. cTnI and CK-MB

concentrations were measured within 30 min after the blood collection using automated machines (Tosoh AIA 360 and ILab 650 with ISE). Machines measured cTnI concentrations with a sandwich ELISA method and CK-MB concentrations with an immuno-inhibition method. The detection range of cTnI and CK-MB tests is 0.01–50 ng/ml and 2–1000 U/l, respectively. The canine reference intervals for cTnI and CK-MB tests for dogs are 0.03–0.07 ng/ml and 5–25 IU/L, respectively (Sleeper et al. 2001; Aktas et al. 1993). The manufacturer's quoted coefficient of variation for cTnI and CK-MB tests is 3.0 and 3.5 % with minimum detection limits 0.01 ng/ml and 2 U/L, respectively. The samples were run three times in the same batch to evaluate the intra-assay precision. Also, the samples were run each day for 3 days to evaluate the inter-assay precision. Three different concentrations of cTnI and CK-MB control samples were prepared by spiking serum from that of healthy dog. The linearity was evaluated by spiking pooled canine serum with cTnI and CK-MB standards. The concentration of cTnI and CK-MB was ranged from 0.01 to 10 ng/ml and 1 to 1000 U/l, respectively. The unspiked serum was prepared from the same pooled canine serum.

Statistical analysis

The signalment and history of all dogs were reported descriptively. The normality of the continuous data was tested with the Shapiro-Wilk test. The data with normal distribution were presented as mean ± standard deviation (SD). Non-normally distributed data were presented as median [25th–75th interquartile range]. The difference between cTnI and CK-MB concentrations between DKA and normal dogs was evaluated by the Mann-Whitney *U* test. The correlation between cTnI and CK-MB concentrations and other variables including age, weight, hematology and blood chemistry values, pH, and electrolytes was analyzed using a Spearman's rank correlation. Multiple regression analysis models were constructed to determine whether variables predicted an elevation of cTnI and CK-MB concentrations. A univariate Cox regression was performed to evaluate the risk of death from DKA by using cTnI or CK-MB concentrations or the presence of pancreatitis as a predictor. The result was presented in the hazard ratio (HR). The statistical significance was considered with a p value less than 0.05. Statistical analyzes were performed using computer-based software (Normality, Mann-Whitney *U* test, Spearman's rank correlation, Multi-regression, Kaplan-Meier analysis (Minitab 17, State College, PA, USA) and univariate Cox regression (SAS 9.3, Cary, NC, USA)).

The intra- and inter-precision of cTnI and CK-MB assays were calculated as coefficient of variation (CV). The linearity was determined via least-squares regression analysis. If the correlation coefficient was >0.95, the linearity of the assay was assumed (Oyama and Phillip 2004).

Results

Dogs

Plasma was collected from 46 dogs. Two DKA dogs were excluded because of an evidence of cardiac disease. Of the remaining 44 dogs, 20 were normal dogs and 24 were dogs with DKA. The normal group consisted of 4 males and 16 females. Breeds of dogs were Shih Tzu (n = 5), Pomeranian (n = 4), Poodle (n = 3), French Bulldog (n = 2), West Highland White Terrier (n = 1), Beagle (n = 1), Chihuahua (n = 1), Miniature Pinscher (n = 1), and mixed breed (n = 2). The DKA group included 12 males and 12 females. Breeds of dogs were Poodle (n = 12), Shih Tzu (n = 3), Chow Chow (n = 1), Miniature Pinscher (n = 1), Siberian (n = 1), Dachshund (n = 1), Rottweiler (n = 1), and mixed breed (n = 4). The average age of dogs in normal (8.9 ± 2.6 years) and DKA groups (10.7 ± 3.3 years) were not significantly different (p = 0.118). There was no difference between weight of normal (6.20 [3.68–9.37] kg) and DKA (6.00 [4.25–9.78] kg) dogs (p = 0.383). The median age of dogs at the time DM was diagnosed was 10 [8–13] years. The median age of dogs at the time DKA was diagnosed was 11 [9–15] years. Fourteen dogs (58.33 %) were diagnosed with DKA at the time of initial diagnosis of DM.

