

# Time-frequency and complexity analyses for differentiation of physiologic murmurs from heart murmurs caused by aortic stenosis in Boxers

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**Objective**—To investigate whether time-frequency and complexity analyses of heart murmurs can be used to differentiate physiologic murmurs from murmurs caused by aortic stenosis (AS) in Boxers.

**Animals**—27 Boxers with murmurs.

**Procedures**—Dogs were evaluated via auscultation and echocardiography. Analyses of time-frequency properties (TFPs; ie, maximal murmur frequency and duration of murmur frequency > 200 Hz) and correlation dimension ( $T_2$ ) of murmurs were performed on phonocardiographic sound data. Time-frequency property and  $T_2$  analyses of low-intensity murmurs in 16 dogs without AS were performed at 7 weeks and 12 months of age. Additionally, TFP and  $T_2$  analyses were performed on data obtained from 11 adult AS-affected dogs with murmurs.

**Results**—In dogs with low-intensity murmurs, TFP or  $T_2$  values at 7 weeks and 12 months did not differ significantly. For differentiation of physiologic murmurs from murmurs caused by mild AS, duration of murmur frequency > 200 Hz was useful and the combination assessment of duration of frequency > 200 Hz and  $T_2$  of the murmur had a sensitivity of 94% and a specificity of 82%. Maximal murmur frequency did not differentiate dogs with AS from those without AS.

**Conclusions and Clinical Relevance**—Results suggested that assessment of the duration of murmur frequency > 200 Hz can be used to distinguish physiologic heart murmurs from murmurs caused by mild AS in Boxers. Combination of this analysis with  $T_2$  analysis may be a useful complementary method for diagnostic assessment of cardiovascular function in dogs. (*Am J Vet Res* 2007;68:962–969)

Physiologic (innocent) heart murmurs are detectable in young dogs of many breeds, and in most instances, they diminish or resolve by the age of 6 months.<sup>1,2</sup> However, in Boxers, the prevalence of heart murmurs in the adult population is high, reportedly 50% to 80%.<sup>3–5</sup> Many of these murmurs are mild (often referred to as soft) with uncertain etiogenesis; however, the smaller size of the left ventricular outflow tract and increased stroke volume in Boxers, compared with findings in other dog breeds, may be important factors.<sup>6</sup> Among possible diagnoses, mild AS is the most common in this breed.<sup>3,7</sup> In Newfoundlands, subvalvular AS can

## ABBREVIATIONS

AS	Aortic stenosis
TFP	Time-frequency property
$T_2$	Correlation dimension
2D	Two-dimensional
LDA	Linear discriminant analysis

develop and progress during the first year of life.<sup>8</sup> The general recommendation has therefore been to reevaluate dogs at 1 year of age if a murmur was detected at a younger age. Analysis of murmurs can be made by standard phonocardiographic examination,<sup>9</sup> but a highly detailed characterization of the murmurs is not possible by this method. Whether heart murmurs in Boxers are associated with characteristics that could be used for differentiation between physiologic murmurs and murmurs caused by mild AS has not been investigated to our knowledge.

A sound spectral averaging technique has recently proved useful in distinguishing physiologic murmurs from those caused by AS in adult humans.<sup>10</sup> Sound spectrography of phonocardiographic signals was suggested for characterization of heart murmurs more than 50 years ago by McKusick et al,<sup>11</sup> but because of technical difficulties, the technique was abandoned as a bedside diagnostic tool.<sup>12</sup> With the advent of modern electronic

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stethoscopes, heart sounds can easily be recorded digitally for subsequent analysis of TFPs, which has resulted in a revival of this technique.<sup>12</sup> In the recent report by Tavel and Katz,<sup>10</sup> assessment of maximal murmur frequency as well as duration of murmur frequency > 200 Hz was used for differentiation of physiologic murmurs from those caused by AS in humans.

Another means of sound analysis is via investigation of the complexity of the sound signal. Normal heart sounds have a certain sound structure, whereas murmurs are more complex and background noise has no structure at all.<sup>13</sup> The fractal dimension of a signal is 1 method by which its complexity can be measured. It has been suggested that the multiscale structure associated with turbulence in fluids can be described by fractals<sup>14</sup> and that there is a strong interaction between the turbulence and its radiated sound field.<sup>15</sup> It is thus reasonable to believe that the flow behavior of an ejection murmur could be characterized by the fractal dimension. It is possible to express the fractal dimension as the  $T_2$ .<sup>16</sup> If this murmur characterization method can be used in dogs, it could aid in differentiation between physiologic murmurs and murmurs caused by AS and could possibly be used for estimation of the severity of AS. To our knowledge, neither TFP nor  $T_2$  analyses have been used in evaluation of heart murmurs in dogs.

The purpose of the study reported here was to investigate whether time-frequency and complexity analyses of heart murmurs could be used to differentiate physiologic murmurs from murmurs caused by AS in Boxers. We hypothesized that TFP and  $T_2$  analyses could be used for evaluation of murmurs in dogs. Because it has been claimed that some mild murmurs that are detected in Boxers early in life can progress to AS, our first aim was to assess 7-week-old puppies and compare those findings with data from the same individuals at the age of 1 year (experiment 1). A second objective was to investigate whether TFP and  $T_2$  analyses could be used to distinguish physiologic murmurs from those caused by AS in adult dogs (experiment 2).

