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ARFI (Acoustic Radiation Force Impulse) elastography and B-mode ultrasonography of the adrenal glands in healthy dogs of different ages

[Elastografia ARFI (Acoustic Radiation Force Impulse) e ultrassonografia modo-B das glândulas adrenais em cães hígidos e de diferentes idades]

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ABSTRACT

Sixty adrenals of 30 dogs were evaluated, divided into three groups, according to age: Group 1 = healthy youngsters (10 animals) – from one to three years old; Group 2 = healthy adults (10 animals) – four to nine years old; and Group 3 = healthy senile animals (10 animals) – older than ten years old. The experimental design was randomized, with a significance of 5%. There was a significant difference (P=0.017) in length between young and elderly dogs. The thickness of the cranial pole showed a significant difference (P=0.001) between young and adults when compared with the elderly group. The thickness of the caudal pole was not significant for adults when compared to elderly and young people. Between elderly and young, the difference was significant (P=0.024). The mean speeds differed between the groups of young and adult animals regarding the left adrenal and its cranial pole. The hardness pattern and its measurements can vary in healthy dogs of different ages, being more rigid in the cranial pole of the left adrenal in young dogs.

Keywords: canine, stiffness, ultrasound, adrenal

RESUMO

Foram avaliadas 60 adrenais de 30 cães, divididos em três grupos, de acordo com a idade: grupo 1 = jovens saudáveis (10 animais) – de um a três anos de vida; grupo 2 = adultos saudáveis (10 animais) – de quatro a nove anos de vida; e grupo 3 = senis saudáveis (10 animais) – a partir de 10 anos de vida. O delineamento experimental foi ao acaso, com significância de 5%. No comprimento, houve diferença significativa (P=0,017) entre os cães jovens e os idosos. A espessura do polo cranial apresentou diferença significativa (P=0,001) entre jovens e adultos quando comparados com o grupo de idosos. A espessura do polo caudal não foi significativa para adultos quando comparadas com idosos e jovens. Entre idosos e jovens, a diferença foi significativa (P=0,024). As médias das velocidades diferiram entre os grupos de animais jovens e adultos quanto à adrenal esquerda e a seu polo cranial. O padrão de dureza e suas medidas podem variar em cães saudáveis de diferentes idades, sendo mais rígidas no polo cranial da adrenal esquerda de cães jovens.

Palavras-chave: caninos, rigidez, ultrassom, adrenal

INTRODUCTION

The adrenal gland function is vital for the production and secretion of important hormones responsible for the maintenance of homeostasis in the animal`s body (Singh, 2019; Klein, 2021). Within the most common adrenal gland pathologies in small animals are hypoadrenocorticism, feochromocitoma,

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incidental adrenal masses, cists, abscesses and hyperadrenocorticism (Cushing syndrome), which deserves special attention due to its prevalence. (Nürnberg *et al*., 2011; Nelson e Couto, 2015).

Ultrasound examination is considered a diagnostic imaging technique, it is noninvasive, non-ionizing and useful to diagnose alterations and diseases that affect the adrenal glands, and hyperadrenocorticism (HAC) can be detected in dogs with adenomegaly with up to 95,6% sensitivity (Melián *et al*., 2021), sensitivity and specificity of 75 and 94%, respectively, for detecting pituitary-dependent HAC (PDH) (Choi *et al*., 2011) , the adrenal glands appear significantly reduced in hypoadrenocorticism (Klein & Peterson, 2010) and malignant neoplasm should be suspected when any mass ≥ 2 cm or with evidence of vascular invasion is observed. (Cook *et al*., 2014). Definitive diagnosis of some pathologies, like those involving adrenal glands, depends on a more invasive exam and has a wide operator-dependent variation. Regarding these limitations, elastography is a relatively new technology that allows a quantitative and qualitative evaluation. It is objective, allows repetition and adds new information related to biomechanics and physiology of the affected tissue. (Simões *et al*., 2020; Hu *et al*., 2023)

The ARFI elastography evaluates tissue elasticity by generation of acoustic radiation force associated with B-mode evaluation, providing quantitative (stiff tissue with high velocities) and qualitative (stiffness represented in a color map) values without evaluator or repetition interference, in a non-invasive and safe manner. (Nightingale *et al*., 2001; Carvalho *et al*., 2015; Garcia *et al*., 2015).

