

Acoustic radiation force impulse elastography of prostate and testes of healthy dogs: preliminary results

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OBJECTIVES: To describe the use of acoustic radiation force impulse elastography to evaluate the prostate and testes in healthy dogs and establish reference values for these organs.

METHODS: Thirty dogs were divided into three groups according to their age: juvenile, adult and senior. Echotexture, size, contours and margins of prostate and testes were assessed via ultrasound. The presence of deformities and tissue stiffness (greyscale and homogeneous or heterogeneous) were evaluated by qualitative acoustic radiation force impulse. The shear velocity was evaluated quantitatively.

RESULTS: The B mode findings were normal. The qualitative elastography demonstrated that the testes and prostate tissues were hard, homogeneous and not pliable. The shear velocity values were: left testes – juveniles: 1.28 m/s, adults: 1.23 m/s and seniors: 1.23 m/s; right testes – juveniles: 1.28 m/s, adults: 1.28 m/s and seniors: 1.28 m/s; left prostatic lobe – juveniles: 1.74 m/s, adults: 2.03 m/s and seniors: 1.82 m/s; right prostatic lobe – juvenile: 1.62 m/s, adults: 1.87 m/s and seniors: 1.90 m/s with no significant differences between groups.

CLINICAL SIGNIFICANCE: Acoustic radiation force impulse elastography of the testes and prostate in dogs was easily implemented. This study provides baseline data for these organs.

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INTRODUCTION

Elasticity is the ability of a body or substance to return to its original size or shape after it is deformed by an external force. The amount of deformity is inversely proportional to the stiffness and breakthrough times for tissue repair (Goddi *et al.* 2012). Elastography was first developed in the early 1990s for studying the hardness of tissues. This technique has been used in humans for identifying and differentiating between mammary tumours, diagnosing prostate tumours, assessing the testes (D'Anastasi *et al.* 2011, Duda *et al.* 2011) and, recently, evaluating mammary neoplasms in female dogs (Feliciano *et al.* 2014).

Acoustic radiation force impulse (ARFI) elastography is an imaging technique that provides quantitative and qualitative measurements of tissue stiffness with reduced inter-observer variability. Qualitative ARFI uses short, high intensity acoustic pulses to deform the tissue and create a static greyscale map (elastogram) that represents the relative stiffness of the tissue in the imaged region. The elastogram can then be compared to the corresponding conventional ultrasound image. In general, lighter areas represent more deformable tissue compared with darker areas (Goddi *et al.* 2012).

Quantitative ARFI involves directing a primary acoustic pulse towards a region of interest to create propagating pressure waves

that are capable of quantitatively deforming the tissues (pressure wave velocity of propagation or shear velocity). The velocity of propagation and attenuation of the waves are related to the rigidity and viscoelasticity of the tissue (Comstock 2011).

Tissue damage (e.g. in the testis and prostate) of any (physiological or pathological) nature immediately promotes a series of events related to tissue repair and can present itself in two forms: tissue regeneration with recomposition of functional activity of the tissue or tissue healing with the formation of a fibrotic matrix. In cases of chronic and neoplastic lesions, the healing process negatively interferes and promotes a loss of normal functional tissue patterns (Balbino *et al.* 2005). This cicatricial characteristic is related to tissue stiffness and currently can be studied by qualitative and quantitative ARFI elastography allowing the differentiation between benign and malignant processes in tissues, such as mammary neoplasms in bitches (Feliciano *et al.* 2014).

Canine testicular and prostatic ultrasonography is an important and complementary diagnostic method for identifying reproductive diseases in this species (Karmazyn 2010). However, the definitive diagnosis of benign or malignant lesions is made only by histopathology after castration or ultrasound-guided biopsy. These methods are considered invasive and may cause complications, such as urethral haematuria, haemorrhage, peritonitis secondary to abscess or dissemination of cancer cells during prostate biopsy (Smith 2008). Therefore, by standardising ARFI elastography measurements of the prostate and testes of dogs, this pilot study sought to provide a non-invasive alternative for the diagnosis of prostatic and testicular alterations in small animals that can be used in future experimental studies and routine veterinary examinations.

