Evaluation of liver stiffness in a population of client owned healthy dogs using two-dimensional shear wave elastography: intraobserver reliability

Evaluatie van de lever van gezonde eigenaarshonden via tweedimensionele “shear wave”-elastografie: intraobserver-betrouwbaarheid

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ABSTRACT

Sonoelastographic techniques can complement conventional grey-scale and Doppler ultrasonography by evaluating tissue elasticity, which has the prospect to provide differentiation between malignant and benign conditions. Both technical and patient-related confounding factors are known to influence the reliability and reproducibility of elastographic methods. Therefore, the study was designed to assess liver stiffness in a diverse population of client-owned healthy dogs, evaluate the effect of patient characteristics on the elastography measurements and to assess intraobserver reliability. A total of forty dogs underwent two-dimensional shear wave elastography (2D SWE) twice, performed by the same operator, one to six days apart. The average, median and maximum 2D SWE velocities for the linear probe were 2.50 +/- 0.067 m/s; 2.46 +/- 0.067 m/s; 3.58 +/- 0.13 m/s, respectively, and for the convex probe 0.99 +/- 0.11 m/s; 0.98 +/- 0.12 m/s and 1.34 +/- 0.21 m/s, respectively. No statistically significant effect (P> 0.05) on the 2D SWVs was seen of the dog-related characteristics sex, age, body weight and body condition score. The intraobserver agreement of 2D SWE was moderate with the intraclass correlation coefficient (ICC) for the average, median and maximum 2D SWE being 0.69; 0.71 and 0.74, respectively. The moderate ICC and discrepant results obtained with different probes mandates standardization of patient-related and technical factors to overcome excessive variability in measurements in order to implement this technique in clinical practice.

SAMENVATTING

Met elastografietechnieken kan de weefselelasticiteit geëvalueerd worden. Op deze manier kunnen ze een standaard B-mode en dopperechografisch onderzoek aanvullen. Mogelijk kunnen ze ook helpen bij de differentiatie tussen goed- en kwaadaardige processen.

Zowel technische als patiëntgerelateerde factoren die eerder al tegenstrijdig bleken, kunnen de betrouwbaarheid en de reproduceerbaarheid van elastografieresultaten beïnvloeden. Daarom werd de voorliggende studie opgezet om de lever te beoordelen bij een gevarieerde populatie van gezonde eigenaarshonden, het effect van patiënteigenschappen te evalueren middels elastografiemetingen en om de intraobserver-betrouwbaarheid te bepalen. In totaal ondergingen veertig honden tweemaal een tweedimensioneel “shear wave” elastografisch onderzoek (2D SWE), uitgevoerd door dezelfde operator met een tussenspanne van één tot zes dagen. De gemiddelde, mediaan en maximum 2D SWE-snelheden voor de lineaire sonde waren respectievelijk 2,50 +/- 0,067 m/s; 2,46 +/- 0,067 m/s; 3,58 +/- 0,13 m/s, voor de convex sonde waren ze respectievelijk 0,99 +/- 0,11 m/s; 0,98 +/- 0,12 m/s en 1,34 +/- 0,21 m/s. Er werd geen statistisch significant effect (P> 0,05) gezien op de 2D SWV met betrekking tot het geslacht, de leeftijd, het lichaamsgewicht en de lichaamsconditiescore van de honden. De intraobserver-overeenkomst van de 2D SWE was matig waarbij de intraclass-correlatiecoëfficiënt (ICC) voor de gemiddelde, mediaan en maximum 2D SWE respectievelijk 0,69; 0,71 en 0,74 bedroeg. De matige ICC en de tegenstrijdige resultaten verkregen met verschillende sondes nopen tot standaardisatie van patiëntgerelateerde en technische factoren. Dit om overmatige variabiliteit van de resultaten te voorkomen teneinde deze techniek te kunnen implementeren in de klinische praktijk.

