Evaluation of liver and spleen stiffness of healthy dogs by use of two-dimensional shear wave elastography

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Received April 12, 2018.
Accepted September 10, 2018.

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OBJECTIVE
To assess liver and spleen stiffness in healthy dogs by use of a novel 2-D shear wave elastography (SWE) technique and to investigate the repeatability and reproducibility of the technique.

ANIMALS
8 healthy adult Beagles.

PROCEDURES
2-D SWE was performed on each dog to assess liver and spleen stiffness. Repeatability (intraday variability) and reproducibility (interday variability) of 2-D SWE were investigated. For all 8 dogs, 2-D SWE was performed 3 times in 1 day (4-hour intervals) and on 3 separate days (1-week interval). Data were expressed as mean ± SD values for shear wave velocity and the Young modulus in the liver and spleen. Intraday and interday coefficients of variation were assessed for all variables.

RESULTS
Mean ± SD shear wave velocity obtained for the liver and spleen was 1.51 ± 0.08 m/s and 2.18 ± 0.27 m/s, respectively. Mean value for the Young modulus obtained for the liver and spleen was 6.93 ± 0.79 kPa and 14.66 ± 3.79 kPa, respectively. Elasticity values were significantly higher for the spleen than for the liver. Intraday and interday coefficients of variation for all variables were < 25% (range, 3.90% to 20.70%).

CONCLUSIONS AND CLINICAL RELEVANCE
2-D SWE was a feasible technique for assessing liver and spleen stiffness of healthy dogs. Future studies on the application of 2-D SWE for dogs with chronic hepatitis, cirrhosis, and portal hypertension are needed to evaluate the clinical applicability of 2-D SWE. (Am J Vet Res 2019;80:378–384)

Chronic hepatitis is a common liver disease in dogs and is histologically defined by the presence of hepatocellular apoptosis or necrosis, inflammatory cell infiltration, and fibrosis. Cirrhosis is the end stage of chronic hepatitis, and the progression of chronic hepatitis leads to portal hypertension as a result of fibrosis, increased resistance to blood flow, or increased blood flow in the portal circulation. The clinical consequences of portal hypertension include acquired portosystemic collateral vessels, ascites, hepatic encephalopathy, and coagulopathy. Conventional methods for the diagnosis of chronic hepatitis include serum biochemical analysis (eg, liver enzyme activity) and conventional abdominal ultrasonography. Although these methods provide necessary information for the diagnosis of chronic hepatitis of dogs, they do not necessarily reflect severity of the disease. Histologic examination of liver tissues currently is the criterion-referenced standard for the diagnosis and assessment of the severity of chronic hepatitis. The histologic severity of chronic hepatitis is mainly evaluated on the basis of the extent of fibrosis and degree of inflammation. However, collecting liver biopsy specimens is associated with a number of limitations, including the risk of hemorrhage and anesthetic complications, invasiveness of the procedure, and possibility of sampling errors. Furthermore, repeated liver biopsies are required to assess the progression of disease and efficacy of treatment, but serial biopsies are rarely performed on dogs. Therefore, a noninvasive alternative to liver biopsy is desirable in veterinary medicine.

Although examination of liver biopsy specimens remains the criterion-referenced standard for assessing liver fibrosis in humans, alternative noninvasive and repeatable methods have been extensively evaluated. A promising method is ultrasonographic elastography, which is a relatively recent imaging technique that can be used to noninvasively evaluate tissue stiffness. Similar to conventional B-mode ultrasonography, elastography is noninvasive, does not involve radiation, and can be easily learned. Two major categories of elastography techniques are currently widely used in clinical settings for humans.
Strain imaging is an elastography technique in which stress is applied by external compression with an ultrasound transducer or internal physiologic pulsation, such as a heartbeat or respiration. Strain imaging is used to measure the amount of lesion deformation relative to the surrounding normal tissue; it involves use of a color display and provides qualitative or semiquantitative evaluations. In veterinary medicine, strain imaging has been performed on the liver, spleen, and kidneys of clinically normal dogs and cats. However, strain imaging cannot be used to quantitatively evaluate tissue stiffness.