Adrenal gland qualitative elastography in healthy adult dogs demonstrated that these structures are homogenous (medium gray color) and non-deformable. Although elastography can be considered a viable technique, of easy implementation, that generates reference values for structural studies (Fernandez *et al*., 2017), there isn`t any information regarding its applicability in adrenal glands of dogs in a wide range of ages yet. Considering what was presented above, it is evident the importance of new research of ARFI elastography for adrenal glands of dogs with focus on normal value variation in a a wide range of ages to understand normal elastographic behavior. This would help further studies evaluating elastography alterations of the adrenal glands of animals to occur. The goal of this study is to assemble data on normal adrenal glands ARFI elastographic values in dogs of different ages and to describe either quantitative or qualitative features.

MATERIAL AND METHODS

This study was submitted and approved by the Ethics Committee on Animal Use (CEUA protocol No. 8547/15) of the Faculty of Agricultural and Veterinary Sciences (FCAV-UNESP/Jaboticabal, Brazil). The group of animals used for the study consisted of 30 healthy beagle dogs selected after anamnesis, physical examination, hemogram, and biochemical profile. The animals were divided into three experimental groups, each composed of 10 dogs. Group 1 (GC1, 1 to 3 years old), Group 2 (GC2, 4 to 9 years old), and Group 3 (GC3, 10 years old and above) represented young, adult, and elderly dogs, respectively.

The ultrasound examination was performed after extensive trichotomy of the abdominal region. The animals were positioned in dorsal and lateral recumbency with the head towards the monitor and the body parallel to the device. Then, specific coupling gel for ultrasound exam was applied on the skin in the area of interest. The ACUSON S2000/SIEMENS equipment (Siemens, Munich, Germany) was used to perform the B-mode ultrasound with a 9.0MHz linear matrix transducer. The echotexture (homogeneous or heterogeneous) and echogenicity (hypoechoic, hyperechoic, or mixed compared to the surrounding adipose and connective tissue) of the parenchyma, size (length and width of the cranial and caudal poles), contours, and margins (regular or irregular) were evaluated in longitudinal sections. Quantitative and qualitative elastography were performed using the same equipment with the Virtual Touch Tissue Imaging and Quantification (VQT) - Acoustic Radiation Force Impulse (ARFI) elastography software.

Qualitative ARFI provided elastograms in color maps that were evaluated based on the subjective deformity (deformable or non-deformable) of the observed tones. Bluish areas indicated elastic or less rigid tissues, while reddish areas indicated non-deformable or rigid tissues. The software itself was able to provide quality control of the acquired images, homogeneous greenish images indicated high technique quality and yellowish and heterogeneous images indicated low technique quality. In the quantitative evaluation, the system automatically determined the shear wave velocity (SWV) of the regions of interest (ROI) defined by the user through the positioning of a 25mm caliper on the elastogram. Immediately after B-mode scanning, qualitative and quantitative ARFI assessments were carried out. In the qualitative, images with specific focus, white areas (more elastic, soft, and deformable and less rigid tissue) and dark areas (more rigid, harder, and non-deformable) were evaluated. In the qualitative stage, the absolute speed capture function was activated and the caliper was positioned in the area of interest of the adrenals (interest box dimensions were 10 mm × 10 mm); six measurements in each portion of the left and right adrenal glands (body, caudal and cranial pole) were measured (depth between 1.3 and 3.1 cm) to determine the sheer velocities (means and standard deviations [SD]), following what was reported by Fernandez *et al*. (2017).

Statistical analysis was performed using IBM SPSS Statistics 20® (International Business Machines Corp, São Paulo, Brazil). A confidence level of 95% was considered for all tests conducted ($P<0.05$). The analysis of echogenicity and echotexture was performed descriptively (percentages), and the homogeneity of the measurements of total length, cranial and caudal pole heights, and shear velocities were assessed using the Kolmogorov-Smirnov test. Means and standard derivations were then obtained for each of these variables, and differences between groups were assessed using ANOVA and Tukey's post-hoc tests. The sample size was defined based on the already published paper, "Acoustic radiation force impulse (ARFI) elastography of adrenal glands in healthy adult dogs" by Fernandez *et al*. (2017).