Twenty-one dogs (87.5 %) had hypersthenuria, 3 dogs (12.5 %) had isosthenuria, and no dog had hyposthenuria. Fifteen dogs (62.5 %) had azotemia (BUN >20 and creatinine >1.4) (IRIS 2013). One dog with azotemia had isosthenuria. None of the dogs had abnormal thoracic radiographic finding. Sixteen dogs (66.7 %) had concurrent disorders diagnosed at the time of evaluation of DKA including pancreatitis (10/24), fatty liver (2/24), adrenal mass (1/24), splenic mass (1/24), left renal mass (1/24), hepatic mass (1/24), cystic calculi (1/24), hyperadrenocorticism (1/24), and urinary tract infection (1/24). The median duration of hospitalization was 5 [3–7] days. Seven dogs (29.2 %) died during hospitalization. Two dogs had electrocardiographic abnormalities including ST segment depression and ventricular tachycardia. None of the dogs had structural cardiac abnormalities assessed by radiography and echocardiography. All dogs had echocardiographic values within a normal limit.

The linearity of cTnI and CK-MB assays used in this study were 0.976 and 0.954, respectively. The mean intra-assay precision for cTnI and CK-MB assays were 4.2 ± 1.5 and 4.8 ± 1.8, respectively. The mean inter-assay precision for cTnI and CK-MB assays were 4.5 ± 1.7 and 5.1 ± 1.9, respectively

Serum cTnI and CK-MB concentrations

The median serum cTnI concentration of the DKA group (0.053 [0.034–0.200] ng/ml) was higher than that of the normal group (0.020 [0.015–0.043] ng/ml) (p < 0.0001) (Fig. 1).

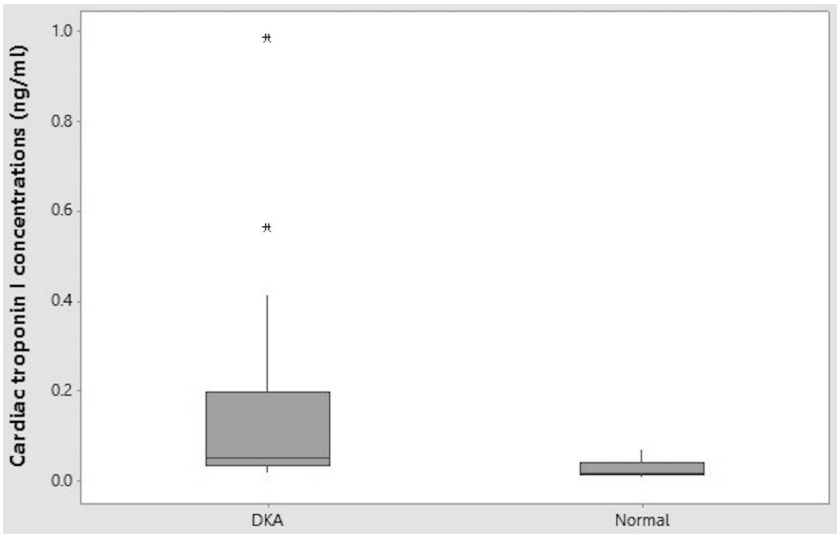

Fig. 1 Box plots of cardiac troponin I (cTnI) concentrations in diabetic ketoacidosis (*DKA*) and normal dogs. The line dissecting the box represents the median and outer lines of the box represent the 25th and 75th interquartile. Comparison of the two groups shows a significant difference of the median cTnI concentrations ($p < 0.0001$). The outliers are presented as *asterisk*

The median serum CK-MB concentration was also higher in the DKA group (203.5 [142.8–290.0] U/l) than the normal group (90 [78–134.5] U/l) ($p = 0.008$) (Fig. 2). A negative correlation was found between cTnI concentrations and red blood cell count (RBC) ($r = -0.746$, $p < 0.0001$). cTnI concentrations correlated positively with BUN ($r = 0.497$, $p = 0.014$) and creatinine ($r = 0.749$, $p < 0.0001$). CK-MB concentrations correlated positively with the white blood cell count (WBC) ($r = 0.556$, $p = 0.039$). cTnI concentrations did not correlate with CK-MB concentrations ($p = 0.311$). Multiple regression analysis of the cTnI concentration as an outcome variable and including RBC, BUN, creatinine, pancreatitis (yes/no), and death (yes/no) found an effect of creatinine on serum cTnI concentrations ($p = 0.037$). The adjusted R (Ammann et al. 2003) of the final model was 0.47. Multiple regression analysis of the CK-MB concentration as an outcome variable and including WBC, pancreatitis (yes/no), and death (yes/no) was not associated with serum CK-MB concentrations. Dogs with higher cTnI concentrations had a higher risk of death from DKA than those with lower cTnI concentrations (HR 12.34; 95 % CI 1.66, 91.97) (Table 1). The concentrations of CK-MB and the presence of pancreatitis were not associated with the risk of death from DKA.