A more recently developed and more quantitative elastography technique is shear wave imaging, which overcomes the limitation of strain imaging and consists of ultrasonographic-based transient elastography and SWE. Shear wave imaging involves the use of dynamic stress to generate shear waves in parallel or perpendicular dimensions. Measurements of the shear wave propagation speed result in qualitative and quantitative estimates of tissue elasticity. Furthermore, SWE by use of ARFI has been developed. For SWE, shear wave propagation speed is measured within tissues and allows tissue stiffness to be evaluated as shear wave velocity (reported in meters per second) or the Young modulus (reported in kilopascals). Two types of SWE are currently used in human medicine: point SWE and 2-D SWE. Point SWE is used to measure tissue stiffness with the shear wave generated from a fairly limited area of an ROI, which needs to be in a homogeneous area. In contrast, 2-D SWE generates a 2-D image of stiffness over a larger region of tissue. An arbitrarily sized ROI may be used in 2-D SWE. Furthermore, 2-D SWE provides 2-D color velocity maps and also has the advantage of the placement of arbitrary ROIs during real-time imaging.

Chronic liver damage results in an increase in the extracellular matrix produced by fibroblast-like cells, and the progression of liver fibrosis leads to stiffer liver parenchyma. Additionally, portal hypertension, which is caused by fibrotic hepatopathies, often results in splenomegaly and a stiffer spleen in humans. Severity of liver fibrosis and portal hypertension may be evaluated by the quantification liver and spleen stiffness by use of 2-D SWE in human patients with chronic liver disease. In contrast to the literature for humans, the usefulness of shear wave imaging in veterinary medicine has been reported in only a few studies.

Assessments of repeatability (intraday variability) and reproducibility (interday variability) of 2-D SWE in the evaluation of liver and spleen stiffness are important prerequisites before 2-D SWE can be used in clinical settings. Therefore, the objective of the study reported here was to evaluate the applicability, repeatability, and reproducibility of 2-D SWE of the liver and spleen in healthy dogs. Furthermore, results of the study could provide reference values for tissue stiffness that might be used in the evaluation of dogs with liver and splenic diseases.

**Materials and Methods**

**Animals**

Eight healthy Beagles (5 sexually intact females and 3 sexually intact males) were included in the study. Dogs were between 1 and 4 years of age, and body weight was between 9.7 and 15 kg. All dogs were confirmed to be healthy on the basis of results of a physical examination, CBC, serum biochemical analysis, electrocardiography, and abdominal ultrasonography. All animal experimental procedures were conducted in accordance with standard protocols of the institutional animal experimental committee reviewed by the Association for Assessment and Accreditation of Laboratory Animal Care International. All animal experiments were approved by the Animal Experimentation Committee of the Graduate School of Veterinary Medicine at Hokkaido University (accession No. 16-0094).

**Measurement of 2-D SWE**

Food was withheld from dogs for 12 hours, and conventional B-mode ultrasonography and 2-D SWE then were performed with an ultrasound scanner. Only 1 ultrasonographer (MT) performed examinations to ensure consistent imaging conditions throughout the study. Conventional B-mode ultrasonography with a 7.0-MHz convex transducer was performed before 2-D SWE; it was used for general imaging of the liver and spleen. The ultrasonographer performed 2-D SWE with a 3.5-MHz convex transducer 3 times in 1 day (4-hour intervals) and on 3 separate days (1-week interval). Dogs were not sedated for ultrasonography or 2-D SWE.

In accordance with recommended guidelines for the clinical use of elastography in humans and results of studies on dogs, all dogs were initially positioned in left lateral recumbency for imaging of the right lobe of the liver. The probe was placed parallel to and within the intercostal space with a sufficient amount of gel to minimize rib shadowing. The probe was positioned to acquire images of the parenchyma of the right lobe of the liver during visual assessment in 2-D B-mode ultrasonography; measurements were obtained at depths of up to 45 mm. All dogs were positioned in dorsal recumbency for acquisition of spleen images. The probe was maintained perpendicular to the curve of the body surface, and measurements were obtained in the parenchyma of the main portion of the spleen at depths of up to 35 mm. The 2-D SWE of the liver and spleen was performed during the end-expiratory phase or respiration to minimize effects of respiratory motion.