RESULTS

The mode-B ultrasound and ARFI elastography (qualitative and quantitative) examinations were successfully performed. In the B-mode ultrasound evaluation, both the right and left adrenal glands appeared hypoechoic compared to the adjacent fat, with homogeneous echotexture, regular contours, and margins in all animals. However, the size of the left and right adrenal glands varied according to age as well as the elastographic evaluation of the cranial pole of the left adrenal gland (Table 1).

In B-mode evaluation of the left adrenal gland, there was a significant difference ($P = 0.017$) between the ages of the animals and the thickness of the cranial pole, caudal pole, and total length. It was found that elderly animals had greater adrenal length than young animals. In adult dogs there was no significant difference in length values. The thickness of the cranial pole showed a significant difference ($P = 0.001$) between young animals and adults when compared to the elderly group. The thickness of the caudal pole was not significant for adults compared to the elderly and young groups. There was a significant difference ($P = 0.024$) between the elderly and young groups.

In B-mode evaluation of the right adrenal gland, there was a difference among the three groups assessed regarding the thickness of the cranial pole, caudal pole, and total length of the gland. The measurement of length showed a significant difference ($P = 0.001$) between the elderly group and adults compared to the young group. The cranial and caudal poles showed a significant difference ($P = 0.001$) in the measurements of young animals compared to adult animals and elderly animals. There was no difference between adult and elderly groups for both the cranial and caudal poles.

Table 1. Statistical values of the variables of the left adrenal gland (LAG) and right adrenal gland (RAG), including measurements of their lengths, cranial and caudal poles, and shear wave velocities (SWV) obtained from elastography

B-mode Ultrasound Variable	Groups	Mean ± standard deviation (cm)	P value (ANOVA)
Length LAG	Young Adults Elderly	1.39±0.19[b] 1.64±0.35[ab] 1.78±0.346[a]	0.017*
Cranial pole of LAG	Young Adults Elderly	0.44±0.06[b] 0.48±0.06[b] 0.58±0.10[a]	< 0.001*
Caudal pole of LAG	Young Adults Elderly	0.45±0.08[b] 0.52±0.07[ab] 0.59±0.15[a]	0.024*
Shear Wave Velocities (SWV)	**Groups**	**Mean ± standard deviation (m/s)**	**P value (ANOVA)**
SWV cranial LAG	Young Adults Elderly	1.79±0.45[a] 1.38±0.18[b] 1.56±0.28[ab]	0.028*
SWV caudal LAG	Young Adults Elderly	1.57±0.40[a] 1.45±0.32[a] 1.53±0.41[a]	0.769
B-mode Ultrasound Variable	**Groups**	**Mean ± standard deviation (cm)**	**P value (ANOVA)**
Lenght RAG	Young Adults Elderly	1.45±0.25[b] 1.85±0.47[a] 2.22±0.28[a]	< 0.001*
Cranial pole RAG	Young Adults Elderly	0.44±0.11[b] 0.58±0.13[a] 0.68±0.08[a]	< 0.001*
Caudal Pole RAG	Young Adults Elderly	0.44±0.09[b] 0.59±0.14[a] 0.70±0.10[a]	< 0.001*
Shear Wave Velocities (SWV)	**Groups**	**Mean ± standard deviation (m/s)**	**P value (ANOVA)**
SWV cranial RAG	Young Adults Elderly	1.90±0.42[a] 1.66±0.45[a] 1.60±0.46[a]	0.205
SWV caudal RAG	Young Adults Elderly	1.71±0.39[a] 1.67±0.58[a] 1.55±0.62[a]	0.779

a, b: Different lowercase letters in the columns indicate statistical differences by ANOVA and Tukey's post hoc test. Equal letters do not differ significantly from each other.

In the elastography exam of the left adrenal gland, there was a significant difference (P = 0.028) between the groups of young animals and adults in the cranial pole. The values of the elderly group showed no difference when compared to the young and adult animals. Regarding the caudal pole of the left adrenal gland (P = 0.769) and the cranial (P = 0.205) and caudal (P = 0.779) poles of the right adrenal gland, there was no significant difference between the groups.

B-mode ultrasound and elastography images illustrating the measurements of length, cranial pole, caudal pole, and SWV are shown in Fig. 1.

