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Aplicabilidade da elastografia *acoustic radiation force impulse* (ARFI) na avaliação de neoplasias em caninos

Pirassununga
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RESUMO

FELICIANO, M.A.R. **Aplicabilidade da elastografia acoustic radiation force impulse (ARFI) na avaliação de neoplasias em caninos**. 109p., 2022. Tese (Livre Docência). Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022. Na veterinária existem vários estudos que demonstram a aplicabilidade da elastografia *acoustic radiation force impulse* (ARFI), sendo que os principais relacionam o aumento da rigidez tecidual de tumores em caninos com a presença de malignidade, apresentando com alta sensibilidade, especificidade e acurácia diagnóstica para tal finalidade. Como pioneiro na utilização do método na veterinária, apresento nesta tese alguns dos trabalhos mais importantes que desenvolvi nos últimos anos da minha carreira (2014-2022), relacionando a aplicabilidade da ARFI na detecção de malignididade em tumores de cães. **Artigo 1:** Avaliou-se a aplicabilidade da elastografia ARFI como método complementar no diagnóstico das neoplasias mamárias em cadelas, verificando-se que os tumores malignos eram mais rígidos do que nódulos benignos. **Artigo 2:** Buscou-se descrever a utilização da ARFI na avaliação de afecções testiculares em cães e verificar que as neoplasias foram as afecções mais rígidas. **Artigo 3:** Objetivou-se verificar eficácia das diferentes modalidades da ultrassonografia na predição de malignidade dos tumores de mama em cadelas e, especificamente à ARFI, obteve-se valores de velocidade de cisalhamento > 2,57 m/s como indicativos de malignidade tecidual, com sensibilidade 94,7%, especificidade 97,2% e acurácia de 95,04%. **Artigo 4:** Estudou-se características ultrassonográficas dos carcinomas mamários em cadelas, diferentes tipos e graus de carcinomas mamários em cadelas, verificando-se que a elastografia auxilia na identificação de neoplasias do tipo especial. **Artigo 5:** Buscou-se verificar a acurácia das diferentes técnicas de ultrassom na identificação de metástases em linfonodos loco-regionais de cadelas com neoplasias mamárias e a ARFI detectou metástase nessas estruturas, permitindo também a diferenciação entre tecidos normais, reativos e metastáticos. **Artigo 6:** Verificou-se a aplicabilidade de técnicas ultrassonográficas na avaliação de tumores cutâneos e subcutâneos de cães, sendo que a ARFI demonstrou que tumores malignos são mais rígidos que benignos. **Artigo 7:** Estudou-se a aplicabilidade da ARFI na detecção de malignidade em lesões esplênicas de cães, verificando-se acurácia de 97% da elastografia para detecção de lesões malignas do baço canino.

Palavras-chave: cães, ultrassom, rigidez tecidual

ABSTRACT

FELICIANO, M.A.R. **Applicability of acoustic radiation force impulse (ARFI) elastography in the evaluation of canine neoplasms**. 109p., 2022. Tese (Livre Docência). Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022. In veterinary, there are several studies that demonstrate the applicability of acoustic radiation force impulse (ARFI) elastography, the main ones relating the increase in tissue stiffness of tumors in canines with the presence of malignancy, presenting high sensitivity, specificity and diagnostic accuracy for this. goal. As a pioneer in the use of the method in veterinary, I present in this thesis some of the most important works that I developed in the last years of my career (2014-2022), relating the applicability of ARFI to the detection of malignancy in tumors in canines. **Article 1:** The applicability of ARFI elastography as a complementary method in the diagnosis of mammary neoplasms in bitches was evaluated, verifying that the malignant tumors were more rigid than benign nodules. **Article 2:** We sought to describe the use of ARFI in the evaluation of testicular disorders in dogs and it was verified that neoplasms were the most rigid disorders. **Article 3:** The objective was to verify the effectiveness of the different modalities of ultrasound in the prediction of malignancy of breast tumors in bitches and, specifically for ARFI, shear velocity values > 2.57 m/s were obtained as indicative of tissue malignancy, with 94.7% sensitivity, 97.2% specificity and 95.04% accuracy. **Article 4:** Ultrasonographic characteristics of mammary carcinomas in bitches, different types and degrees of mammary carcinomas in bitches were studied, verifying that elastography helps in the identification of neoplasms of the special type. **Article 5:** We sought to verify the accuracy of different ultrasound techniques in identifying metastases in lco-regional lymph nodes of bitches with mammary neoplasms and ARFI detected metastasis in these structures, also allowing the differentiation between normal, reactive and metastatic tissues. **Article 6:** The applicability of ultrasound techniques was verified in the evaluation of skin and subcutaneous tumors in dogs, and the ARFI showed that malignant tumors are more rigid than benign. **Article 7:** The applicability of ARFI in the detection of malignancy in splenic lesions of dogs was studied, verifying an accuracy of 97% of elastography for the detection of malignant lesions of the canine spleen.

Keywords: dogs, ultrasound, tissue stiffness

SUMÁRIO

INTRODUÇÃO

Baseado no meu histórico científico abaixo descrito, conjunto de disciplinas que ministro na graduação e pós-graduação e minha área de pesquisa, esta tese de livre-docência foi escrita no formato de Artigos, de acordo com a Portaria interna FZEA nº 7/2015, e apresentará os resultados obtidos nos últimos anos de pesquisa.

Desde meu doutorado, tenho trabalhado com a ultrassonografia e sua aplicação na avaliação de tecidos neoplásicos em pequenos animais, visto a importância dessas anormalidades nos pacientes, prognóstico reservado à desfavorável que os tumores malignos apresentam em caninos e felinos e a necessidade de se realizar um diagnóstico acurado, rápido e o menos invasivo possível para os animais, visando aumento da sobrevida dos pacientes.

Em Medicina, novas técnicas de imagem têm sido implementadas e, dentre elas, ressalta-se a elastografia com resultados bastante promissores, o que faculta a utilização desse método em Medicina Veterinária, particularmente no cão pela sua importância econômica, afetiva e de similaridade cientifica para o homem.

A elastografia é um método recente que avalia a rigidez dos tecidos, fornecendo informações sobre a elasticidade tecidual. Esta técnica tem demonstrado singular aplicabilidade na avaliação de tecidos crônicos e detecção de malignidade dos tumores, conforme será demonstrado ao longo desta tese, incremetando o poder diagnóstico da ultrassonografia no estudo de tecidos neoplásicos em animais de companhia.

A técnica elastográfica ARFI – *Acoustic Radiation Force Impulse* tem grandes vantagens sobre os outros métodos elastográficos e traz informações valiosas sobre aspectos biológicos e estruturais de diferentes tecidos em pacientes humanos e animais. Na veterinária, eu e meu grupo de pesquisa temos o ineditismo das pesquisas envolvendo esse método em diversas espécies e somos responsáveis pela publicação dos principais trabalhos nacionais e internacionais utilizando a ARFI, o que nos dá grande responsabilidade no que desenvolvemos e importância junto ao cenário da ultrassonografia veterinária brasileira e internacional.

Os resultados aqui apresentados foram obtidos de auxílio à pesquisa e de bolsa Jovem Pesquisador FAPESP (2013/06443-1 e 2012/16635-2, respectivamente), projeto intitulado “Elastografia, ultrassonografia com contraste por microbolhas e Doppler como métodos de diagnóstico das neoplasias mamárias, afecções prostáticas e testiculares em cães”, do qual fui responsável, e outros resultados alcançados em projetos desenvolvidos por mim e meu grupo de pesquisa.

Apresento em seguinda o meu histórico científico, fundamentação teórica abordando uma revisão geral sobre a elastografia e sete artigos relacionados ao tema proposto na tese:

ARTIGO 1: O primeiro artigo publicado com a elasgrafia ARFI na espécie canina, avaliando a capacidade do método em detectar malignidade de tumores mamários em cadelas. Os resultados desse manuscrito demonstram que a técnica ARFI pode indicar a presença de malignidade nas neoplasias mamárias, pelo aumento da rigidez tecidual nas massas.

ARTIGO 2: No segungo manuscrito, a técnica ARFI foi utilizada para avaliação de diferentes anormalidades dos testículos de cães e, como resultados, demontrou grande aplicabilidade na diferenciação de tecidos begninos e malignos, sendo esses últimos com maior rigidez tecidual.

ARTIGO 3: Este artigo é o resultado do projeto Jovem Pesquisa da FAPESP, utilizando um considerável n amostral de tumores mamários em cadelas, confirmando os resultados obtidos no primeiro artigo. A elastografia ARFI permite a detecção de malignidade em tumores mamários de cadelas com sensibilidade de 94,72%, especificidade de 97,22% e acurácia de 95,04%.

ARTIGO 4: Após comprovação da aplicabilidade da elastografia ARFI na detecção de malignidade dos tumores mamários de cadelas nos artigos 1 e 3, este manuscrito demonstra que na caracterização dos diferentes tipos e graus de carcinomas mamários em cadelas, neoplasias consideradas do tipo especial são deformáveis quando comparadas com as simples e complexas, à elastografia.

ARTIGO 5: Neste manuscrito, aplicou-se a técnica elastográfica na avaliação de linfonodos loco-regionais inguinais e axilares de cadelas com tumores mamários, verificando-se que a ARFI é aplicável na detecção de linfonodos metastáticos e diferenciação entre tecidos normais, reativos e neoplásicos, sendo que os últimos apresentam aumento de rigidez tecidual quando comparado com os demais.

ARTIGO 6: Na avaliação de tumores cutâneos e subcutâneos, este manuscrito demonstra que a elastografia ARFI permitiu verificar que a malignidade está associada a tecidos não deformáveis e valores de velocidade de cisalhamento maiores que 3,52 m/s, ou seja, tumores malignos são mais rígidos que benignos.

ARTIGO 7: Este último manuscrito finaliza a tese e traz uma acurácia de 97% da ARFI na detecção de malignidade em lesões esplênicas de cães, credenciando o método como a principal técnica de imagem para essa finalidade no baço canino, possibilitando um direcionamento clínico mais rápido e não invasivo para os pacientes com anormalidades neste órgão.

HISTÓRICO - TRAJETÓRIA CIENTÍFICA DO AUTOR

Meu primeiro “contato com a pesquisa”, posso dizer assim, foi na farmácia do meu pai em São Sebatião do Paraíso! Gostava muito de fazer “experiências” com medicamentos, misturar produtos químicos e ver os resultados! E nem imaginava que isso era o início da minha curiosidade de pesquisador.

Em 1999, iniciei meu curso de Medicina Veterinária pela Universidade Federal de Lavras – UFLA e, desde o começo, me inseri em atividades científicas, primeiramente durante minha monitoria no Laboratório de Fisiologia e as atividades de pesquisa que aconteciam no laboratório. Após essa monitoria, iniciei minha iniciação científica, como bolsista CNPq, com projeto intitulado “Caracterização química, atividade anti-helmíntica e estudos de toxicidade de óleos extraídos de sementes de abóboras (Cucurbita moschata Dush., C. maxima e C. pepo L.)”, sob orientação da Profa. Dra. Maria das Graças Cardoso e coorientação do Prof. Dr. Raimundo Vicente de Sousa (julho de 2002 a julho de 2003). Como resultado deste projeto, publiquei meu primeiro resumo científico nos Anais do XVI CICESAL, em Lavras, 2003.

Após minha graduação, ingressei em 2005 na Residência Médico-Veterinária em Diagnóstico por Imagem em Pequenos Animais da UFLA, sob orientação do Prof. Dr. Carlos Artur Lopes Leite. Durante os dois anos de Residência, além das atividades de ensino e extensão, minha produção cientifica merece ressalva (considerando meu ínicio como pesquisador), com a confecção e publicação de resumos e artigos científicos, com resultados importantes de pesquisas e relatos de casos inéditos atendidos, até a produção de atlas radiográficos, apostilas e outros materiais. Nesse período, publiquei 6 resumos simples e 12 resumos expandidos.

Ao final da Residência Médico-Veterinária, como forma de avaliação, apresentei minha monografia intitulada "Ultrassonografia bidimensional convencional, de alta resolução e tridimensional no acompanhamento da gestação em cadela". Essa obra foi um marco para minha carreira e me fez descobrir uma das coisas que mais gosto de fazer, pesquisar e escrever resultados que um dia vão ser importantes para outras pessoas e animais. Como resultado da minha monografia, publiquei durante o mestrado o meu primeiro artigo como autor principal, "divisor de águas" para uma das minhas linhas de pesquisa – ultrassonografia gestacional:

- FELICIANO, M.A.R.; MUZZI, L.A.L.; LEITE, LOPES, C.A.; JUNQUEIRA, M.A. Ultra-sonografia bidimensional convencional, de alta resolução e tridimensional no acompanhamento da gestação em cadela. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.59, p.1333-1337, 2007.

Em março de 2007, ingressei no Mestrado em Zootecnia pela UFLA, área de Nutrição de Cães e Gatos, continuando minha linha de pesquisa em Diagnóstico por Imagem, sob orientação da Profa. Dra. Flávia Maria de Oliveira Borges Saad e coorientação do Prof. Dr. Carlos Arthur Lopes Leite. Além de dar continuidade aos trabalhos iniciados durante a Residência Médico-Veterinária, nessa fase colaborei com a implementação de pesquisas clínicas no Núcleo de Pesquisa em Nutrição Animal e desenvolvimento de outros projetos em paralelo, produzindo de modo substancial trabalhos relevantes para a área. Neste período participei de 10 seminários, 9 congressos e simpósios, sendo publicados 2 resumos simples, 13 expandidos.