The display mode could be changed among 3 options after data acquisition: speed mode, elasticity mode, and propagation mode (Figure 1). The speed mode displayed shear wave velocity with a map (range, 0.5 to 6.5 m/s). In contrast, the elasticity mode displayed the Young modulus with another map (range, 0 to 120 kPa). Both maps were obtained so that we could display distributions of shear wave...
propagation speed in semitransparent 2-D color images, which was overlaid on the B-mode image. Shear wave velocity and the Young modulus were displayed as a gradation of colors on the 2 maps, with increasing stiffness in an ascending order of blue, green, yellow, and red. Regions that were not color coded on elasticity images indicated absence of a shear wave.

The propagation mode could be used to provide guidance to evaluate whether ROI placement was accurate in the speed or elasticity mode. A tissue ROI generally needs to be placed in an area with parallel contour lines. The ROI was set at 8 to 10 mm in diameter and excluded regions that were not color coded. The ROI was positioned in the parenchyma of the right lobe approximately 10 mm deep to the liver capsule and in the parenchyma of the spleen approximately 5 mm deep to the spleen capsule (Figure 2). At least 10 valid measurements were obtained for each dog. Mean values of shear wave velocity and the Young modulus were considered representative of elasticity values in the liver and spleen.

To compare liver stiffness between the right and left lobes of the liver, the left lobe of the liver of each dog was evaluated by use of 2-D SWE. All dogs were positioned in right lateral recumbency for imaging of the left lobe of the liver, and liver stiffness was measured by use of the same protocol used for the right lobe of the liver. Assessment with 2-D SWE was performed 3 times in 1 day (4-hour intervals).

**Statistical analysis**

All data of continuous variables were expressed as mean ± SD. Statistical analyses were performed with commercially available software. A fixed-effect linear model was used to analyze intraday and interday variabilities as follows:

$$Y_{ijk} = \mu + \text{dog}_i + \text{day}_j + (\text{dog} \times \text{day})_{ij} + \varepsilon_{ijk}$$

where $$Y_{ijk}$$ is the first value measured for dog $$i$$ on day $$j$$, $$\mu$$ is the overall mean, $$\text{dog}_i$$ is the differential effect of dog $$i$$, $$\text{day}_j$$ is the differential effect of day $$j$$, $(\text{dog} \times \text{day})_{ij}$ is the interaction between dog $$i$$ and day $$j$$, and $$\varepsilon_{ijk}$$ is the model error. The SD of intraday variability was estimated as the residual SD of the model, and the SD of interday variability was estimated as the SD of the differential effect of day. The CV was obtained by dividing each SD by the mean. A small CV indicates a highly reliable measurement, and a CV < 30% was considered clinically acceptable on the basis of a study on SWE in humans. The 95% CI was calculated by multiplying SD by 2.069. The CIs provided an estimated range of values that were likely to include an unknown population parameter from the data obtained, and they represented a probability of < 0.05 that a true change for an individual dog would be detected. Comparison between the right lobe of the liver and spleen stiffness was conducted by use of a linear mixed model, with the measurement number (6 measurements) for each dog, organ (right lobe of the liver and spleen), and interaction term as categorical fixed effects and dog as a fixed effect. An F test was performed to assess effects of measurement number and organ. A t test was performed to assess significant differences between organs. Bland-Altman analysis with modifications for repeated measures was performed to assess the agreement of liver stiffness between the right and left lobes of the liver. Mean of the difference (bias) and the 95% CI for bias were calculated. The 95% CI for bias was compared with 0 and was consid-

![Figure 1](image-url) — Images of the right lobe of the liver of a dog for the speed mode (A and B) and elasticity mode (C and D) of 2-D SWE. Images for the speed mode are the shear wave velocity map (A) and map for the propagation mode (B); images for the elasticity mode are the shear wave elasticity map (C) and map for the propagation mode (D). Notice the consistent parallel contour lines for the speed and elasticity modes. Tick marks on the side of the images are at intervals of 5 mm.
erred significant when it did not contain 0. The 95% limits of agreement (mean of the difference ± 1.96 X SD) were also calculated. For all statistical comparisons, values of $P < 0.05$ were considered significant.