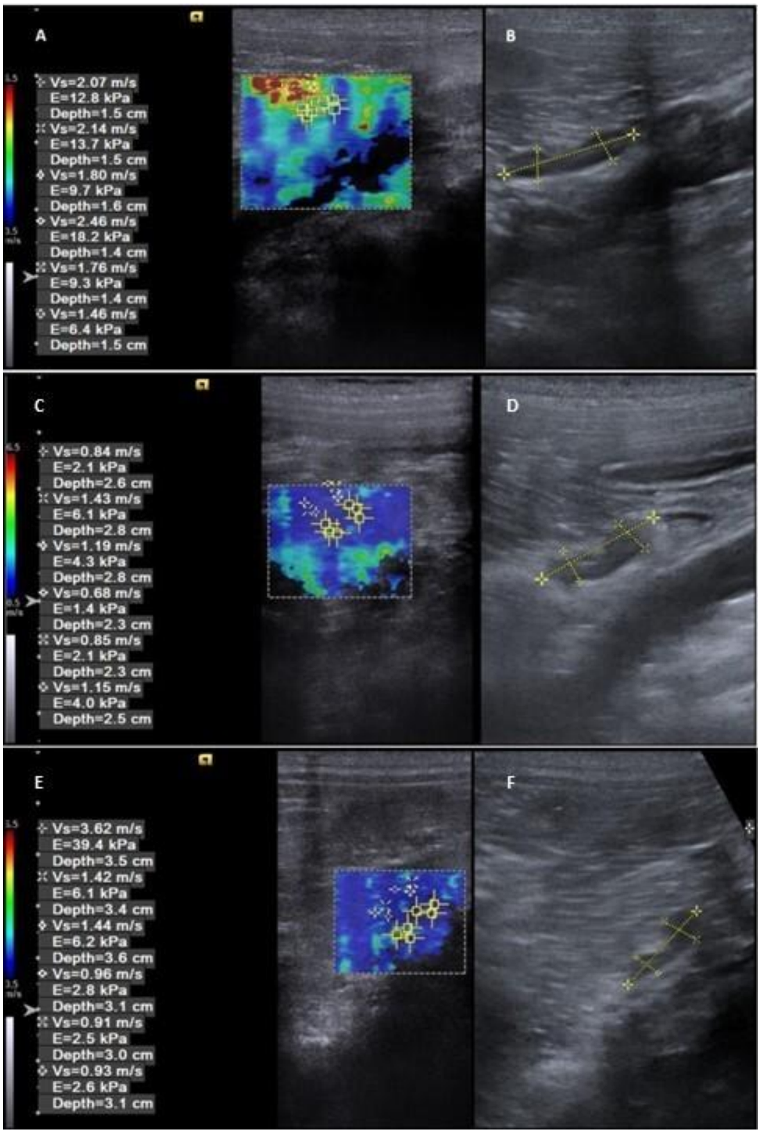

Figure 1. A: Measurement of SWV in the adrenal gland of a young animal in six different areas (ROI's). B: Measurements of length (larger dashed line), caudal pole (dashed line on the right), and cranial pole (dashed line on the left) of the adrenal gland of a young animal. C: Measurement of SWV in the adrenal gland of an adult animal in six different areas (ROI's). D: Measurements of length (larger dashed line), caudal pole (dashed line on the right), and cranial pole (dashed line on the left) of the adrenal gland of an adult animal. E: Measurement of SWV in the adrenal gland of an elderly animal in six different areas (ROI's). F: Measurements of length (larger dashed line), caudal pole (dashed line on the right), and cranial pole (dashed line on the left) of the adrenal gland of an elderly animal.

DISCUSSION

According to the results of this study, it was possible to gather quantitative and qualitative data through ARFI elastography technique and to evaluate the adrenal glands biometry through B-mode exam of healthy Beagle dogs of different ages. This is the first known study that determines reference values of ARFI technique for adrenal glands evaluation in a wide range of ages group of dogs, allowing the use of tissue stiffness study to a more precise diagnosis.

The B-mode ultrasound is the most used

modality to evaluate and identify possible affections of the adrenal gland in dogs. According to Soulsby *et al*. (2015), the absence of adrenal glands alteration does not exclude the presence of a disease. Factors that may influence the results are the experience of the observer/examiner, age, weight and breed of the animals (Mogicato *et al*., 2011; Cook *et al*., 2014).