In humans, elastography of the prostate is used to visualise focal prostatic structures because better contrast is obtained compared with B-mode ultrasound, and there is greater precision of orientation during biopsies of this tissue (D'Anastasi *et al.* 2011, Zai *et al.* 2012). The qualitative technique has 100% sensitivity in detecting testicular tumours when values indicate an increased stiffness of the testicular parenchyma. This technique also facilitates the diagnosis of cystic alterations, haematomas, calcification, necrosis and other alterations that disrupt the homogeneity of the testicular tissue (Aigner *et al.* 2012) and can detect malignant lesions (Lorenz *et al.* 2000). These results demonstrate the importance of elastography as a diagnostic tool for assessing and qualitatively characterising images and for determining normal shear velocity reference values in the testes and prostates of dogs.

Given the importance of this new ultrasound technique for studying the stiffness of various tissues and its recent application in veterinary medicine, the aims of this study were to describe the use of ARFI elastography for evaluating the prostatic and testicular parenchyma in dogs and to establish normal reference values for these tissues.

MATERIALS AND METHODS

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the School of Agrarian and

Veterinary Sciences of Sao Paulo State University, Brazil (protocol No 023705/12). Thirty male dogs of different breeds from the same kennel and owners (dachshund, poodle, Doberman, shih-tzu, pug and mixed breed dogs) aged between 5 weeks and 15 years and weighing between 2.0 and 12.0 kg were included in this study. To minimise the effects of anatomical variations (weight and abdominal depth and prostatic size) on the elastography measurements of the prostate, this study included smaller animals with minimal weight variations (weighing between 2.0 and 12.0 kg) as described by Holdsworth *et al.* (2014).

General and specific physical examinations (inspection of the scrotum, testicular palpation, rectal palpation and digital assessment of the prostate) were performed on all animals to determine which animals were healthy and met the inclusion criteria for the study. After examination, the animals were divided into three experimental groups: group 1 (G1, n=5) – dogs aged five weeks to one year (juvenile; 9.2 ± 1.92 months); group 2 (G2, n=13) – dogs aged one year to five years (adults; 2.69 ± 1.16 years) and group 3 (G3, n=12) – dogs aged more than five years (senior; 8.16 ± 2.08 years).

After the animals were selected, the abdomen and scrotal sac were clipped for ultrasonography. Before the examination, gel was applied for the ultrasound. No sedation was needed. To facilitate the evaluation of the prostate, the animals were restricted from food for 8 hours before performing an enema.

The ultrasonography was performed by a single evaluator experienced in ultrasonographic examinations. B-Mode ultrasonography was performed with a 9.0 MHz linear transducer using ACUSON S2000/SIEMENS ultrasound equipment (Siemens, Munich, Germany). The echotexture (homogeneous or heterogeneous; hypo, hyperechoic or mixed) of the parenchyma, size (increased, decreased or normal), contours and margins (regular or irregular) of the prostate and testes (right and left) were assessed and categorised via B-Mode ultrasound in longitudinal and transverse sections.

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

After B-mode ultrasonography, qualitative ARFI was performed to obtain greyscale images of the testicular and prostatic tissue in longitudinal sections. The images were evaluated for the presence of deformities, white areas (indicative of more elastic tissue that is less rigid, softer and more pliable) and dark regions (more rigid, harder and not pliable).

The quantitative analysis was performed after scanning the testes and prostate with the B-mode ultrasound to determine the mean shear velocity values for these tissues. To obtain the shear velocity, the calliper was positioned in the right and left testicular parenchyma in the longitudinal section (excluding the mediastinal portion). In the prostate, the measurements were obtained from transverse sections of the right and left lobes. Six measurements were recorded in each region (testicular, left and right lobe). The depth utilised for the testicular measurements was 0.5 to 1.0 cm and 1.5 to 2.5 cm for the prostate measurements.

