Use of signal analysis of heart sounds and murmurs to assess severity of mitral valve regurgitation attributable to myxomatous mitral valve disease in dogs

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Objective—To investigate use of signal analysis of heart sounds and murmurs in assessing severity of mitral valve regurgitation (mitral regurgitation [MR]) in dogs with myxomatous mitral valve disease (MMVD).

Animals—77 client-owned dogs.

Procedures—Cardiac sounds were recorded from dogs evaluated by use of auscultatory and echocardiographic classification systems. Signal analysis techniques were developed to extract 7 sound variables (first frequency peak, murmur energy ratio, murmur duration > 200 Hz, sample entropy and first minimum of the auto mutual information function of the murmurs, and energy ratios of the first heart sound [S1] and second heart sound [S2]).

Results—Significant associations were detected between severity of MR and all sound variables, except the energy ratio of S1. An increase in severity of MR resulted in greater contribution of higher frequencies, increased signal irregularity, and decreased energy ratio of S2. The optimal combination of variables for distinguishing dogs with high-intensity murmurs from other dogs was energy ratio of S2 and murmur duration > 200 Hz (sensitivity, 79%; specificity, 71%) by use of the auscultatory classification. By use of the echocardiographic classification, corresponding variables were auto mutual information, first frequency peak, and energy ratio of S2 (sensitivity, 88%; specificity, 82%).

Conclusions and Clinical Relevance—Most of the investigated sound variables were significantly associated with severity of MR, which indicated a powerful diagnostic potential for monitoring MMVD. Signal analysis techniques could be valuable for clinicians when performing risk assessment or determining whether special care and more extensive examinations are required. (*Am J Vet Res* 2009;70:604–613)

A systolic heart murmur is a prominent finding in dogs with regurgitation through the mitral valve (ie, MR) attributable to MMVD. This condition is the most common cardiac disorder in dogs,[1–4] and the highest prevalence is found in small- to medium-size breeds.[5–8] The disease is characterized by progressive degeneration of the mitral valve,[3,4,9] which can progress to heart failure and lead to an increase in the number of deaths caused by cardiac disease before the age of 10 years in affected breeds.[10]

In general, the intensity of a murmur typically increases with the severity of valve lesions,[11] and af-

Abbreviations

AUC	Area under the receiver operating characteristic curve
LA:Ao	Ratio of left atrium to aortic root
LVIDd	End-diastolic left ventricular internal diameter
$LVIDd_{inc}$	Percentage increase in end-diastolic left ventricular internal diameter
LVIDs	End-systolic left ventricular internal diameter
$LVIDs_{inc}$	Percentage increase in end-systolic left ventricular internal diameter
MMVD	Myxomatous mitral valve disease
MR	Mitral regurgitation
PCG	Phonocardiogram
ROC	Receiver operating characteristic
S1	First heart sound
S2	Second heart sound

Received March 14, 2008.
Accepted August 20, 2008.

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Supported by the Swedish Agency for Innovation Systems, the Health Research Council in the Southeast of Sweden, and the Swedish Research Council.

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fected dogs progress from mild to severe MR without any outward clinical signs. Thus, a typical dog in which MMVD has been diagnosed echocardiographically is often monitored by use of murmur assessment until a high-intensity murmur is diagnosed or overt signs of congestive heart failure develop. Although the characterization of acoustic findings is based on established physiologic principles, their interpretation is highly dependent on the experience of the evaluator, and there is considerable interobserver variation.[12–15] Furthermore, some information in the cardiac sound signal is inaccessible by standard auscultation because of limitations in the human auditory system.[16] The PCG, which is a quantitative graphic representation of cardiac sound signals, does not have the same limitations. However, detailed characterization of sounds is not possible via manual interpretation of the PCG. Therefore, a signal analysis tool with the ability to objectively perform analysis of heart sounds and murmurs is desirable.

Studies[11,13] in dogs with MR attributable to MMVD have revealed that increasing severity of MR is associated with certain characteristic features on the PCG recording. The murmur increases in duration from early or late systole to holosystole, the amplitude of the murmur increases, and there is a shift in the amplitude ratio between S1 and S2. However, those evaluations were conducted on standard PCG recordings. Newer techniques for signal analysis allow a more objective characterization of heart sounds and murmurs.[17] Extensive research has been performed on signal processing of human cardiac sounds, and feasible analysis techniques include a number of linear and nonlinear approaches operating in the time or frequency domains.[18–21,a] However, those studies primarily focused on distinguishing various types of cardiac diseases, differentiation between physiologic and pathologic murmurs, or assessment of the severity of aortic stenosis. To our knowledge, signal processing of heart sounds and murmurs has never been applied for assessment of MR in any species. The objective of the study reported here was to investigate whether linear and nonlinear signal analysis of cardiac sounds can be used to assess MR severity in dogs with MMVD.

Materials and Methods

Animals—Client-owned dogs examined at the cardiology unit of the Faculty of Veterinary Medicine and Animal Sciences in Uppsala, Sweden, were used in the study. Written consent was obtained from the owners prior to participation of their dogs in the study. The study was approved by the Local Ethical Committee in Uppsala, Sweden.

A database consisting of an owner interview, a physical examination, a PCG, an ECG, and an echocardiographic examination, which were performed during the same consultation, was established for each dog. Inclusion criteria were that the dogs had to have evidence of MMVD or be free from physical or echocardiographic evidence of cardiac disease. Dogs also had to have a sinus rhythm. Dogs with congenital heart disease, other acquired cardiovascular disorders, or major organ-related or systemic diseases were not included in the study.

