

Acoustic radiation force impulse (ARFI) elastography of kidneys in healthy adult cats: preliminary results

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OBJECTIVES: To describe acoustic radiation impulse force elastography in evaluation of kidneys of adult cats.

MATERIALS AND METHODS: Ten healthy adult short-haired cats were included. Echogenicity and texture, cortico-medullary relationship, size and edges of kidney were assessed by B-mode and by qualitative elastography to detect the presence of deformities and shear velocities of different portions (cranial, middle and caudal of cortex and medulla).

RESULTS: Findings of ultrasonography were normal in all cats. Qualitative elastography demonstrated that the renal cortex was not deformable and had homogeneous dark gray areas; the renal pelvis had lower stiffness (white); and the medulla showed a mosaic pattern. The results of shear wave velocity were different in cranial, middle and caudal regions of cortex and medulla: 2.46 ± 0.45 , 2.46 ± 0.48 and 2.37 ± 0.42 ($P=0.795$) in cortex and 1.61 ± 0.69 , 1.75 ± 0.66 and 2.00 ± 0.55 m/s ($P=0.156$) in medulla, respectively.

CLINICAL SIGNIFICANCE: Quantitative and qualitative acoustic radiation impulse force elastography of the kidney in adult cats was easily performed and this study provides base line data to allow the use of acoustic radiation impulse force in diseased animals.

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INTRODUCTION

Renal ultrasonography in small animals provides important information about the anatomy of the kidneys, including dimensions, contours and internal architecture, regardless of their function (Silva *et al.* 2008), enabling the identification of cortical, medullary and renal pelvis regions (Yeager & Andreson 1989, D'Anjou 2011). Specifically in cats, the accumulation of vacuoles

of fat in the renal cortex can be confused with a pathological increase in echogenicity of the cortex (Yeager & Andreson 1989, D'Anjou 2011).

Modification of renal echogenicity is only one of several parameters for assessing kidney disease in cats. The definitive diagnosis of different kidney diseases in small animals requires renal cytology and histopathology, both of which are invasive methods that carry a degree of risk (Seyrek-Intas & Kramer 2008).

Elastography is a promising ultrasound technique that evaluates tissue elasticity. Various methods for assessing tissue elasticity have been proposed, such as compression, acoustic radiation force impulse (ARFI) and real-time shear velocity (RSV) (Feliciano *et al.* 2014a).

Compression elastography is based on the change in sound wave speed (and so the change in time for echoes to return to the transducer) induced by the application of a compressive force to the organ or structure under examination, and is performed by comparing images obtained prior to and following the application of pressure through the transducer (Saftoiu *et al.* 2007, Hoskins 2012, Destounis & Gruttadauria 2013). However the technique is operator-dependent and is sensitive to body conformation (weight, tissue depth), particularly when evaluating deeper organs or obese patients (Carvalho & Chammas 2013).

ARFI elastography is safe and non-invasive and provides both quantitative and qualitative measures of tissue rigidity (Dudea *et al.* 2011) and is based on the propagation of a force capable of causing the displacement of the tissue without the need for manual compression. Compared to other methods, this technique is more accurate with less inter-observer variation and with greater reproducibility (Carvalho & Chammas 2013).

ARFI elastography involves the generation of shear waves using radiation force impulse or shear force associated with a B-mode image (Carvalho *et al.* 2015). For qualitative ARFI, short acoustic pulses and high intensities are utilised to deform the elements of the tissue and create a static map (elastogram) of the relative stiffness of the tissue. In general, the lighter areas represent the more deformable tissues (Goddi *et al.* 2012, Feliciano *et al.* 2014a). The quantitative study uses a primary acoustic impulse on a region of interest, promoting the formation of pressure waves capable of deforming the tissues and raising the speed of propagation of the pressure waves (shear). The velocity of propagation is related to the rigidity and viscoelasticity of the tissue. The waves have a higher velocity in rigid tissues (Comstock 2011).

In veterinary medicine, the ARFI technique is a recent development and has been used to evaluate focal liver lesions in rats (Carvalho *et al.* 2012), mammary tumours in female dogs (Feliciano *et al.* 2014b), and the spleen, liver and kidneys of adult dogs (Holdsworth *et al.* 2014), splenic tissue in cats (Feliciano *et al.* 2014a) and prostate and testes in dogs (Feliciano *et al.* 2015).