Em janeiro de 2018, fui aprovado na defesa da minha dissertação, intitulada: "Suplementação de probiótico para filhotes cães da raça beagles recebendo alimentos comerciais". Como resultado deste trabalho, foram publicados posteriormente 3 artigos, sendo dois de grande relevância:

- FELICIANO, M.A.R.; SAAD, F.M.O.B.; LOGATO, P.V.R.; AQUINO, A.A.; JOSE, V.A.; ROQUE, N.C. Efeitos de probióticos sobre a digestibilidade, escore fecal e características hematológicas em cães. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.61, p.1268-1274, 2009.

- FELICIANO, M.A.R.; SAAD, F.M.O.B.; LEITE, C.A.L.; VICENTE, W.R.R.; NEPOMUCENO, A.C.; SILVEIRA, T. Avaliações ultrassonográfica e radiográfica dos efeitos da suplementação com dois tipos de probióticos sobre o intestino de cães filhotes. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.62, p.1109-1116, 2010.

Iniciei meu Doutorado em março de 2008 na Faculdade de Ciências Agrárias e Veterinárias UNESP/Jaboticabal, sob orientação do Prof. Wilter e coorientação da Profa. Dra. Paula Diniz Galera. O doutorado veio consolidar todo meu aprendizado obtido durante as etapas anteriores e nortear ainda mais minha carreira para a docência e pesquisa. Nos três anos do doutoramento participei de diversos projetos, 12 congressos, publicamos 12 resumos simples, 7 expandidos e 22 artigos científicos. Ressalto aqui a publicação dos meus primeiros resumos internacionais na área de Diagnóstico por Imagem no 15th Congress of the International Veterinary Radiology Association e Conference of European Society for Domestic Animal Reproduction – ESDAR.

Minha Tese de Doutorado, intitulada "Ultrassonografia convencional e modo Doppler em cores e Power na avaliação da neoplasia mamária em cadelas" foi defendida e aprovada em dezembro de 2010. O projeto que resultou nesta obra trouxe vários resultados singulares para minha carreira, desde a concessão da minha bolsa de doutorado e auxílio pesquisa FAPESP, possibilitando a compra de um novo aparelho ultrassonográfico para pesquisas do nosso grupo (que utilizamos até hoje – como ferramenta de pesquisa: Ultrassonografia modo B e Dopplerfluxometria materno fetal de cabras gestantes, projeto de uma orientada minha de doutorado), como também permitiu iniciar uma nova linha de pesquisa em ultrassonografia mamária e sobre o uso de técnicas ultrassonográficas não convencionais. Foram publicados 2 artigos, sendo que um deles é de grande relevância e aplicabilidade em ultrassonografia mamária e outro dentro da oncologia veterinária:

- FELICIANO, M.A.R.; VICENTE, W.R.R.; SILVA, M.A.M. Conventional and Doppler ultrasound for the differentiation of benign and malignant canine mammary tumours. **Journal of Small Animal Practice**, v. 53, p. 332-337, 2012.

- FELICIANO, M.A.R.; SILVA, A.S.; PEIXOTO, R.V.R.; GALERA, P.D.; VICENTE, W.R.R. Estudo clínico, histopatológico e imunoistoquímico de neoplasias mamárias em cadelas. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.64, p.1094-1100, 2012.

Ao final do meu doutorado fui contemplado com a aprovação de uma bolsa de Pós-Doutoramento junto à FAPESP, com o projeto intitulado “Ultrassonografia convencional e Dopplerfluxometria materno fetal na avaliação pré-natal de cadelas gestantes”, pela Faculdade de Ciências Agrárias e Veterinárias UNESP/Jaboticabal, sob supervisão do Prof. Wilter, iniciado em 5 de janeiro de 2011 e finalizado em março de 2013. Como principais resultados desta pesquisa, foram publicados 6 artigos científicos, sendo um de grande relevância para a literatura veterinária:

- FELICIANO, M.A.R.; NEPOMUCENO, A.C.; CRIVELARO, R.M.; OLIVEIRA, M.E.F.; COUTINHO, L.N.; VICENTE, W.R.R. Foetal echoencephalography and Doppler ultrasonography of the middle cerebral artery in canine foetuses. **Journal of Small Animal Practice**, v.54, p.149-152, 2013.

Posteriormente, junto ao meu auxílio e bolsa de Jovem Pesquisador, realizei meu 2º Pós-Doutoramento vinculado à UNESP/Jaboticabal, sob supervisão do Prof. Wilter, iniciado em julho de 2013 e finalizado em julho de 2015; e o 3º Pós-doutorado, também sob supervisão do Prof. Wilter, iniciado em 2016 e finalizado em 2017/2018.

Um dos grandes marcos da minha carreira científica foi alcançado em junho de 2013, conquistando parte de um grande sonho: implementar um Centro de Pesquisa em Diagnóstico por Imagem em uma grande instituição, onde poderia além de trabalhar no ensino, desenvolver intensamente pesquisa de qualidade e assim contribuir para a comunidade científica do meu país. Fui contemplado com a aprovação do meu Jovem Pesquisador (JP) e uma bolsa JP junto à Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, para implementação de um Centro de Pesquisa no Departamento de Reprodução Animal da Faculdade de Ciências Agrárias e Veterinárias UNESP/Jaboticabal, sendo finalizado em 2017.

O projeto intitulado “Elastografia, ultrassonografia com contraste por microbolhas e Doppler como métodos de diagnóstico das neoplasias mamárias, afecções prostáticas e testiculares em cães” e a aquisição deste JP permitiu a criação do Laboratório de Ultrassonografia na Reprodução Animal, sob minha responsabilidade, juntamente com a compra de materiais de consumo e permanentes, de modo especial um aparelho de ultrassonografia de ultima geração contendo as novas tecnologias abordadas no projeto.

Desde então, meu grupo de pesquisa tem promovido a formação de recursos humanos nos Programas de Pós-Graduação da UNESP-Jaboticabal e uma maior visibilidade internacional da ultrassonografia veterinária nacional, com a realização de pesquisas inovadoras utilizando métodos não convencionais, como a ultrassonografia contrastada e elastografia, obtendo resultados científicos singulares, de importância e aplicabilidade em pesquisa e para rotina veterinária. Adicionalmente, tem proporcionado parcerias com Instituições internacionais, como exemplo o trabalho que desenvolvemos com o Prof. Dr. Pawel M. Bartlewski do Ontario Veterinary College, University of Guelph, Canadá, Prof. Dr. Marco Russo da Universidade de Napoli e Prof. Dr. Ricardo Andrés Ramirez UScategui da Universidade de Medelin, Colômbia,

Durante o Jovem Pesquisador, foram publicados 64 artigos científicos, 2 livros na área de Diagnóstico por Imagem, 18 capítulos, 7 resumos expandidos e 61 simples, além de orientações e coorientações de mestrado e doutorado. Dentre as publicações, cabe citar as de singular relevância:

- FELICIANO, M.A.R.; CANOLA, J.C.; VICENTE, W.R.R. **Diagnóstico por Imagem em Cães e Gatos**. 1.ed. São Paulo: MedVet, 2015. v.1. 760p.
- FELICIANO, M.A.R.; OLIVEIRA, M.E.F.; VICENTE, W.R.R. **Ultrassonografia na Reprodução Animal**. 1.ed. São Paulo: MedVet, 2013. v.1. 191p.
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Os três Pós-doutoramentos e o Jovem Pesquisador foram essenciais para os meus propósitos em pesquisa e contribuíram para me firmar como pesquisador, responsável por um grupo de pesquisa, Bolsista Produtividade CNPq PQ2 com o projeto intitulado “Ultrassonografia contrastada por microbolhas na avaliação do sistema reprodutor de cadelas” e, atualmente, Bolsista Produtividade CNPq PQ1D, com projeto intitulado “Aplicabilidade da elastografia transtorácica no diagnôstico de doenças pulmonares em cães”.

Em 2013, me credenciei como Docente Permanente nos Programas de Pós-Graduação em Medicina Veterinária e em Cirurgia Veterinária da Faculdade de Ciências Agrárias e Veterinárias UNESP/Jaboticabal. Nestes Programas, sou responsável por ministrar três disciplinas: Métodos Ultrassonográficos Avançados em Veterinária, Métodos de Fecundação, Diagnóstico e Acompanhamento Gestacional em Pequenos Animais e Biotécnicas da Reprodução em Pequenos Ruminantes. Até o momento fui orientador de 11 mestrandos e 12 doutorandos e coorientei 8 mestrandos e 7 doutorandos. Atualmente sou orientador de 5 mestrandos e 3 doutorandos e coorientador de 3 doutorandas e 2 mestrandos.

Atualmente, após minha passagem como Professor Efeitvo do Magistério Superior pela UFRB (agosto de 2016 à janeiro de 2019) e UFSM (janeiro de 2019 à abril de 2022), ingressei como Professor Doutor na FZEA-USP em abril de 2022 e, desde então, ministro as disciplinas de Diagnóstico por Imagem, Tópicos em Diagnóstico por Imagem em Cães e Gatos e Diagnóstico por Imagem em Equinos no curso de Medicina Veterinária, além da disciplina de Pós-Graduação Imaginologia Veterinária, ministrada junto ao programa de Pós-Graduação em Biociência Animal.

Como resultado deste meu histórico como docente e pesquisador, publiquei até o momento 4 livros, 24 capítulos em livros, 178 artigos científicos completos e 184 trabalhos publicados em anais de eventos. Conforme supracitado, contribui com a formação de 38 pós-graduandos e em andamento a orientação de mais 13 orientandos, com possibilidade de novos alunos de pós-graduação a partir de 2023 (maiores informações – link currículo lattes: https://lattes.cnpq.br/4890046762900137). Cabe salientar que toda essa inserção na pesquisa me capacita para ser um docente cada vez melhor na graduação, fornecendo aos alunos de graduação conhecimento técnico de alta qualidade para seu desenvolvimento.

Diante desse breve histórico científico e hoje como Professor Doutor na área de Diagnóstico por Imagem em Medicina Veterinária, pela Faculdade de Zootecnia e Engenharia de Alimentos – FZEA USP, me sinto mais preparado para desenvolver minhas pesquisas, alcançar resultados que irão beneficiar proprietários e seus animais, pesquisas que podem ser extrapoladas à medicina com resultados singulares e aplicáveis à pesquisas médicas e, por fim, formar recursos humanos de qualidade na graduação e pós-graduação, seres humanos com conhecimento consistente e raciocínio lógico na Medicina Veterinária.

FUNDAMENTAÇÃO TEÓRICA

Introdução e Histórico

Em 1981, o cientista chinês Yuan-Cheng Fung e seus colaboradores estudaram as propriedades mecânicas de vários tecidos por meio da resposta a uma força aplicada aos mesmos. Estes pesquisadores verificaram que a elasticidade, dureza e mobilidade dos tecidos dependem da sua constituição molecular (gordura, fibras colágenas, elastina, água e entre outros) e organização estrutural macroscópica e microscópica (Fung *et al*. 1993).

Sabe-se que modificações nas propriedades mecânicas dos tecidos estão diretamente correlacionadas com anormalidades teciduais, como por exemplo: carcinomas mamários que são rígidos, devido ao aumento na densidade estromal; esteatose ou hialinose, processos degenerativos que envolvem lipídios e colágeno, aumentando ou diminuindo a elasticidade do tecido; presença de microcistos difusos e imperceptíveis em exames de rotina, promovendo menor rigidez tecidual; afecções crônicas, com a substituição do tecido normal por conjuntivo ou fibroso, como a cirrose hepática que causa redução significativa da elasticidade do fígado; entre outras (Ophir *et al*. 1991).

Uma forma de avaliar os tecidos, suas propriedades mecânicas e possíveis alterações é realizada por uma das mais antigas técnicas de identificação de lesões em medicina, a palpação, que é um método semiológico empregado para identificar anormalidades como aumento de volume, alteração de formato e da consistência dos tecidos examinados (Maronezi *et al*. 2019). O uso da palpação como técnica diagnóstica é conhecido desde os primórdios da civilização, descrita em papiro de Edwin Smith (Allen 2005) e atribuído ao médico e padre Imhotep, em 3000 e 2500 a.C. (Breasted 1991).

Apesar da sua importância, a palpação é muita subjetiva e dependente da experiência do examinador. Alguns métodos de imagem, como a ultrassonografia modo-B, tomografia computadorizada e ressonância magnética veem substituindo a palpação na avaliação de vários órgãos na medicina e veterinária, porém em muitos casos não permitem a distinção entre vários tipos de lesões e detecção de malignidade, uma limitação importante dentro da rotina clínica (Garra 2015).

Considerando a descoberta de Yuan-Cheng Fung e colaboradores, a elasticidade pode ser definida como a propriedade de um corpo ou substância a qual permite que eles sejam deformados quando sujeitos a uma força externa e possam retomar a sua forma original ou o tamanho, quando essa força é removida e que essa deformação é inversamente proporcional à rigidez e ao tempo de reparo do tecido (Goddi *et al*. 2012). Essa afirmação foi fundamental para que em 1991, Jonathan Ophir e seus colaboradores, da Universidade do Texas, desenvolvessem uma nova técnica de imagem - a Elastografia! O método descrito pelos pesquisadores utilizou um estímulo axial externo juntamente com técnicas de correlação cruzada para gerar uma tensão axial nos tecidos (Ophir *et al*. 1991). A deformação obtida foi convertida em módulos elásticos e assim criada uma imagem.