## Materials and Methods

**Animals**—The study was approved by the Local Ethical Committee in Uppsala, Sweden. Sixteen privately owned Boxer puppies (10 females and 6 males) were included in experiment 1. Informed consent was obtained from the owners prior to participation of their dogs in the study. Inclusion criteria were the presence of a low-intensity heart murmur (grade 1 to 2/6) at the left heart base and no morphologic evidence of AS via 2D echocardiography (ie, no evidence of subvalvular or valvular changes) at 7 weeks of age. Puppies with intermittent low-grade murmurs detected via thoracic auscultation were also included if a murmur was registered during phonocardiographic examination. Cardiac diseases other than AS were excluded on the basis of results of echocardiographic examination. Flow murmurs attributable to anemia were excluded by analysis of hemoglobin concentration. The dogs were examined at 7 weeks and reexamined at 12 months of age.

Two groups of dogs were included in experiment 2. Group A consisted of the same 16 dogs included in ex-

periment 1; data from the examination at 12 months of age were used. Group B consisted of 11 privately owned Boxers; there were 5 females and 6 males, and the mean  $\pm$  SD age of the dogs was  $3.7 \pm 2.8$  years (range, 1 to 9 years). Informed consent was also obtained from these owners prior to participation of their dogs in the study. Inclusion criteria for group B dogs were detection via auscultation of heart murmurs of grades 2 to 6/6 and morphologic evidence of AS via 2D echocardiography (ie, evidence of subvalvular or valvular changes consistent with AS). Cardiac diseases other than AS were excluded on the basis of results of echocardiographic examination. Seven of the dogs had subvalvular AS, and 4 had valvular AS.

The groups included in experiment 2 were further divided into 4 subgroups on the basis of peak aortic flow velocity. Dogs in group A (no AS) were allocated to subgroup A1 (dogs with peak aortic flow velocities < 1.8 m/s) or subgroup A2 (dogs with peak aortic flow velocities  $\geq$  1.8 m/s). Dogs in group B (AS affected) were allocated to subgroup B1 (dogs with peak aortic flow velocities  $\leq$  3.2 m/s [mild AS]) or subgroup B2 (dogs with peak aortic flow velocities > 3.2 m/s [moderate to severe AS]). The subgroup classification was based on categorization described in the veterinary medical literature<sup>17,18</sup> as well as our own clinical experience.

**Procedures**—Physical examination of each dog was performed. Established cardiovascular diagnostic methods were used for characterization of the dogs and for group classification in experiment 2. All examinations were performed by individuals (KH, JH, and CK) who were experienced in thoracic auscultation and echocardiographic evaluation of Boxers. Thoracic auscultation and phonocardiographic examination took place in a quiet room, with each dog in a standing position. For both examinations, an electronic stethoscope<sup>a</sup> was used. The audio output of the electronic stethoscope system is unfiltered, giving a flat frequency response within the audible range. However, for low frequencies, the sensor has a 3-dB cutoff frequency at 30 Hz. In experiment 1, the auscultation was performed by 2 independent examiners (KH and JH or CK) because of the high variability of low-intensity murmurs in Boxers.<sup>19</sup> The grade of heart murmur was determined from the mean value of the grades assigned by the 2 examiners. In group B of experiment 2, the dogs were auscultated by 1 examiner (KH). For the phonocardiographic examinations, the stethoscope was connected to a laptop computer<sup>b</sup> equipped with an analysis software program.<sup>c</sup> The chest piece of the stethoscope was placed over the aortic area at the left heart base, giving the loudest and clearest heart murmur. The signal was digitized at 44.1 kHz with 16 bits/sample by use of a sound card.<sup>d</sup> The recordings were stored for later analyses.

The echocardiographic examination took place in a quiet examination room with each dog positioned in right and then left lateral recumbency on the examination table. The ultrasonographic examinations were performed by use of a system with a 5-MHz transducer.<sup>e</sup> A complete echocardiographic examination involving standardized imaging planes<sup>20</sup>

was performed; particular attention was given to 2D changes indicative of AS or pulmonic stenosis. The mitral, tricuspid, aortic, and pulmonic valves were screened for regurgitation via color flow Doppler analysis. Pulsed-wave Doppler was used for measurement of the pulmonic flow velocity. The aortic flow velocity was measured via continuous wave Doppler analysis with the subcostal transducer location recommended by Lehmkuhl and Bonagura.<sup>21</sup>

**Analysis of TFPs and  $T_2$** —All recorded phonocardiographic signals were manually segmented by use of a mathematical computer program.<sup>1</sup> Four markers per heart cycle were determined: the beginning of the first heart sound, the end of the first heart sound, the beginning of the second heart sound, and the end of the second heart sound. The recording lasted for 10 seconds, and noisy or corrupted signal segments were excluded from further evaluation. Hence, the mean  $\pm$  SD number of analyzed heart cycles per dog was  $11 \pm 3$  (range, 4 to 18), and the number of excluded heart cycles was  $5 \pm 3$  (range, 0 to 14). Only the systolic part of the signal, defined as the period from the end of the first heart sound to the beginning of the second heart sound, was used for the analysis. All signals were adjusted to unit amplitude, and prior to  $T_2$  analysis, a fifth-order Butterworth high-pass filter with a cutoff frequency of 50 Hz was used to remove low-frequency components from the signal. All filtering was performed by zero-phase digital filters, which process the input data in both forward and reverse directions.