In human nephrology, elastography has been used in the study of chronic kidney disease (Hu *et al.* 2014), for the detection of areas of renal fibrosis (Fahey *et al.* 2005, Syversvee 2010) and classification of renal masses (Guo *et al.* 2014), which suggests the possibility of using this technique for evaluating renal diseases in animals. However, reference values for healthy feline and canine renal ARFI elastography (including use of the shear wave) would be required to allow assessment of renal pathology.

In feline medicine, a pilot study (White *et al.* 2014) used the combination of B-mode ultrasound and elastography by compression to aid in the diagnosis of alterations in abdominal organs, including the kidney. Considering this pilot information and the reports of ARFI elastography in tissue evaluation of small animals, the aims of this study were to describe the technique

of ARFI elastography in the evaluation of the kidney of adult cats, to evaluate the stiffness of healthy renal parenchyma, and to determine the quality and quantity (shear velocity) standards of the ARFI technique, which have not yet been described in feline medicine.

MATERIAL AND METHODS

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Universidade Estadual Paulista, Campus Jaboticabal, Brazil (protocol No 018.897/13). The kidneys of 10 healthy adult male domestic short-haired cats (20 kidneys in total), 2–6 years of age (mean age = 4.7 ± 1.62 years) and weighing between 2.9 and 4.3 kg (mean = 3.45 ± 0.7 kg) were selected for this study following a normal physical examination, haematological and biochemical profiles (urea and creatinine), urinalysis and urinary protein creatinine to ratio (UPC).

After the animals were selected, the abdominal hair was shaved liberally to allow for ultrasonography. Before the examination, coupling gel was applied to the skin. The animals sedated by intramuscular injection of 0.5 mg/kg chlorpromazine and 5 mg/kg tramadol hydrochloride as premedication. After 15 minutes, anaesthesia was induced with 10 mg/kg ketamine injected intramuscularly, and subsequently an intravenous catheter was placed in the cephalic vein.

The ultrasonography was performed by a single, experienced ultrasonographer. B-Mode ultrasonography was performed with a 9.0 MHz linear matrix transducer using ACUSON S2000/SIEMENS ultrasound equipment (Siemens, Munich, Germany). The echotexture (homogeneous or heterogeneous), echogenicity (hypoechoic, hyperechoic or mixed) of the parenchyma, size (length and width) and cortico-medullary relationship of the kidney were assessed and categorised.

For the elastography, software for qualitative and quantitative analysis was compared using the ARFI method (Virtual Touch Tissue Quantification) and a 9.0 MHz linear matrix transducer (Syversveen *et al.* 2011, Feliciano *et al.* 2014b).

After performing B-mode ultrasonography, the qualitative ARFI technique was applied, resulting in the formation of greyscale images of the cranial and caudal portions of the kidney. The images were evaluated with specific focus on the presence of deformities, white areas (indicative of more elastic tissue that is less rigid, softer, and more deformable) and dark areas (more rigid, harder and non-deformable regions within the kidney).

Quantitative evaluation was also performed after scanning the kidney with the B-mode ultrasound. The function for obtaining shear velocity was activated, and the caliper was positioned in the splenic parenchyma; the fixed region of interest box dimensions were 10×10 mm. Six measurements in each position (cranial, medial and caudal portions in the renal cortex and medulla) of the kidney were obtained (with depth between 0.6 and 2.4 cm for the cortex and 0.9 and 2.7 cm for the medulla) and used to determine the shear velocities (mean and standard deviation (sd)) (Fig 1).

