ORIGINAL RESEARCH

Electrophoretic fractionation of creatine kinase isoenzymes and macroenzymes in clinically healthy dogs and cats and preliminary evaluation in central neurologic disease

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Background: Information about the electrophoretic distribution of CK-MM, CK-MB, and CK-BB, serum creatine kinase (CK) isoenzymes that are indicators of skeletal muscle, cardiac muscle, and brain lesions, respectively, and CK macroenzymes (macro-CK1 and macro-CK2) in dogs and cats with and without central neurologic disease is scant and equivocal.

Objectives: The objectives of this study were to describe the electrophoretic distribution of CK isoenzymes and macroenzymes in healthy dogs and cats and to provide a preliminary assessment of the utility of CK enzymatic electrophoresis in dogs and cats with central neurologic disease.

Methods: Electrophoretic separation of serum CK isoenzymes and macroenzymes was performed on freeze-thawed serum samples from 20 healthy dogs and 3 dogs with central neurologic disease and from 14 healthy cats and 6 cats with neurologic feline infectious peritonitis (FIP). Electrophoretic separation was also performed on supernatants of homogenized brain, skeletal muscle, and cardiac muscle from both species, to assess the tissue distribution of isoenyzmes in dogs and cats.

Results: CK-MM was the predominant isoenzyme in the serum of healthy dogs and cats, followed by macro-CK2 and CK-BB in dogs and by both macroenzymes in cats. In dogs, CK-MB was essentially absent from both serum and homogenized hearts. CK-BB increased in dogs with neurologic disease. In cats, CK-BB was essentially absent from serum, but was present in brain homogenates. Two of 6 cats with FIP had increased macro-CK1 and increased CK-BB activity.

Conclusions: This study identified the electophoretic distribution of CK isoenzymes and macroenzymes of dogs and cats and provided encouraging data about the possible use of CK-BB as a biomarker for canine neurologic disorders, but not for FIP.

Introduction

Creatine kinase (CK; EC 2.7.3.2) catalyzes the reversible reaction between ADP and phosphocreatine to form creatine and ATP. Phosphocreatine acts as a phosphate donor for the formation of ATP, which is needed for muscle contraction. At rest CK contributes to restoration of phosphocreatine concentration using ATP in excess of that needed by muscle. In people, tissues with the highest CK activity include skeletal muscle and cardiac muscle, followed by neural tissue, although its activity per gram of neural tissue is low owing to the large amount of myelin in the central nervous system (CNS). Low CK activity can be detected in other tissues, such as gastrointestinal tract, urinary bladder, kidney, and thyroid.[1] CK is primarily cytoplasmic and leakage from injured or inflamed tissues leads to increased CK activity in blood, permitting assay of plasma or serum CK activity as a biomarker of tissue damage. CK is a dimer composed of B monomers, M monomers, or both, with a moderate degree of structural homology between the 2 subunits and between different species.[2] Combinations of these monomers generate isoenzymes CK-BB, CK-MB, and CK-MM, which can be found predominantly in brain,

cardiac muscle, and skeletal muscle, respectively.[3] CK-BB, CK-MB, and CK-MM are also called CK1, CK2, and CK3, respectively, according to their electrophoretic migration. In addition to the 3 dimeric CK isoenzymes, there is a structurally different mitochondrial isoform, CK-MT or CKm, detectable in tissues, and 2 macroenzymes, macro-CK1 and macro-CK2, detectable in blood. Macro-CK1 is dimeric CK-BB bound to immunoglobulins macro-CK2 consists of oligomers of CK-MT.[3]

Total CK activity can be measured in plasma or serum using an enzymatic method that utilizes CK-*N*-acetylcysteine (CK-NAC).[4] Although this method does not identify specific isoenzymes or isoforms, it provides an estimate of CK-MM activity, which is responsible for the majority of plasma CK activity in many species. CK-MB can be measured by immunoenzymatic methods designed for diagnostic purposes in people with myocardial diseases; these methods have also been used in dogs, although definitive validation studies have not been done.[5–7] By contrast, CK-BB and macroenzymes can be measured only by more expensive and labor-intensive methods, such as chromatography, immunoprecipitation, immunoinhibition, differential enzyme activation, and electrophoresis.[3]