**Results**

**Assessment of repeatability and reproducibility**

The 2-D SWE was successfully performed on the liver and spleen of all 8 dogs. All variables were summarized (Table 1). There was high repeatability and reproducibility (ie, low CVs) for 2-D SWE of the right lobe of the liver. Intraday and interday CVs for shear wave velocity in the right lobe of the liver were 3.90% and 4.60%, respectively, whereas intraday and interday CVs for the Young modulus in the right lobe of the liver were 8.70% and 10.00%, respectively. There was also high repeatability and reproducibility for 2-D SWE of the spleen. Intraday and interday CVs for shear wave velocity in the spleen were 8.00% and 6.10%, respectively, whereas intraday and interday CVs for the Young modulus in the spleen were 20.70% and 12.20%, respectively.

**2-D SWE analysis between the right lobe of the liver and spleen**

Mean ± SD values for 2-D SWE of the right lobe of the liver were 1.51 ± 0.08 m/s and 6.93 ± 0.79 kPa. Mean values for 2-D SWE of the spleen were 2.18 ± 0.27 m/s and 14.66 ± 3.79 kPa. Stiffness was compared between the right lobe of the liver and spleen by use of a linear mixed model and reported as the least squares mean and 95% CI (Figure 3). Results of 2-D SWE for both shear wave velocity and the Young modulus were significantly ($P < 0.001$) higher in the spleen than in the right lobe of the liver.

![Figure 2](image)

**Figure 2**—Images of the right lobe of the liver (A and B) and spleen (C and D) of a dog used to measure stiffness for the speed mode of 2-D SWE. Stiffness was measured as shear wave velocity (meters per second) in the parenchyma of the right lobe of the liver and as the Young modulus (kilopascals) in the parenchyma of the spleen. Notice the ROIs (T1, T2, and T3; 10 mm) used for the measurements (circles).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Intraday</th>
<th>Interday</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CI*</td>
<td>SD†</td>
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<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Shear wave velocity (m/s)</td>
<td>1.51 ± 0.08</td>
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<tr>
<td>Young modulus (kPa)</td>
<td>6.93 ± 0.79</td>
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<td>Spleen</td>
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<tr>
<td>Shear wave velocity (m/s)</td>
<td>2.18 ± 0.27</td>
<td>0.62</td>
<td>0.30</td>
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<tr>
<td>Young modulus (kPa)</td>
<td>14.66 ± 3.79</td>
<td>8.83</td>
<td>4.27</td>
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</tbody>
</table>

For all 8 dogs, 2-D SWE was performed 3 times in 1 day (4-hour intervals) to determine intraday CV and on 3 separate days (1-week interval) to determine interday CV.

*Calculated as SD X 2.069. †Estimated as the residual SD of the model. ‡Estimated as the SD of the differential effect of day.
Comparison between the right and left lobes of the liver

Stiffness was compared between the right and left lobes of the liver. Mean ± SD values for 2-D SWE of the left lobe of the liver lobe were 1.42 ± 0.07 m/s and 6.02 ± 0.61 kPa. Results of Bland-Altman analysis with modifications for repeated measures were graphically illustrated (Figure 4). Mean bias for 2-D SWE between the right and left lobes of the liver by use of shear wave velocity was 0.09 m/s (95% CI, 0.02 to 0.17 m/s). Mean bias for 2-D SWE between the right and left lobes of the liver by use of the Young modulus was 0.83 kPa (95% CI, 0.15 to 1.51 kPa). Elasticity values for both the shear wave velocity and Young modulus were significantly higher in the right lobe of the liver than in the left lobe of the liver.

Discussion

The objective of the present study was to evaluate the applicability, repeatability, and reproducibility of assessment of liver and spleen stiffness in healthy dogs by use of 2-D SWE. The goal was the application of 2-D SWE as a noninvasive method to assess liver fibrosis and predict the onset or severity of portal hypertension in dogs with hepatic diseases.