Ophir e colaboradores (1991) verificaram que o tecido se desloca em resposta à uma pequena pressão aplicada e essa diferença de deslocamento – "antes da pressão" e "depois da pressão", está relacionada à elasticidade ou deformidade tecidual, podendo ser calculada matematicamente e expressa em função da distância (Garra 2011). Em 1996, este mesmo grupo de pesquisa revisou os fundamentos teóricos e técnicas de medição empregadas na análise elástica tecidual e desenvolveu uma análise qualitativa descrita como elastograma (Ophir *et al*. 1996).

A elastografia pode ser definida como a técnica ultrassonográfica que avalia a elasticidade dos tecidos, sendo capaz de estudar sua dureza ou rigidez. É considerado um método de imagem promissor e que se baseia em modelos elásticos para o imageamento de propriedades mecânicas dos tecidos (Dudea *et al*. 2011; Maronezi *et al*. 2019).

Após determinação *in vitro* dos modelos elastográficos e aperfeiçoamento da técnica, Ophir e outros colaboradores (Garra *et al*. 1997) avaliaram 46 lesões mamárias de mulheres, com o intuito de verificar o potencial diagnóstico da elastografia no estudo dessas anormalidades. Neste primeiro estudo *in vivo* descrito na literatura utilizando a elastografia, os pesquisadores observaram que os tecidos macios - adiposo, apresentavam áreas mais claras no elastograma e que tecidos mais rígidos - parênquima e nódulos eram mais escuros na imagem elastográfica. Os autores também verificaram que nódulos malignos foram estatisticamente mais escuros do que as lesões benignas, demonstrando um possível potencial diagnóstico da elastografia na detecção de malignidade em tumores mamários.

Desde então, vários métodos para avaliação da elasticidade tecidual têm sido propostos em elastografia: a primeira técnica criada - por compressão;

sonoelastografia ou elastografia transitória; acoustic radiation force impulse – ARFI; e a supersonic shear wave imaging (Dudea *et al*. 2011).

Após a criação da elastografia por compressão, em 2003 iniciou-se a utilização de um novo método elastográfico - sonoelastografia, também denominada elastografia transitória, permitindo avaliar a rigidez do tecido e fornecer dados quantitativos com medidas em KiloPascal (kPa). Este método tem sido aplicado rotineiramente na medicina para avaliação de tecido fibrótico de paciente humanos com suspeita de cirrose hepática (Patel & Wilder 2014).

Em 2009, foram descritos os primeiros resultados sobre a utilização de uma nova técnica elastográfica em tecidos humanos, utilizando ondas sonoras para avaliar a rigidez dos tecidos, por meio da compressão tecidual promovida por uma energia acústica e a captação ou mensuração da velocidade das ondas de cisalhamento, geradas pelo deslocamento do tecido - elastografia ARFI (Fierbinteanu-Braticevic *et al*. 2009). Nesse primeiro estudo, os pesquisadores verificaram uma correlação muito boa da ARFI com resultados histopatológicos de amostras hepáticas, apresentando boa sensibilidade e excelente especificidade para detecção de fibrose nos pacientes humanos.

Em medicina veterinária, a elastografia ARFI foi descrita pela 1ª vez na avaliação dos tumores mamários de cadelas (Feliciano *et al*. 2014) e no estudo da rigidez de alguns órgãos abdominais de cães (Holdsworth *et al*. 2014). Feliciano e colaboradores (2014) em seu estudo preliminar, verificaram que a ARFI demonstrou aplicabilidade na detecção de malignidade em tumores mamários, sendo que os tecidos malignos eram mais rígidos do que os benignos. Holdsworth e colaboradores (2014) descrevem uma avaliação preliminar da ARFI em tecidos abdominais de alguns cães adultos, demonstrando que a técnica pode ser utilizada para o estudo da rigidez de órgãos abdominais e sugeriram que novas pesquisas deveriam ser realizadas, visando padronizar a técnica com um maior número de animais e de diferentes idades.

Atualmente, a técnica ARFI tem sido amplamente estudada pelo grupo de pesquisa coordenado pelo Prof. Dr. Marcus Antônio Rossi Feliciano, da Universidade de São Paulo, sendo avaliados diferentes tecidos animais normais e com anormalidades, em diferentes espécies animais (Maronezi *et al*. 2019).

Tipos de Elastografia

A elastografia está relacionada com a imagem quantitativa do módulo E de Young – um parâmetro físico relacionado com a rigidez. Esse módulo apresenta as diferenças físicas entre os tecidos biológicos e caracteriza a rigidez das estruturas avaliadas. Para avaliar o módulo de Young do tecido, as técnicas de elastografia se baseiam na aplicação de uma força externa ao tecido e os movimentos resultantes obtidos (Sarvazyan *et al*. 1995; Sigrist *et al*. 2017).

De modo geral, existem dois tipos de elastografia (Garra 2015; Zaleska-Dorobisz *et al*. 2014; Ozturk *et al*. 2018) (Figura 1) (Quadro 1):

1) Elastografia de deformação: técnicas que criam "imagens de deformação" e envolvem uma ou mais compressões graduais do tecido e são chamadas de "estáticas" ou "quase-estáticas" (elastografia por compressão ou tensão); A compressão é realizada pela aplicação da pressão da probe ou por meio de força mecânica endógena (como por exemplo – pulsação de algum vaso).

2) Elastografia por ondas ou velocidade de cisalhamento - Shear wave elastography (SWE)**:** técnicas que criam imagens baseadas em ondas emitidas e são denominadas de "dinâmicas" (sonoelastografia, SWE-2D, supersonic shear wave imaging e a ARFI). Uma onda ou velocidade de cisalhamento do tecido é induzida pelo sistema de imagem por meio de força de radiação acústica.

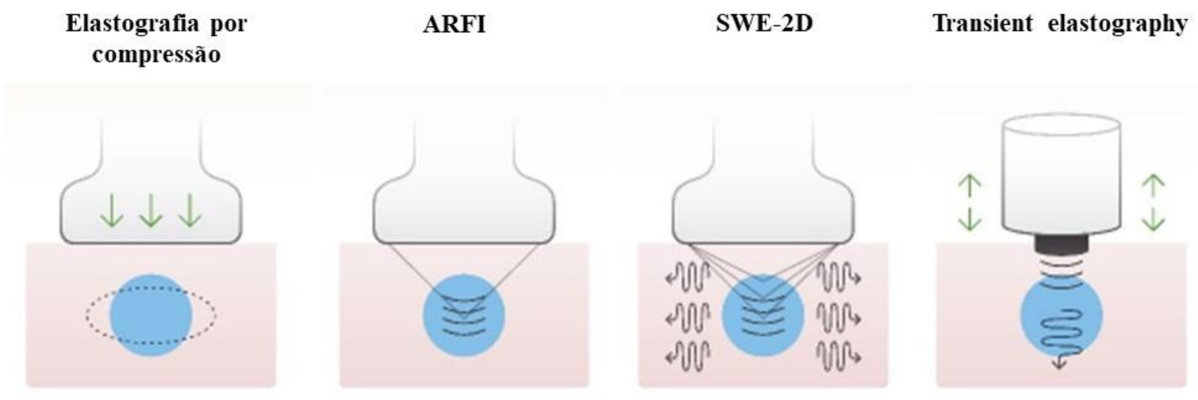

Figura 1: Imagem ilustrativa dos tipos de elastografia estática (elastografia por compressão) e dinâmicas (ARFI, SWE-2D e transient elastography). Nessa imagem é possível verificar como os transdutores agem para deformação dos tecidos (imagem modificada de Sigrist *et al*. 2017).

Quadro 1: Tipos de Elastografia: comparação das características das técnicas

Técnica	Força aplicada	Fonte de Vibração	Vantagens	Desvantagens
Elastografia por Compressão	Compressão Mecânica	Manual ou Automática	Comercialmente acessíve;	Operador dependente; Sensível a condições corporais: ascite e pacientes obesos;
Elastografia Transitória	Compressão Automática	Força mecânica transitória	Validado em humanos: fibrose hepática;	Alto custo; Longa curva de aprendizado; Sensível a condições corporais: ascite e pacientes obesos;
Supersonic Image	Onda de cisalhamento	Impulso de força de radiação acústico	Mais rápida; Não operador dependente;	Alto custo; Pouca padronização; Poucos estudos;
ARFI	Onda de cisalhamento	Impulso de força de radiação acústico	Usado em todos os órgãos; Não é operador dependente; Usado em pacientes obesos e com ascite; Melhor precisão;	Alto custo; Poucos estudos e em padronização;

ARFI: acoustic radiation force impulse; adaptado de Carvalho *et al*. (2015), Gennisson *et al*. (2013) e Maronezi *et al*. (2019).

Elastografia por Compressão

A elastografia por compressão é baseada na obtenção de ecos de radiofrequência digitalizados da região de interesse, por meio da compressão realizada pelo próprio operador ou por meio do deslocamento criado por movimentos fisiológicos internos (Gennisson *et al*. 2013; Sigrist *et al*. 2017). São obtidos ecos antes da compressão ou deslocamento e, em seguida, ecos de radiofrequência lineares são criados após uma leve compressão realizada pelo operador utilizando a probe, ao longo da mesma direção do feixe sonoro. Os dados obtidos são comparados e a mudança gerada na região de interesse após a compressão é calculada (Sigrist *et al*. 2017, Maronezi *et al*. 2019).

São necessárias várias compressões com o transdutor na região de interesse, seguidas de momentos de “relaxamento”, criando assim ciclos de compressão e relaxamento. A compressão deve ser suave para comprimir de 2 - 5 mm. As medições de deformação dos tecidos são exibidas como mapa de cores - elastograma, que é sobreposto à imagem em modo-B. Tecidos macios se deformam mais quando sujeitos a compressão, enquanto os rígidos se deformam menos (Sigrist *et al*. 2017; Maronezi *et al*. 2019). A deformidade tecidual é apresentada em escala em cores conforme a variação elástica, sendo que tecidos de baixa tensão (rígido) a imagem é exibida em tonalidades claras e de alta tensão (macio) é exibida em tonalidades escuras (Figura 2). A escala de cores depende do aparelho de ultrassom e fornecedor (Bhatia *et al*. 2013; Gennisson *et al*. 2013).

Ainda é possível determinar uma razão de deformação, considerada avaliação pseudo-quantitativa, que é calculada pela razão da tensão obtida em uma região de interesse normal (ROI) e outro ROI da lesão avaliada. Razão de deformação > 1 indica que a lesão alvo deforma menos do que o tecido adjacente normal, indicando menor tensão e maior rigidez (Choi *et al*. 2015).

Algumas considerações importantes para se obter imagens elastográficas por compressão adequadas: necessidade de tricotomia da região de interesse; manter o transdutor perpendicularmente à superfície de avaliação, evitando movimentação lateral; o exame deve ser iniciado com a probe apenas tocando a pele, sem compressão; obtenção dos ciclos de compressão e relaxamento adequadamente (Dietrich *et al*. 2017; Maronezi *et al*. 2019).

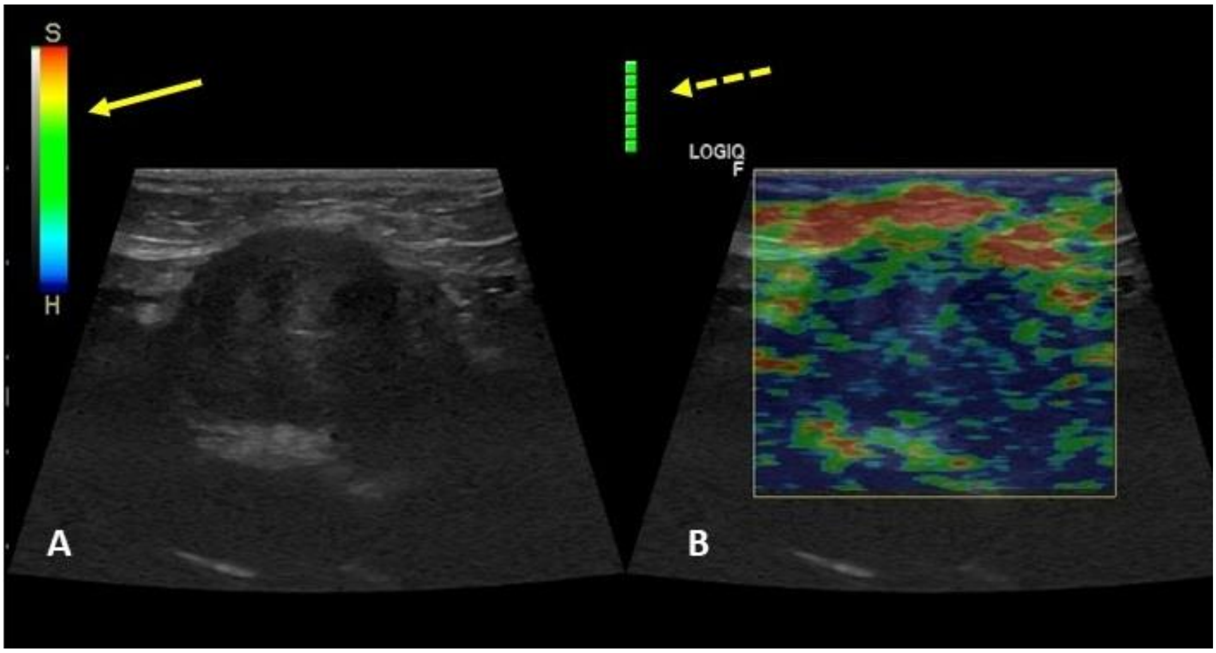

Figura 2: Imagem de elastografia por compressão de nódulo em tireoide de cão. Em (A), imagem da massa em modo-B e em (B), elastograma, com maior predominância de tonalidades claras no nódulo avaliado. Na seta amarela contínua, observa-se a escala de rigidez, onde S significa soft (macio) e H é hard (rígido), indicando que o nódulo se apresenta rígido (tonalidades azuis) quando comparado com tecido adjacente (tonalidades vermelhas e verdes). A seta amarela tracejada indica a qualidade do exame obtido, que nessa imagem foi muito boa.