The limited information about CK isoenzymes in dogs is focused on CK-MM and CK-MB.[5–13] Only one study reported serum CK-BB activity in dogs with neurologic disease.[12] To our knowledge there are no studies of canine and feline macro-CK1 and macro-CK2, which have been associated with immune-mediated and neoplastic conditions, respectively, in people,[14] or of electrophoretic fractions of CK in feline serum. The aim of this study was to describe the electrophoretic distribution of CK isoenzymes and macroenzymes in healthy dogs and cats and to determine if changes in CK distribution in a low number of animals with central neurologic disorders justifies future studies on CK-BB in animals with CNS diseases.

Materials and Methods

Animals, samples, and routine testing

Healthy dogs and cats were identified based on normal physical examination and lack of abnormalities detected on laboratory tests, including CBC, serum biochemical profile, serum protein electrophoresis (SPE), and urinalysis. Inclusion criteria for dogs and cats with central neurologic disease were the presence of neurologic signs and relevant laboratory test results as detailed below.

Blood samples were collected into tubes containing EDTA and tubes without anticoagulant (Venoject,

Terumo Italia Srl, Rome, Italy) by the referring veterinarians from client-owned animals after obtaining informed consent from the owners as requested by the Institutional Animal Care Committee (Comitato Etico Tutela degli Animali). Samples were submitted to the Department of Veterinary Pathology, Hygiene and Public Health, Unit of General Pathology and Parasitology, University of Milan for routine screening or diagnostic tests. Samples that were grossly hemolyzed or lipemic were excluded from the study. Serum was obtained by centrifugation ($1100g \times 8$ minute) of blood collected in plain tubes. After collection and separation of serum the basic panel of diagnostic tests described below was immediately performed, and then samples were stored at $-20°$C. Electrophoretic separation of CK isoenzymes was performed within 1 month on batched samples to reduce possible interassay variability.

A CBC and a panel of serum biochemical tests, including, urea, creatinine, glucose, total protein, albumin, calcium, and phosphorus concentrations and alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase, γ-glutamyl transferase (GGT), and CK (CK-NAC method) activities, was performed on all samples using an automated spectrophotometer (Cobas Mira, Roche Diagnostic, Basel, Switzerland) with reagents from Real Time Diagnostic System (Viterbo, Italy). SPE on agarose gel strips using an automated apparatus (Hydrasis, Sebia Italia srl, Florence, Italy) was performed for 1 dog with suspected leishmaniasis and for all cats suspected of having the dry form of feline infectious peritonitis (FIP). For these cats, serum concentration of α_1-acid glycoprotein (AGP) was also measured using a commercially available radial immunodiffusion kit (Tridelta Development Ltd., Maynooth, Ireland). The diagnosis of leishmaniasis was based on detection of increased α_2 and γ-globulins by SPE, increased protein concentration and mixed pleocytosis in CSF, high-antibody titers (direct immunofluorescence assay), and positive polymerase chain reaction test. The diagnosis of FIP was based on detection of increased α_2 and γ-globulins by SPE and increased concentration of serum AGP,[15] in most cases associated with renal failure (based on increased concentrations of creatinine, urea, and phosphate) or hepatobiliary damage (based on increased ALT, ALP, and GGT activities), on detection of typical gross and microscopic lesions, and on positive immunohistochemical staining for feline coronavirus within the lesions.