For TFP analysis, all calculations were made by use of a mathematical computer program.<sup>1</sup> Time-frequency representation of sound includes frequency on the vertical axis, time on the horizontal axis, and intensity by use of colors (Figure 1). A phase-corrected wavelet, denoted as the S transform,<sup>22</sup> was used to calculate the time-frequency representation. By use of the methods outlined by Tavel and Katz,<sup>10</sup> 2 variables were defined to characterize the murmur. The logarithm of the time-frequency representation first reached the threshold at  $-2.5$ , a level at which the boundaries of the murmur were emphasized and the influence of noise was minimized. The first variable registered the highest frequency in the murmur at the threshold level, thereby representing the maximal frequency content of the murmur. The second variable was determined as the duration of murmur frequency  $> 200$  Hz (Figure 2). Both variables were automatically calculated for each heart cycle and averaged over available heart cycles, providing a single mean value per dog. To investigate the influence of respiratory sinus arrhythmia, the coefficient of variation between heart beats in each recording was calculated.

The fractal dimension of the murmur was estimated as  $T_2$ ,<sup>16</sup> derived by use of a mathematical computer program.<sup>1</sup> Correlation dimension is a measure of the probability that 2 randomly chosen states in a reconstructed state space are close to each other. For the purposes of the study, the reconstructed state space of the signal was set up on the basis of an embedding dimension of 5 and a time delay of 147 samples. These variables were automatically estimated by use of average mutual information<sup>23</sup> and the Cao method.<sup>24</sup> Closeness was defined as  $< 0.13$ , relative to the adjusted (normalized) attractor diameter. To avoid bias from temporal correlations, samples that were temporally located  $< 80$  samples (1.8 milliseconds) apart were omitted from the search. All available murmur segments were concatenated into 1 large set of murmur data for each dog, thereby providing a single value of the signal's fractal dimension. Detailed information regarding  $T_2$  calculations is available elsewhere.<sup>16,23</sup>

**Statistical analysis**—A statistical program<sup>8</sup> was used for all statistical analyses, except the LDA. Differences in maximal murmur frequency, duration of murmur frequency  $> 200$  Hz, and  $T_2$  as well as heart murmur grade and peak aortic flow velocity in dogs at 7 weeks and 12 months were evaluated by use of the Wilcoxon signed rank test. A 1-way ANOVA was used to identify significant differences in maximal murmur frequency, duration of murmur frequency  $> 200$  Hz, and  $T_2$  between groups A and B. For evalu-

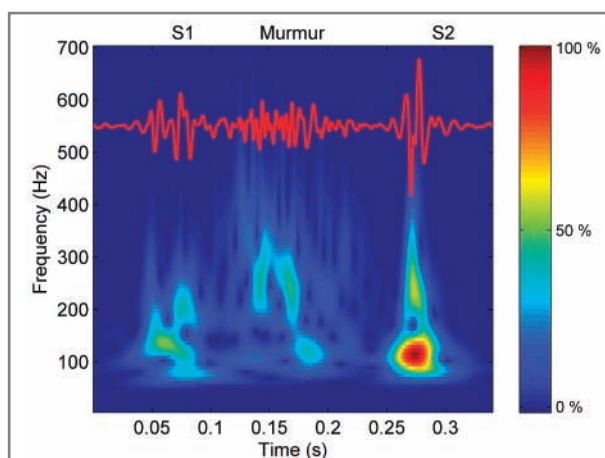


Figure 1—Representative color spectral display of heart sounds and murmur in a Boxer with mild AS (allocated to subgroup B1). The standard phonocardiogram is superimposed, showing the first heart sound (S1), the murmur, and the second heart sound (S2). Notice that the frequency content in the signal changes over time. The color scale at the right represents adjusted (normalized) sound intensity.

Table 1—Results (mean  $\pm$  SD) of TFP and  $T_2$  analyses as well as heart murmur grade and peak aortic flow velocity in 16 Boxers examined at 7 weeks and 12 months of age.

Variable	7 weeks	12 months	P value
Maximal murmur frequency (Hz)	390 $\pm$ 120	424 $\pm$ 73	0.50
Duration of murmur frequency $> 200$ Hz (milliseconds)	53.7 $\pm$ 36.0	30.3 $\pm$ 30.4	0.32
$T_2$ of murmur	3.05 $\pm$ 0.58	2.87 $\pm$ 0.67	0.35
Heart murmur grade (scale of 1–6)	1.25 $\pm$ 0.77	0.94 $\pm$ 0.77	0.31
Peak aortic flow velocity (m/s)	1.76 $\pm$ 0.15	1.83 $\pm$ 0.24	0.23

A value of  $P < 0.05$  was considered significant.

ation of significant differences in maximal murmur frequency, duration of murmur frequency > 200 Hz, and  $T_2$  of the murmur among the 4 subgroups in experiment 2, a multiple comparison Tukey-Kramer  $t$  test was used. Equal variances between groups were assessed by use of the  $F$  test (variance equal test). In instances of unequal variances, the nonparametric Wilcoxon test was used instead (applied only for the maximal murmur frequency). By use of a mathematical computer program,<sup>f</sup> LDA<sup>25</sup> was applied to investigate the differentiation between groups A and B in experiment 2. The 3 variables under investigation (maximal murmur frequency, duration of murmur frequency > 200 Hz, and  $T_2$  of the murmur) were analyzed in a pairwise manner. Because of the limited study population, a leave-1-out approach was used to create the training and the test sets.<sup>26</sup> In this approach, data from all but 1 dog are used as training data to construct the discriminant functions, and data from the excluded dog are used for validation. This procedure, in which a different dog is excluded each time, is iterated for all dogs. By use of the Wilcoxon signed rank test, differences in peak aortic flow velocity as well as maximal murmur frequency, duration of murmur frequency > 200 Hz, and  $T_2$  of the

murmur between dogs with subvalvular and valvular AS were evaluated. Results are reported as mean  $\pm$  SD. The level of significance was set at a value of  $P < 0.05$ .