Elastografia transitória

A sonoelastografia ou transient elastography ou Fibroscan é a técnica mais utilizada e validada para estudo da fibrose hepática em humanos, sendo frequentemente utilizada na rotina clínica (Sigrist *et al*. 2017). Esta técnica permite quantificar a fibrose dando uma pontuação geral de elasticidade em uma determinada profundidade (Gennisson *et al*. 2013).

O transdutor desse método é um dispositivo único que funciona como um vibrador. O operador seleciona a área de imagem para avaliação e, ao pressionar um botão que aciona a técnica, vibrações mecânicas de amplitude e frequência baixas são transmitidas ao tecido avaliado, induzindo a formação de uma onda de cisalhamento elástica que se propaga através do tecido. A mesma probe utiliza a técnica Doppler para calcular a velocidade da onda de cisalhamento, recuperando um módulo E de Young e fornecendo dados para avaliação qualitativa (imagem elastográfica) e quantitativa (valores de rigidez tecidual em kPa) (Wong & Chan 2010; Dhyani *et al*. 2015; Maronezi *et al*. 2019).

Na avaliação qualitativa, a imagem está relacionada com a amplitude de vibração do tecido, medida em resposta às vibrações aplicadas. Tecidos mais rígidos correspondem a pouca amplitude, enquanto tecidos mais macios caracterizam alta vibração. Por meio do método quantitativo, é determinada a velocidade de cisalhamento, expressa em kPa, sendo maior em tecidos mais rígidos (Maronezi *et al*. 2019).

Elastografia shear wave 2D (SWE-2D) e Supersonic shear wave imaging (SWI)

A SWE-2D e a SWI são métodos baseados na mensuração de valores para velocidade de propagação da onda de cisalhamento em tecidos moles, que fornecem informações qualitativas e quantitativas das regiões avaliadas (Zaleska-Dorobisz *et al*. 2014).

Ondas de cisalhamento são geradas após a escolha da região de interesse. O transdutor cria uma energia de radiação, induzindo a formação das ondas de cisalhamento que se propagam diretamente para o tecido de interesse. Os métodos possibilitam a criação de um mapa de cores bidimensional e valores para elasticidade tecidual em kPa (Zaleska-Dorobisz *et al*. 2014; Maronezi *et al*. 2019).

Atualmente, a SWE-2D é o método SWI mais recente e que usa uma força de radiação acústica. Nessa técnica, a força de radiação acústica desloca o tecido em vários pontos, ou seja, avalia várias regiões focais – ROIs, criando um cone de onda de cisalhamento quase cilíndrico, permitindo o monitoramento em tempo real das ondas de cisalhamento em 2D. O elastograma é apresentado como um mapa de exibição colorido, com resultados quantitativos disponíveis como velocidade de propagação da onda de cisalhamento em m/s ou em kPa. Os mapas de cores da rigidez tecidual, em tempo real, adicionados à imagem em modo-B permitem que o operador evite confundir estruturas anatômicas, como vasos sanguíneos (Bamber *et al*. 2013; Ozturk *et al*. 2018).

Elastografia ARFI

A elastografia ARFI é uma técnica ultrassonográfica que fornece medidas quantitativas e qualitativas da rigidez dos tecidos com uma variabilidade interobservador reduzida. Esse método permite quantificar as propriedades mecânicas do tecido sem compressão manual, obtendo valores para velocidade da onda de cisalhamento induzida por radiação e propagação acústica do tecido (Goddi *et al*. 2012; Maronezi *et al*. 2019).

Para realização da ARFI, se utiliza um impulso acústico primário em direção a região a ser examinada, promovendo a formação de ondas de pressão em propagação que deformam os tecidos, com posterior captação da velocidade de cisalhamento (Maronezi *et al*. 2019). A velocidade de propagação e a atenuação das ondas estão relacionadas com a rigidez e viscoelasticidade do tecido, sendo que as ondas apresentam maior velocidade em tecidos rígidos, registrada em m/s (Comstock 2011).

Em relação à avaliação qualitativa, o método utiliza impulsos acústicos curtos e de alta intensidade para deformar os tecidos e criar o elastograma para rigidez tecidual relativa. O elastograma pode estar disposto direto na imagem modo-B ou como uma imagem ao seu lado, para melhor comparação, sendo que áreas de tonalidades claras são indicativas de tecidos mais elásticos (menos rígidos) e mais deformáveis, enquanto áreas de tonalidades escuras são de tecidos mais rígidos (duros) e não deformáveis (Goddi *et al*. 2012) (Figura 3).

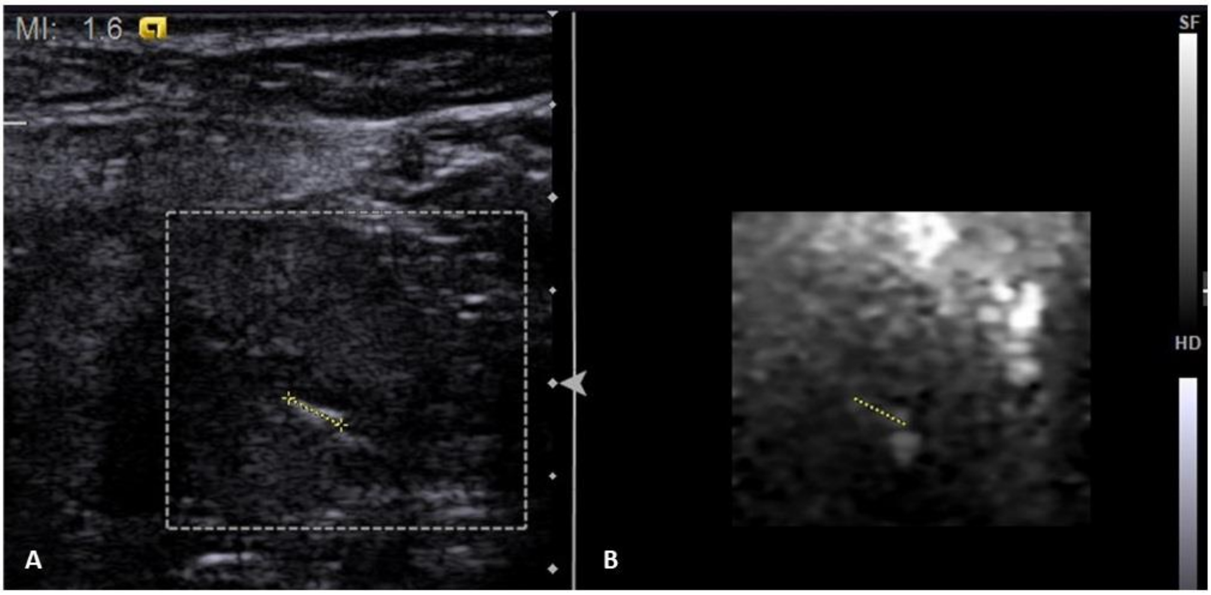

Figura 3: Imagem da avaliação elastográfica ARFI do tecido prostático de cão. Em (A), tecido prostático ao modo-B, com presença de área hiperecoica (traço descontínuo) e, em (B), elastograma obtido pela técnica ARFI, demonstrando o tecido prostático rígido com tonalidades cinza escura, não deformável e com pequena área

(traço descontínuo e relacionada com a hiperecogenicidade observada ao modo-B) menos rígida, com tonalidade clara.

Para realização do exame, o transdutor é colocado na região de interesse com gel o suficiente para minimizar curvas de sombreamento, controlando a imagem pelo método ultrassonográfico convencional. Após acionado, um ou vários volumes de amostras podem ser colocados nas regiões de interesse e as velocidades de cisalhamento são obtidas imediatamente (Figura 4). Sofwares recentes de ARFI apresentam uma função para avaliar a qualidade do exame (Maronezi *et al*. 2019).

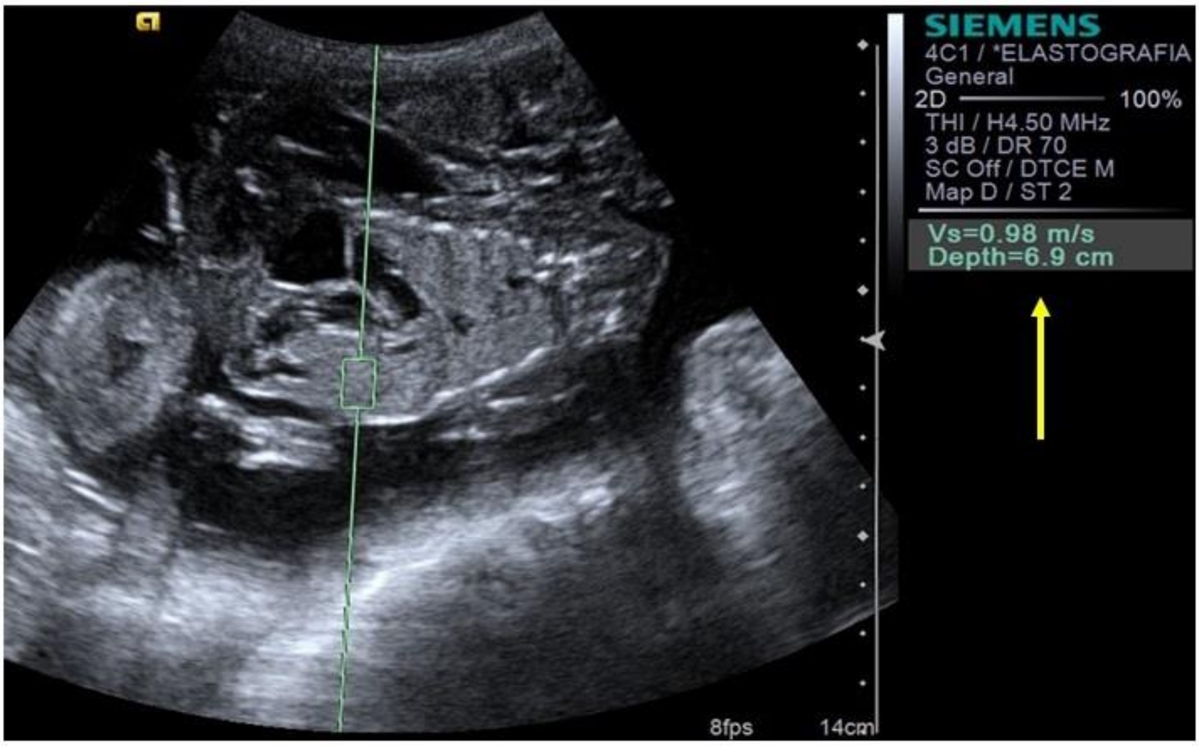

Figura 4: Imagem da elastografia ARFI de um feto ovino, com amostra de volume em tecido hepático fetal, com aquisição da sua velocidade de cisalhamento – 0,98m/s (seta amarela)

ARFI na Veterinária

Conforme supracitado na Introdução e Histórico, os dois primeiros relatos literários da aplicação da ARFI em veterinária foram em um estudo realizado para avaliação dos tumores mamários de cadelas (Feliciano *et al*. 2014) e outro para descrever a aplicabilidade do método no estudo da rigidez de alguns órgãos abdominais de cães (Holdsworth *et al*. 2014). Posteriomente, vários outros trabalhos foram desenvolvidos com a técnica ARFI, em diferentes espécies animais, seja para padronização do método e obtenção de dados qualiquantitativos para rigidez tecidual de tecidos saudáveis, como para verificar a aplicabilidade da técnica na avaliação de tecidos anormais, apresentando até o momento resultados excelentes para rotina veterinária (Maronezi *et al*. 2019).

Em relação aos achados normais de rigidez tecidual obtidos pela padronização da técnica ARFI em pacientes saudáves, os manuscritos encontrados na literatura veterinária descrevem avaliações em:

- *caninos*: baço (Holdsworth *et al*. 2014; Maronezi *et al*. 2015), fígado (Holdsworth *et al*. 2014), pâncreas (Holdsworth *et al*. 2014; Avante *et al*. 2020), rins (Holdsworth *et al*. 2014), adrenais (Fernandez *et al*. 2017), bulbo oculares (Abreu *et al*. 2018), próstata e testículos (Feliciano *et al*. 2015b), em tecidos materno-fetais de gestantes (Simões *et al*. 2018; Simões *et al*. 2020a; Simões *et al*. 2020b), músculo pectíneo (Rossignoli *et al*. 2020) e joelhos (Izique *et al*. 2020);

- *felinos*: baço (Feliciano *et al*. 2015a), rins (Garcia *et al*. 2015), testículos (Brito *et al*. 2015);

- *ovinos*: em tecidos materno-fetais de ovelhas com gestação a termo (Silva *et al*. 2019) e prematuras (Rodrigues *et al*. 2020);

- *equinos*: tendões flexores de equinos (Bernardi *et al*. 2020).