Tissue distribution of CK electrophoretic fractions

In order to assess tissue distribution of isoenzymes and to confirm that isoenzymes have the same

electrophoretic migration of their human counterparts, electrophoretic separation was performed on tissues homogenized using a modification of the original procedure.[1] Specifically, slices of brain (temporal cortex and parietal regions), skeletal muscle (semitendinosus and semimebranosus), and cardiac muscle (left ventricular wall), approximately 1 cm^3 each, were collected during necropsy from a cat with chronic renal failure and a dog with a ruptured splenic hemangiosarcoma. Slices were immersed in liquid nitrogen immediately after sampling and stored frozen at $-20°C$ until use 1 day later. After thawing, tissues were placed in 1 mL of saline solution and manually homogenized using a tissue grinder (Glass/Teflon Potter Elvehjem Wheaton, Millville, NJ, USA). Following homogenization, fluid was transferred to an Eppendorf tube and centrifuged at 2500g for 15 minutes. Supernatant was then transferred to another tube and frozen at $-20°C$ until use 1 week later. According to the manufacturer's instructions, if total CK activity is > 750 U/L the sample should be diluted before electrophoretic fractionation of isoenzymes to prevent overlap of large bands and permit identification of single bands. Therefore, the total CK activity of each supernatant was determined using the CK-NAC assay, and each sample was diluted to obtain CK activity of approximately 500 U/L. Electrophoresis of the supernatants of homogenized tissue was then performed using the same procedure for serum samples described below.

Electrophoretic identification of CK isoenzymes and macroenzymes

Electrophoresis was performed according to the manufacturer's instructions using a commercially available kit (Hydragel ISO-CK, Sebia Italia Srl) and an automated apparatus (Hydrasys, Sebia Italia Srl) equipped with specific accessories (Standard Mask Accessories for ISO-CK/LD). The principle of the assay is that isoenzymes and macroenzymes have characteristic motility owing to the electric charge of the subunits. After migration, CK fractions are visualized based on a reaction catalyzed by all the CK fractions and based on the conversion of creatine phosphate to a chromogenic compound (formazan) through a series of intermediate reactions involving substrates and cofactors included in the kit. Briefly, 200 µL of serum or supernatant from homogenized tissues were mixed with 2 µL of activating solution containing β-mercaptoethanol and incubated for 10 minutes at approximately 20°C. For feline samples, 10 µL of activated serum or supernatant from homogenized tissues were placed in the wells of the applicator provided with the kit. For

canine samples, 20 µL were applied as preliminary testing revealed that weak bands were generated when 10 µL were applied. Agarose gel (8%, pH 8.40 ± 0.05, included in the kit) and the applicator were placed in the migration chamber, and the automated migration program was then selected. After migration (10–20 W, 27 V h, 20°C) CK substrate containing chromogenic solution was applied and the reaction was stopped using blocking solution. Gels were then washed, dried by heating, and placed on the scanner provided with the instrument. Scanned images were analyzed using Phoresis software (Sebia Italia Srl) and visually inspected for possible errors in separation, which, if present, were manually corrected using the appropriate software utilities.

Intra- and interassay precision of the method was assessed in test runs on pooled canine and feline serum samples, randomly received by our diagnostic laboratory, irrespective of the presence or absence of disease or laboratory abnormalities. Specifically, intraassay imprecision was tested by running each pooled serum on 3 different lanes of the same gel. Interassay imprecision was tested by running aliquots of each pooled serum in 3 different runs, with 2 intervals of approximately 15 days. In the first run fresh pooled serum was used; aliquots frozen at $-20°C$ were thawed and used in the other 2 runs. Mean activities and standard deviations (SDs) were calculated for both intra- and interassay precision tests, and coefficients of variation (CV) were calculated using the formula: CV% = (SD/mean) × 100.

Statistical analysis

Statistical analyses were done using an Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) and Analyse-it software (Analyse-it Software Ltd., Leeds, UK). Activities were expressed as median values, with additional reporting of minimum and maximum activities. Although this approach does not provide precise information about reference intervals, it has been suggested to estimate reference intervals in small groups of animals.[16] After determining that the data were not normally distributed, the results from dogs and cats with central neurologic disease were compared with those of the healthy control animals using a Mann–Whitney U-test with $P \leq .05$ considered significant.
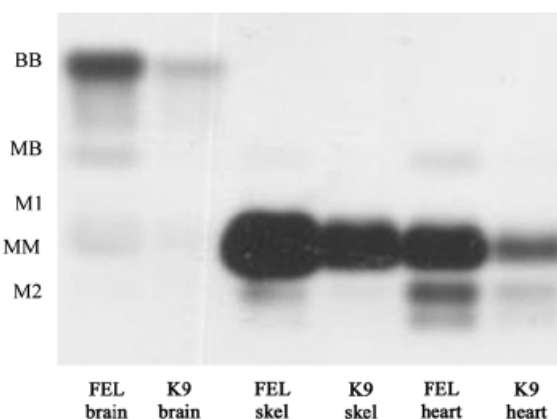
Results