## Results

In experiment 1, there were no significant differences in maximal murmur frequency, duration of murmur frequency > 200 Hz, and  $T_2$  of the murmur; auscultated murmur grade; or peak aortic flow velocity between dogs at 7 weeks and 12 months of age (Table 1).

Of the 27 dogs in experiment 2, 8 were allocated to subgroup A1, 8 were allocated to subgroup A2, 5 were allocated to subgroup B1, and 6 were allocated to subgroup B2. Dogs in subgroups A1 and A2 had no morphologic evidence of AS and grade 1 to 2/6 heart murmurs. Dogs in subgroups B1 and B2 had morphologic evidence of AS and grade 2 to 4/6 and grade 3 to 5/6 heart murmurs, respectively. In dogs of subgroups A1 and A2, mean  $\pm$  SD aortic flow velocity was  $1.65 \pm 0.09$  m/s (range, 1.52 to 1.73 m/s) and  $2.02 \pm 0.19$  m/s (range, 1.84 to 2.41 m/s), respectively. In dogs of subgroups B1 and B2, mean aortic flow velocity was  $2.82 \pm 0.36$  m/s (range, 2.40 to 3.20 m/s) and  $4.68 \pm 0.57$  m/s (range, 4.00 to 5.50 m/s), respectively. The values of maximal murmur frequency, duration > 200 Hz,

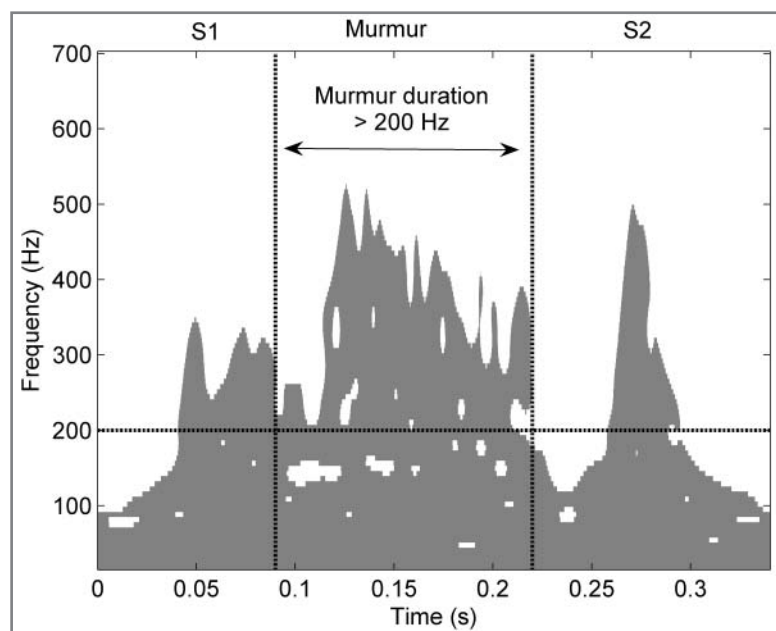


Figure 2—Changes in frequency content of the recorded sound from the dog with mild AS in Figure 1 after the time-frequency representation has had a threshold applied at  $\sim 2.5$ . The vertical markers indicate the period (0.13 seconds) during which the murmur frequency exceeds 200 Hz (threshold marked by the horizontal line). The peak frequency is approximately 530 Hz.

as well as  $T_2$  of the murmur were compared between dogs without (group A) and dogs with (group B) morphologic evidence of AS. Values of maximal murmur frequency, duration of murmur frequency > 200 Hz, as well as  $T_2$  of the murmur were significantly higher in group B dogs ( $483 \pm 55$  Hz,  $105 \pm 38$  milliseconds, and  $3.90 \pm 0.52$ , respectively), compared with values in group A dogs ( $424 \pm 73$  Hz [ $P = 0.03$ ],  $30.3 \pm 30.4$  milliseconds [ $P < 0.001$ ], and  $2.87 \pm 0.67$  [ $P < 0.001$ ], respectively).

Results of TFP and  $T_2$  analyses in the 4 subgroups of experiment 2 were assessed (Table 2). Compared with findings in subgroup A2, the maximal frequency of the murmur was significantly higher in subgroups A1 and B2. In subgroups B1 and B2, the duration of murmur frequency > 200 Hz was significantly greater than the value in subgroups A1 and A2. The  $T_2$  of the murmur was significantly higher in subgroup B2, compared with subgroups A1 and A2, and was also significantly higher in B1, compared with A1 (but not A2; Figure 3).

