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Blood Chemistry Profile and Cardiac Troponin T Concentration in Thai Stray Dogs Infected with Heartworms

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ABSTRACT

To investigate blood chemistry profile and to determine levels of myocardial marker proteins in canine dirofilariasis, measurements of biochemical parameters in liver and renal profile as well as the activity of the enzyme creatine kinase (CK) and cardiac troponin T (cTnT) concentration were performed on 72 Thai stray dogs with and without the presence of heartworms. The results showed that, with the exception of the low levels of gamma-glutamyl transferase (GGT) and uric acid, levels of other blood chemistry variables are comparable to those obtained from human subjects. The relatively high activity of CK observed was probably due to skeletal muscle injury occurred during restraint of animals. The majority of dogs, on the other hand, exhibited virtually non-detectable cTnT concentration comparable to those observed in apparently healthy persons. Furthermore, there was no statistically significant difference in cTnT levels between asymptomatic dogs with and without the presence of heartworms. These results may provide a basis for future clinical studies on assessing the extent of myocardial cell damage in symptomatic heartworm disease.

Key words : blood chemistry parameters, myocardial markers , canine heartworm infection

INTRODUCTION

The infection of dogs with heartworms appears to be common throughout the world. In many parts of the United States, there was a high prevalence of canine dirofilariasis (Ciferri, 1982), and in Japan, as high as 47% canine heartworm infection rate has been reported (Nogami and Sato, 1997). Although many of these animals remain asymptomatic, severe forms of canine heartworm disease may occur which result in multiple organ dysfunctions. To monitor the course of such diseases, several biochemical parameters have been used for testing of liver and renal malfunctions including the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as blood urea nitrogen (BUN) and creatinine respectively. Most of these organ dysfunctions, however, are secondary from circulatory disturbance due to lesions in pulmonary arteries and right-sided heart failure (Rawlings, 1986 ; Kitagawa *et al.*, 1991).

In human medicine, serum activities of the enzyme creatine kinase (CK) have long been used as an indicator of cardiac damage in patients with ischemic myocardial injury. This enzyme usually

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shows, however, a relatively low cardiospecificity due to its presence in a significant amount in skeletal muscle (Lott and Wolf, 1986). With the advent of sophisticated test method and the development of immunoassay system for determining levels of the more heart-specific protein troponin T (cTnT), several recent studies performed on human (reviewed by Mercer, 1997 and Wu, 1997) as well as on animals (Carrier *et al.,* 1994 ; Bachmaier *et al.*, 1995) reported a higher diagnostic performance of cTnT than CK in detecting damage to the heart muscle.

The present study was conducted, therefore, to comparatively determine levels of cardiac marker proteins in Thai stray dogs with or without the presence of heartworms. The results obtained would be served as baseline data for future studies of the canine model of myocardial damage in general, and of the severity of cardiac dysfunction in dogs presenting with symptomatic heartworm disease in particular.

MATERIALS AND METHODS

Study animals

The study comprised 72 stray dogs brought to the Department of Veterinary Medicine, Kasetsart University in the year 1997 for anatomical and physiological study purposes. There were 30 male and 42 female animals, most of which were older than 1 year of age. All animals studied showed no clinical signs and symptomes of severe cardiac dysfunction (e.g. caval syndrome).

Laboratory analysis

Blood samples were drawn from saphenous veins of each animal on the day of arriving and the heparinized plasma obtained were frozen at -20°C until analysis of blood chemistry profile and cardiac troponin T (cTnT) concentration performed within 5 days after blood sampling procedure. The activity of the enzymes AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and CK as well as levels of BUN, creatinine, uric acid, total cholesterol and triglycerides were measured with standard enzymatic methods. Total protein and albumin levels were determined by Biuret method and Bromocresol green method respectively. Measurements of all these variables were performed using the fully-automated blood chemistry analyzer Hitachi 717 (Boehringer Mannheim)

Cardiac troponin T concentration was determined by a second-generation cTnT immunoassay (Enzymun TnT) performed on ES 300 analyzer (Boehringer Mannheim). This newly developed test is based on a single-step sandwich ELISA principle with streptavidin coated tubes as the solid phase and two cardiacspecific monoclonal cTnT antibodies directed against two different epitopes on cTnT molecules. The intraassay precision and between-day imprecision of this assay showed a coefficient of variation (CV) < 4.1 and < 5.8 %, respectively. The cross-reactivity of cTnT with skeletal muscle TnT was reported to be $< 0.3\%$ (Mueller-Bardorff *et al.,* 1997). The monoclonal antibodies used in this immunoassay system were recently shown to recognize both human and murine cardiac but not skeletal muscle TnT (Bachmaier *et al.,* 1995), and were applicable across other animal species including those used in the canine model of cardiac injury (Beck *et al.,* 1997).