Procedures—All physical examinations, including auscultation and PCG recordings, were conducted and evaluated by 2 investigators (KH and CK) who were veterinarians experienced in veterinary cardiology. No sedatives were used in the study. After completion of the owner interview, a physical examination (which included thoracic auscultation), followed by PCG examination, was conducted in a quiet examination room with the dog in a standing position. The intensity of heart murmurs was graded on a scale of 1 to 6 in accordance with established guidelines.[22] An electronic stethoscope system,[b] which was connected to a laptop computer[c] with accompanying acquisition software,[d] was used for auscultation and for recording the PCG signal and ECG. The electronic stethoscope had an unfiltered audio output (which resulted in a flat frequency response within the audible range), with a 3-dB cutoff value at 30 Hz. The ECG and PCG were digitized at 44.1 kHz with 16 bits/sample and stored in the computer for later analyses. During recording, the flat diaphragm chest piece of the electronic stethoscope was placed firmly on the left side of the chest wall over the costochondral junctions between the fifth and sixth intercostal spaces to provide the loudest and clearest heart murmur possible. The PCG signals were recorded for 10 seconds. To improve the sound quality, the right side of the chest wall was pushed slightly toward the stethoscope on the left side. Background noise was minimized, and the mouths of panting dogs were gently closed during recording to reduce ventilation artifacts. The ECG (lead II) recording was used as a time reference and for verification of a sinus rhythm.

Echocardiography, which was the last part of the examination, was performed to verify the diagnosis of MMVD and to exclude other primary or secondary cardiac diseases. Dogs were placed in right and then left lateral recumbency on an ultrasound examination table. The echocardiographic evaluation was conducted by use of an ultrasonographic unit[e] equipped with a 5-MHz phased-array transducer and ECG monitoring. Images obtained by use of standardized imaging planes[23] were digitally stored. Assessment of mitral valve structures was conducted from the right parasternal long-axis view and the left apical 4-chamber view. Additionally, the same views were used for assessing the degree of MR by use of color Doppler mapping. The MR was subjectively assessed as the area of regurgitant jet relative to the area of the left atrium. The MR was scored as described elsewhere,[24] with slight modifications. Scores were recorded as no regurgitation, mild (< 30%) regurgitation, moderate (30% to 50%) regurgitation, and severe (> 50%) regurgitation. Screening of potential regurgitation through the tricuspid, aortic, and pulmonic valves was performed in a routine manner by use of color Doppler ultrasonography. Furthermore, the LA:Ao was quantified from a right 2-dimensional short-axis view, as described elsewhere.[25] The M-mode measurements of the left ventricle were obtained by use of standard techniques[26] from a right parasternal short-axis view. The M-mode values were used to derive fractional shortening, LVIDd, $\mathrm{LVIDd_{inc}}$, LVIDs, and $\mathrm{LVIDs_{inc}}$. Values for $\mathrm{LVIDd_{inc}}$ and $\mathrm{LVIDs_{inc}}$ (ie, the percentage increase) were calculated as follows: ([observed dimension – expected normal dimension])/expected normal dimension) X 100. Expected normal dimensions were calculated for LVIDd (ie, expected normal LVIDd = body weight$^{0.294}$ X 1.53) and LVIDs (ie, expected normal LVIDs = body weight$^{0.315}$ X 0.95).[27] Evaluation of the echocardiographic data was performed by an investigator (JH) experienced in echocardiography who was unaware of the auscultatory findings.

Two systems were used to classify the severity of MR. In the auscultatory classification system, dogs were classified into the following murmur groups: clinically normal (no audible cardiac murmur), low intensity (grades 1/6 and 2/6), moderate intensity (grades 3/6 and 4/6), and high intensity (grades 5/6 and 6/6). In the echocardiographic classification system, estimation of MR severity was based on the obtained LA:Ao and the MR jet size; dogs were classified as follows: clinically normal (LA:Ao < 1.5 and no MR jet), mild (LA:Ao ≤ 1.5 and MR jet < 30%), moderate (LA:Ao < 1.8 and MR jet < 50%), and severe (LA:Ao ≥ 1.8 and MR jet ≥ 50%).

Signal analysis—All recorded PCG signals were manually segmented by use of the ECG recordings, which were used as an aid for timing of the heart sounds. Four markers (beginning of S1, end of S1, beginning of S2, and end of S2) were determined for each heart cycle. Noisy or corrupted signal segments (as determined by visual inspection of the data) were excluded from further analyses. The median number of heart cycles analyzed per dog was 13 (interquartile range, 11 to 16), and the number of excluded heart cycles per dog was 5 (interquartile range, 3 to 8).

To reduce computational complexity and to remove high-frequency noise, data were adjusted to 4.4 kHz by use of a polyphase interpolation structure and an antialiasing (low-pass) finite-impulse-response filter. A fifth-order zero-phase digital Butterworth high-pass filter with a cutoff frequency of 30 Hz was applied to emphasize the discerning information in the PCG and to reduce low-frequency noise. All processing of PCG signals was performed by use of a mathematic computer program.[f] Seven sound variables were automatically derived from the segmented PCG signals.

The first frequency peak is a measure of the harshness of the murmur and was estimated as the minimum angle of the complex roots from a fourth-order autoregressive model determined with the Burg method.[28,a] All systolic segments within each recording were linked together in a series, and 1 value/dog was determined by use of this batch of data (**Figure 1**).

The murmur energy ratio quantifies the percentage of higher frequencies in the spectrum.[19] A periodogram was calculated for all systolic segments; each segment was extended by adding zeros (zero padded) to obtain the same duration. The mean of the available periodograms was then calculated to yield a final spectral estimate for each dog. This approach is similar to the Welch spectral estimation method.[28] The murmur energy was calculated in 2 frequency bands (murmur energy 1 [ie, E1] from 20 to 50 Hz and murmur energy 2 [ie, E2] from 50 to 500 Hz; **Figure 2**). The murmur energy ratio was then defined as (E2/[E1 + E2]) X 100.

Murmur duration > 200 Hz measures the duration of sounds exceeding 200 Hz as a fraction of the duration of systole. A joint time-frequency representation was determined with a phase-corrected wavelet, which was denoted as the S-transformation.[29] Threshold for the logarithm of the joint time-frequency representation was set at –25 dB of the sound intensity, which was a value at which the boundaries of the murmur were emphasized and the influence of noise was minimized.[20] The duration was measured in each cardiac cycle and the mean value determined across available heart cycles (**Figure 3**).