Quanto aos achados anormais de rigidez obtidos pela ARFI em pacientes animais, os manuscritos encontrados na literatura veterinária descrevem em:

- *caninos*: avaliações de neoplasias mamárias (Feliciano *et al*. 2014; Feliciano *et al*. 2017; Gasser *et al*. 2018; Feliciano *et al*. 2018), linfonodos loco-regionais de cadelas com neoplasias mamárias (Silva *et al*. 2018), neoplasias cutâneas e subcutâneas (Cruz *et al*. 2022), anormalidades testiculares (Feliciano *et al*. 2016) e prostáticas (Cintra *et al*. 2020), pulmão fetal de fetos caninos com hidropsia (Maronezi *et al*. 2018), doenças pancreáticas (Avante *et al*. 2020), lesões esplênicas (Maronezi *et al*. 2022), doença renal crônica (Cruz *et al*. 2021), tecido hepático e esplênico em pacientes com síndrome dos braquicefálicos (Facin *et al*. 2020), lentes com catarata (Abreu *et al*. 2021), plexo retino-coroide e nervo óptico de pacientes com glaucoma (Madruga *et al*. 2021) e linfoma intraocular (Madruga *et al*. 2022), músculo pectíneo em animais com displasia coxofemoral (Rossignoli *et al*. 2020);

- *felinos*: avaliação de neoplasias mamárias (Feliciano *et al*. 2015c);

- *equinos*: tendões flexores de animais com lesão induzida (Bernardi *et al*. 2022).

Todas as informações obtidas na ARFI podem ser extrapoladas para a interpretação dos achados de outros métodos de elastografia. De modo geral, os resultados observados nos tecidos anormais em todos os estudos já realizados trazem informações complementares e importantes aos outros exames de imagem, auxiliando no melhor entendimento das alterações morfofisiopatológicas de lesões, aumentando o valor diagnóstico da ultrassonografia na detecção das anormalidades, fornecendo achados para detecção de malignidade em processos tumorais, com alta sensibilidade, especificidade e acurácia, e detecção de processos degenerativos, entre outros.

OBJETIVOS

Tendo em vista o apresentado, os objetivos dos artigos subsequentes são:
I. Descrever a utilização da elastografia ARFI na avaliação de tumores mamários em cadelas e sua aplicabilidade.
II. Descrever o uso da elastografia ARFI na avaliação de distúrbios testiculares em cães.
III. Avaliar e comparar a eficácia do modo-B, Doppler, ultrassonografia contrastada e elastografia ARFI na predição de malignidade em tumores mamários caninos.
IV. Avaliar e comparar a precisão diagnóstica do modo-B, Doppler, ultrassonografia contrastada e elastografia ARFI na determinação dos tipos e graus dos carcinomas mamários.
V. Avaliar e comparar a precisão diagnóstica da ultrassonografia modo-B, Doppler e elastografia ARFI na identificação de metástase em linfonodos axilares e inguinais de cadelas com neoplasias mamárias.
VI. Descrever os resultados preliminares sobre a precisão de técnicas ultra-sonográficas, como elastografia ARFI, ultrassonografia contrastada e Doppler na determinação de alterações pancreáticas.
VII. Verificar a precisão da ultrassonografia modo-B, Doppler e elastografia ARFI para prever malignidade em neoplasias subcutâneas caninas.
VIII. Avaliar a acurácia da ultrassonografia modo-B e da elastografia ARFI na detecção de malignidade das lesões esplênicas caninas.

ARTIGO 1

ARFI elastography as a complementary diagnostic method for mammary neoplasia in female dogs – preliminary results

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BSAVA BRITISH SMALL ANIMAL VETERINARY ASSOCIATION

JSAP JOURNAL OF SMALL ANIMAL PRACTICE

PAPER

ARFI elastography as a complementary diagnostic method for mammary neoplasia in female dogs – preliminary results

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Objectives: **To evaluate the applicability of acoustic radiation force impulse elastography as a complementary method in diagnosing mammary neoplasia in dogs.**

Methods: **Mammary tumours from 50 female dogs were evaluated and divided into two groups: G1 (benign tissue) and G2 (malignant tumours). The nodules were assessed by B-Mode ultrasonography, qualitative and quantitative acoustic radiation force impulse elastography and histopathology.**

Results: **B-Mode ultrasound examination was ineffective at separating the tumours into the two groups. Likewise, there was no correlation between the grayscale images of the mammary tissue by qualitative elastography. A difference was found in the deformity of the mammary masses between the malignant and benign groups (P=0·002). Using quantitative elastography, the mean values of shear velocity were 3·33 m/s for malignant tumours and 1·28 m/s for benign tissue (P<0·0001).**

Clinical Significance: **The use of acoustic radiation force impulse elastography may help to differentiate between malignant and benign mammary neoplasms.**

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INTRODUCTION

Elastography was first developed in the early 1990s for studying the hardness of tissues, and this technique has been used in humans for identifying and differentiating between mammary tumours, diagnosing prostate tumours, monitoring focal fibrotic lesions and studying the structural properties of kidneys (Srinivasan *et al.* 2004, Dudea *et al.* 2011).

Among the available elastography techniques, the acoustic radiation force impulse (ARFI) imaging method provides quantitative and qualitative measures of tissue rigidity with reduced inter-observer variability (Dudea *et al.* 2011, Goddi *et al.* 2012).

Quantitative ARFI involves directing a primary acoustic pulse towards a region of interest, promoting the formation of propagating pressure waves capable of deforming the tissues that can be measured (pressure wave velocity of propagation or shear).

The velocity of propagation and attenuation of the waves are related to the rigidity and viscoelasticity of the tissue. The waves exhibit higher velocities in rigid tissues (Comstock 2011).

Qualitative ARFI elastography is an imaging method that can be used to evaluate tissue stiffness, facilitating the diagnosis of breast lesions in humans. More deformable tissues are likely to be benign, and more rigid tissues are likely to be malignant (Tavassoli and Deville 2003).

There are no reports on the use of ARFI elastography for the assessment and differentiation of benign and malignant mammary neoplasms in bitches, as reported in human patients. Therefore, the aims of the current preliminary study were to describe the use of this new sonographic technique in the evaluation of mammary tumours in bitches and the applicability of ARFI elastography to the differentiation of mammary neoplasms.

MATERIALS AND METHODS

Animals

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the School of Agrarian and Veterinary Sciences of the São Paulo State University, Brazil (protocol No 023705/12). Fifty female dogs of different breeds, aged between two and eight years old were included in this study.

Anamnesis, general and specific physical examination (localisation, size, presence of adherence, regularity of the surface tumour and presence of ulceration), regional lymph node palpation and radiographic surveys were performed in all animals to meet the inclusion criteria for the study (Feliciano *et al.* 2012a). Thoracic radiographs were performed to detect the presence of pulmonary metastases and assess the cardiac silhouette.

Experimental design

Using a prospective study design, 50 female dogs with mammary neoplasms were recruited. After an ultrasound examination of the mammary masses and a histopathological diagnosis of the type of neoplasm, the animals were divided into two experimental groups: dogs with benign tissue (tumours and hyperplasia; group 1) and dogs with malignant tumours (group 2) (Misdrop *et al.* 1999).

Diagnostic imaging

The ultrasonography was performed by a single evaluator experienced in ultrasonographic examinations. The ultrasound examination was performed prior to histological identification of the tumour type according to Misdrop *et al.* (1999). B-Mode ultrasonography was performed with a 9·0 MHz linear transducer using ACUSON S2000/SIEMENS ultrasound equipment (Siemens, Munich, Germany). Via ultrasound, the echotexture and echogenicity of the parenchyma and the contours and margins of the mammary masses were scanned (Feliciano *et al.* 2012a).

For the elastography, an ACUSON S2000 ultrasound equipment with software for qualitative and quantitative analysis was used, that was evaluated with the ARFI method (virtual touch tissue quantification) and a 9·0 MHz linear transducer (Syversveen *et al.* 2011).

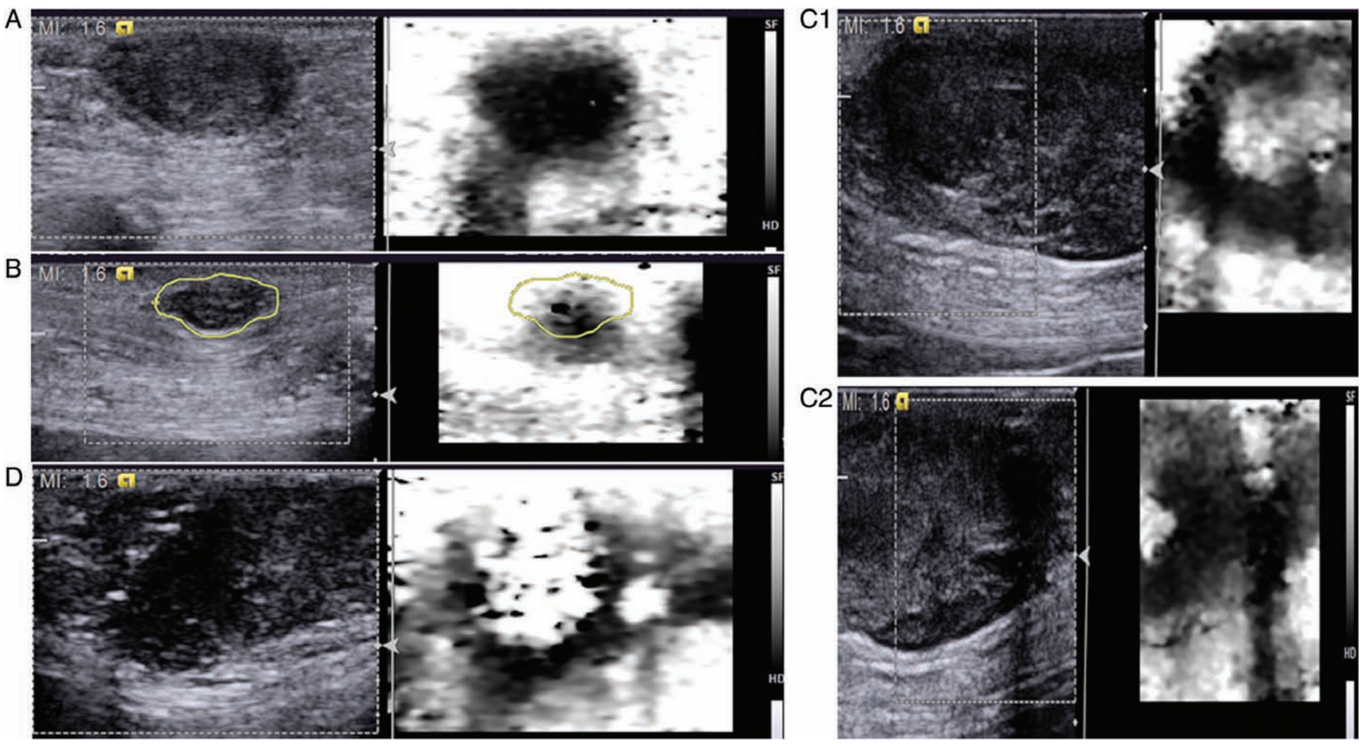

FIG 1. Image of the qualitative acoustic radiation force impulse (ARFI) elastography, which shows the characteristics of the stiffness in the mammary tumours. In (A) the image is heterogeneous and hard (black) with a cystic hard area; (B) the image is heterogeneous and soft (white) with a hard central area; (C1 and C2) the image is heterogeneous with hard to soft areas and (D) the image is heterogeneous and hard with a soft central area

After performing B-Mode ultrasonography, the qualitative ARFI technique was applied, resulting in the formation of grayscale images of the mammary tissue. The images were evaluated according to the presence of deformity and white areas (indicative of more elastic tissue that is less rigid, softer, and more deformable) and dark regions (more rigid, harder and not deformable) (Fig 1).

The quantitative evaluation was performed after scanning the mammary tumour with the B mode ultrasound. The function for obtaining the shear velocity was activated, and the calliper was positioned in the tumour parenchyma. Six measurements for each tumour were obtained and used to determine the mean and standard deviation (sd) shear velocities (Fig 2).

Histopathological analyses

Samples of the mammary tumours were collected after mastectomy for histopathology. The macroscopic appearance of each neoplasm was evaluated. Multiple fragments were fixed in 10% formaldehyde solution buffered with phosphates to a pH of 7·4, routinely processed and embedded in paraffin. The tissue was sectioned at 5 µm with a microtome. The slides were stained with haematoxylin and eosin. Optical microscopy was used to classify the neoplasms histologically as benign or malignant according to the criteria recommended by the World Health Organization (Misdrop *et al.* 1999). The samples were assessed by a Senior Professor (Ph.D.) of the Department of Veterinary Pathology of the College of Agricultural and Veterinary Sciences, São Paulo State University.

Statistical analysis

The data were tested for normality and homogeneity of variances (*F* test). The gross and transformed averages were evaluated by analysis of variance. In addition, a *t* test for paired samples was applied for all shear velocities.