Detection of heartworm infection

The presence of heartworms was defined as the detection of microfilariae by wet blood smear and Knott concentration test in the peripheral blood smear examination. In case of a negative blood smear result, an immunodiagnostic test based on enzyme immunoassay for the semi-quantitative detection of Dirofilaria immitis antigen (Snap® canine heartworm PF, Idexx) was used to diagnose

the presence of adult heartworn infection.

Statistical analysis

The results are expressed as mean ± standard deviation. The difference in the mean between subgroups was examined with unpaired Student's t test. For all comparisons p values of < 0.05 were considered significant.

RESULTS

Routine blood chemistry profile

Blood samples for determinations of BUN and creatinine concentration were obtained from all 72 animals studied. Due to a low sample volume obtained by blood sampling, the activity of AST, ALT and levels of uric acid, total cholesterol and triglycerides as well as the activity of ALP,GGT and the concentration of albumin and total protein were measured in 60 and 45 dogs respectively. Table 1 shows, in comparison, levels of these biochemical parameters in male and female animals. A wide range of activities and concentrations was observed across the sexes in nearly all variables measured. On the average, female dogs demonstrated higher concentrations of uric acid and triglycerides and higher activities of ALP and GGT than male animals, whereas higher activities of ALT and higher BUN, creatinine, total cholesterol, albumin and total protein concentrations were observed in male dogs. However, all of these differences did not reach statistical significance (Table 1).

Cardiac marker proteins.

Measurements of the activity of CK and cTnT concentration were performed in all 72 animals studied. Male animals exhibited higher activities ($p > 0.05$) of CK than female animals. (Table 1). Determinations of cTnT concentration, on the other hand, showed comparable results between animals of both sexes. Figure 1 demonstrates the distribution of cTnT values in 72 dogs. It can be seen that the majority of animals (53%, n = 38) displayed virtually non-detectable cTnT concentration of 0.00 ng/ml, with a mean value of 0.016 ng/ml. None of the animals studied had cTnT levels exceeding the upper limit of reference range of 0.10 ng/ml reported for human subjects.

There was no significant correlation between the activity of CK and cTnT concentration and no statistically significant difference in both CK activity and cTnT concentration between dogs with and without the presence of heartworms (Table 2).

DISCUSSION

Several blood biochemical parameters have been used in veterinary medicine for diagnosing and assessing the extent and severity of a disease state. With the exception of the enzyme GGT and uric acid, most of the reported normal ranges in blood chemistry profile in dogs (Meyer *et al.,* 1992 ; Willard *et al.,* 1994) are comparable with those determined in human subjects (Henry, 1996). In the present study, although some parameters in liver and renal profile showed a wide range in activities and concentrations across the sexes, the results with Thai stray dogs are on average in agreement with those stated in the literature (Meyer *et al.,* 1992 ; Willard *et al.,* 1994), and are also similar to the values obtained from 15 heartworm-free dogs reported recently by Kitagawa *et al.* (1997). However, these authors observed, as in our study, a much lower activity of GGT compared with that usually found in apparently healthy persons. The reason for this difference is at present not known but may reflect a biochemical peculiarity unique for dogs and cats, since higher values of GGT have been observed in other animal species (Schmid and Forstner, 1986). In contrast, a low

Table 1 Comparison of mean blood chemistry levels (range in parenthesis) in female and male dogs.