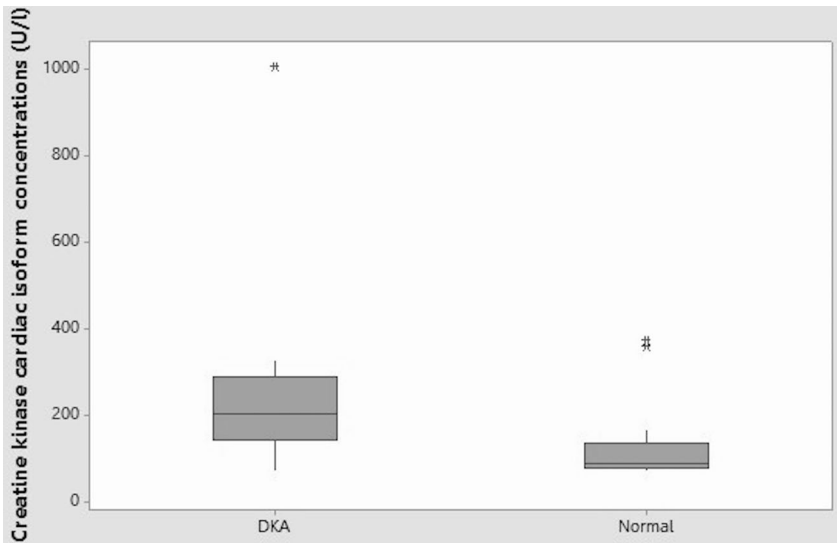

Fig. 2 Box plots of creatine kinase cardiac isoform (*CK-MB*) concentrations in diabetic ketoacidosis (*DKA*) and normal dogs. The line dissecting the box represents the median and outer lines of the box represent the 25th and 75th interquartile. Comparison of the two groups shows a significant difference of the median CK-MB concentrations ($p = 0.008$). The outliers are presented as *asterisk*

Table 1 The univariate Cox regression analysis for predicting death

Variable	Hazard ratio	95 % CI (low, high)	*p* value
Pancreatitis	0.41	0.08, 2.14	0.291
cTnI[a]	12.34	1.66, 91.97	0.014
CK-MB[a]	0.99	0.99, 1.004	0.816

[a] The result was for 1 unit increment of cTnI or CK-MB concentration

Discussion

The main finding of this study was an increase in plasma cTnI and CK-MB concentrations in dogs with DKA. Also, an increase in cTnI concentrations was associated with a higher risk of death.

The most popular breed affected with DKA in the present study was Poodle, similar to a previous study (Hume et al. 2006). The majority of dogs with DKA (70.83 %) were small breed dogs. Most DKA dogs had concurrent disorders, and 25 % of these dogs had more than one concurrent disorder at the time of DKA diagnosis. Similar to a previous study (Hume et al. 2006), the most popular concurrent condition was pancreatitis. Concurrent disorders such as pancreatitis are associated with increased mortality in human patients with DKA (Efstathiou et al. 2002). With statistical insignificance, dogs with pancreatitis in this study tended to have a higher risk of death than dogs without pancreatitis.

The present study showed that cTnI and CK-MB concentrations were increased in dogs with DKA. These findings were similar to those reported in human patients with DKA (Atabek et al. 2004; Eubanks et al. 2012). One dog with DKA in this study developed ventricular tachycardia that had disappeared after successful treatment of DKA. It was unclear whether the cardiac injury, metabolic disorder-induced sympathetic overactivity, or electrolyte imbalance was the cause of this condition. None of the structural changes was detected by echocardiography in the population of dogs in this study. However, echocardiography is not a sensitive tool to evaluate the structural myocardial injury. Other diagnostic methods such as magnetic resonance imaging (MRI) or an invasive technique like histopathology need to be performed to detect myocardial abnormalities and injury.