The data were tested for normality and homogeneity of variances (F test). The gross and transformed mean were evaluated by analysis of variance. In addition, a one-way analysis of variance (ANOVA) for paired samples was applied to all shear velocities (values of testicular and prostatic lobes). A P value <0.05 was considered significant. Non-parametric data (echotexture, echogenicity size, greyscale images and the presence of deformities of the testes) were evaluated using descriptive analysis.

RESULTS

None of the owners reported or observed alterations that would compromise the health of the animals during the review of the medical history or the clinical examination. Assessment of the testes and prostates in all dogs using B-mode ultrasound revealed no abnormalities in the shape, size, margins and surface of the parenchyma (echogenicity and echotexture). Although it is possible that adults and senior animals [older than five years (2 adults and 12 seniors)] had benign prostatic hyperplasia, the clinical and ultrasonography evaluations did not indicate this alteration.

During qualitative elastography, for all age groups, the dogs demonstrated a prostatic and testicular parenchyma that was homogeneous and not pliable even though the prostatic parenchyma has a higher stiffness than the testes (Fig 1). There were no observed qualitative differences in the stiffness characteristics of these tissues between the experimental groups.

For quantitative elastography (Fig 2), the mean shear velocity values for the testes and prostate are described in Table 1. There was no significant difference between the right and the left testes ($P=0.1867$) or the right and the left prostate lobes ($P=0.1474$).

DISCUSSION

The canine testicular homogeneity and stiffness values obtained from qualitative ARFI in this study can aid the future use of elastography in the detection of tissue heterogeneity (soft or hard areas) resulting from pathological processes in dogs. According to previous reports, the diagnosis and characterisation of testicular lesions in dogs are determined after physical examination (palpation) and ultrasonography (Nyland & Matton 2002, Davidson & Baker 2009, Silva *et al.* 2012). These are dependent on the veterinarian's expertise because small and deep nodules might remain undetected even by an experienced veterinarian, and the characterisation of the consistency and type of lesion by ultrasound is almost impossible (Grasso *et al.* 2010). In humans, it is suggested that elastography could be important for the detection and characterisation of these tissues allowing for early diagnosis of pathological testicular alterations (Grasso *et al.* 2010, Aigner *et al.* 2012).

Ultrasound examination of the canine prostate aids evaluation of the echogenic characteristics of healthy parenchyma and detection of inflammatory processes, such as prostatitis or neoplastic growths. If the homogeneous nature of the prostatic image is lost, it is difficult to differentiate between the types of

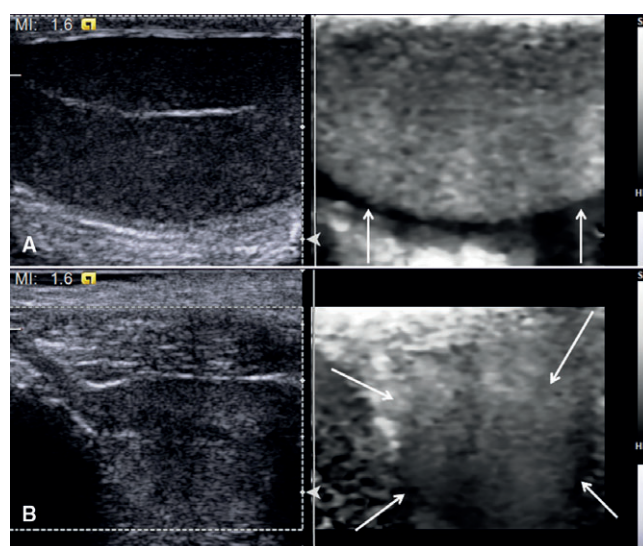


FIG 1. Image of the qualitative ARFI elastography analysis showing the stiffness in the testicular and prostatic parenchyma of the dogs. (A) B-mode ultrasound image and qualitative ARFI elastography (arrows) of the testicle of a dog showing a homogeneous image (mid-grey) that is typical and not pliable. (B) B-mode ultrasound image and qualitative ARFI elastography (arrows) of the prostate of a dog showing a homogeneous image (black) that is typical and not pliable. Note the increased stiffness of the prostate compared to the testes