By use of LDAs, significant models for differentiation between physiologic mur-

Table 2—Results (mean  $\pm$  SD [range]) of TFP and  $T_2$  analyses in dogs without AS in subgroup A1 (dogs with peak aortic flow velocities < 1.8 m/s;  $n = 8$ ) and subgroup A2 (dogs with peak aortic flow velocities  $\geq 1.8$  m/s; 8) and dogs with AS in subgroup B1 (dogs with peak aortic flow velocities  $\leq 3.2$  m/s [mild AS]; 5) and subgroup B2 (dogs with peak aortic flow velocities > 3.2 m/s [moderate to severe AS]; 6).

Variable	Subgroup			
	A1	A2	B1	B2
Maximal murmur frequency (Hz)	469 $\pm$ 79 (360–568)	378 $\pm$ 21 (344–404)	447 $\pm$ 61 (367–535)	514 $\pm$ 26 (484–545)
Duration of murmur frequency > 200 Hz (milliseconds)	40.0 $\pm$ 37.5 (3.5–98.3)	20.5 $\pm$ 18.8 (0–51.4)	93.5 $\pm$ 45.1 (20.5–139.9)	114.9 $\pm$ 30.7 (55.5–145.1)
$T_2$ of murmur	2.65 $\pm$ 0.75 (1.70–3.78)	3.09 $\pm$ 0.54 (1.98–3.74)	3.68 $\pm$ 0.53 (2.94–4.28)	4.09 $\pm$ 0.47 (3.58–4.71)

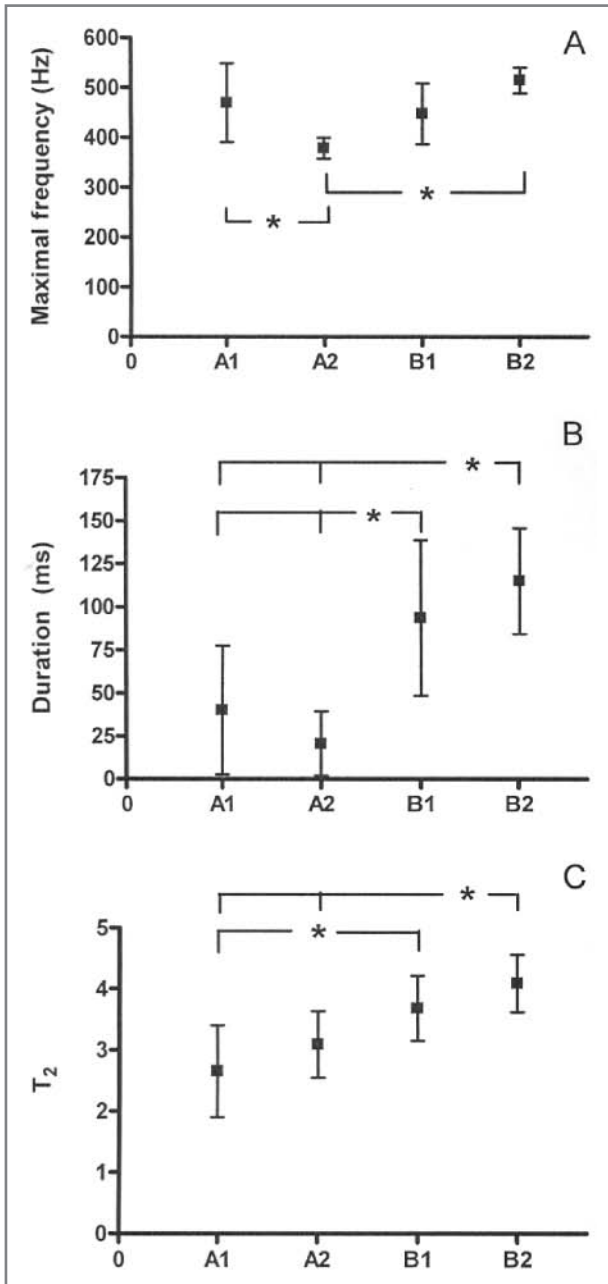


Figure 3—Mean  $\pm$  SD values for TFP and  $T_2$  data in dogs without AS in subgroups A1 (dogs with peak aortic flow velocities  $< 1.8$  m/s;  $n = 8$ ) and subgroup A2 (dogs with peak aortic flow velocities  $\geq 1.8$  m/s; 8) and dogs with AS in subgroups B1 (dogs with peak aortic flow velocities  $\leq 3.2$  m/s [mild AS]; 5) and subgroup B2 (dogs with peak aortic flow velocities  $> 3.2$  m/s [moderate to severe AS]; 6). Maximal murmur frequencies (A) did not differ among subgroups A1, B1, and B2. Duration of murmur frequency  $> 200$  Hz (B) was significantly longer in dogs with AS (subgroups B1 and B2) than it was in dogs without AS (subgroups A1 and A2). The  $T_2$  values (C) indicate greater complexity of murmurs in dogs with AS, compared to dogs without AS. \*Bracketed values are significantly ( $P < 0.05$ ) different.

murs and murmurs caused by AS were identified for 3 pairs of data sets: maximal murmur frequency versus duration of murmur frequency  $> 200$  Hz, maximal murmur frequency versus  $T_2$ , and duration of murmur frequency  $> 200$  Hz versus  $T_2$ . The canonical correlation was 0.81, 0.71, and 0.82 for the 3 pairs of data sets,