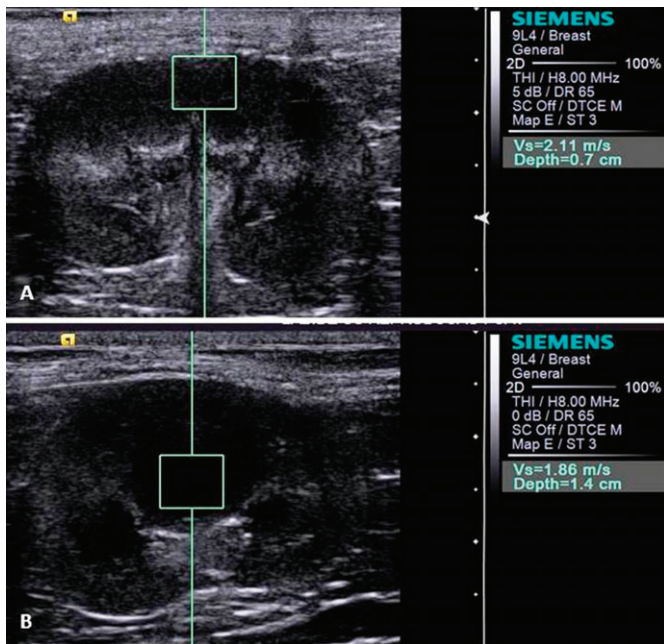


FIG 1. Ultrasound image of feline kidney during its quantitative ARFI evaluation of the cortical (A) and medullary (B) portion. Note the measurement of the shear velocity of renal tissue, with the presence of a caliper for the portion assessed in each renal region

The data were analysed for normality of residuals and homogeneity of variances (F test). PROC MEANS-SAS™ and Graph-Pad Prism 4™ were used for analysis. The significance level was set $P < 0.05$ for all tests. Raw and transformed averages were evaluated by an analysis of variance. In addition, a t-test for paired samples was applied for all shear velocities and depth from the different renal portions. Using the Pearson test, a correlation was evaluated in the weight of animals with shear rates obtained in the various portions of the feline kidney. Qualitative data (echotexture, echogenicity, size and edges; greyscale images and presence of deformities) were listed.

RESULTS

Assessment of the kidneys in all cats using B-mode ultrasound showed no abnormalities in echotexture, echogenicity of the parenchyma, size and cortico-medullary relationship.

On qualitative elastography, the evaluated renal segments were non-deformable, with cortical and pelvic areas appearing homogeneous. Medullary areas appeared heterogeneous (with a mosaic appearance) due to the presence of urine. The cortical regions appeared harder (with a mid-grey echogenicity) than the renal pelvis (white echogenicity) and medullary regions (mosaic-like echogenicity) (Fig 2).

Using quantitative elastography, the mean shear velocity values (Fig 2) (for twenty renal units) were: 2.46 m/s for the cranial, 2.46 m/s for the middle and 2.37 m/s for the caudal cortical portion (95% confidence interval: 2.31 and 2.54 m/s); and 1.61 m/s for cranial, 1.75 m/s for middle and 2.00 m/s for caudal medullary portion (95% confidence interval: 1.62 and 1.96 m/s);

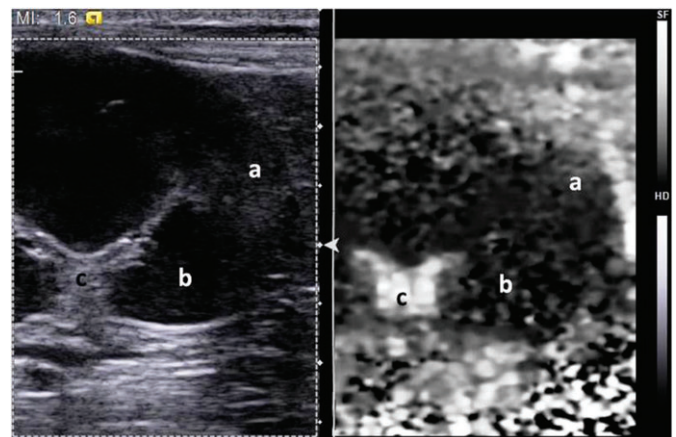


FIG 2. Ultrasound image of feline kidney during its qualitative ARFI evaluation. Note the B-mode image (left) and the image of the elastography of the kidney (right) with the characterisation of the stiffness of the pelvis (c), medullary (b) and cortical (a) portions of tissue in greyscale and the evaluation of the deformity

and these values were not significantly different (cortical portion: $P = 0.795$; and medullary portion: $P = 0.156$) (Table 1). The quantitative elastography of the cortical region (2.43 ± 0.45 m/s) was approximately 35% higher ($P < 0.001$) than the medullary region (1.79 ± 0.65 m/s).

By assessing the left and right renal structures of cats the mean shear velocity values were not significantly different in renal portions (cranial, middle and caudal) and regions (cortical and medullary) ($P > 0.05$; Table 2).