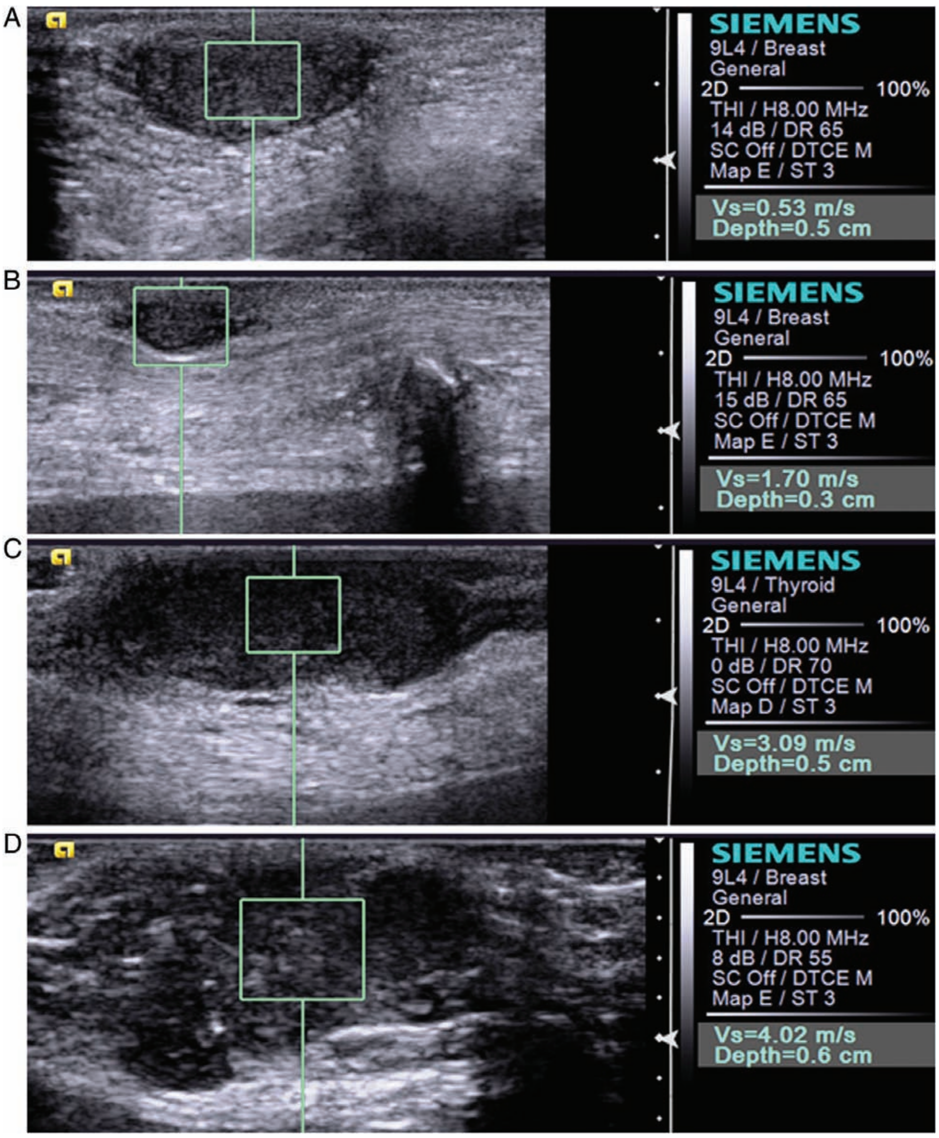

FIG 2. Image of the quantitative acoustic radiation force impulse (ARFI) elastography in mammary tumours. Note (A, B, C and D) the presence of the calliper within of the neoplasm's parenchyma, which was used to measure the shear velocity

Non-parametric data (echotexture, echogenicity, contours and margins, grayscale images and presence of deformity of the mammary masses) were evaluated using descriptive analysis and percentages and the Mann-Whitney U test was performed for the presence of deformities in the mammary masses. A P value $<0{\cdot}05$ was considered significant.

RESULTS

None of the owners reported or observed alterations that would compromise the health of the animals during anamnesis or the clinical examination. Equally none of the animals had physical or radiographic abnormalities (areas resembling pulmonary metastasis) that would contraindicate surgical treatment or tumour sampling.

After mastectomy and histopathological diagnosis, 14 nodules were identified as benign (23%) and 36 as malignant (77%). Each dog had only one tumour. The benign group (group 1) was composed of mammary hyperplasia (n=4), adenoma (n=2), fibroadenoma (n=1) and mixed benign tumour (n=7). The malignant group (group 2) included tubular carcinoma (n=6), complex tubulopapilliferous carcinoma (n=5), mixed carcinoma (n=15), simple solid carcinoma (n=8) and complex solid carcinoma (n=2).

Regarding the echogenicity of mammary parenchyma nodules, 1 benign nodule was anechoic; 6 benign and 8 malignant nodules were hypoechoic; 1 malignant nodule was hyperechoic; 5 benign and 15 malignant nodules were mixed with solid components; 1 benign nodule was mixed with a liquid component; and 1 benign and 12 malignant nodules were mixed with liquid and solid components.

With respect to the echotexture of the tumour parenchyma, 7 benign and 8 malignant tumours had a homogeneous parenchymal echotexture, while 7 benign and 28 malignant tumours had a heterogeneous parenchymal echotexture. Regarding the contours and margins, the benign and malignant mammary tissues presented defined and non-invasive margins.

Qualitative and quantitative elastography of the mammary masses in these cases was performed without difficulty. In the qualitative elastography, 12 (85%) benign and 5 (13%) malignant neoplasms were deformable, and 2 (15%) benign and 29 (87%) malignant neoplasms were not deformable. This difference was significant (P=0·002).

With respect to the grayscale images, the images presented homogenous white areas in 4 (28%) benign and 8 (22%) malignant nodules; homogenous dark areas in 7 (50%) benign and 17 (47%) malignant nodules and heterogeneous areas (white and dark areas) in 3 (22%) benign and 11 (31%) malignant nodules.

Using quantitative elastography, the mean shear velocity values were 3·33 m/s (95% confidence interval: 2·83 and 3·83 m/s) for malignant tumours and 1·28 m/s (95% confidence interval: 0·85 and 1·71 cm/s) for benign tissues which was significantly ($P<0{\cdot}0001$) different. In seven malignant mammary tumours, the shear velocity values were 'XX' (values more than 9 m/s).

DISCUSSION

In the present preliminary study, the clinical findings (anamnesis, general and specific physical examination) in the bitches with mammary neoplasia were not helpful in distinguishing between malignant and benign masses. It is well known that mammary tumours in dogs do not present with characteristic reproductive histories or clinical examination findings that would otherwise assist in differentiating malignant from benign tumours (Feliciano *et al.* 2012b).

The data obtained in the present study describe the use of novel imaging tools for epidemiological and clinical studies for each type of mammary neoplasm in female dogs. The classification used in the present study is in agreement with previous reports (Misdrop *et al.* 1999).

B-Mode ultrasonography is not a useful method for diagnosing the malignancy of mammary tumours in female dogs, possibly because of the substantial variety of tumour types found in dogs (Feliciano *et al.* 2012b). In the study presented here, ultrasonographic characteristics, such as echotexture, echogenicity and contours and margins of the mammary masses were shared in benign and malignant mammary tumours, demonstrating the ineffectiveness of the B-Mode technique in differentiating between benign and malignant tumours.

Regarding the elastography, the ARFI technique produced reproducible images of the mammary masses in female dogs, and other operators should be able to achieve the same image quality without limitation. On the basis of the qualitative assessment, elastography enhances B-Mode ultrasonographic evaluation of mammary neoplasms. Elastography increases the sensitivity of detecting tissue heterogeneity and malignant *versus* benign tumours. There was higher heterogeneity in the elastographic images of malignant mammary neoplasms than in images of benign mammary tissues. These findings are in agreement with the results obtained in human patients with breast cancer (Hiltawsky *et al.* 2001, Gweon *et al.* 2013, Yoon *et al.* 2013).

In a previously published qualitative ARFI study, the benign mammary lesions in women were observed as deformable, and the malignant tissues were rigid (Tavassoli and Deville 2003). Likewise, in the present report, the benign mammary nodules were deformable, and the malignant masses were not deformable.

Quantitative analysis demonstrated that ARFI verified that the shear velocity values in malignant neoplasms were higher than in benign masses. This difference is similar to that observed in humans (Zhou *et al.* 2013), where shear velocities of 2·68 ±1·20 m/s were found in benign masses *versus* 5·62 ±3·26 m/s in malignant masses.

In seven malignant mammary tumours, the shear velocity values were 'XX' m/s. This characteristic of malignant tumours demonstrates a greater stiffness in these tissues, corroborating a preliminary study in humans (Zhou *et al.* 2013) where values more than 9 m/s were demonstrated by the ultrasound machine as 'XX' m/s.

The increase in the stiffness of the malignant lesions observed in the present study and in another published study (Zhou *et al.* 2013) may be explained by the stromal reaction induced by mammary carcinoma, which is associated with increased levels of collagen.

Conclusions

The use of ARFI in this preliminary study helped differentiate between malignant and benign mammary neoplasms. Future studies with a larger number of samples should be performed to determine the sensitivity and specificity of this imaging technique in the evaluation of mammary tumours in female dogs compared with other imaging techniques, such as the Doppler mode.

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ARTIGO 2

Acoustic radiation force impulse (ARFI) elastography of testicular disorders in dogs: preliminary results

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Acoustic radiation force impulse (ARFI) elastography of testicular disorders in dogs: preliminary results

[*Elastografia* acoustic radiation force impulse *(ARFI) de afecções testiculares em cães: resultados iniciais*]

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ABSTRACT

The aim of this study was to describe the use of acoustic radiation force impulse (ARFI) elastography in the evaluation of testicular disorders in dogs. Eighteen dogs with testicular disorders (thirty-six testicles) were assessed. Echotexture, size, contours and margins of testes were analysed by ultrasonography. Deformities and tissue stiffness (greyscale and homogenous or heterogeneous) were evaluated by qualitative elastography and shear velocity was determined quantitatively. Subsequent to orchiectomy, testicular samples were collected for histopathology analysis and thirty-six disorders were identified. Qualitative elastography revealed that normal healthy testicular tissues were homogenous and not pliable while the affected testicles had alterations in tissue stiffness and homogeneity. The values obtained for quantitative elastography of the testicular tissues were: normal/healthy - 1.30±0.12 m/s; degenerated - 0.97±0.08 m/s; atrophied - 2.00±0.35 m/s; hypoplastic - 0.82±0.2 m/s; cystic - 1.32±0.18 m/s; orchitis - 2.68±0.42 m/s; interstitial cell tumours - 3.32±0.65 m/s; sertolioma - 2.99±0.07 m/s and leydigoma - 2.73±0.37. ARFI elastography of abnormal testes proved to be an applicable and complementary technique in the diagnosis of testicular disease in dogs.

Keywords: canine, stiffness, testicular disease

RESUMO

O objetivo deste estudo foi descrever o uso da elastografia ARFI (acoustic radiation force impulse) para avaliar as afecções testiculares em cães. Dezoito cães com distúrbios testiculares (36 testículos) foram avaliados. Ecotextura, tamanho, contornos e margens dos testículos foram avaliados por meio da ultrassonografia modo-B. A presença de deformidades e a rigidez tecidual (escala de cinza; homogênea ou heterogênea) foram avaliadas pela elastografia qualitativa; e a velocidade de cisalhamento foi determinada pela avaliação quantitativa. Amostras dos tecidos testiculares foram coletadas após orquiectomia para o diagnóstico histopatológico. Após ultrassonografia, orquiectomia e histopatologia, foram identificados 36 distúrbios em tecidos testiculares. Durante a elastografia qualitativa, os tecidos normais apresentaram-se homogêneos e não deformáveis; os testículos alterados demonstraram alterações na rigidez tecidual e de sua homogeneidade. Para a elastografia quantitativa, os valores obtidos foram: tecidos normais – 1,30±0,12m/s; degenerados – 0,97±0,08m/s; atrofiados – 2,00±0,35m/s; hipoplásicos – 0,82±0,2m/s; cistos – 1,32±0,18m/s; orquite – 2,68±0,42m/s; tumores de células intersticiais – 3,32±0,65m/s; sertolioma – 2,99±0,07m/s; e leydigoma – 2,73±0,37m/s. A elastografia ARFI de testículos anormais em cães demonstrou ser uma técnica aplicável e complementar para o diagnóstico de doenças testiculares nessa espécie animal.

Palavras-chave: canino, rigidez, doença testicular

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INTRODUCTION

The testicles, also defined as gonads, are the primary sexual organs of males and their function is to produce sperm and sexual hormones, especially testosterone. Studies on the normal testicular pattern of canines and on testicular alterations and their influence on fertility are important for early diagnosis of the main testicular disorders and in the selection of breeders (Kennedy and MacLachlan 2002; Feldman and Nelson 2004; Nelson and Couto 2006; Domingos and Salomão 2011).

Testicular ultrasonography in dogs enables the determination of the size, volume, position and internal constitution of the testicles. It aids the detection of small lesions or those inaccessible by palpation and provides details of the testicular tissue that cannot be obtained by radiography (Brandão *et al.* 2006; Davidson and Baker 2009; Goddi *et al.* 2012; Souza and Silva 2014). However, in most cases, ultrasonography is unable to differentiate the various canine testicular disorders and thus it must be used in conjunction with the animal's reproductive history, complimentary laboratory tests and invasive diagnostic techniques such as fine needle aspiration cytology, testicular biopsy and histopathology (Gradil *et al.* 2007).