Histologic examination of liver biopsy specimens is the criterion-referenced method for diagnosis and monitoring of chronic hepatitis and cirrhosis, but it is sometimes difficult to obtain biopsy specimens because of a patient's poor condition and complications associated with the procedure. For humans, investigators of many studies have reported that shear wave imaging is an alternative noninvasive diagnostic modality to liver biopsy and is useful in noninvasively assessing the severity of liver fibrosis through evaluation of liver stiffness. Although the cause of chronic hepatitis in dogs currently remains unknown, some histopathologic features of chronic hepatitis in dogs are similar to those of humans with chronic hepatitis, such as mixed inflammatory cell infiltration and fibrosis. The usefulness of shear wave imaging for the diagnosis of liver fibrosis has been investigated in veterinary medicine. Investigators of 1 study reported that transient elastography is applicable for dogs with experimentally induced hepatic disease. Those authors concluded that the best measurements of liver stiffness are obtained in the right lobe of the liver with a dog positioned in left lateral recumbency. However, transient elastography, which is a 1-D modality, cannot display an anatomic B-mode image, and its performance is inferior to techniques that involve the use of ARFI. Investigators reported that transient elastography is less accurate than 2-D SWE for the assessment of severe fibrosis in humans with chronic hepatitis C. In another study that involved the use of 2-D SWE, liver stiffness obtained in the right lobe of the liver was positively correlated with the stage of liver fibrosis in dogs with CCI4-induced liver fibrosis. However, 2-D SWE was performed on anesthetized dogs in that study, which did not provide reference values for liver stiffness in healthy dogs. In accordance with the recommended guidelines for the clinical use of elastography in humans and results for studies of dogs, we initially evaluated the feasibility of 2-D SWE for use on the abdomen of conscious dogs, including assessing repeatability and reproducibility of measurements, and also obtained reference values for tissue stiffness for both the shear wave velocity and Young modulus of the right lobe of the liver and spleen in healthy dogs.

Mean shear wave velocity for the right lobe of the liver of the healthy dogs of the present study was 1.51 m/s, which is similar to findings obtained by use of 2-D SWE for the right lobe of the liver of healthy humans (1.4 m/s). The liver is a highly vascular organ with large-diameter capillaries lined by endothelial cells between rows of plates or cords of he-
patocytes. The sinusoids also contain Kupffer cells of the reticuloendothelial system. This arrangement imparts relative softness to the liver.

Results obtained in the study reported here indicated that shear wave velocity of 2-D SWE was highly repeatable and reproducible in conscious dogs; the CV for all variables was <10%. We speculated that the clinically acceptable high repeatability and reproducibility of shear wave velocity of 2-D SWE in the present study relied on the proper propagation mode, which is a component of the novel technology for 2-D SWE. The proper propagation mode was displayed as arrival time contours with the shape of contour lines. This mode allowed operators to preliminarily confirm whether shear waves propagated as expected and verify the reliability of the data obtained. When the contour lines were nearly straight and consistently parallel to each other, reliability of the data was high. In contrast, when the contour lines were irregularly distorted and chaotic, the reliability of data was low. When the latter was encountered, elastography had to be repeated to yield reliable results. Because shear wave propagation speed becomes faster as tissue stiffness increases, the distance between contour lines is wider (ie, blue to green) in regions with greater tissue stiffness and narrower (ie, blue to red) in regions with lower tissue stiffness. The reliability of data may be verified by examining the propagation map. In diffuse liver disease, the shear wave propagation speed does not change, and the intervals between the contour lines are constant. Thus, use of this novel technology may allow objective assessment of the reliability of data simply by the examination of contour lines and may enable investigators to select suitable areas for measurements of shear wave propagation speed.

On the other hand, intraday and interday CVs were higher for the Young modulus than for the shear wave velocity of 2-D SWE and indicated moderate repeatability and reproducibility. The stiffness of tissue may be estimated and expressed in kilopascals by a physical quantity called the modulus of elasticity (ie, the Young modulus), which is defined as the ratio of the applied stress to the introduced strain from the Hooke law. However, accurate data on the modulus of elasticity have not yet been obtained because it cannot directly measure applied stress. On the other hand, shear wave propagation speed may be calculated as the displacement of localized tissue under short-duration ARFI; therefore, SWE may be used to measure the elasticity of tissue by use of the following formula: $E = \frac{3\rho c_s^2}{\rho}$, where $E$ is tissue stiffness measured in kilopascals, $\rho$ is density of the tissue, and $c_s$ is shear wave velocity measured in meters per second. By use of this principle, shear wave velocity of 2-D SWE is proportionally related to $E$, and it also offers quantitative elastic information. The value for $E$ may be calculated by use of the aforementioned formula when the density of tissue is the same as that of water (ie, 1 g/mL); however, soft tissues are inherently nonlinear, viscoelastic, and heterogeneous. In addition, because $E$ is calculated by use of the value for shear wave velocity, we speculated that all CVs were higher for the Young modulus of 2-D SWE than for the shear wave velocity of 2-D SWE. Authors of a recent report advocate the reporting of findings for humans as the shear wave velocity as part of a standardized approach to enable comparison among modalities and machines. Results of the present study also supported the use of shear wave velocity of 2-D SWE from the aspect of repeatability and reproducibility.