Sample entropy is useful for investigating dynamics of time series.[30] Higher values of sample entropy indicate more disorder in the system. Hence, high-intensity murmurs will have higher sample entropy values. Sam-

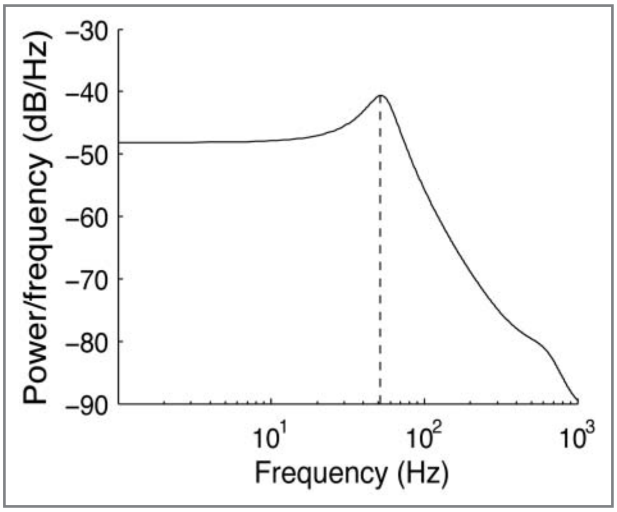

Figure 1—An example of a power spectrum estimated by use of the Burg method[28] for a representative dog with moderate MR, as determined by use of the echocardiographic classification. The first frequency peak of the murmur (vertical dashed line) is in the lower frequency range.

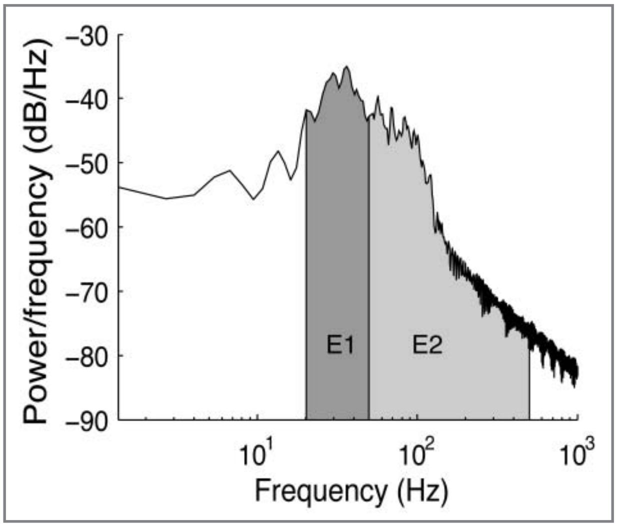

Figure 2—An example of a power spectrum estimated by use of the Welch method[28] for a dog with moderate MR, as determined by use of the echocardiographic classification system. The power spectrum has been separated into a lower frequency portion that ranges from 20 to 50 Hz (E1 [dark gray shading]) and a higher frequency portion that ranges from 50 to 500 Hz (E2 [light gray shading]). The murmur energy ratio is defined as (E2/[E1 + E2]) X 100.

ple entropy is derived as the negative natural logarithm of the probability that 2 similar sequences of length d will remain similar at length d + 1. In this study, sequences of length d = 2 were included, and similarity was determined with a tolerance set to 0.2 times the SD of the signal. All systolic segments within each recording were linked together in a series, and 1 value for sample entropy was determined for each dog by use of this batch of data.

The auto mutual information function represents the mean predictability of future samples on the basis of previous samples in a signal. The faster the auto mutual information function decays, the harder it will be to predict the signal.[31] In the study reported here, the first local minimum of the auto mutual information function was used to quantify how fast it decayed. All systolic segments within each recording were linked together in a series, and 1 value was determined for each dog by use of this batch of data. Because severe murmurs are more irregular and thus more difficult to predict, a decrease in auto mutual information reflected an increase in MR severity (**Figure 4**).

The energy ratio of S1 was calculated as the standardized energy within the S1 segment. The value was determined in each heart cycle, and the final variable was determined as the means across available heart cycles. To obtain values for comparison, the variable was standardized against the energy in diastole, as described in another study.[32] The energy ratio of S2 was calculated in a manner similar to that for the energy ratio of S1.

Statistical analysis—A mathematic computer program[d] was used for all statistical analyses, except for the multiple regression analysis. Values were reported as median and interquartile range.

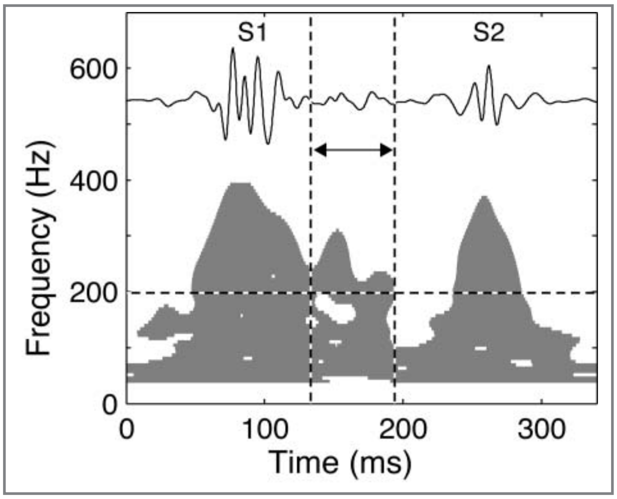

Figure 3—An example of the joint time-frequency representation of heart sounds and a murmur in a dog with mild MR, as determined by use of the echocardiographic classification system. The standard PCG signal is superimposed at the top, indicating S1, the murmur (area between the 2 vertical dashed lines), and S2. The threshold of the logarithm of the joint time-frequency representation was set at –25 dB of the sound intensity, and the signal components exceeding this threshold are indicated (gray shading). A threshold of 200 Hz is indicated (horizontal dashed line). Murmur duration > 200 Hz denotes the duration of sounds exceeding 200 Hz (gray shading in the space indicated by the double-headed arrow) and is measured as a fraction of the duration of systole.