INTRODUCTION

Ultrasonography is a widely available, non-invasive and sensitive imaging modality used in the diagnostic work-up process of hepatobiliary diseases. However, due to similarities in the sonographic appearance of different disease processes and the fact that liver can appear unremarkable even in the presence of severe pathology (Feeney et al., 2008), liver cytology or biopsy are often needed for a definitive diagnosis (Marolf, 2017). Liver biopsy is an invasive technique associated with possible complications such as anesthetic risk, hemorrhage, air-embolism and vagotonic shock, and sampling errors can occur due to the variability in disease presence and/or severity in different parts of the liver (Bedossa et al., 2003; Cole et al., 2002; Kemp et al., 2015; Lidbury, 2017). In recent years, liver elastography has been researched in veterinary medicine (Cha et al., 2022; Holdsworth et al., 2014; Jung et al., 2020; K. Kim et al., 2020; Park et al., 2021; Tamura et al., 2021; Tamura, Ohta, Nisa, et al., 2019; Tamura, Ohta, Shimbo, et al., 2019), as studies in human medicine have shown promise of this non-invasive elastography method in both the diagnosis and monitoring of liver pathologies (Dietrich et al., 2017; Feng et al., 2016; Sigrist et al., 2017). With sonoelastography techniques, the firmness of tissues which might indicate the presence of pathological tissues, can be evaluated (Ophir et al., 1999; Sigrist et al., 2017). There are four types of ultrasound-based elastography techniques available: strain elastography, transient elastography, point-shear wave elastography and two-dimensional shear wave elastography (2D SWE) (Ozturk et al., 2018). The last method is most frequently used as it enables to sample a large area, change the sampling area quickly under B-mode ultrasound observation, while displaying a color map of shear wave values over a B-mode image (Dietrich et al., 2017; Naganuma et al., 2020). In 2D SWE, the ultrasound transducer produces focused push pulses, which mechanically produce small perpendicular tissue movements (shear waves). The shear wave speed can be measured and quantitative information regarding tissue elasticity is thus obtained (Dietrich et al., 2017; Ophir et al., 1999; Ozturk et al., 2018). It is known from human medicine that elastography can be affected by various technical (ultrasound system, probe, scanning approach, etc.) and patient-related factors (positioning, breathing, fasting, anesthesia) (Dietrich et al., 2017; Naganuma et al., 2020; Zelesco et al., 2018). Therefore, specific guidelines and recommendations by EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology) have been implemented for performing elastography studies in human patients (Dietrich et al., 2017). In recent years, several veterinary studies have been published pertaining to liver elastography of both normal dogs (Cha et al., 2022; Holdsworth et al., 2014; Jeon et al., 2015; Jung et al., 2020; Kim et al., 2020; Tamura, Ohta, Nisa, et al., 2019; White et al., 2014) and dogs with various pathological hepatobiliary diseases (Facin et al., 2020; Feng et al., 2016; Huaijantug et al., 2020; Tamura et al., 2021; Tamura, Ohta, Shimbo, et al., 2019). However, many of these above-mentioned studies have been performed on a small number of purpose-bred research colony dogs, specific population groups (e.g. brachycephalic dogs) or single disease entity patients (e.g. dogs with extrahepatic biliary obstruction). The findings on sources of variability and inter- and intraobserver variability have been conflicting. Therefore, the aim of this study was to evaluate liver stiffness by 2D SWE in a larger cohort of healthy client-owned dogs without the use of anesthesia or breath-holding methods. Furthermore, intraobserver variability and the effect of patient characteristics (sex, age, body weight, body condition score) on the 2D SWE values was assessed.

MATERIALS AND METHODS

Animals

All procedures were approved by and conducted in accordance with the local ethical and deontological committee (EC 2020-009, 2020-061 and DWZ/KF/20/1.15/44). Clinically healthy client-owned dogs, not receiving any medication, were recruited. Inclusion criteria mandated normal physical examination, the absence of any abnormalities on hematology, serum biochemistry, B-mode abdominal ultrasound, and the presence of normal preprandial serum bile acids (SBA). Dogs were excluded in case any abnormality was found during any of the examinations or analysis. Maltese dogs and brachycephalic dogs were excluded as Maltese dogs and dogs with respiratory disease can have increased serum bile acid concentrations without having liver disease (Bauer et al., 2006; Tisdall et al., 1995). Overweight and obesity were not a criterion for exclusion. Signalment, that consisted of sex, age, body weight and body condition score (BCS) on a nine-point scale was recorded at the time of recruitment.