Twenty healthy dogs (10 female and 10 male, 2–14 years with a median age of 7 years) were included in

the study. Two additional dogs (one 14-year-old male and one 10-year-old female) had intracranial tumors detected with magnetic resonance imaging (in 1 case by a private veterinary clinic and in the other by the radiology unit of the Department of Veterinary Clinical Sciences, University of Milan), and another dog (6-year-old male) had neurologic leishmaniasis. Fourteen healthy cats (7 female and 7 male, 1–10 years with a median age of 3.5 years) were included in the study. Six cats (3 female and 3 male, 1–7 years with a median age of 1.5 years) were diagnosed with neurologic disease secondary to FIP. Necropsies were performed on the 6 cats, and in all cases lesions consistent with FIP were present along with immunohistochemical positivity for the virus in at least 1 abdominal organ. However, examination of the brain for typical pyogranulomatous lesions was investigated in only 3 cats.

CK electrophoretic fractions in supernatants of homogenized tissues

Analysis of homogenized tissue supernatants revealed that CK-BB and CK-MM were present in brain and skeletal muscle, respectively, in both dogs and cats (Figure 1). Supernatants from feline brain also contained 2 weak bands with migration distances similar to those of CK-MB and CK-MM. Supernatants from homogenized cardiac tissue from both cats and dogs mainly expressed CK-MM. A minor CK-MB band was detectable in cats, but not in dogs; however, CK-MB was detected in both dogs and cats in undiluted samples (CK activity > 500 U/L; data not shown). Macro-

Table 1. Intra- and interassay precision of total CK activity (U/L) and of electrophoretic fractions (percentage of total CK activity).

	Total CK Activity (U/L)	CK-BB (%)	CK-MB (%)	Macro-CK1 (%)	CK-MM (%)	Macro-CK2 (%)
Intra-assay						
Dog						
Mean	48	15.7	1.5	1.6	60.2	21.0
CV (%)	3.2	2.4	3.8	7.4	1.4	1.7
Cat						
Mean	278	1.1	0.8	11.9	80.6	5.6
CV (%)	3.4	5.1	7.5	3.0	0.8	3.6
Inter-assay						
Dog						
Mean	44	13.7	1.2	2.2	64.4	18.4
CV (%)	9.3	2.9	9.4	12.0	1.4	2.0
Cat						
Mean	258	1.8	2.4	15.4	69.8	10.6
CV (%)	6.9	8.6	8.8	6.0	1.7	4.4

Intra- and interassay precision was evaluated based on 3 runs.
Total CK was determined by the CK-NAC method.
CV, coefficient of variation; CK, creatine kinase; NAC, N-acetylcysteine.

CK2 was detectable in supernatants from homogenized cardiac and skeletal muscle in both species.

Distribution of CK electrophoretic fractions in serum

Intra- and interassay imprecision for total CK activity in canine and feline serum was comparable (Table 1). In dogs, both intra- and interassay CVs for fractionated enzymes were < 3% except for the fractions with the smallest quantities (CK-MB and macro-CK1). In cats, both intra- and interassay assay CVs were ≤ 6% except for the fractions with the smallest quantities (CK-BB and CK-MB).

Activities of fractionated CK of healthy animals were compared with those of dogs and cats with central neurologic disease (Table 2, supporting information Figures S1 and S2). CK-MM was the predominant electrophoretic fraction in serum samples from healthy dogs followed by macro-CK2 and CK-BB, whereas CK-MB and macro-CK1 were essentially absent. CK-MM was the predominant electrophoretic fraction in serum samples from healthy cats. Macro-CK1 and macro-CK2 were detected in varying proportions, whereas CK-BB and CK-MB were essentially absent in healthy cats.