The Cuzick test[33] for ordered groups was used to investigate associations between the sound variables and the 4 MR severity groups. In variables in which a significant ($P < 0.05$) association was detected, a pairwise comparison was also performed by use of the Mann-Whitney *U* test with Bonferroni adjustment, for which a value of $P < 0.008$ was considered significant. The Kolmogorov-Smirnov test was used to ensure similar variable distribution among the MR severity groups before group comparisons were made. These statistical tests were performed on data for both the echocardiographic and the auscultatory classification systems.

Effects of sex, age, breed (Cavalier King Charles Spaniel [yes vs no]), body weight, heart rate, and LA:Ao on each of the sound variables were investigated by use of multiple regression analysis in a statistical program.[g] Analyses were performed in a backward stepwise manner, starting with all variables included in the model and then removing the variable with the highest *P* value until all the remaining variables had a value of $P < 0.05$. All variables were assessed only as main effects; no interaction terms were considered in the model. The adjusted R^2 is defined as the percentage of the total sum of squares that can be explained by the regression, and it also takes into consideration the degrees of freedom for variables that are added.

Linear discriminant analysis was used to investigate whether a combination of sound variables could be used to distinguish severe MR from the other 3 severity groups. Because of the limited number of dogs with severe MR, a leave-1-out approach was used for cross validation. 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. The optimal number of variables to be used in linear discriminant analysis was investigated with an exhaustive search, in which all possible combinations of sound variables were tested. These analyses were performed by use of

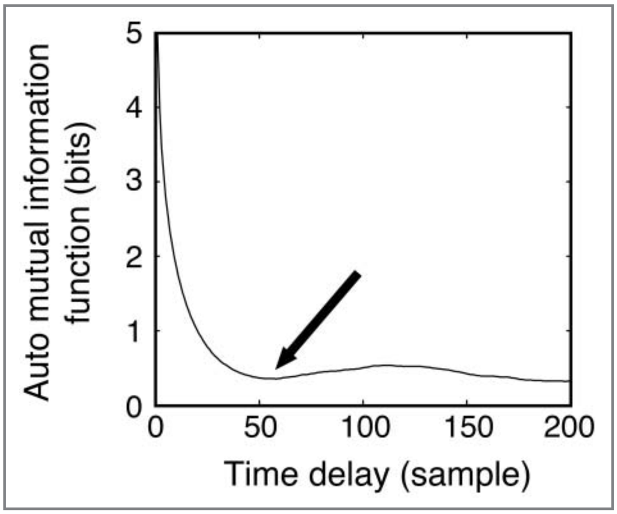

Figure 4—An example of a typical profile of the auto mutual information function obtained for a dog with mild MR, as determined by use of the echocardiographic classification system. The first local minimum (arrow) indicates the auto mutual information.

data for both the echocardiographic and the auscultatory classification systems for characterization of severe MR. The diagnostic efficacy of the optimal combinations of sound variables (which were obtained from the linear discriminant analysis) was further evaluated by use of ROC curves. In particular, sensitivity, specificity, and the AUC were investigated.

Results

Animals—Eighty-two dogs were initially recruited for the study. Five dogs were excluded from the study because of difficulties encountered in segmentation of heart sounds. There were 77 dogs (41 females and 36 males) with a median age of 8.7 years (interquartile range, 6.7 to 10.8 years) and median body weight of 9.5 kg (interquartile range, 8.4 to 11.4 kg) that met the inclusion criteria and were enrolled for further investigation. The most commonly represented breeds were Cavalier King Charles Spaniel (n = 59) and Dachshund (5). Thirteen other breeds (1 dog/breed) were also represented in the study.

Of the 77 dogs, 17 did not have audible cardiac murmurs, 23 had a low-intensity murmur, 23 had a murmur of moderate intensity, and 14 had a high-intensity murmur. Five dogs had unremarkable results for echocardiography, 38 had mild MR, 17 had moderate MR, and 17 had severe MR **(Table 1)**. Because there were only 5 dogs included in the clinically normal echocardiographic group, these dogs were excluded from the multiple comparison test among groups but not from the overall statistical analysis. Echocardiographic data were summarized **(Table 2)**.

Significant ($P < 0.001$; Cuzick test) associations were detected between the severity of MR and all but one of the investigated sound variables **(Figure 5)**. A significant ($P = 0.048$) association was detected between the energy ratio of S1 and severity of MR by use of the auscultatory classification. However, this sound variable was not significantly ($P = 0.143$) associated with severity of MR by use of the echocardiographic classification.

First frequency peak—Significantly higher values were found in dogs with moderate- and high-intensity murmurs, compared with values in the clinically normal dogs. Additionally, dogs with high-intensity murmurs had higher values than did dogs with low-intensity murmurs. By use of the echocardiographic classification, the first frequency peak was significantly higher in dogs with moderate MR, compared with values in dogs with mild MR. Significantly higher values were also detected in dogs with severe MR, compared with values in dogs with mild or moderate MR (Figure 5).

Murmur energy ratio—Significantly higher murmur energy values were detected in dogs with moderate- and high-intensity murmurs, compared with values in clinically normal dogs. Furthermore, significantly higher values were obtained in dogs with high-intensity murmurs, compared with values in dogs with low-intensity murmurs. By use of the echocardiographic classification, significantly higher murmur energy val-

Table 1—Agreement between the auscultatory and echocardiographic classification systems for assessment of severity of MR in client-owned dogs.