Two-dimensional shear wave elastography examination of the liver

The recruited dogs were also part of a study assessing the influence of the type and quantity of food on the postprandial SBA concentrations. Therefore, all dogs underwent pre- and postprandial serum bile acid measurements and the recommended eight hours fasting prior to the ultrasonographic examination was not achieved. The food quantity administered was however small: two teaspoons of either recovery diet (RD), two teaspoons of a liver-supportive diet (LD), 10% RER RD, 10% RER LD, or 50% RER LD. Routine ultrasonography was performed on non-sedated dogs with an ultrasonography scanner (Philips EPIQ7) after clipping the subject's hair and applying coupling gel. At first, a conventional B-mode study was performed using either a linear probe (eL18-4) or a convex transducer (C8-5) after which the 2D SWE measurements were taken using Philips ElastQ Imaging (ElastQ, software version 3.0.3, Philips, Brussels, Belgium) and a linear (eL18-4) or convex (C5-1) probe. The measurements were taken in accordance with recommended guidelines for the clinical use of elastography in humans (Dietrich et al., 2017; Sigrist et al., 2017) and published results in dogs (Holdsworth et al., 2014; Jung et al., 2020; Tamura, Ohta, Nisa, et al., 2019). Using the visual control of the 2D B-mode image, an artifact-free image of the liver parenchyma was chosen for subsequent 2D SWE measurements, avoiding interfering structures such as blood vessels. The elastograms were displayed alongside the greyscale image with a superimposing confidence map, which highlighted areas with optimal shear wave propagation. The confidence interval was placed at 50%, which set the areas of stiffness/velocity image with a confidence value less than 50% as transparent, thus the transparent areas were not measured. At least ten 2D SWE velocity measurements were performed at end-expiration phase to minimize the effect of breathing motion. One region of interest (ROI) per elastogram was assessed, which was set at 5-10 mm in diameter, and excluded regions that were not color-coded. The ROI was positioned in the parenchyma of the liver at least 10 mm deep to the liver capsule. In each ROI, shear wave velocities (SWVs) were generated by the ElastQ software (mean, median, maximum SWV and interquartile range). For the consistency of measurements, the interquartile range (IQR) ratio to median was set to be <30%. The IQR is the spread of 50% of the measurements around the median and thus the IQR/median is an effective way to assess the quality of the range of measurements (Barr, 2014). On each dog two-dimensional SWE measurements were acquired by one ECVDI second-year resident (M.T.) on two separate days (maximum of six days between both 2D SWE studies). The sub-xiphoidal and/or left lateral recumbency intercostal approach was used for obtaining the 2D SWE measurements from the right liver lobes. The right liver lobes are used as per recommendations from human medicine to decrease the influence from cardiac motion to the velocity results (Dietrich et al., 2017; Sigrist et al., 2017; Zelesco et al., 2018). The decision on the intercostal or subxiphoidal approach was based on the best hepatic image accessibility with the differentiation between measurement approaches not recorded.

Statistical Analysis

Statistical analysis was performed with SAS (SAS V.9.4, SAS institute). The Shapiro-Wilk test was used to assess whether the response variables (average, median and maximum 2D SWE velocities) followed a normal distribution. A mixed model was fitted with animal as random effect, and probe, sex, age, weight and BCS as fixed effect factors; F-tests were used to assess the effect of the different variables. Probe, sex and weight were introduced as continuous variables and probe and sex as nominal categorical variables. BCS is an ordinal categorical variable, and its score was put in as a number to assess the linear relationship between BCS and the response variables. The intraobserver intraclass correlation coefficient (ICC) was calculated to assess the reproducibility of the assessment. The agreement results were defined as follows: poor < 0.5, moderate 0.5 to 0.75, good 0.75 to 0.9, and greater than 0.90 excellent (Koo and Li, 2016). Finally, a logistic regression model was fitted to assess whether particular dog characteristics had an effect on the choice of the probe type.