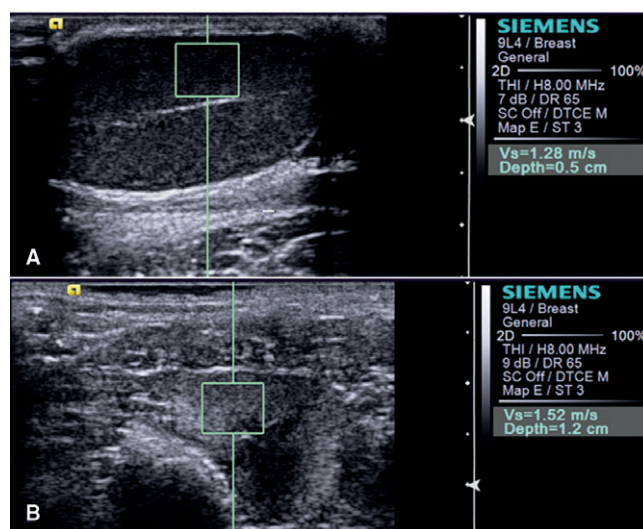


FIG 2. Image of quantitative ARFI elastography in the prostate and testis of a dog. Note the presence of the calliper within the testicular parenchyma (A) and prostate (B) for measuring the shear velocity

pathology present in this tissue (Smith 2008), which decreases the specificity of this imaging method. Similar to elastography of the human prostate (Barr *et al.* 2012, Zai *et al.* 2012), measuring canine prostatic stiffness, homogeneity and lack of pliability may assist in the identification of possible aetiologies for increased or decreased stiffness (for example, cystic areas with benign hyperplasia (cystic) or calcification in chronic inflammatory or neoplastic processes). This may increase the sensitivity of ultrasonography for establishing a diagnosis and prognosis of prostate diseases in dogs.

Table 1. Mean and standard deviation for shear velocity of the left and right testes and prostatic lobe of dogs with quantitative ARFI elastography

Samples	Shear velocity (m/s)			P value
	Group 1	Group 2	Group 3	
Left testis	1.28 ±0.14	1.23 ±0.15	1.23 ±0.14	0.7802
CI	1.10 to 1.47	1.14 to 1.32	1.14 to 1.32	
Right testis	1.28 ±0.14	1.28 ±0.14	1.28 ±0.14	0.9989
CI	1.09 to 1.27	1.12 to 1.24	1.07 to 1.30	
Left prostatic lobe	1.74 ±0.23	2.03 ±0.37	1.82 ±0.22	0.1867
CI	1.52 to 1.96	1.74 to 2.32	1.46 to 2.17	
Right prostatic lobe	1.62 ±0.20	1.87 ±0.31	1.90 ±0.19	0.1474
CI	1.43 to 1.82	1.63 to 2.10	1.58 to 2.21	

CI Confidence interval 95% One-way analysis of variance (ANOVA) at 5% significance level. Group 1: juvenile; group 2: adults and group 3: senior

Quantitative ARFI has been used to assess various abdominal organs in humans. The testes are extra-abdominal organs with minimal overlying tissue, which is suitable for ARFI assessment (D'Anastasi *et al.* 2011). In veterinary research, recent studies have described this technology with significant results (Feliciano *et al.* 2014, Holdsworth *et al.* 2014), but this is the first study to evaluate the testicular and prostatic parenchyma of dogs.