	Echocardiographic classification of MR severity*				
Murmur intensity†	**Clinically normal**	**Mild**	**Moderate**	**Severe**	**Total**
Not detected	5	12	0	0	**17**
Low	0	19	4	0	**23**
Moderate	0	7	11	5	**23**
High	0	0	2	12	**14**
Total	**5**	**38**	**17**	**17**	**77**

*In the echocardiographic classification system, estimation of MR severity was based on the obtained LA:Ao and the MR jet size; dogs were classified as follows: clinically normal (LA:Ao < 1.5 and no MR jet), mild (LA:Ao ≤ 1.5 and MR jet $< 30\%$), moderate (LA:Ao < 1.8 and MR jet $< 50\%$), and severe (LA:Ao ≥ 1.8 and MR jet $\geq 50\%$).†In the auscultatory classification system, dogs were classified into the following murmur groups: normal (no audible cardiac murmur), low intensity (grades 1/6 and 2/6), moderate intensity (grades 3/6 and 4/6), and high intensity (grades 5/6 and 6/6).

Table 2—Summary of signalment and basic echocardiographic variables obtained by use of the echocardiographic classification in 77 client-owned dogs.

	Echocardiographic classification of MR severity*			
Variable	**Clinically normal (n = 5)**	**Mild (n = 38)**	**Moderate (n = 17)**	**Severe (n = 17)**
Sex (female vs male)	4/1	24/14	8/9	5/12
Cavalier King Charles Spaniel (yes vs no)	4/1	32/6	10/7	13/4
Age (y)†	3.73 (3.02 to 6.94)	8.03 (6.01 to 10.20)	10.10 (7.25 to 11.20)	10.30 (8.20 to 11.70)
Body weight (kg)†	8.70 (7.45 to 10.90)	9.30 (8.25 to 10.80)	10.70 (8.28 to 11.50)	11.00 (8.80 to 12.10)
Heart rate (beats/min)†	124 (104 to 164)	106 (97 to 121)	120 (94 to 136)	139 (115 to 150)
LA:Ao†	1.10 (1.09 to 1.16)	1.20 (1.16 to 1.26)	1.53 (1.48 to 1.70)	2.05 (1.97 to 2.35)
LVIDs (mm)†	1.87 (1.75 to 1.96)	2.11 (1.94 to 2.35)	2.12 (2.04 to 2.76)	2.39 (2.05 to 2.60)
$LVIDs_{inc}$ (%)†	–2.49 (–9.45 to 6.34)	6.59 (2.02 to 21.50)	10.80 (3.37 to 21.80)	12.50 (7.03 to 29.40)
LVIDd (mm)†	2.76 (2.62 to 2.81)	3.16 (2.91 to 3.43)	3.67 (3.21 to 4.21)	4.22 (4.05 to 4.77)
$LVIDd_{inc}$ (%)†	–6.86 (–11.20 to –4.97)	5.22 (–1.89 to 11.10)	23.90 (7.05 to 29.40)	36.00 (27.70 to 55.20)
Fractional shortening (%)†	29.5 (26.8 to 37.1)	31.5 (27.7 to 36.7)	37.1 (31.7 to 42.2)	44.9 (43.1 to 47.7)

†Values are reported as median (interquartile range).
See Table 1 for remainder of key.