RESULTS

Study population

Dogs that fulfilled all prerequisites were enrolled until a total of forty dogs were included. This number was chosen in accordance with the ASVCP guidelines (Friedrichs et al., 2012) in order to be able to determine reference intervals for pre- and postprandial SBA. As it is not known from the literature which variability in elastography measurement is to be expected, all these dogs were also included for the 2D SWE procedure and study.

The causes for exclusion were mostly related to abnormalities on blood analysis (n=29). Two dogs had abnormalities on abdominal ultrasonography, two dogs refused to eat, and four dogs were uncooperative. Eight intact females, 19 neutered females, 7 intact males and 6 neutered males and the following breeds were included: seven cross breed dogs, five Labrador retrievers, four Border collies, four golden retrievers, two Jack Russell terriers, two Nova Scotia Duck Tolling retrievers, two Shetland sheepdogs and one each of Australian shepherd dog, Dutch sheepdog, English Cocker spaniel, Great dane, husky, Kooikerhondje, Lagotto Romagnolo, Lakeland terrier, Miniature Australian shepherd dog, Miniature schnauzer, Pomeranian, Samoyed, Tamaskan dog, White Swiss shepherd dog. Age and body weight of the dogs were not normally distributed. The median age of the dogs was 48 months (range, 7-146 months). The median body weight of the dogs was 19.5 kg (range: 4.9-65.0 kg). The median body condition score was 5/9 (range 4-9), with the majority having a normal weight (BCS 4-5/9, n=29), six having a BCS of 6/9, four having a BCS of 7/9 and one dog with a BCS of 9/9.

Two-dimensional shear wave elastography measurements of the liver

A representative 2D SWE image of the right lobes of the liver using the linear and convex probe is seen on Figures 1 A and B.

The linear probe was used for 85% of the measurements (n=34) and the curvilinear probe for 22.5% of the measurements (n=9). In three dogs, the 2D SWE acquisition was performed with both probes separately.

Weight was a significant predictor (P= 0.03) for transducer type selection with a more frequent use of the curvilinear probe associated with a higher weight.

The mean 2D SWE velocities in the liver parenchyma according to the probe used are illustrated in Figure 2. The mean values for the average, median and maximum 2D SWE velocities for the linear probe were 2.50 +/- 0.067 m/s; 2.46 +/- 0.067 m/s; 3.58 +/- 0.13 m/s, respectively, and for the convex probe 0.99 +/- 0.11 m/s; 0.98 +/- 0.12 m/s and 1.34 +/- 0.21 m/s, respectively (Table 1).

The ICC for intraobserver variability for the average (AvgVel), median (MedVel) and maximum (MaxVel) 2D SWVs was 0.69, 0.71 and 0.74, respectively.

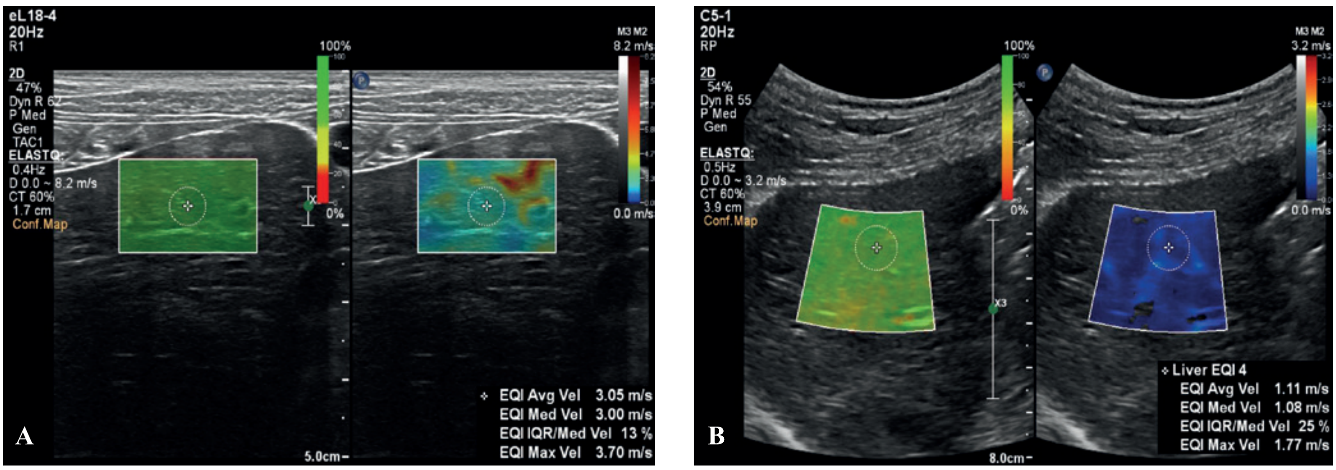
The categorical variable sex did not have an effect on the average, median and maximum 2D SWVs (Table 2), nor did the variables age, weight and BCS (Table 3).