Stiffness of the spleen also was evaluated in the present study. The spleen is a good target for the assessment of stiffness by ultrasonographic elastography. There is a change in spleen stiffness with portal hypertension because human patients commonly have splenomegaly secondary to portal hypertension. Spleen stiffness measured by use of transient elastography is increased in patients with hepatitis C virus–induced cirrhosis and portal hypertension, and transient elastography values may be used to predict the onset or severity of portal hypertension. On the other hand, splenomegaly is a rare finding in dogs with portal hypertension. However, splenic congestion may occur secondary to portal hypertension and lead to an increase in spleen stiffness. Thus, similar to the situation for humans, evaluations of spleen stiffness may predict the onset or severity of portal hypertension in dogs with chronic hepatitis and cirrhosis. The value of $E$ for the spleen of healthy dogs in the present study was similar to that in the spleen of healthy humans. Anatomically, the spleen comprises the parenchyma, including red and white pulp, and also fibroelastic supporting tissue that forms the capsule, trabeculae, and a fine reticulum. This arrangement causes the spleen to be a relatively stiff organ. Elasticity values for both the shear wave velocity and Young modulus were significantly higher in the spleen than in the right lobe of the liver of the dogs of the present study.

In the present study, stiffness in the left lobe of the liver was measured to enable us to evaluate the feasibility of this technique for assessment of the left lobe of the liver and to compare liver stiffness between the right and left lobes of the liver. Values for both the shear wave velocity and Young modulus were significantly higher in the right lobe of the liver than in the left lobe of the liver. Studies of humans have revealed differences in liver stiffness between sides, and those investigators suggested that imaging of the left lobe in humans is often influenced by the movement of body organs, such as the heart, lungs, diaphragm, and stomach. Furthermore, findings of that study indicate that ARFI elastography of the right lobe of the liver is more accurate for diagnosing liver fibrosis in humans than is ARFI elastography of the left lobe of the liver. However, that type of study has not been performed in veterinary medicine. Although 2-D SWE can be used to assess liver stiffness in the right and left lobes of the liver of...
dogs, differences in tissue stiffness between the right and left lobes of the liver should be expected, and the same lobe of the liver should be evaluated throughout follow-up assessments in dogs.

The present study had several limitations. Cyto logic or histologic examinations of the liver of the dogs were not performed. Thus, we cannot completely exclude the possibility of the existence of liver disease. However, the laboratory Beagles used in the present study had no clinical signs of illness or abnormalities at the time physical examination, clinicopathologic examinations (including measurement of fasting and postprandial total bile acid concentrations), and abdominal ultrasonography were performed for the study. Additionally, occult disease was ruled out on the basis of follow-up examinations conducted for 6 months. Furthermore, all dogs used in the study were Beagles. Additional studies with smaller and larger breeds are needed to characterize liver and spleen stiffness in dogs of various body sizes.

Results of the present study indicated that intraday and interday CVs for elasticity values obtained for healthy conscious dogs by use of a novel 2-D SWE technique were clinically acceptable. Elasticity values obtained for healthy dogs in the present study may serve as reference values for the assessment of liver fibrosis and for predicting the onset or severity of portal hypertension in dogs with hepatic diseases.

Acknowledgments

No third-party funding or support was received in connection with this study or the writing or publication of the manuscript. The authors declare that there were no conflicts of interest with the sources or companies mentioned in the manuscript.

Footnotes

a. Aplio 500, Canon Medical Systems, Tochigi, Japan.
b. PVT 712 BT, Canon Medical Systems, Tochigi, Japan.
c. PVT 775, Canon Medical Systems, Tochigi, Japan.
d. JMP Pro, version 13.1.0, SAS Institute Inc, Cary, NC.

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