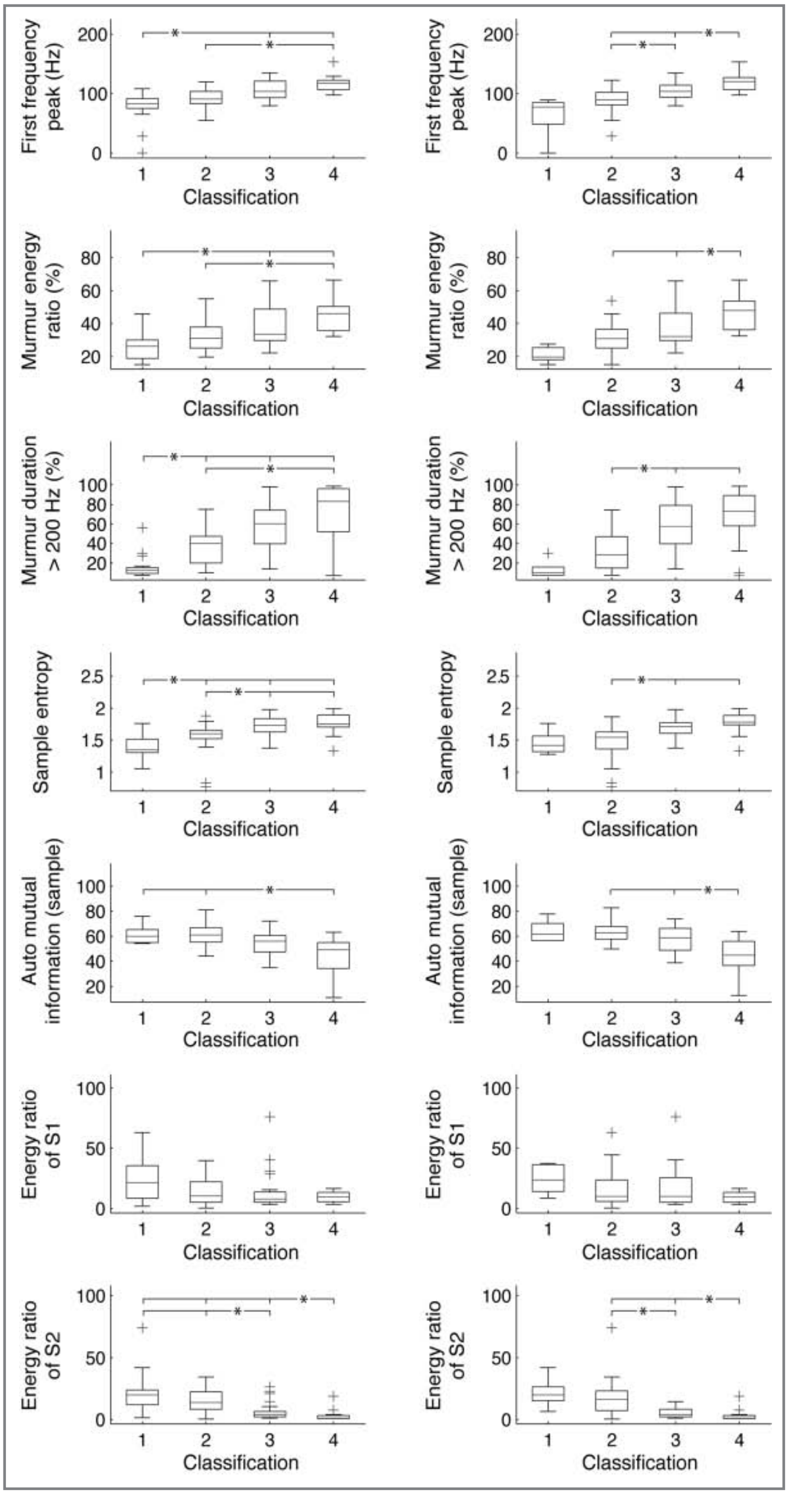

Figure 5—Box-and-whisker plots of 7 sound variables evaluated against the auscultatory (left column) and echocardiographic (right column) classification systems. The upper and lower limits of each box represent the lower quartile and upper quartile values, respectively; the horizontal line within each box represents the median. The whiskers represent the extent of the data (1.5 times the interquartile range). Outliers are indicated (plus signs). The scale for the auscultatory classification system was as follows: 1= no audible murmur; 2 = low-intensity murmur; 3 = moderate-intensity murmur; and 4 = high-intensity murmur. The scale for the echocardiographic classification system was as follows: 1 = clinically normal; 2 = mild MR; 3 = moderate MR; and 4 = severe MR. Because of the low number of dogs in the clinically normal group for the echocardiographic classification system, these dogs were excluded from the multiple groupwise comparison test. Severity groups that differed significantly ($P < 0.008$) are indicated (asterisk).

ues were detected in dogs with severe MR, compared with values in dogs with mild or moderate MR (Figure 5).

Murmur duration > 200 Hz—Significantly higher values were detected in dogs with murmurs, compared with values in clinically normal dogs. Comparing murmur groups, significantly higher values were detected in dogs with high-intensity murmurs than in dogs with low-intensity murmurs. By use of the echocardiographic classification, significantly higher values were detected in dogs with moderate or severe MR, compared with values in dogs with mild MR (Figure 5).

Sample entropy—Significantly higher values were detected in dogs with murmurs, compared with values in clinically normal dogs. Comparing dogs with differing severity of murmurs, significantly higher values were detected in dogs with moderate- or high-intensity murmurs, compared with values in dogs with low-intensity murmurs. By use of the echocardiographic classification, significantly higher values were detected in dogs with moderate or severe MR, compared with values in dogs with mild MR (Figure 5).

Auto mutual information function—Significantly lower values were detected in dogs with high-intensity murmurs, compared with values in clinically normal dogs and dogs with low-intensity murmurs. By use of the echocardiographic classification, auto mutual information was significantly lower in dogs with severe MR, compared with values in dogs with mild or moderate MR (Figure 5).

Energy ratio of S1—The energy ratio of S1 did not differ significantly among groups with differing MR severity (Figure 5).

Energy ratio of S2—Analysis of the energy ratio of S2 revealed significantly lower values in dogs with moderate-intensity murmurs, compared with values in clinically normal dogs and dogs with low-intensity murmurs. Additionally, dogs with high-intensity murmurs had a lower energy ratio of S2, compared with values in dogs in the other severity groups. By use of the echocardiographic classification, the energy ratio of S2 was significantly lower in dogs with moderate MR, compared with values in dogs with mild MR. Dogs with severe MR had a lower energy ratio of S2, compared with values in dogs with mild or moderate MR (Figure 5).

Multiple regression analysis—The 7 sound variables were dependent variables, and signalment variables (age, sex, breed,

Table 3—Summary of prediction equations obtained from the backward stepwise multiple regression analysis that used echocardiographic variables as independent variables to predict each of the sound variables and that included the LA:Ao and heart rate obtained from the echocardiographic examination and signalment variables (sex, age, breed, and body weight) in the initial model.

Sound variable	Prediction equation	Adjusted R^2 of model	*P* value of model*
First frequency peak (Hz)	$(70.7 - [0.24 \times HR]) + (36.7 \times LA{:}Ao)$	0.40	< 0.001
Murmur energy ratio (%)	$10 + (17 \times LA{:}Ao)$	0.34	< 0.001
Murmur duration > 200 Hz (%)	$-11 + (37 \times LA{:}Ao)$	0.30	< 0.001
Sample entropy (arbitrary units)	$1.2 + (0.27 \times LA{:}Ao)$	0.23	< 0.001
Auto mutual information (arbitrary units)	$83.1 - (18.2 \times LA{:}Ao)$	0.36	< 0.001
Energy ratio of S1	$26.2 - (7.49 \times LA{:}Ao)$	0.04	0.046
Energy ratio of S2	$32.7 - (14.1 \times LA{:}Ao)$	0.24	< 0.001

*Values were considered significant at $P < 0.05$.
HR = Heart rate.