DISCUSSION

The appeal to use the non-invasive 2D SWE technique in veterinary medicine is clear; but similarly to human medicine, the impact of technical and patient-related confounders have been conflicting (Cha et al., 2022; Facin et al., 2020; Holdsworth et al., 2014; Jung et al., 2020; K. Kim et al., 2020; Tamura, Ohta, Nisa, et al., 2019; Tamura, Ohta, Shimbo, et al., 2019) regarding approach (intercostal versus subxiphoidal), depth of ROI placement, breathing phase versus sedation/anesthesia, sex and body weight. Therefore, the aim of this study was to perform 2D SWE measurements under common clinical situations and circumstances in a population of healthy dogs consisting of different dog breeds.

Good quality elastography images could be obtained in all dogs without anesthesia or breath-holding due to the rapid measurement process and real-time evaluation of the B-mode image in conjunction with the confidence map with the IQR/median ratio not exceeding 0.3. All 2D SWE systems have built-in indicators of the quality of the shear-wave speed estimate and take appropriate action to adjust the display when the quality falls too low, which typically occurs as the signal-to-noise ratio deteriorates with depth (Dietrich et al., 2017).

The ICC for intraobserver variability in the present study was moderate (0.69-0.74). Based on the 95%



Figures 1 A and B. Representative 2D SWE images of the right lobes of the liver for a speed mode in a dog using the linear (eL18-4) and convex (C5-1) probes. The green confidence map and the elastogram image are displayed concurrently over the B-mode image.

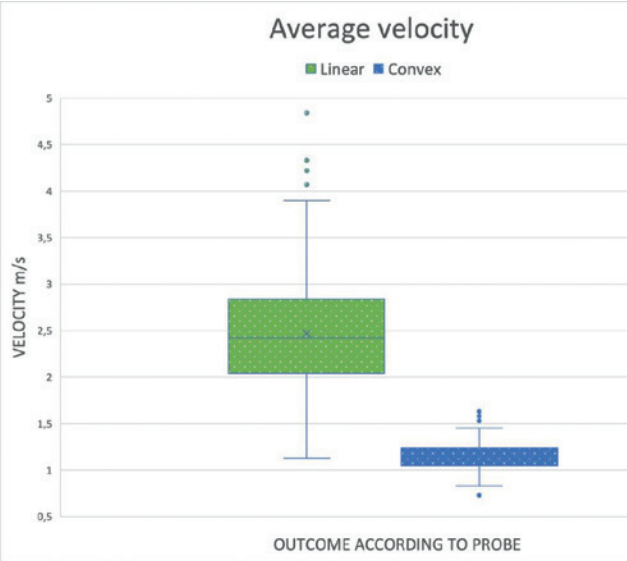


Figure 2. Box and Whiskers plot for average two-dimensional shear wave velocities in healthy dog livers according to probe.

confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively (Koo and Li, 2016). Previous studies pertaining to liver stiffness measurements in dogs and cats have shown better agreement (Jung et al., 2020; Kim et al., 2020; Tamura, Ohta, Nisa, et al., 2019). For example, in the study by Jung et al. (2020), the ICC for the liver in healthy beagle dogs was excellent: 0.864–0.948. It could be argued that the poorer ICC in the present study could be attributed to the fact that both intercostal and subxiphoidal approaches were used interchangeably, no breath-holding method was applied, and a variety of dog breeds were included. In humans, excessive panting, for example due to stress, is known to cause less saturated 2D SWE boxes (shear wave propagation map or wavefront map), resulting in inaccurate estimation of liver stiffness (Naganuma et al., 2020; Pellot-Barakat et al., 2016; Zelesco et al., 2018). However, in pediatric medicine, which in many technical aspects is similar to the small animal scenario, it has been shown that mean elasticity values do not reveal any significant difference between free breathing and breath-holding (Dietrich et al., 2018, 2019). Nevertheless, the moderate agreement in the current study indicates that without firm measurement guidelines to decrease result variability, the potential implementation of this method in clinical practice will not give sufficient advantages over more invasive diagnostic methods.

The mean liver 2D SWE velocities in this study differ according to the probe used with the measurements obtained with the convex probe showing lower velocity values. The convex probe was more frequently used in heavier patients to obtain reliable measurements, as maximum possible tissue depth of the linear probe is inferior to the convex probe. The logistic regression model showed that weight was a significant predictor (P= 0.03) for probe selection and increase in weight made it more likely to choose a curvilinear probe. In human studies, the data regarding the different values obtained with the linear versus the convex transducer are conflicting. In a study by Zelesco et al. (2018), a higher frequency linear transducer (10 MHz) in comparison to lower frequency convex transducer (5 MHz) produced considerably higher SWVs for the liver (15.3 kPa versus 5.8 kPa, respectively). In a study using phantoms and normal liver tissue, Chung et al. (2013) demonstrated that SWVs with a lower frequency probe had a tendency to be higher at the same depth when compared to the linear probe (Chang et al., 2013). Also, in pediatric medicine, conflicting results have been reported. Hanquinet et al. (2013) demonstrated similar mean SWVs between probes; however, in that study, the choice of the transducer was based on the age of the child and no child was examined with both probes. Contrarily, Fontanilla et al. (2014) and Franchi-Abella et al. (2016) showed that the SWV means tended to be lower with the higher frequency linear transducer when compared to the convex low frequency probe. The mean liver 2D SWE velocities in the present study ranged from 2.46-3.58 m/s (linear transducer) and 0.98 -1.34 m/s (convex transducer). The measurements obtained with the linear transducer

Table 1. Mean (+/- standard error) of the two-dimensional shear wave velocities (2D SWV) of healthy dog livers using the linear (eL18-4) or convex (C8-5) transducer.

2D SWV	Average (m/s)	Median (m/s)	Maximum (m/s)
Linear probe (n=33)	2.50 +/- 0.07	2.46 +/- 0.07	3.57 +/- 0.13
Convex probe (n=8)	0.99 +/- 0.12	0.98 +/- 0.12	1.34+/-0.22
P-value	< 0.0001	< 0.0001	< 0.0001

Table 2. Mean (+/- standard error) of the two-dimensional shear wave velocities (2D SWV) of healthy dog livers according to gender.

2D SWV	Average (m/s)	Median (m/s)	Maximum (m/s)
Female intact (n=8)	1.78 +/- 0.14	1.75 +/- 0.14	2.58 +/- 0.26
Female neutered (n=19)	1.78 +/- 0.09	1.75 +/- 0.09	2.64 +/- 0.17
Male intact (n=7)	1.71 +/-0.15	1.69 +/- 0.15	2.30 +/- 0.28
Male castrated (n=6)	1.71 +/-0.15	1.69 +/-0.15	2.31 +/-0.29
P-value	0.96	0.97	0.60

Table 3. The change in the two-dimensional shear wave velocities (2D SWV) for every unit increase in age, body condition score (BCS) and body weight (slope +/- standard error).