No significant correlation was found between body weight and shear velocity values in renal tissue ($P = 0.707$; Fig 3) and of the depth used for evaluation of the left (1.58 ± 0.48 cm) and right kidney (1.72 ± 0.54) ($P = 0.15$).

DISCUSSION

The diagnosis of renal disorders in cats is made through the association of clinical findings, laboratory tests and other techniques, such as ultrasonography. However, the diagnosis of renal disorders is commonly made at a fairly advanced stage of the disease process due to the functional reserve capacity of the kidney structures, compromising the animal's prognosis (Heine 2008). This exemplifies the need for the study of new diagnostic techniques such as ARFI elastography. This is the first study using ARFI technique in the evaluation of renal structures of cats and obtains reference values for this technique, and suggests that the study of the stiffness can be used to increase the sensitivity of a diagnosis of chronic renal alterations.

Ultrasonography is an important diagnostic test performed for renal evaluation of cats, but renal ultrasonography is not a specific diagnostic technique because of the difficulty of identifying diffuse abnormalities and differentiating between malignant and benign tissues (Vescoci *et al.* 2009, Garcia *et al.* 2012). Preliminary results from the ARFI elastographic evaluation of mammary neoplasms in female dogs demonstrated that malignancy was

Table 1. Mean values ($\pm$ standard deviation) (m/s) and 95% confidence intervals (CI 95%) for shear velocities of different portions in the anatomic feline kidney determined using the acoustic radiation force impulse (ARFI) quantitative elastography

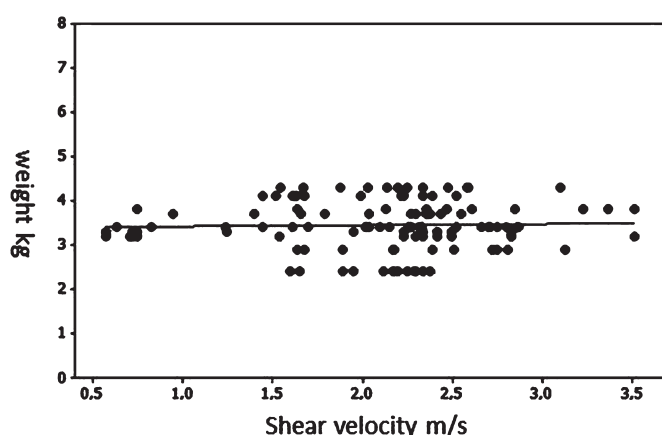
Kidney region	Cranial	Medial	Caudal	IC 95%	P value
Cortical	2.46 $\pm$ 0.45	2.46 $\pm$ 0.48	2.37 $\pm$ 0.42	2.31–2.54	0.795
Medullary	1.61 $\pm$ 0.69	1.75 $\pm$ 0.66	2.00 $\pm$ 0.55	1.62–1.96	0.156

One-way ANOVA at 5% of significance level
CI: confidence interval 95%

Table 2. Mean values ($\pm$ standard deviation) (m/s) and 95% confidence intervals (CI 95%) for shear velocities of the feline's right and left kidney (cranial, medial and caudal) determined using the acoustic radiation force impulse (ARFI) quantitative elastography

Kidney region	Cranial	Medial	Caudal	P value
Left cortical	2.41 $\pm$ 0.33	2.25 $\pm$ 0.49	2.52 $\pm$ 0.55	0.42
(CI 95%)	2.17–2.64	1.89–2.60	2.13–2.92	
Left medullary	1.63 $\pm$ 0.72	2.14 $\pm$ 0.32	1.51 $\pm$ 0.64	0.052
(CI 95%)	1.11–2.14	1.91–2.37	1.05–1.97	
Right cortical	2.50 $\pm$ 0.55	2.49 $\pm$ 0.31	2.39 $\pm$ 0.40	0.80
(CI 95%)	2.10–2.90	2.27–2.72	2.09–2.68	
Right medullary	1.58 $\pm$ 0.70	1.85 $\pm$ 0.70	1.98 $\pm$ 0.62	0.41
(CI 95%)	1.08–2.09	1.35–2.35	1.53–2.43	

One-way analysis of variance – 5% of significance level
CI: confidence interval 95%

**FIG 3. Graphic illustration showing the correlation between renal shear velocity and weights of the felines evaluated**

suggested by rigid and non-deformable tissue with high shear-wave velocity (Feliciano *et al.* 2014b). It is possible that renal disease (e.g. neoplasms) may produce alterations that are detectable by ARFI elastography, and this may aid diagnosis of renal abnormalities in the future.