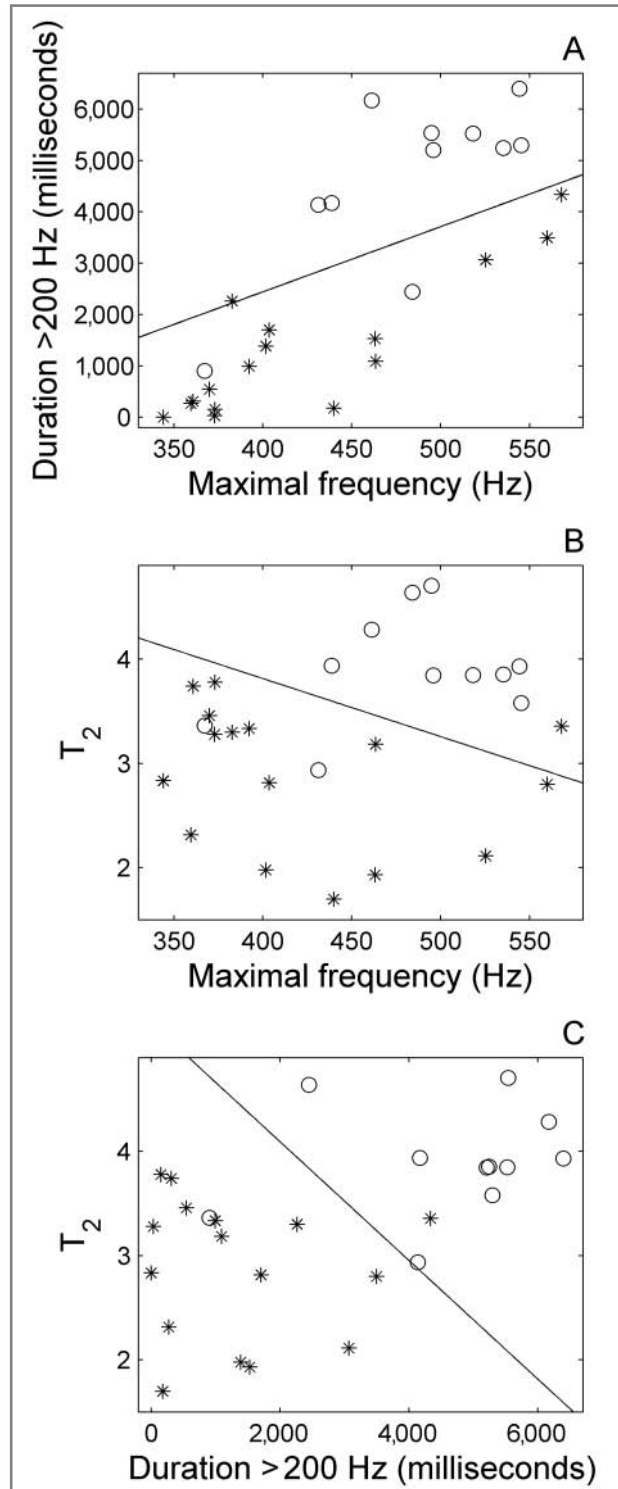


Figure 4—Linear discriminant analysis of TFP and  $T_2$  data obtained from 16 dogs without AS (group A; asterisks) and 11 dogs with AS (group B; circles). In each panel, the line indicates differentiation between the groups. Notice that optimal differentiation was obtained by combining duration of murmur frequency  $> 200$  Hz with  $T_2$  analysis data (C).

respectively; the cross-validated classification scores were 85.1%, 85.1%, and 88.9%, respectively. Among the 3 data set pairs, the sensitivities for differentiation of a physiologic murmur from an AS-related murmur in

Boxers were 87.5%, 87.5%, and 93.8%, respectively, and the specificities were 81.8%, 81.8%, and 81.8%, respectively (Figure 4).

With regard to peak aortic flow velocity, there was no significant ( $P = 0.34$ ) difference between dogs with subvalvular ( $n = 7$ ) and valvular (4) AS. Neither the maximal frequency ( $P = 0.57$ ) nor the duration of frequency  $> 200$  Hz of the murmur ( $P = 0.13$ ) differed between those groups. However,  $T_2$  of the murmur was significantly ( $P = 0.008$ ) higher in dogs with subvalvular AS, compared with dogs with valvular AS. The coefficient of variation between heart beats was 25% for maximal murmur frequency and 22% for duration of murmur frequency  $> 200$  Hz.

## Discussion

Signal analysis techniques have been used extensively for characterization of human phonocardiographic signals.<sup>27</sup> Signal characteristics that have been investigated include time domain properties,<sup>28</sup> frequency properties,<sup>10,29,30</sup> and parametric modeling techniques.<sup>31</sup> Several of these characteristics have been used for classification of various murmurs, and the artificial neural network (a computer-assisted classification technique) is commonly used.<sup>28,29</sup>

Both time and frequency properties of heart murmurs in Boxers were investigated in the present study. The selected properties—maximal murmur frequency and duration of murmur frequency  $> 200$  Hz—were chosen because of their usefulness in differentiating physiologic murmurs from those caused by AS in humans.<sup>10</sup> In the dogs included in experiment 2, the duration of murmur frequency  $> 200$  Hz was significantly longer in group B, compared with group A. On analysis of the subgroups, the duration of murmur frequency  $> 200$  Hz was significantly longer in subgroup B2, compared with findings in subgroups A1 and A2. However, most importantly, the duration of murmur frequency  $> 200$  Hz was significantly longer in subgroup B1, compared with subgroups A1 and A2. Dogs in subgroup B1 (those with mild AS) had auscultated murmur grades of 2 to 4/6, whereas murmur grades in subgroups A1 and A2 were either 1 or 2/6. In dogs with a grade 2/6 murmur, it is not possible to determine the cause of the murmur via cardiac auscultation alone.<sup>32</sup> If a larger number of dogs with grade 2/6 murmurs had been available, it would have been interesting to apply the TFP and  $T_2$  analyses to their murmur data to investigate whether differentiation of physiologic murmurs and murmurs caused by mild AS is possible in that group of dogs.