Red blood cell count, BUN, and creatinine levels significantly correlated with cTnI concentrations. Red blood cell counts negatively correlated with cTnI concentrations with an unknown cause. A previous study also noted an increase of cTnI concentrations in dogs with moderate to severe anemia (Serra et al. 2010). However, the mechanisms causing an increase of cTnI concentrations in anemic dogs have not been explained. One possible mechanism may be an anemic hypoxia-induced myocardial tissue hypoxia resulting in myocardial injury (Smith et al. 2016). Multivariable regression analysis demonstrated that only creatinine levels significantly correlated with cTnI concentrations. High BUN and creatinine concentrations can be caused by either renal or pre-renal causes in case of dogs affected with DKA (Kerl 2001). Pre-renal azotemia may be caused by volume depletion secondary to osmotic diuresis (Chiassen et al. 2003). The renal clearance can affect the cTnI concentrations. It has been reported that human patients with chronic renal failure (creatinine >8.0 mg/dL) can have a high cTnI concentration secondary to decreased renal clearance (Christenson et al. 1998). In veterinary medicine, a previous study revealed that dogs with renal failure had elevated cTnI concentrations compared to healthy dogs (Sharkey et al. 2009). However, not all dogs with renal failure have cTnI concentrations increased above reference intervals of normal dogs (Porciello et al. 2008). One dog in this study had azotemia with inappropriate urine concentration suggesting a renal failure condition that might affect an increase in cTnI concentrations. The remaining dogs had pre-renal azotemia with hypersthenuria suggesting a normal renal function. However, specific renal function tests were not performed in this study. Thus, a possible profound effect of impaired renal clearance on cTnI concentrations cannot be ruled out.

Venous pH, electrolyte, and glucose levels did not correlate with cTnI concentrations. These findings are different from previous studies in humans that cTnI concentrations negatively correlated with blood pH and serum bicarbonate levels (Atabek et al. 2004; Eubanks et al. 2012). Another study in human patients with DM also showed a correlation between blood glucose and cardiac troponin T (cTnT) (Zheng et al. 2012).

A previous study noted that dogs with pancreatitis had elevated cTnI concentrations (Serra et al. 2010). However, the concentration of neither cTnI nor CK-MB was related to the presence of pancreatitis in DKA dogs in this study.

The present study showed that CK-MB concentrations increased with increased white blood cell count. In the case of myocardial infarction in human patients, the white blood cell count usually rises early after infarction, at the same time as CK-MB increases, before the cTnI concentration do (Willerson and Armstrong 2015).

The present study demonstrated that elevated CK-MB concentrations were independent predictors of elevated cTnI concentrations, similar to a previous study in humans (Eubanks et al. 2012). As cTnI, but not CK-MB concentrations, were associated with a higher risk of death, cTnI might be a more sensitive prognostic indicator of mortality in dogs with DKA.

The limitation of this study was a small sample size of dogs recruited in the study. The study should be repeated with a larger number of dogs. Also, there was a lack of MRI examination or histopathological study to evaluate cardiac and myocardial structure in this population of dogs. Thus, evidence of cardiac injury could not be assessed. Some DKA dogs had abdominal mass that may affect the cTnI and/or

CK-MB concentrations. Further investigations should be performed to clarify the effect of abdominal mass on cardiac biomarker concentrations. Lastly, the profound effect of renal clearance to cTnI concentrations cannot be eliminated because the renal function tests were not performed in this study.

In conclusion, cTnI and CK-MB concentrations increase in dogs with DKA. With higher cTnI concentrations, dogs have a higher risk of death. An increase of cTnI and CK-MB concentrations in dogs with DKA may be due to several causes such as anemia, azotemia, pancreatitis, and/or cardiac injury. Further studies evaluating cardiac and myocardial structure should be performed to find evidence of cardiac injury morphologically.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University, Thailand (Animal Use Protocol No. 1431087).

This article does not contain any studies with human participants performed by any of the authors.

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