2D SWV	Effect	Slope	P-value
AvgVel	Age (months)	-0.00044 (+/-0.0018)	0.81
	BCS (scale 1-9)	0.002660 (+/-0.058)	0.96
	Weight (kg)	0.002493 (+/-0.0054)	0.65
MedVel	Age (months)	-0.00040 (+/-0.0018)	0.83
	BCS (scale 1-9)	-0.00032 (+/-0.0585)	0.99
	Weight (kg)	0.002185 (+/-0.0054)	0.69
MaxVel	Age (months)	-0.00192 (+/-0.0034)	0.58
	BCS (scale 1-9)	0.05128 (+/-0.1104)	0.64
	Weight (kg)	0.005963 (+/- 0.0102)	0.56

differ considerably from the results obtained in studies by Holdsworth et al. (1.18-1.88 m/s), Jung et al. (1.60-1.69 m/s) and Tamura et al. (1.51± 0.08 m/s) on healthy dogs' livers (Holdsworth et al., 2014; Jung et al., 2020; Tamura, Ohta, Nisa, et al., 2019). In the first two studies, a linear probe was used, whereas in the last one, a curvilinear probe was used for SWV measurement acquisition. The present study design did not include acquisition of 2D SWV measurements using both probes on all the enrolled dogs. Although the linear probe could not have been used in all the dogs due to their weight and BCS, measurements with convex probe would have been feasible in all recruited dogs. In retrospect, this would have been interesting data to record and compare. It is known that comparison of data between the different SWE technologies from different manufacturers should not be done, as measured values of shear wave velocity will vary with a number of system factors, in particular shear wave vibration frequency and bandwidth (Dietrich et al., 2017; Ozturk et al., 2018; Sigrist et al., 2017). Other known causes of measurement variability between ultrasound systems are the depth, shape, strength and irradiation time of the push pulse and the shape and size of the ROI (Kishimoto et al., 2022). Nevertheless, manufacturers do not completely disclose the processing algorithms and scanner sequencing used in their devices, which is considered an underlying factor of the problem; therefore, standardization among systems is required (Kishimoto et al., 2022).

In the current study, an (P=0.65) effect of body weight on SWE velocity measurements could not be demonstrated. This is contrary to Holdsworth's et al. (2014) findings who demonstrated a statistically significant positive effect for body weight, with the shear wave velocity value increasing by 0.03 m/s for every kg increase in body weight. The latter study is the only one so far that also included different dog breeds and sizes. However, due to their low sample size (n=15), their correlation observed with weight can be due to type I error or natural variation between individuals. The study populations in other veterinary studies by Tamura et al. (2019) and Jung et al. (2020) included purpose-bred beagle dogs only, hence no proper association with body weight could be drawn. In human medicine, both obesity and undernutrition (increased or decreased body mass index) have shown to cause increased liver stiffness values (Dietrich et al., 2019; Ferraioli et al., 2015). Additionally, morbid obesity is also known to contribute to the likelihood of increased data variability (Dietrich et al., 2017, 2019; Ferraioli et al., 2015). The present study also assessed the effect of BCS on SWV results, which, to the authors' knowledge, has not been documented in other veterinary studies thus far. Although a positive trend was identified, with the 2D SWV value increasing by 0.0026-0.051 m/s for every unit increase in the BCS scale system, this was not statistically significant (P=0.96). An additional parameter, not assessed in the present study, that can influence the 2D SWE velocities is the depth of the ROI placement. In all the cases in the current study, the ROI was placed, as per recommendations, at least 10 mm below the hepatic capsule in order to avoid reverberation artefacts and increased subcapsular stiffness (Dietrich et al., 2017; Ferraioli et al., 2015; Zelesco et al., 2018). However, the maximum depth of ROI placement could have varied to some degree. Nevertheless, as per recommendations in human hepatic medicine, the optimal depth of 4-5 cm from the transducer and never deeper than 6-7 cm was followed (Dietrich et al., 2017). There is, however, no clear recommendation in dogs regarding the preferred depths of measurement in the liver. In a study by Holdsworth et al. (2014), it was shown that considering the median and IQR values of the liver, the SWE velocity value decreased as depth increased (Holdsworth et al., 2014), whereas Jung et al. (2020) showed that SWV of the liver tended to be higher with an increasing depth (Jung et al., 2020). This is similar to pediatric studies, where the depth of measurements has shown contradictory influence (Dietrich et al., 2018).