There are many techniques available for investigation of changes in the frequency content of a signal over time. Tavel and Katz<sup>10</sup> used the short-time Fourier transform in their study. However, that approach is affected by the uncertainty principle because the frequency resolution decreases as the time resolution increases, and vice versa. In the present study, the S transform (a phase-corrected wavelet transform) was used instead, thereby allowing better time resolution in the murmur frequency range and greater accuracy in determining the duration of murmur frequency  $> 200$  Hz.

Among the dogs in experiment 2 of the present study, the maximal murmur frequency was significantly higher in group B (dogs with AS), compared with group

A (dogs without AS). On analysis of the subgroup data, murmurs in subgroup B2 dogs had significantly higher maximal murmur frequency than those in subgroup A2 dogs, which is in accordance with findings of a previous investigation.<sup>10</sup> However, in contrast to findings in humans,<sup>10</sup> the maximal frequency of the murmur was also significantly higher in subgroup A1 dogs, compared with subgroup A2 dogs. In adult humans, AS is most commonly an age-related degenerative calcific valvular disease, although discrete subvalvular stenosis has been detected.<sup>33</sup> In dogs, AS is considered a congenital disease, of which the subvalvular form is most common.<sup>17</sup> The differences in disease characteristics between humans and dogs might affect murmur properties and thus the maximal frequency. Another possible explanation could be found in anatomic differences between the species. The human thorax is flat, whereas in a dog, the thorax is narrow and deep; this may result in differences in positioning of the heart as well as in distance between the heart and the thoracic wall. Thus, variation in dampening of the heart murmurs between humans and dogs may be expected. The difference in body size between humans and dogs might also affect the results.

Correlation dimension is a novel property in heart sound evaluation that allows analysis of nonlinear signal patterns. This technique has previously been used to characterize various dynamic systems. Calculation of  $T_2$  is notoriously difficult because the number of datum points is often too small to permit an accurate determination of the correlation sum.<sup>16</sup> To increase the accuracy of the calculations, all available murmur segments were concatenated into 1 large set of murmur data for each dog in the present study. To further counteract the effect of a sparsely sampled state space, the Takens estimator<sup>16</sup> was used to calculate the  $T_2$  value. This approach was suitable for our purposes because it provides good results for sparse, high-dimensional, and noisy data.

In experiment 2 of the present study,  $T_2$  of the heart murmur was significantly higher in group B dogs, compared with group A dogs, indicating a higher complexity of murmurs in dogs affected by AS. As the stenosis becomes more severe, blood flow generally becomes more turbulent. Correlation dimension assesses the dynamics of the underlying flow system. With increasing turbulence, the reconstructed state space will depict a more complicated system; thus,  $T_2$  will increase. Among 11 dogs with AS in the present study, 7 dogs had subvalvular lesions and 4 had valvular changes. Because there was no difference in aortic flow velocity between these dogs, disease severity was considered similar. On comparison of the murmur characteristics of those 2 groups, there was no difference in maximal murmur frequency or duration of murmur frequency  $> 200$  Hz, although  $T_2$  was significantly higher in dogs with subvalvular AS. The small sample size should give rise to caution in interpretation of these results. However, from a fluid dynamic point of view, subvalvular AS is likely to cause more complicated flow behaviors than valvular AS. Because subvalvular stenoses are often asymmetric, dynamically changing jets develop that impinge with 1 or several of the leaflets, causing interaction between

the flow and several anatomic structures. If  $T_2$  is able to reflect these differences, it would be a very interesting fact; objective analysis of this finding will, however, require a controlled in vitro investigation and is therefore left for future studies.

With regard to the subgroups of experiment 2,  $T_2$  of the murmur was significantly higher in both subgroups B1 and B2, compared with subgroup A1, whereas the value in subgroup A2 was significantly different only from that of subgroup B2. Accordingly, on the basis of results of the present study,  $T_2$  alone cannot be used for differentiation of heart murmurs caused by mild AS and physiologic murmurs. The LDA was used to investigate whether a pairwise combination of maximal murmur frequency, duration of murmur frequency > 200 Hz or  $T_2$  would be useful for differentiation of dogs in group A from those in group B. The classification score of the dogs improved when 2 variables instead of only 1 were used, with the highest accuracy rate for duration of murmur frequency > 200 Hz and  $T_2$  (89.9%). Bhatikar et al<sup>29</sup> used a nonlinear classifier with 252 input variables to achieve a sensitivity of 93% and a specificity of 90% for differentiation of physiologic murmurs and murmurs caused by ventricular septal defect in children. In the present study, the combination of duration of murmur frequency > 200 Hz with  $T_2$  proved most accurate for distinction of physiologic murmurs and murmurs caused by AS with a sensitivity of 94% and a specificity of 82%.