Assessment of the renal parenchyma of cats with qualitative elastography by compression suggested that the cortical region is less rigid than the medullary and pelvis (White *et al.* 2014). By contrast, it was observed in this study that the cortical region is more rigid than the medullary and pelvis regions, a characteristic that was confirmed by ARFI quantitative assessment. These differences in stiffness of the renal region are explained by differences in histological and structural characteristics of the portions, because the cortical portion comprises renal corpuscles and proximal convoluted tubules (promoting a consistent characteristic), the medullary is composed of the loop of Henle and the

collecting ducts and the presence of the deposition of adipose tissue in pelvis (Ellenport 1986, Colville 2010). It is expected that this technique could be performed to detect the intraparenchymal areas (hard/soft or deformable/non-deformable) that might alter the overall homogeneity and different renal portions.

It is important to mention that the observed difference in the results obtained in this study and that of White *et al.* (2014) in cats can be explained by the type of technique used and the selection of animals. White *et al.* (2014) used the compression technique and evaluated renal structure depending on differences in compressibility. This is ultrasonographer dependent and sensitive to body conformation. Additionally, the animals used in that study were not evaluated for renal disorders (clinical history and haematological and biochemical profiles and urinalysis). In this study, ARFI elastography is automatic (without the need for manual compression) with less inter-observer variation and with greater reproducibility; and the animals were evaluated clinically (haematological examination, urea and creatinine concentrations, urinalysis and UPC evaluation) and were all considered healthy.

To minimise the effects of body conformation (weight and abdominal depth), age and sex cited by Holdsworth *et al.* (2014) in the elastography of canine spleens, male animals of the same breed, with little variation in weight and age, were used. It is noteworthy, that in this study there was no correlation ($P>0.05$) between the weight of the animals and the depth of renal structures with the values of the quantitative elastography.

The quantitative ARFI verified that the shear velocity values in the renal portions (cortical and medullary) were similar ($P<0.05$). The results obtained for shear velocity in the adult feline kidney were similar to those observed in healthy human subjects – mean values were 2.49 ± 0.81 m/s for right and 2.36 ± 0.75 m/s for left kidney (Bota *et al.* 2015). These results were also similar to the

reported values found in adult dogs for the interquartile range: 2.2–3.31 m/s (Holdsworth *et al.* 2014).

These quantitative values obtained by ARFI of the kidney in adult cats are the first that have been described in feline medicine, and further studies in animals with renal disease should be performed to evaluate the applicability of the technique in a veterinary clinical setting. This technique has proven application for assessment of mammary tissue of humans (Tozaki *et al.* 2011, Bai *et al.* 2012) and female dogs (Feliciano *et al.* 2014b), in which high shear rates (compared to healthy state mammary tissue) are related to a greater stiffness of mammary tissue and increased likelihood of malignancy.

The reference values determined for the shear velocity of the kidney of adult cats may assist in the diagnosis of malignant lesions in these tissues by favouring an early diagnosis and accelerating the institution of therapy in diseased animals.

It is important to comment on some limitations observed in this study. First poor patient compliance may hamper the acquisition of measurements. Although sedation was used in this study, obtaining an appropriate window for the qualitative assessment may be hampered by respiration or movement by the patient. There were a limited number of animals included with little breed, age or sex variation. However, this pilot study provides important information about the validation of the technique and about obtaining reference values for adult male cats. As noted by Holdsworth *et al.* (2014) and Feliciano *et al.* (2014a) in their study with dogs and cats, respectively, it is necessary to conduct a study with a larger number of animals and, specifically, to evaluate cats of different breeds, weights, sex and age ranges to verify the interference of these variables on the findings of elastography in the cat kidney.

It is believed that after the standardisation of the reference values for the kidney elastography in healthy cats and with a larger sample size, the differences in values of shear velocity from the damaged tissues can be evaluated for the applicability of ARFI elastography for differentiating benign and malignant renal injuries in cat.

Quantitative and qualitative ARFI elastography of the kidney in adult cats was easily implemented and may provide base line data in the study of this organ to allow the use of ARFI in diseased animals.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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