	Female	Male	p - value
BUN (mg/dl)*	15 ± 15 (4 - 34)	18 ± 7 (5 - 36)	0.12
Creatinine (mg/dl)*	1.0 ± 1.0 (0.5 - 1.7)	1.1 ± 0.2 (0.8 - 1.6)	0.07
AST (U/L)$^{+}$	35 ± 25 (13 - 161)	35 ± 20 (21 - 111)	0.99
ALT (U/L)$^{+}$	28 ± 16 (11 - 84)	31 ± 19 (15 - 104)	0.51
ALP (U/L)$^{++}$	64 ± 55 (15 - 226)	54 ± 41 (13 - 143)	0.49
GGT (U/L)$^{++}$	0.3 ± 0.8 (0 - 3)	0.2 ± 0.5 (0 - 2)	0.54
Uric acid (mg/dl)$^{+}$	0.6 ± 0.3 (0.1 - 2.0)	0.5 ± 0.2 (0.0 - 0.8)	0.13
Total cholesterol (mg/dl)$^{+}$	139 ± 37 (63 - 237)	151 ± 45 (62 - 234)	0.28
Triglycerides (mg/dl)$^{+}$	53 ± 17 (22 - 109)	47 ± 15 (21 - 75)	0.21
Albumin (g/dl)$^{++}$	3.1 ± 0.6 (1.9 - 4.4)	3.3 ± 0.3 (2.5 - 3.8)	0.30
Total protein (g/dl)$^{++}$	8.7 ± 1.5 (5.6 - 11.5)	8.9 ± 1.3 (6.6 - 11.6)	0.70
CK (U/L)*	167 ± 167 (55 - 750)	210 ± 208 (56 - 1125)	0.27
cTnT (ng/ml)*	0.016 ± 0.023 (0.00 - 0.09)	0.016 ± 0.025 (0.00 - 0.10)	0.96

Number of female / male : * 42/30, $^{+}$ 37/23, $^{++}$ 26/19

Table 2 Mean activity of CK and cTnT concentration (range in parenthesis) in asymptomatic dogs with and dogs without the presence of heartworms.

	Presence (n = 35)	Absence (n = 37)	p Value
CK (U/L)	162 ± 133 (62 - 750)	207 ± 188 (55 - 1125)	0.25
cTnT (ng/ml)	0.017 ± 0.023 (0.00 - 0.10)	0.015 ± 0.024 (0.00 - 0.09)	0.65

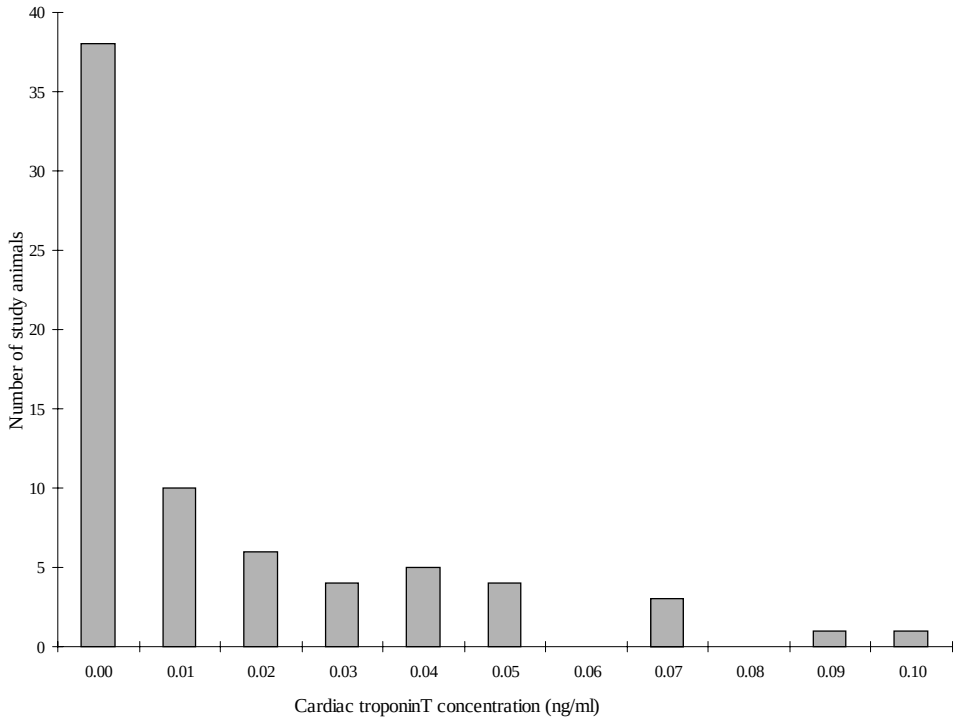

Figure 1 Distribution of cardiac troponin T concentration from 72 Thai stray dogs.

concentration of uric acid was reported in all domestic animals (Clarenburg, 1992) as compared with that in humans (Henry, 1996), and was also found in 72 dogs and 15 heartworm-free dogs in the present study and in the study by Kitagawa *et al.* (1997) respectively.