Acoustic radiation force impulse (ARFI) elastography is an ultrasonographic technique that provides quantitative and qualitative measurements of tissue stiffness, with reduced inter-observer variability (Feliciano *et al.* 2015a). Qualitative ARFI creates a static greyscale map (elastogram) that represents the relative stiffness of the tissue in the scanned area and which can be compared to a corresponding conventional ultrasound scan. In general, lighter areas represent more pliable tissue than darker areas (D'Anastasi *et al.* 2011; Goddi *et al.* 2012). Quantitative ARFI creates propagating pressure waves that are capable of deforming the tissue quantitatively (pressure wave velocity of propagation or shear velocity). The velocity of propagation and attenuation of the waves are related to the stiffness and viscoelasticity of the tissue (Comstock 2011; Dudea *et al.* 2011).

In Veterinary Medicine, elastography has been used in the standardization of reference values of various tissues such as feline spleen (Feliciano *et al.* 2015b), canine spleen, liver and kidneys (Holdsworth *et al.* 2014) and in the differentiation of mammary neoplasms in female dogs (Feliciano *et al.* 2014). Feliciano *et al.* (2015a) conducted the first study on the use of the ARFI technique in the evaluation and standardization of reference values for canine testicles and obtained important results on the qualitative and quantitative analysis of healthy tissues in animals from different age groups.

In human obstetrics, elastography is a new ultrasound technique that has proved to be important in the detection of testicular alterations. According to some studies, the qualitative technique has 100% sensitivity in detecting testicular tumours when values indicate an increased stiffness of the testicular parenchyma. Furthermore, it enables the diagnosis of cysts, haematomas, calcifications, necrosis and other alterations that disrupt the homogeneity of the testicular tissue (Aigner *et al.* 2012; Huang and Sidhu 2012) as well as detect malignant lesions (Lorenz *et al.* 2000). In Veterinary Medicine, there are currently no studies on the use of this technique in the evaluation of testicular diseases in animals.

Analysis of the stiffness of canine testicular tissue by ARFI elastography provides a non-invasive alternative in the diagnosis of testicular alterations in small animals (Feliciano *et al.*, 2015a). Given the importance of this new ultrasound technique in determining tissue stiffness and its recent application in small animal medicine, the aim of this study was to describe the use of ARFI elastography in the evaluation of abnormal testicular parenchyma in dogs.

MATERIALS AND METHODS

This study was approved by the Animal Ethics and Welfare Committee of the School of Agrarian and Veterinary Sciences of the Univ Estadual Paulista- UNESP, Jaboticabal-SP, Brazil (protocol 023705/12). Using a prospective study design, eighteen dogs with testicular disorders (thirty-six testicles), of various breeds and aged from 5 months to 12 years, were included in this study.

Anamnesis, general and specific physical examination (inspection of the scrotum and testicular and abdominal palpation), palpation of the regional lymph nodes and ultrasound scans (testicular and abdominal) were performed in all animals prior to inclusion in the study. Ultrasonography was used to identify ectopic testicular structures in the abdomen and subcutaneous and testicular lesions.

The animals from the present study did not have any physical conditions that could contraindicate surgical treatment (orchiectomy). Subsequent to ultrasound assessment and orchiectomy, the testicular tissues were subjected to histopathology analysis and classified according to the disorder observed.

The abdomen and scrotal sac were clipped and gel applied locally prior to ultrasound scanning. No sedation was needed.

Ultrasonography was carried out by a single experienced evaluator. 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 and the size (increased, decreased or normal), contours and margins (regular or irregular) of the testes (right and left) were assessed and categorised by longitudinal and transverse B-Mode ultrasound sections.

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

Subsequent to B-mode ultrasonography, qualitative ARFI was performed to obtain greyscale images of the testicles in longitudinal sections. The images were evaluated for deformities, white (indicative of a less rigid, more elastic and pliable tissue) and dark areas (a more rigid, harder and less pliable tissue) (Feliciano *et al.* 2015a).

The quantitative analysis to determine mean shear velocity was performed after B-mode ultrasound scanning of the testes. To determine shear velocity, the calliper was positioned in the right and left testicular parenchyma and in focal lesions in the longitudinal sections (excluding the mediastinal portion). Six measurements were recorded for each area. The depth used for the testicular measurements was 0.5 to 1.0 cm (Feliciano *et al.* 2015a).

Animals were subjected to orchiectomy and samples of the testicular tissues collected for histopathology analysis. The macroscopic appearance of each testicular tissue was evaluated. Multiple fragments were fixed in 10% buffered formaldehyde solution (pH 7.4), processed and embedded in paraffin for routine histopathology analysis. Sections of 5µm were obtained using a microtome, mounted onto slides and stained with Hematoxylin & Eosin. Light microscopy was used to analyse the histological characteristics of the tissues and for diagnosis of the disorders. The samples were assessed by a Senior Professor (PhD) of the School of Agrarian and Veterinary Sciences, Univ Estadual Paulista – UNESP, Department of Veterinary Pathology, Jaboticabal-SP, Brazil.

Non-parametric data (echotexture, echogenicity, size, greyscale images and the presence of deformities in the testes) were evaluated using descriptive analysis. The mean values and standard deviation obtained for shear velocity of the testicular tissue were compared to the reference values for healthy testicular tissue reported by Feliciano *et al.* (2015a).

RESULTS

Physical and specific examination of the 18 dogs with testicular alterations identified six cases of unilateral cryptorchidism (subcutaneous location), two cases of bilateral cryptorchidism (subcutaneous location), one case of bilateral cryptorchidism (abdominal location), one case of testicular enlargement and one case of decreased testicular size. Seven animals did not show any testicular alterations upon physical or specific examination.

Histopathology analysis of the thirty-six testicular tissues revealed normal (8), degenerated (15) and atrophied (3) testicles; testicular hypoplasia (2) and cyst (2); orchitis (3); interstitial cell tumours (2); sertolioma (1) and leydigoma (1).

The assessment of six testicles using B-mode ultrasound showed no abnormalities in the shape, size, margins or parenchyma surface (echogenicity and echotexture).

The following alterations in sonographic findings were observed in 30 testicular samples: 1) Testicular degeneration - normal or reduced size, gross echotexture, normal or hypoechoic echogenicity and regular or irregular contours and margins; 2) Testicular atrophy - reduced size, heterogeneous echotexture, mixed echogenicity (hyperechoic areas) and regular or irregular contours and margins; 3) Testicular hypoplasia - reduced size, homogeneous echotexture, hypoechoic echogenicity and regular contours and margins; 4) Testicular cysts - cystic structures < 1cm in diameter; 5) Orchitis - increased size, heterogeneous echotexture, mixed echogenicity (hypoechoic, hyperechoic and anechoic areas), irregular contours and margins, mediastinal obliteration and hydrocele; 6) Interstitial cell tumour - increased size, heterogeneous echotexture, diffuse pattern with mixed echogenicity (hypoechoic and hyperechoic areas) or focal hypoechoic lesion (0.96cm of diameter), regular or irregular contours and margins and mediastinal obliteration; 7) Sertolioma - normal size, heterogeneous echotexture, focal hypoechoic lesion (0.98cm of diameter), regular contours and margins; and 8) Leydigoma - normal size, heterogeneous echotexture, diffuse pattern with mixed echogenicity (hypoechoic and hyperechoic areas), irregular contours and margins and mediastinal obliteration.

Qualitative elastography showed that healthy testicular parenchyma was homogenous (mid-grey) and not pliable. On the other hand, abnormal testicles were stiff and not homogeneous (Table 1) (Figure 1 and 2).

For quantitative elastography, the mean shear velocity value of normal healthy testicles was 1.30±0.12m/s. The results obtained for shear velocity of abnormal testicles are detailed in Table 1 (Figure 3 and 4).

Table 1. Characteristics of tissue stiffness and shear velocity of abnormal testicles of dogs evaluated by qualitative and quantitative elastography

Disorders	Characteristics of tissue stiffness	Shear velocity (m/s)
Testicular degeneration	heterogeneous parenchyma, not pliable	0.97±0.08
Testicular atrophy	heterogeneous parenchyma, not pliable	2.00±0.35
Testicular hypoplasia	heterogeneous parenchyma, light-grey, not pliable	0.82±0.2
Testicular cysts	areas in mosaic	1.32±0.18 (peri-cystic region)
Orchitis	heterogeneous parenchyma, not pliable	2.68±0.42
Interstitial cell tumour	heterogeneous parenchyma, not pliable, with hard focal lesion	3.32±0.65
Sertolioma	heterogeneous parenchyma, not pliable, with hard focal lesion	2.99±0.07
Leydigoma	heterogeneous parenchyma, not pliable	2.73±0.37

m/s: meters per second

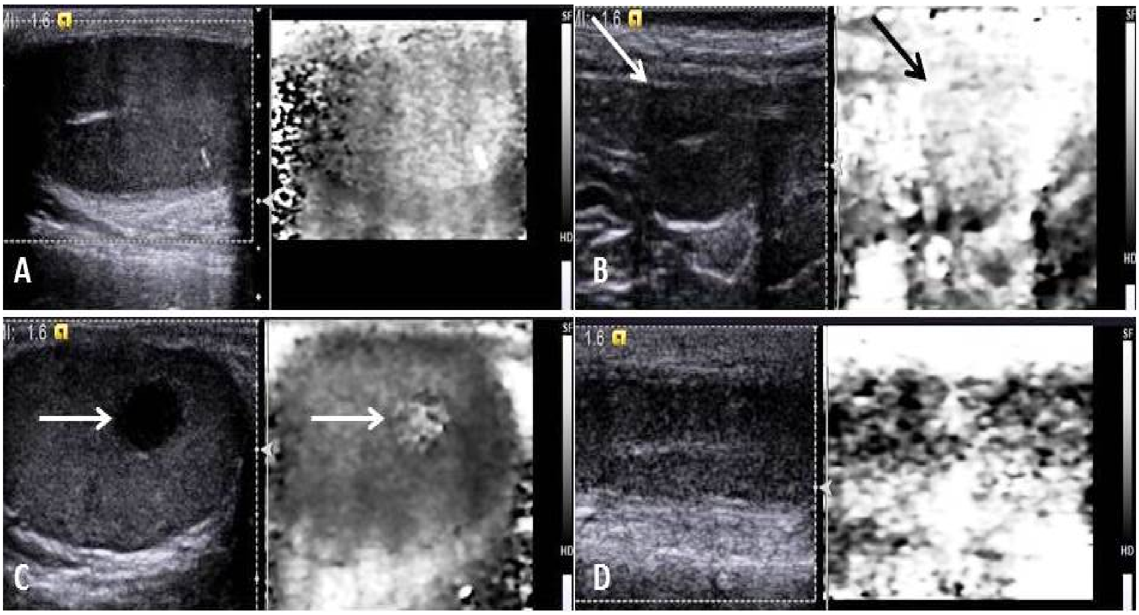

Figure 1. Qualitative ARFI elastography image illustrating the stiffness in testicular disorders in dogs: (A) heterogeneous parenchyma and not pliable, testicular degeneration; (B) heterogeneous parenchyma (arrows), light grey and not pliable, testicular hypoplasia; (C) areas in mosaic, testicular cysts (arrows) and (D) heterogeneous parenchyma and not pliable, testicular atrophy.

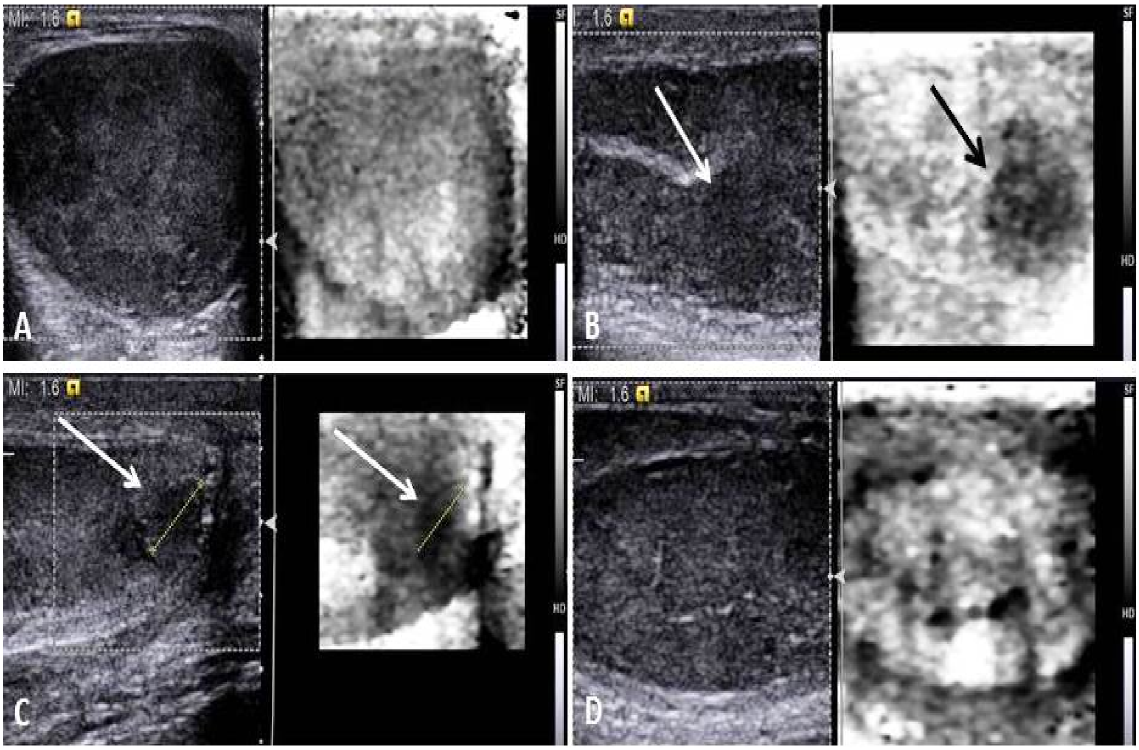

Figure 2. Qualitative ARFI elastography images illustrating the stiffness in testicular disorders in dogs: (A) heterogeneous parenchyma and not pliable, orchitis; (B) heterogeneous parenchyma with hard focal lesion and not pliable (arrows), interstitial cell tumour; (C) heterogeneous parenchyma with hard focal lesion and not pliable, sertolioma (arrows); and (D) heterogeneous parenchyma and not pliable, leydigoma.