In one published study, the effect of selected variables on the values obtained for the shear velocity of the abdominal organs of dogs was observed (Holdsworth *et al.* 2014). To minimise the effects of body conformation (weight, abdominal volume and examination depth) cited by Holdsworth *et al.* (2014) in the elastography of healthy dogs, this study examined animals of the same approximate size with little variation in weight.

The shear velocities obtained for the testes in juvenile (1.28 m/s), adult (1.23 m/s) and senior (1.23 m/s) dogs were similar and higher than those observed in humans (0.62 to 1.01 m/s) (D'Anastasi *et al.* 2011), most likely because dogs have more testicular fibrous tissue. For the prostate, the mean values for shear velocity in humans are 1.32 ±0.36 m/s (Goertz *et al.* 2011), which is similar to that observed in dogs.

It was verified that the values for shear velocity of the canine prostate were similar for animals of different ages. However, 100% of canines (uncastrated) develop histological evidence of hyperplasia with advanced age (Shimomura *et al.* 2009). The present study included adult and senior animals. In addition, the dogs in these experimental groups did not demonstrate any clinical or imaging signs consistent with this alteration. These observations may be explained by the presence of two different patterns for benign prostatic hyperplasia in dogs: (1) initial glandular hyperplasia (condition suggested for animals in the present study) is histologically characterised by the proliferation of secreting cells and the projection of hypertrophic and hyperplastic epithelium without structural involvement of the gland and (2) cystic hyperplasia is characterised by the formation of cavities, areas with intercalated glandular hyperplasia and foci of atrophy, causing a change in the prostatic structure. The presence of cystic structures and prostatic atrophy present in advanced cases of prostatic hyperplasia (as well as cases of prostatitis and neoplasm) are easily identified by ultrasound (Shimomura *et al.* 2009) and may alter the tissue stiffness of the prostatic parenchyma and subsequently promote alterations in the shear velocity values.

The reference values determined for the shear velocity of the testes and prostate of dogs can assist the diagnosis of malignant lesions in these tissues by favouring early diagnosis and accelerating the institution of therapy in diseased animals. This statement is corroborated by data in human and veterinary studies (D'Anastasi *et al.* 2011, Dudea *et al.* 2011, Feliciano *et al.* 2014) where quantitative ARFI techniques were used to differentiate between benign and malignant tumours (rigid and non-pliable tissues with high shear velocity values are indicative of malignancy).

In the present study, the ARFI technique yielded reproducible images of the testes and prostate in dogs. Other operators should also be able to obtain similar image quality to increase the sensitivity of tissue heterogeneity detection (Grasso *et al.* 2010, Aigner *et al.* 2012, Barr *et al.* 2012, Zai *et al.* 2012).

It is important to comment on some of the limitations observed in this study.

First, the qualitative and quantitative elastography of the testes and prostate in dogs was performed without difficulty and without sedation. Due to the location of the evaluated structures, there was no interference from movements (e.g. respiration) that hindered the acquisition of the measurements as cited by Holdsworth *et al.* (2014) during abdominal elastography in canines. Secondly, this pilot study provides important data about the validation of the technique and sets reference values for dogs, but it is necessary to conduct a study with a larger number of animals of different breeds and weights to verify how these variables affect the elastography values in the prostate and testes of canines. Thirdly, because the animals were considered healthy breeding animals by clinical examination and B-mode ultrasound, the collection of tissue samples (prostate and testes) was not allowed. Future studies should conduct histological evaluation of testicular and prostatic tissues in dogs, especially in diseased animals.

Fourthly, only one person was responsible for imaging, and future studies should verify any inter- or intra-observer variability in the acquisition and values obtained for qualitative and quantitative elastography of the canine prostate and testis.

After standardising the reference values for testicular and prostatic elastography in healthy dogs, the differences in shear velocity values of diseased tissues can be evaluated to differentiate between benign and malignant tumours in canines.