The primary location of dirofilaria immitis, the dog heartworm, is the right ventricle and pulmonary arteries where the adult nematode lodges (Rawlings, 1986). The adult female sheds up to several thousand microfilariae daily and circulating microfilariae can be detected in the peripheral blood examination. In human medicine, the infection with heartworms was rarely reported and man is usually a dead-end host (Ciferri, 1982 ; De Campos *et al.*, 1997). Many cases of heartworm disease in animals have also a benign course but complications may develope resulting in right-sided heart failure and circulatory disturbances (Rawlings, 1986 ; Kitagawa *et al.*, 1991).

In the past, the enzyme CK has been used as an indicator of myocardial damage. However, the variable normal levels of CK and its presence in a significant amount in non-cardiac muscle tissue have limited its diagnostic value. Increased CK activity has been observed in patients with skeletal muscle injury of various etiologies including muscular dystrophies, myopathies, surgery, poisoning and all types of trauma (Lott and Wolf,1986). In the present study, male animals exhibited, although not with significant difference, a higher CK activity than females. This difference is comparable with the result obtained from human subjects and may reflect the higher muscle mass in male animals. On the other hand, 10 of 30 males (33%) and 12 of 42 female animals (29%) demonstrated a higher activity of CK than the upper limit of normal determined in human of 195

U/L. Furthermore, the mean CK activity in both sexes (female = 167 ± 167 U/L ; male = 210 ± 208 U/L) are also higher than those reported by Kitagawa *et al.* (1997) obtained from heartworm-free dogs (101 ± 37 U/L). The relatively high CK activity found in the study may possibly be due to skeletal muscle injury occured during catching and restaint of animals, and again points to the limitation of CK as a reliable diagnostic marker of myocardial cell injury.

Troponin T belongs to the troponin complex located on the thin filament of myofibrils that regulates the calcium-dependent adenosine triphosphate hydrolysis of actomyosin. To improve the cardiospecificity of a test as cardiac marker, an immunoassay using two cardiospecific monoclonal TnT antibodies has recently been developed, which can detect the cardiac isoform of TnT with a high analytical specificity (Mueller-Bardorff *et al.*, 1997). This novel assay system was also shown to have a high clinical specificity as cTnT was found to be not elevated in patients with skeletal muscle injury, in whom a marked increase in CK activity was observed (Collinson *et al.*, 1995). Determinations of cTnT concentration have been performed to evaluate the diagnostic performance in detecting myocardial injury in several animal species including mice and rats (Bachmaier *et al.*, 1995 ; Vorderwinkler *et al.*, 1996), chickens (Maxwell *et al.*, 1995), pigs (Valen *et al.*, 1997) and dogs (Carrier *et al.*, 1994 ; Carrier *et al.*, 1996). However, to the best of our knowledge, there has been thus far no clinical data demonstrating the normal distribution of cTnT concentration in animals. The results obtained from 72 Thai stray dogs showed that most of the animals studied displayed virtually non-detectable cTnT concentration comparable to those observed in apparently healthy persons. In addition, there was no statistically significant difference in cTnT concentration between the sexes and also between asymptomatic dogs with and without the presence of heartworms. These results indicated that the presence of heartworms in the right ventricle per se, without causing any symptoms suggestive of right-sided heart failure, is not associated with a significant myocardial cell injury. Whether measurements of cTnT concentration may provide a reliable test method for assessing the degree of cardiac damage in symptomatic heartworm disease remains to be determine in future studies.

LITERATURE CITED

Bachmaier, K., J. Mair, F. Offner, C. Pummerer, and N. Neu. 1995. Serum cardiac troponin T and creatine kinase-MB elevations in murine autoimmune myocarditis. Circulation 92 : 1927-1932.

Beck, M.L., G.W. Dameron, Y.J. Kang, B.K. Erickson, T.H. Di Battista, K.E. Miller, K.N. Jackson, S. Mittelstadt, and P.J. O'Brien. 1997. Cardiac troponin T is a sensitive and specific biomarker of cardiac injury in laboratory animals. Clin. Chem. 43 : S192.

Carrier, M., A. Khalil, A. Tourigny, B.C. Solymoss, and L.C. Pelletier. 1996. Effect of L-arginine on metabolic recovery of the ischemic myocardium. Ann. Thorac. Surg. 61 : 1651-1657.

Carrier, M., A.Tourigny, N. Thoribe, M. Montpetit, A. Khalil, B.C. Solymoss, and L.C. Pelletier. 1994. Effects of cold and warm blood cardioplegia assessed by myocardial pH and release of metabolic markers. Ann. Thorac. Surg. 58 : 764-767.