In colony-bred Newfoundlands, AS has been shown to develop during the postnatal period.<sup>8</sup> On the basis of that study, there is a widespread clinical belief that puppies with mild heart murmurs but without morphologic evidence of AS (determined via 2D echocardiography) are at risk of developing the disease if they are of a breed of a high predisposition, such as Boxers, Rottweilers, or Golden Retrievers.<sup>17</sup> In experiment 1 of the present investigation, no significant differences in TFP or  $T_2$  data obtained from dogs at the examinations at 7 weeks versus 12 months of age were detected. Neither the mean heart murmur grade nor the peak aortic flow velocity differed between the age groups, and none of the dogs developed morphologic evidence of AS (determined via 2D echocardiography) during their first year of life. To further evaluate this, a larger prospective study on the progression of heart murmurs and development of AS in young Boxers with low-intensity murmurs would be useful.

There are some important limitations to our study. The gold standard for diagnosis of subvalvular AS is necropsy,<sup>8</sup> a procedure that, for obvious reasons, is not possible to perform for research purposes in privately owned dogs. The best clinical diagnostic method available to date is echocardiography. However, the mildest subvalvular aortic lesions may not be detectable via this method.<sup>17</sup> It is therefore possible that 1 or more of the dogs in subgroup A2 actually had mild subvalvular AS and should have been allocated to subgroup B1. However, in Boxers with low-grade murmurs, the left ventricular outflow tract is smaller than that in other dog breeds,<sup>6</sup> which is a likely cause of the heart murmurs in subgroups A1 and A2. Whether this anatomic difference is genetically associated with AS is an interesting question that remains to be investigated.

Because of anxiety or resistance to restraint, echocardiographic examinations are not possible to perform without sedation in all dogs. In a study by Johnson et al,<sup>34</sup> it was possible to perform spectral analysis in 100% of examined humans. In our study conditions, recording of the phonocardiographic sound files was a simple and rapid procedure and analyses of the sound files could be performed for all of the study dogs. There was no correction for individual reactions to the recording procedure in terms of sympathetic stimulation. Because this could have affected the murmur intensity<sup>19</sup> and, possibly, the frequency content of the murmur, it was a potential study limitation.

To our knowledge, this is the first study to use a mathematical model for evaluation of time-frequency and complexity characteristics of heart murmurs in dogs. However, this investigation only included Boxers with or without AS. Further studies are needed to investigate murmur characteristics in other breeds of dog with other diseases. The effects of differences in thoracic shape, nutritional status (eg, obesity), and other physiologic variables on murmur recordings and murmur data analyses remain to be evaluated.

The 3 variables used in the present study make use of a few invariant features in the murmur signal. However, murmurs stem from turbulent blood flow, which varies in time as a result of the pulsatile flow. Tracking changes in various properties such as maximum and mean frequency over time would provide additional information about the characteristic features and the underlying dynamics of the murmur. Such time-variable properties would probably be better suited for detecting subtle pathologic changes in anatomic structures; however, such time-variable features would be considerably more complicated to interpret, both for the veterinarian and for an automated computer analysis.

To investigate the influence of respiratory sinus arrhythmia, the coefficient of variation between heart beats in each recording was calculated in our study. For both maximal frequency and duration of frequency > 200 Hz of the murmur, the coefficient of variation was approximately 20%. Assessment of the mean of several heart beats should somewhat compensate for the sinus arrhythmia.

The intra-observer variation in recording of phonocardiographic sound files in dogs remains to be investigated. Baykal et al<sup>35</sup> performed a study on the reproducibility of second heart sound recordings in humans with mechanical prosthetic aortic valves. In that study, a coefficient of variation  $\leq$  5% of modal frequencies over 12 recording sites on the surface of the thorax was identified.

The TFP and  $T_2$  analyses available presently require advanced mathematical computer systems, which are not available in most veterinary clinics. Nevertheless, results of the present study provided interesting diagnostic information from heart murmur analyses. With further development of the methods, the analyses could be performed quickly and promptly by use of a preprogrammed computer, thereby providing a useful diagnostic tool in clinical practice.

The data obtained in our study suggested that the duration of murmur frequency > 200 Hz can be used

for differentiation between physiologic murmurs and murmurs caused by mild AS in Boxers. The combination of the duration of murmur frequency > 200 Hz with T<sub>2</sub> analysis data proved most accurate for differentiation of physiologic murmurs from murmurs caused by AS in the study dogs. Assessment of maximal murmur frequency cannot be recommended for differentiation of dogs with and without AS. Duration of murmur frequency > 200 Hz and T<sub>2</sub> analysis may be useful in the evaluation of low-intensity murmurs in Boxer puppies and, possibly, for prediction of their cardiac status as 1-year-old dogs.

- a. Welsh Allyn Meditron ASA, Medi-Stim ASA, Oslo, Norway.
- b. Dell Latitude D800 laptop, Dell Computer Corp, Limerick, Ireland.
- c. Meditron analyzer, version 4.0V, Welsh Allyn Meditron ASA, Medi-Stim ASA, Oslo, Norway.
- d. Sound card, Welsh Allyn Meditron ASA, Medi-Stim ASA, Oslo, Norway.
- e. GE Vivid 3 ultrasound machine, General Electric Co, Stockholm, Sweden.
- f. MATLAB 7.04, The MathWorks Inc, Natick, Mass.
- g. JMP, version 6.0.0, SAS Institute Inc, Cary, NC.

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