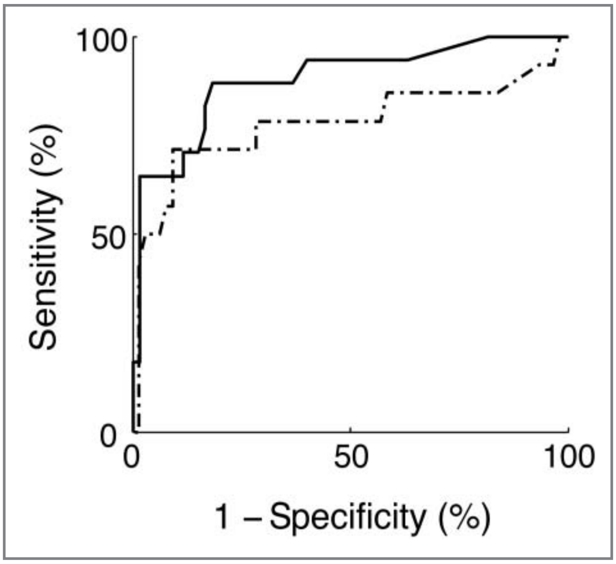

Figure 6—The ROC curves obtained by use of linear discriminant analysis that reveals the diagnostic efficacy for separating severe MR from other disease-severity groups. The 2 curves correspond to results obtained by use of the echocardiographic (solid line) and auscultatory (dotted and dashed line) classification systems, respectively. The energy ratio of S2 and murmur duration > 200 Hz were the optimal combination of sound variables by use of the auscultatory classification. Corresponding variables for the echocardiographic classification were the auto mutual information function, first frequency peak, and energy ratio of S2.

and body weight), heart rate, and LA:Ao were independent variables. Analyses confirmed a major effect of LA:Ao on the sound variables but revealed no effect of any of the signalment variables. The first frequency peak was the sound variable with the highest model R^2 (**Table 3**).

Linear discriminant analysis—The energy ratio of S2 and murmur duration > 200 Hz were the optimal combination of variables for distinguishing dogs with a high-intensity murmur from dogs of the other severity groups. Corresponding variables for the echocardiographic classification were the selection of auto mutual information, first frequency peak, and energy ratio of S2. These optimal variable sets for distinguishing severe MR from the other severity groups resulted in sensitivity of 79%, specificity of 71%, and AUC of 0.78 for the auscultatory classification and sensitivity of 88%, specificity of 82%, and AUC of 0.89 for the echocardiographic classification (**Figure 6**).

Discussion

Most of the sound variables were significantly associated with the severity of MR. A higher severity of MR resulted in greater contribution of higher frequencies, increased signal irregularity, and decreased energy ratio of S2. Hence, the results revealed a diagnostic potential of signal analysis techniques for monitoring dogs with MMVD diagnosed by use of echocardiography until a high-intensity murmur has been detected or overt signs of congestive heart failure have developed. To our knowledge, this is the first study to indicate that sound variables, which were obtained by linear and nonlinear signal analysis, can be related to the severity of MR.

The audible characteristics of a sound depend on its absolute intensity as well as its frequency content.[34] Because frequency content is extremely important for auscultation, 3 techniques were developed to quantify frequency properties. The first frequency peak basically represents the dominant frequency component in the signal and roughly corresponds to the harshness of the sound.[a] Analysis of our results indicated that the first frequency peak, and consequently the frequency spectrum, was shifted toward higher frequencies with an increase in MR severity. The murmur energy ratio quantifies the amount of frequency components above 50 Hz in the frequency spectrum. More severe MR resulted in a murmur with a harsher quality. On the basis of the results, the increased harshness originated from an intensification of frequencies exceeding 50 Hz. With increasing severity of MMVD, the murmur shifts from an early- or late-systolic murmur to a holosystolic murmur,[13] a transition probably reflected in the murmur duration > 200 Hz. Overall, the study reported here revealed that murmurs in dogs with moderate and severe MR contained a higher proportion of high-frequency sounds. Because the frequency content is important for how a murmur is perceived, it may be debated whether the established murmur intensity denomination is a proper term for assessment of murmur severity. It is possible that murmur audibility is in better accord with the actual auscultatory findings.

The cardiac sound signal contains nonlinear information,[35–37] which is not revealed by frequency analysis. Assuming that the recorded sound originates from a dynamic system and that the state of the system gradually changes with MR severity, nonlinear invariant measures can be used to estimate the current disease state. A commonly used measure for such system characterization is the correlation dimension, which requires long-duration stationary recordings with a high signal-to-noise ratio.[38] Because biological data seldom fulfill these requirements, a method called sample entropy was developed.[30] Sample entropy, which is applicable to fairly short-duration data sequences, can be interpreted as a complexity measure in which higher values indicate a more irregular sound signal. In theory, a highly disturbed blood flow will generate a more complex sound signal and consequently a higher sample entropy value. The validity of this hypothesis is strengthened by the results of the study reported here. A complementary complexity measure is the first minimum of the auto mutual information function. Although sample entropy is a statistic that quantifies the regularity in the data, auto mutual information detects nonlinear dependencies in time series. Lower values of auto mutual information were detected in dogs with severe MR, which reflected that the mean predictability decreases with increasing signal complexity. It should be mentioned that the nonlinear measures used in this study are unable to provide absolute information about the state of the system. However, relative differences in the derived quantities are useful for system characterization, as indicated by the results obtained.

During auscultation, S1 is concurrent with the closure of the atrioventricular valves, whereas S2 is attributed to the closure of the semilunar valves. Disagreement still exists whether heart sounds are caused by transient vibrations arising when the valves come to a sudden halt at the end of coaptation or whether they are created by vibrations in the entire cardiac structure. Most likely, the origin of the heart sounds is best described by a combination of these theories.[17] Studies[11,39] in which investigators evaluated heart sounds in dogs with MR have been conducted by use of subjective assessment of the auscultatory findings or estimating the S1-to-S2 ratio determined by analysis of the PCG. We are not aware of any other studies in which researchers used signal analysis techniques to investigate potential alterations of the heart sounds in dogs with MR. Unfortunately, it is not possible to measure an absolute sound intensity because all recordings depend on chest size, skin thickness, and the interface between the skin and stethoscope.[32] One approach to circumvent this problem is to measure the amount of noise during diastole (in the absence of diastolic murmurs) and standardize the energy in S1 and S2 against the amount of diastolic energy.[32] We considered this an appropriate approach because none of the dogs in our study had diastolic murmurs. In dogs with MR, S1 is reported to progressively increase in intensity,[39] possibly indicating chronic volume overload and relatively well-maintained left ventricular contractility. Remarkably, our study determined that the energy ratio of S1 significantly decreased with increasing murmur intensity. By use of the echocardiographic classification system, the energy ratio of S1 was not significantly associated with the severity of MR. These rather unexpected findings may be explained by a potential reduction in ventricular contractility in advanced disease.[40]

Another possible mechanism is that a degenerative insufficient valve may influence vibrations involved in the origin of S1. The energy ratio of S2 decreased with increasing MR severity, which is in accordance with results of another study[11] in dogs with MMVD. The intensity of S2 is primarily dependent on the rate of change in the pressure gradient across the aortic valve.[41] Dogs with severe MR may eject > 75% of the total stroke volume into the left atrium.[42] Thus, the diminished forward stroke volume may explain the decreased energy ratio of S2 detected in dogs with increasing severity of MR. Furthermore, altered anatomic topography as a result of cardiomegaly may influence the propagation of heart sounds through the thoracic system.