Ciferri, F. 1982. Human pulmonary dirofilariasis in the United States : a critical review. Am. J.Trop. Med. Hyg. 31 : 302-308.

Clarenburg, R. 1992. Physiological Chemistry of Domestic Animals. Mosby Year Book, St. Louis. 428 p.

Collinson, P.O., H.A. Chandler, P.J. Stubbs, D.S. Moseley, D. Lewis, and M.D. Simmons. 1995. Measurement of serum troponin T, creatine kinase MB isoenzyme, and total creatine kinase following arduous physical training. Ann. Clin. Biochem. 32 : 450-453.

De Campos, J.R.M., C.S.V. Barbas, L.T.B. Filomeno, A. Fernandez, H. Minamoto, J.V.B. Filho, and F.B. Jatene. 1997. Human pulmonary dirofilariasis : analysis of 24 cases from Sao Paulo, Brazil. Chest 112 : 729-733.

Henry, J.B. 1996. Clinical diagnosis and management by laboratory methods. 19th ed., W.B. Saunders Comp., Philadelphia. 1556 p.

Kitagawa, H., K. Kitoh, T. Iwasaki, and Y. Sasaki. 1997. Comparison of laboratory data in dogs with heartworm caval syndrome surviving and nonsurviving after surgical treatment. J. Vet. Med. Sci. 59 : 609-611.

Kitagawa, H.,Y. Sasaki, K. Ishihara, and M. Kawakami. 1991. Cardiopulmonary function values before and after heartworm removal in dogs with caval syndrome. Am. J. Vet. Res. 52 : 126-132.

Lott, J.A. and P.L.Wolf. 1986. A case-oriented approach : creatine kinase (CK), pp. 149-197. *In* Clinical enzymology. Field, Rich and Associates, Inc., New York.

Maxwell, M.H., G.W. Robertson, and D. Moseley. 1995. Serum troponin T concentrations in two strains of commercial broiler chickens aged one to 56 days. Res. Vet. Sci. 58 : 244-247.

Mercer, D.W. 1997. Role of cardiac markers in evaluation of suspected myocardial infarction : selecting the most clinically useful indicators. Postgrad. Med. 102 : 113-122.

Meyer, D.J., E.H. Coles, and L.J. Rich. 1992. Veterinary Laboratory Medicine : Interpretation and Diagnosis. W.B. Saunders Comp., Philadelphia. 350 p.

Mueller-Bardorff, M., K. Hallermayer, A. Schroeder, C. Ebert, A. Borgya, W. Gerhardt, A. Remppis, J. Zehelein, and H.A. Katus. 1997. Improved troponin T ELISA specific for cardiac troponin T isoform : assay development and analytical and clinical validation. Clin. Chem. 43 : 458-466.

Nogami, S. and T. Sato. 1997. Prevalence of Dirofilaria immitis infection in cats in Saitama, Japan. J. Vet. Med. Sci. 59 : 869-871.

Rawlings, C.A. 1986. Heartworm Disease in Dogs and Cats. W.B. Saunders Comp., Philadelphia. 329 p.

Schmid, M. and V. Forstner. 1986. Laboratory Testing in Veterinary Medicine Diagnosis and Clinical Monitoring. Boehringer Mannheim GmbH, Mannheim. 253 p.

Valen, G., P. Sellei, A. Owall, E. Eriksson, A. Kallner, H. Waldum, B. Risberg, and J. Vaage. 1997. Release of markers of myocardial and endothelial injury following cold cardioplegic arrest in pigs. Scand. Cardiovasc. J. 31 : 45-50.

Vorderwinkler, K.P., J. Mair, B. Puschendorf, A. Hempel, K.D. Schlueter, and H.M. Piper. 1996. Cardiac troponin I increases in parallel to cardiac troponin T, creatine kinase and lactate dehydrogenase in effluents from isolated perfused rat hearts after hypoxia-reoxygenation-induced myocardial injury. Clin. Chim. Acta. 251 : 113-117.

Willard, M.D., H. Tvedten, and G.H. Turnwald. 1994. Small Animal Clinical Diagnosis by Laboratory Methods. 2nd ed., W.B. Saunders Comp., Philadelphia. 377 p.

Wu, A.H.B. 1997. Use of cardiac markers as assessed by outcomes analysis. Clin. Biochem. 30 : 339-350.

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