A less subjective evaluation is to measure the caudal pole of the adrenal glands due to its lower variation compared to the aforementioned factors (Choi *et al*., 2011; Soulsby *et al*., 2015). Choi *et al*. (2011) evaluated the adrenals of dogs weighing less than 10kg and proposed a value of 6 mm for the caudal pole dimension. Soulsby *et al*. (2015) classified the caudal pole dimension of animals up to 10kg (small size) as 5.4 mm, dogs weighing 10-30kg (medium size) as 6.8 mm, and dogs weighing over 30 kg (large size) as 8mm.

The chosen breed (Beagle) can be considered small-sized, as demonstrated by the average weight of Beagles (mean = 8.5±3.19kg) and body condition score of 6 (on a scale of 1 to 9) in the study by Fernandez *et al*. (2017). However, individual specimens may exceed the weight limit of 10 kg (small size), a category established in the study by Soulsby *et al*. (2015). In the present study (young, adult, and elderly animals), the overall mean caudal pole dimensions of the left and right adrenals were 5.2mm and 5.7mm, respectively, which is consistent with previous studies (Soulsby *et al*., 2015; Melián *et al*., 2021) and within the category of small-sized dogs mentioned above. Melián *et al*. (2021), evaluation of the adrenal glands' dimensions in dogs of different breeds and in four weight categories, found high sensitivity in detecting adrenal enlargement when the <10kg category was subdivided into ≥2.5–5kg and >5–10kg (96.9% and 100%, respectively), showing a high correlation between different weight ranges within the same category.

Among the groups, there was a difference in gland size due to the age difference of the patients, indicating the susceptibility of animals to have increased gland size as they get older (Reul *et al*., 1991; Goy-Thollot *et al*., 2007). Regarding the mean length of the adrenals, younger dogs (P=0.001: 1.45 ± 0.25 cm and P=0.017: 1.78 ± 0.346 cm for right and left, respectively) had smaller glands.

According to the qualitative evaluation of elastography in this study, the parenchyma of the adrenal glands in all evaluated dogs was considered non-deformable, consistent with the study by Fernandez *et al*. (2017). The quantitative elastographic evaluation was described in a previous study in healthy animals with mean shear wave velocities as follows: left adrenal gland, cranial pole: 1.42m/s and caudal pole: 1.71m/s; right adrenal gland, cranial pole: 1.42m/s and caudal pole: 1.63m/s, with a 95% confidence interval. There was no difference in shear wave velocity values between the evaluated portions and between the left and right glands (Fernandez *et al*., 2017).

In the present study, the mean shear wave velocities differed between the age groups only between young and adult animals for the left adrenal gland and its cranial pole (1.79m/s and 1.38m/s, respectively), while for other values, there was no significant difference. This difference can be explained by the tendency of mean shear wave velocities of adrenal glands in young dogs, presumably with lower weights, to be higher in this study. However, when only adults and elderly dogs are considered, the results are like those found by Fernandez *et al*. (2017), taking into consideration the weight of each studied animal. Holdsworth *et al*. (2014) had as their main conclusion in their study that there is a significant negative relationship between depth and the value of shear wave velocity (SWV), that is, in all organs evaluated (liver, kidney and spleen), when increased the depth the SWV reduced. There can be many variations, even if we follow the protocol as closely as possible. Furthermore, the exam is not 100% reliable in determining stiffness. It is worth mentioning that, even if a statistical difference was observed between them, the values are very close, which in practice is not very significant.

The importance of this study was to obtain results that can potentially contribute to the identification and diagnosis of lesions or alterations of the adrenal glands by providing information on what is considered normal compared to possible abnormalities. In humans, elastography has been used to differentiate solid and non-malignant cystic adrenal lesions quantitatively (Słapa *et al*., 2014). Feliciano *et al*. (2017) studied ARFI elastography in mammary tumors in female dogs and obtained results consistent with the stiffness of neoplastic mammary tissue, proving to be an effective method in predicting the malignancy of these lesions.

CONCLUSIONS

With the present study, it was possible to conclude that the stiffness pattern and measurements of the right and left adrenals can vary in healthy dogs of different ages. Higher rigidity in the cranial pole of the right adrenal in young dogs differing from adults was the main observation. The obtained results, aiming to standardize the normality of these glands within a single breed (beagle) and according to age categories, can assist in future studies on the same subject, not only in this breed but also in similar-sized breeds, improving the diagnosis of structural alterations of the adrenals.

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