Individual dogs and cats with central neurologic disease had variable changes in the CK fraction activities in serum (Table 3). Serum samples from all 3 dogs had absolute CK-BB activities higher than those of samples from healthy dogs (Table 3, Figure 2) and the

Figure 1. Electrophoretic gels of creatine kinase (CK) in supernatants from homogenized brain, skeletal muscle (skel), and cardiac muscle (heart) of a cat (FEL) and a dog (K9). BB, MB, and MM indicate the 3 isoenzymes, CK-BB, CK-MB, and CK-MM; M1 and M2 indicate the macroenzymes, macro-CK1 and macro-CK2, respectively.

Table 2. Median (minimum–maximum) activities of total and fractionated CK (U/L) in serum (frozen at −20°C for <1 month, then thawed once) from healthy dogs and cats and from dogs and cats with central neurologic disease.

	Dogs		Cats	
Activity	Healthy (n = 20)	CNS Signs (n = 3)*	Healthy (n = 14)	CNS Signs (n = 6)†
CK total	46 (28–172)	116 (44–194)	289 (188–469)	468 (331–804)‡
CK-BB	7 (1–20)	37 (22–75)‡	7 (2–13)	8 (1–20)
CK-MB	1 (0–1)	1 (0–2)	3 (1–15)	3 (0–10)
Macro-CK1	1 (0–7)	2 (1–2)	35 (2–219)	200 (5–550)
CK-MM	27 (11–112)	66 (13–115)	217 (128–374)	285 (109–400)
Macro-CK2	12 (3–53)	7 (3–9)	13 (1–60)	12 (2–87)

*2 dogs with intracranial tumors and 1 with CNS inflammation secondary to leishmaniasis.
†6 cats with neurologic signs associated with feline infectious peritonitis.
‡P <.01 compared with healthy animals.
Total CK activity was determined by the CK-NAC method.
CNS, central nervous system; CK, creatine kinase; NAC, N-acetylcysteine.

median CK-BB activity (37 U/L) was significantly higher than the median CK activity of healthy dogs (7 U/L, P=.006). Total CK activity was significantly greater in cats with FIP (P=.006). Fractionated CK activities for FIP cats were not statistically different from those of healthy cats, but individual increases were observed (Table 3, Figure 2).

Table 3. Total and fractionated CK activities (U/L) in serum of individual dogs* and cats† with central neurologic disease.

|	Total CK	CK-BB	CK-MB	Macro-CK1	CK-MM	Macro-CK2
Dog 1	**194**	**75**	0	1	**115**	<u>3</u>
Dog 2	116	**37**	**2**	2	66	9
Dog 3	44	**22**	1	2	13	7
Cat 1	**517**	16	2	**270**	220	9
Cat 2	331	5	<u>0</u>	203	<u>109</u>	14
Cat 3	411	10	9	24	350	18
Cat 4	419	6	<u>0</u>	5	**400**	7
Cat 5	**600**	1	<u>5</u>	197	**395**	2
Cat 6	**804**	**19**	10	**550**	137	**87**

*Dogs 1 and 2 had intracranial tumors; dog 3 had CNS inflammation secondary to leishmaniasis.
†All 6 cats had feline infectious peritonitis.
Bolded activities are higher than the maximum determined for healthy animals; underlined activities are less than the minimum determined for healthy animals.
Total CK activity was determined by the CK-NAC method.
CNS, central nervous system; CK, creatine kinase; NAC, N-acetylcysteine.
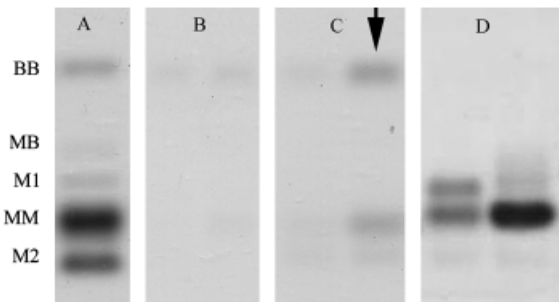
Figure 2. Electrophoretic gels of serum creatine kinase (CK) fractions. (**A**) Mixed sera and supernatants from homogenized tissues, containing all possible bands. (**B**) Serum from 2 dogs (left, healthy dog; right, dog with leishmaniasis) applied according to the standard protocol. Note the weak bands. (**C**) Serum from the same 2 dogs as in (**B**), but applying twice the amount of serum. Weak bands corresponding to CK-MM, CK-BB, and macro-CK2 (M2) are more visible now, especially in the dog with leishmaniasis (arrow). (**D**) Serum from 2 healthy cats. CK-MM is the most abundant band, followed by macro-CK1 (M1).