Aging has been shown to enhance vulnerability to acute liver injury and increase susceptibility to the fibrotic response in human medicine in cases of clinical liver disease (Kim et al., 2015). In the current study, a statistically significant (P=0.81) effect of age on the SWV values could not be demonstrated, which is consistent with findings in human medicine regarding the healthy adult population (Huang et al., 2014; Mulabecirovic et al., 2018). However, in pediatric medicine, the results are contradictory with one study reporting statistically significant difference of SWV values obtained in children <6 years compared to those obtained in older children, while other studies showed no effect (Dietrich et al., 2017; Hanquinet et al., 2013; Matos et al., 2014). Veterinary studies of liver elastography in a population of healthy puppies is lacking and would be an interesting area of research.

Holdsworth et al. (2014) studied 15 dogs (10 females and 5 males) and demonstrated a significant effect (P<0.01) of gender on the liver stiffness measurements, with females showing lower values than males. The results of the present study could not identify any statistically significant effect of sex on the SWV (P=0.39). Other canine studies (Cha et al., 2022; Jung et al., 2020; Tamura, Ohta, Nisa, et al., 2019) also failed to find an association with gender. However, the number of dogs in Holdsworth's et al. study (2014) was small, and the difference could have resulted from the small sample size leading to type I error or natural variation between individuals. Interestingly, in humans, the influence of gender on SWV has been documented in multiple studies (Dietrich et al., 2017; Corpechot et al., 2006; Roulot et al., 2008), suggesting an intrinsic difference between men and women in density of liver extracellular matrix and a possible protective effect of ovarian hormones against the accumulation of extracellular matrix and fibrosis in the liver (Roulot et al., 2008; Yasuda et al., 1999). The situation is somewhat different in veterinary companion animal medicine, where many animals are electively neutered, rendering the hormonal influence on liver stiffness values unsubstantial. In the present study population, from the forty dogs examined, only 15 dogs (8 female, 7 male) were intact and no statistically significant effect of the neuter status on liver stiffness observations was present.

This study has some limitations. First, as the patients in the current study underwent pre- and postprandial bile acid testing, the recommended fasting prior to the ultrasound procedure was not achieved. In human studies, SWE velocity readings are increased for an estimated 120-180 minutes after eating due to increased liver blood flow following food intake (Dietrich et al., 2017; Zelesco et al., 2018). Unfortunately, the authors did not record the exact time when the patients underwent the ultrasound examination compared to eating time. While some of the patients underwent SWE immediately post eating, others had the procedure after the second blood example (that is >2 hours after the meal). The impact of food and influence of time after eating on hepatic elastography results has not been studied in dogs, but a similar physiological influence would be expected. Secondly, only a small number of dogs (n=9) had the SWVs acquired with the convex probe, which may have decreased statistical power and may have led to type II error. Thirdly, healthy hepatic tissue was not confirmed with cytological or histological examination but presumed by physical examination, blood tests and ultrasonography, as liver sampling is invasive and was not considered justifiable in a healthy population of client-owned dogs. Although all important blood parameters to rule out liver disease were determined, bilirubin was not analyzed. Nevertheless, as none of the dogs presented with icterus, increased liver enzymes and increased preprandial SBA, it is unlikely that hyperbilirubinemia was present in any of the dogs (Webster et al., 2019).

CONCLUSION

Two-dimensional SWE is a feasible method to apply on a diverse population of client-owned, healthy, non-anesthetized dogs; however, large discrepancies between measurements obtained with linear versus convex probes and only moderate ICC underline the importance of standardization of technical and patient-related factors to obtain more comparable results between individual dogs, and reliably implement this non-invasive diagnostic method into clinical practice.

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