Quantitative and qualitative ARFI elastography of the testes and prostate in dogs was easily implemented, and this study provides baseline data for the study of these organs with ARFI.

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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.

References

- Aigner, F., De Zordo, T., Pallwein-Prettner, L. *et al.* (2012) Real-time sonoelastography for the evaluation of testicular lesions. *Radiology* **263**, 584-589
- Balbino, C. A., Pereira, L. M. & Curi, R. (2005) Mecanismos envolvidos na cicatrização: uma revisão. *Brazilian Journal of Pharmaceutical Sciences* **41**, 27-51
- Barr, R. G., Memo, R. & Schaub, C. R. (2012) Shear wave ultrasound elastography of the prostate: initial results. *Ultrasound Quarterly* **28**, 13-20
- Comstock, C. (2011) Ultrasound elastography of breast lesions. *Ultrasound Clinics* **6**, 407-415
- D'Anastasi, M., Schneevoigt, B. S., Trottmann, M. *et al.* (2011) Acoustic radiation force impulse imaging of the testes: a preliminary experience. *Clinical Hemorheology and Microcirculation* **49**, 105-114
- Davidson A. P & Baker T. W. (2009) Reproductive ultrasound of the dog and tom. *Topics in Companion Animal Medicine* **24**, 64-70
- Dudea, S. M., Giurgiu, C. R., Dumitriu, D. *et al.* (2011) Value of ultrasound elastography in the diagnosis and management of prostate carcinoma. *Medical Ultrasonography* **13**, 45-53
- Feliciano, M. A. R., Maronezi, M. C., Pavan, L. *et al.* (2014) ARFI elastography as complementary diagnostic method of mammary neoplasm in female dogs - preliminary results. *Journal of Small Animal Practice* **55**, 504-508
- Goddi, A., Bonardi, M. & Alessi, S. (2012) Breast elastography: a literature review. *Journal of Ultrasound* **15**, 192-198
- Goertz, R. S., Amann, K., Heide, R. *et al.* (2011) An abdominal and thyroid status with acoustic radiation force impulse elastometry – A feasibility study acoustic radiation force impulse elastometry of human organs. *European Journal of Radiology* **80**, e226-e230
- Grasso, M., Blanco, S., Raber, M. *et al.* (2010) Elasto-sonography of the testis: preliminary experience. *Archivio Italiano Di Urologia e Andrologia* **82**, 160-163
- Holdsworth, A., Bradley, K., Birch, S. *et al.* (2014) Elastography of the normal canine liver, spleen and kidneys. *Veterinary Radiology & Ultrasound* **55**, 620-627
- Karmazyn, B. (2010) Escrotal ultrasound. *Ultrasound Clinics* **5**, 61-74
- Lorenz, A., Ermet, H., Sommerfeld, H. J. *et al.* (2000) Ultrasound elastography of the prostate. A new technique for tumor detection. *Ultraschall in der Medizin* **21**, 8-15
- Nyland, T. G. & Matton, J. S. (2002) Small Animal Diagnostic Ultrasound W. B. Saunders, Philadelphia, PA, USA p 461
- Silva, L. D. M., Souza, M. B., Barbosa, C. C. *et al.* (2012) Bi-dimensional-ultrasonography and doppler to evaluate the reproductive tract of small animals. *Ciência Animal* **22**, 339-353
- Shimomura, J. Z., Eugênio, F. R., Luvizotto, M. C. R. *et al.* (2009) Benign prostatic hyperplasia in dog: comparison diagnosis methods. *Veterinária e Zootecnia* **16**, 117-126
- Smith J. (2008). Canine prostatic disease: a review of anatomy, pathology, diagnosis, and treatment. *Theriogenology* **70**, 375-383
- Zai, L., Polascik, T. J., Foo, W. C. *et al.* (2012) Acoustic radiation force impulse imaging of human prostates: initial in vivo demonstration. *Ultrasound in Medicine and Biology* **38**, 50-61