Discussion

This study demonstrated that CK isoenzymes and macroenzymes can be separated by electrophoresis in canine and feline serum that has been frozen and thawed. The low activity of CK in canine serum required a modification of the manufacturer's protocol and a doubling of the amount of serum applied to achieve visible bands. The creation of a specific "canine ISO-CK" program with a longer application time, increasing the amount of serum transferred to the gel, is advisable in the future. This low CK activity may have been a storage artifact as a previous study[17] and preliminary experiments in our laboratory (data not shown) demonstrated that freezing and thawing decreases CK activity. This decrease, however, accounts for <10% of native CK activity. Low total CK activity in our canine group was similar to that reported in previous studies,[8,11] and discrepancies between the serum CK activities in healthy animals, especially cats, in this study and other studies[18,19] may result from differences in analytical methods rather than storage artifacts. Similarly, analysis of interassay imprecision for isoenzyme fractions in pooled serum samples did not reveal a common trend (neither an increase nor a decrease of percentages) associated with prior storage at −20°C. It should be emphasized that a complete validation study, which would have provided more detailed information about possible storage artifacts on isoenzyme electrophoresis, was not performed. Specifically, the number of replicates in our preliminary testing was less than is recommended to assess both

intra- and interassay imprecision, and stability should be assessed using a different approach.[20] Despite these limitations, CVs indicated acceptable repeatability and reproducibility of isoenzyme determinations, comparable with those reported by the kit manufacturer for CK-BB, CK-MB, and CK-MM in human serum,[21] independent of storage conditions. A more detailed evaluation of storage-related artifacts, however, is recommended before suggesting clinical application of automated CK gel electrophoresis. Apart from these technical aspects, this study provides useful information on both the distribution of CK isoenzymes and macroenzymes in freeze-thawed serum from healthy animals and on potential applicability of the technique to animals with central neurologic disease.

In healthy dogs, CK-MM was the most abundant electrophoretic fraction, but significant CK-BB activity was also detectable. Other studies have reported similar activities of CK-MM or CK-BB,[9,10] but 1 study demonstrated greater activities of CK-BB than CK-MM.[8] The most likely explanation for this discrepancy is that the technique used in the latter study was based on electrophoretic methods that did not differentiate macroenzymes, which are known to interfere with identification of isoenzymes.[22,23] Alternatively, CK-MM and CK-BB are, respectively, the most and the least stable isoenzymes in serum,[9] and storage, temperature, or pH variations could have caused reductions in CK-BB. However, this is unlikely because in our study all serum samples had identical storage conditions, and CK-BB was clearly detectable in some samples. However, CK-BB could have deteriorated during storage conditions and may have been subsequently detectable in those samples in which CK-BB activity was markedly increased before freezing. Another possible explanation is individual variability, which was detected both in this and in previous studies.[8–10] Individual characteristics of the sampled populations, possibly influenced by physiologic conditions, such as exercise and diet, could have caused discrepant results.