The distribution of dogs in each severity group, especially the clinically normal and mild groups, varied between the auscultatory and echocardiographic classification systems. Several dogs without audible murmurs during auscultation were echocardiographically classified as having mild MR. However, these dogs had minimal leakages, which were likely incapable of inducing audible murmurs. The existence of silent regurgitation murmurs has been reported.[12] Thus, lack of an audible murmur cannot rule out mild regurgitation. Furthermore, the human auditory system may be unable to detect a faint murmur because of the typical intensity of normal heart sounds.[43] In addition, there is a problem in interpretation of small MR jets on color echocardiograms. Some are likely to represent early stages of MMVD, whereas others may be trivial nonpathologic jets.[44,45] To our knowledge, there is no consensus regarding differentiation between these 2 conditions. Therefore, the various sound variables were evaluated by use of both the auscultatory and echocardiographic classification systems. A slightly different approach for the echocardiographic classification would have been to allocate dogs with minimal MR to the same group as the clinically normal dogs, which was performed in another report.[13] However, a minimal jet may create changes in the sound signal, which could influence the results when grouping clinically normal dogs with dogs that have a minimal jet; thus, these groups were not combined. Because of the low number of clinically normal dogs determined by use of the echocardiographic classification system, these dogs were excluded from the multiple comparison test between the groups but not from the overall statistical analysis. Results from the multiple regression analysis, which were determined by use of the continuous variables LA:Ao and heart rate obtained from the echocardiographic examination, validated the use of a grouping system for classification of MR severity. This analysis clearly indicated the effect of MR severity on sound variables. Because of collinearity between the echocardiographic variables,[46] only the LA:Ao was included in the multiple regression analysis. The LA:Ao is considered the most reliable indicator of MR severity in dogs, and this variable has a high prognostic value.[25,42,47] However, because of the fact that the LA:Ao usually is within the reference range for clinically normal dogs during early stages of the disease, an improved separation of clinically normal dogs from dogs with mild MR was obtained by including a regurgitation assessment.

The signal analysis techniques reported here could be valuable for use by clinicians when performing risk assessment or deciding whether special care and more extensive examinations are required. Hence, correct identification of severe MR would be of particular interest. The first frequency peak, murmur energy ratio, auto mutual information, and energy ratio of S2 were the most appropriate variables for this task (Figure 5). These observations (except for the energy ratio of S2) are also supported by the adjusted R^2 values from the multiple regression analysis. To optimize the classification performance, it is possible to combine several sound variables. In the study reported here, this data fusion step was performed by use of a leave-1-out linear discriminant analysis. However, including too many variables in the model will lead to overfitting, which means that the model is consistent with training examples but disagrees on unseen data. It should also be mentioned that the reported classification scores are results obtained from the variable selection process. To properly verify the model equations and assess the classification abilities in the derived variable set, evaluations based on an additional data set are required.

Our study had some limitations. Recorded cardiac acoustic signals can be obscured by respiratory sounds, rumbling sounds from the stomach, friction rubs, and ambient sounds. Furthermore, an increase in stimulation of the sympathetic nervous system as a consequence of the recording procedures may have affected the characteristics of a murmur.[13,15] Time segmentation of various components of the cardiac cycle is a crucial preprocessing step to facilitate the extraction of sound variables. In some instances, difficulties were encountered when separating the end points of S1 from the early part of the murmur. Similarly, low-intensity S2 was sometimes not clearly detectable because of holosystolic murmurs. This overlap between heart sounds and murmurs, particularly for dogs with severe MR, leads to a risk of eliminating early and late components of systole. Signal segments were omitted in dogs with uncertain status, and sound files that were difficult to segment were excluded from the study, which resulted in an exclusion of 5 dogs. The reproducibility of PCG recordings in dogs with MR remains to be investigated. Finally, future multicenter or multiobserver studies should be performed to investigate the validity of this method in a general practice environment.

Most of the investigated sound variables were dependent on MR severity, which indicated a powerful diagnostic potential. Advances in the technical devices that make the technique clinically applicable could provide a valuable complement to traditional cardiac auscultation and may offer a simple and cost-effective method for monitoring dogs with MMVD diagnosed by use of echocardiography until a high-intensity murmur has been diagnosed or overt signs of congestive heart failure have developed.

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a. El-Segaier M. *Digital analysis of cardiac acoustic signals in children*. PhD dissertation, Division of Paediatric Cardiology, Department of Paediatrics, Lund University Hospital, Lund, Sweden, 2007.

b. Welch Allyn Meditron ASA, Medi-Stim ASA, Oslo, Norway.

c. Dell Latitude D800 laptop, Dell Computer Corp, Limerick, Ireland.

d. Meditron analyzer, version 4.0V, Welch Allyn Meditron ASA, Medi-Stim ASA, Oslo, Norway.

e. GE Vivid 3 ultrasound machine, General Electric Co, Stockholm, Sweden.

f. MATLAB, version 7.3, The MathWorks Inc, Natick, Mass.

g. JMP, version 6.0.0, SAS Institute Inc, Cary, NC.

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