CK-MB was essentially absent in the freeze-thawed canine serum in this study. This contrasts with studies that reported detection of CK-MB in canine serum.[9–11] In these studies, however, the presence of macroenzymes may have falsely increased CK-MB activity.[22,23] In support of this hypothesis, another study in which CK-BB activity was possibly overestimated by interference from macroenzymes found the activity of CK-MB was low.[8] In addition, CK-MB was also present in small amounts in supernatants from homogenized heart, in agreement with another study.[9] The evaluation of CK-MB in pathologic conditions is outside the scope of this study, but our results

suggest that the clinical utility of CK-MB measurement is low compared with that of other biomarkers.[6]

CK-BB activity increased in all 3 dogs with central neurologic disease, as reported previously using a different method.[12] Despite the low number of cases, which theoretically limits the statistical power of the comparison, the difference in CK-BB activity between healthy and diseased dogs was statistically significant. Unfortunately, information about histologic brain lesions in the dogs, types of tumors, or degree of invasiveness of the tumors is lacking, precluding conclusions about the utility of CK-BB as a diagnostic or prognostic biomarker for specific CNS diseases in dogs. However, these preliminary results suggest that studies to evaluate which types of CNS disorders are characterized by increases in CK-BB activity would be valuable.

In freeze-thawed serum samples from cats, CK-MM was the largest fraction, followed by macro-CK1 and macro-CK2. Previous reports demonstrated that some areas of feline brain express CK-BB.[24] Analysis of supernatants from homogenized feline brain in our study also confirmed that CK-BB is present in brain tissue and has the same migration pattern as in dogs. Interestingly, CK-BB seems to be almost absent, or below detection limits, in serum from healthy animals and from most cats with FIP. Total CK activity was greater in cats with FIP than in healthy cats, but this finding has poor diagnostic specificity as increased CK activity occurs in several pathophysiologic conditions, including anorexia,[17] myopathy,[18] metabolic syndromes,[25] and CNS disorders.[26] The lack of consistent alterations in CK-BB activity in cats with FIP may have several explanations. Neurologic FIP is a pyogranulomatous meningitis, and CNS tissue is only secondarily affected.[16] Although the blood–brain barrier is usually damaged, it is possible that leakage of CK-BB from neurons is minimal. Another possible explanation is that CK-BB is released by CNS cells but binds to immunoglobulins that are abundant in the serum of cats with FIP[16] and also can be found in cerebrospinal fluid.[27] This would explain the increase of macro-CK1, which is a dimer of CK-BB and IgG.[14] Further studies on central neurologic diseases in cats are required to investigate the potential utility of the CK-BB assay, but based on these results, CK-BB does not appear to have clinical utility in supporting a diagnosis of FIP with CNS involvement.

Macroenzymes were identified based on their migration pattern, which likely corresponds to the migration pattern recorded for human macroenzymes as a high degree of interspecies homology has been reported.[2] It is interesting to note that macroenzymes, which in people are essentially absent from plasma

except in cases of severe disease,[14] can be found in serum from healthy dogs and cats. The possible diagnostic utility of these macroenzymes should be assessed by future studies on animals with a broad spectrum of pathologic conditions.

In conclusion, our study demonstrated that electrophoretic separation of CK isoenzymes and macroenzymes in dogs and cats is feasible, although modification of the original method is required for canine samples, and that CK-MM is the main electrophoretic fraction, followed by CK-BB and macro-CK2 in dogs and by macro-CK1 and macro-CK2 in cats. Promising results about the possible use of CK-BB as a biomarker of central neurologic disorders in dogs were obtained, whereas in cats with FIP the quantification of CK-BB does not seem to have clinical utility. Future studies to investigate the type of CNS disorders responsible for increases in CK-BB activity in dogs and to explore feline central neurologic disorders other than FIP in cats are indicated to define the diagnostic and prognostic potential of CK-BB in a larger number of cases. Additionally, studies on the diagnostic utility of electrophoresis of CK isoenzymes and macroenzymes in cerebrospinal fluid collected from dogs and cats with central neurologic disease may be warranted.

Acknowledgments

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. Dot plots showing the distribution of total CK and CK fraction activities (U/L) for dogs that were healthy and dogs with central neurologic disease.

Figure S2. Dot plots showing the distribution of total CK and CK fraction activities (U/L) for cats that were healthy and cats with central neurologic disease associated with feline infections peritonitis.

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