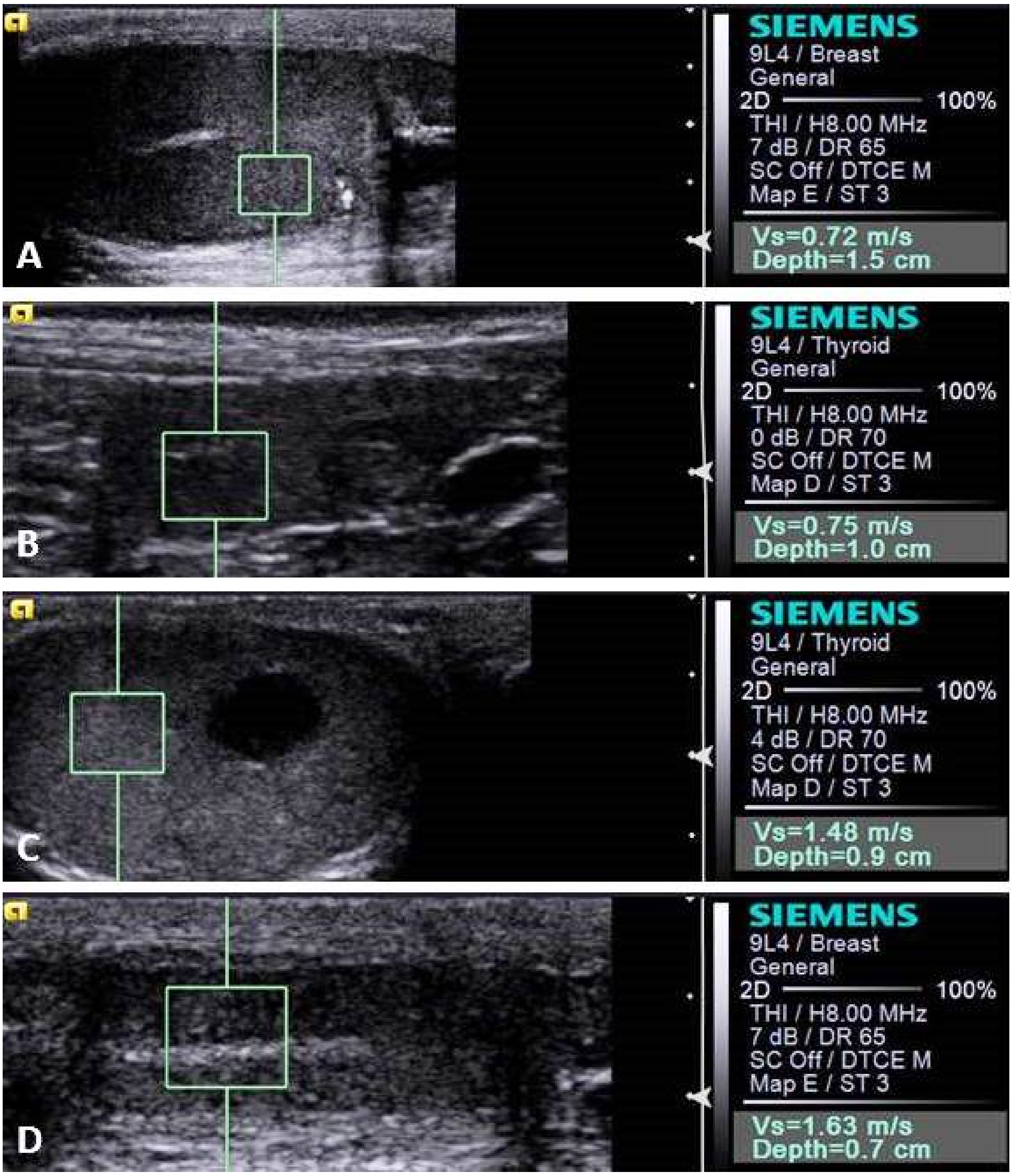

Figure 3. Quantitative ARFI elastography image of testicular disorders in dogs: Note the presence of the calliper in the abnormal testicular parenchyma for shear velocity measurement: (A) testicular degeneration; (B) testicular hypoplasia; (C) testicular cysts; and (D) testicular atrophy.

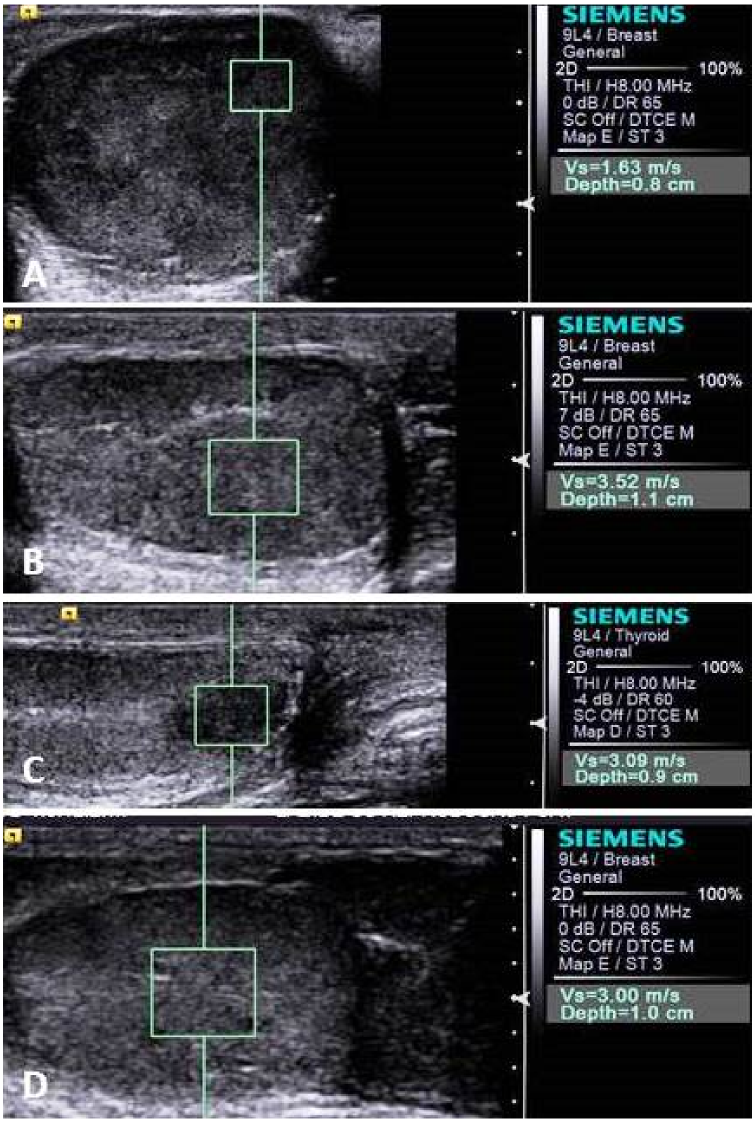

Figure 4. Quantitative ARFI elastography image of testicular disorders in dogs: Note the presence of the calliper in the abnormal testicular parenchyma for shear velocity measurement: (A) orchitis, (B) interstitial cell tumour, (C) sertolioma and (D) leydigoma.

DISCUSSION

In Veterinary Medicine, Feliciano *et al.* (2015a) were the first to use ARFI to study the testicular parenchyma of healthy dogs. Similarly, the present study is the first report on the use of the ARFI elastography on the evaluation of testicular abnormalities in dogs. The results obtained for stiffness of the tissues analysed are promising and can help in the diagnosis of the main testicular diseases in dogs, as well as in humans. Studies have demonstrated that elastography can provide auxiliary and important information in the evaluation of testicular lesions, both for detection of small lesions and for differentiation of benign and malignant disorders (Aigner *et al.* 2012; Goddi *et al.* 2012; Huang and Sidhu 2012).

Ultrasonography has been considered fundamental in the detection of testicular alterations; however, it does not provide conclusive information on the consistency of the tissues or a definitive diagnosis of lesions (Grasso *et al.* 2010; Domingos and Salomão 2011; Goddi *et al.* 2012). In the present study, testicular alterations were detected by B-mode ultrasound and the imaging findings corresponded to those previously reported (Domingos and Salomão 2011). However, some testicular diseases, such as inflammation and neoplasia, showed similar ultrasound characteristics making diagnosis difficult. Aigner *et al.* (2012), when evaluating testicular lesions in humans, obtained considerable values for sensitivity (100%), specificity (81%) and accuracy (94%) for elastography when compared to ultrasonography (sensitivity: 100%, specificity: 75% and accuracy: 92%), confirming the importance of this technique and its use combined with ultrasonography.

For qualitative ARFI elastography, it was generally observed that all abnormal testicles had a heterogeneous parenchyma and were not pliable. The neoplastic (focal or diffuse) and inflammatory lesions showed greater stiffness (dark grey) than benign lesions (light grey), as observed by Huang and Sidhu (2012) in humans. These stiffness characteristics were different from those of healthy testicles (six) observed in this study and from those reported by Feliciano *et al.* (2015a) (homogenous parenchyma, not pliable and of mid-grey tonality).

For quantitative ARFI elastography, the shear velocities obtained for normal healthy canine testicles (1.30±0.12m/s) were similar to the values obtained by Feliciano *et al.* (2015a) in dogs (juvenile: 1.28m/s, adult: 1.23m/s and senior: 1.23m/s) and higher than those observed in humans (0.62 to 1.01m/s) (D'Anastasi *et al.* 2011), probably due to the fact that dogs have greater fibrous tissue in the testicles.

There was a difference between the shear velocity of healthy testicles and that of abnormal testicles (Table 1), demonstrating stiffness in abnormal tissues. This is the first report of shear velocity of testicular disorders in both Veterinary and human Medicine and can thus be used as reference values for the conditions described.

The values of shear velocity in testicular disorders of dogs can assist in the diagnosis of malignant lesions in this organ by enabling early diagnosis and early treatment of animals. This statement is corroborated by data in human and animal studies (D'Anastasi *et al.* 2011; Dudea *et al.* 2011; Feliciano *et al.* 2015b) in which quantitative ARFI techniques were used to differentiate benign and malignant tumours (rigid and non-pliable tissues with high shear velocity values are indicative of malignancy). In the present study, the benign lesions (cysts, hypoplasia, atrophy, degeneration and inflammation) showed lower shear velocity than malignant lesions (testicular neoplasms).

The histopathological characteristics of each testicular disorder observed in this study can explain the different shear velocity values obtained. Testicular degeneration (tissue destruction) and hypoplasia (development failure or early destruction) are characterized by the loss of the normal cellularity of the testicles (Feldman and Nelson 2004; Domingos and Salomão 2011), leading to minor stiffness of these tissues and, consequently, shear velocity values below the normal range (0.97±0.08 and 0.82±0.2 respectively). Testicular atrophy showed shear velocity values above normal (2.00±0.35m/s) due to the thickening of the baseline membrane of the seminiferous tubules and interstitial fibrosis. In cystic cases, the lesions are limited and do not compromise the adjacent tissue (Domingos and Salomão 2011); therefore, the peri-cystic regions had normal shear velocity (1.32±0.18m/s).

Orchitis is characterized by an inflammatory process that promotes tissue disorganization, fibrin deposition, oedema and cell migration (Nelson and Couto 2006); which may be correlated to the increased stiffness (2.68±0.42) observed. Conjunctive septa, hyaline and cellular hyperplasia are the main histopathological characteristics of testicular neoplasms (Kennedy and MacLachlan 2002). They increase tissue stiffness and consequently increase shear velocity (interstitial cell tumour: 3.32±0.65m/s, sertolioma: 2.99±0.07m/s and leydigoma: 2.73±0.37m/s).

It is important to comment on some of the limitations observed this study.
The qualitative and quantitative elastography of the abnormal and normal testes in dogs was performed without difficulty and without sedation. Due to the location of the organ analysed, there was no interference from movements (i.e. respiration) that hindered the acquisition of the measurements, as cited by Holdsworth *et al.* (2014) during abdominal elastography in dogs. This initial study provides

important data on the validation of the technique in testicular diseases in dogs; however, a larger study is needed.

CONCLUSION

Quantitative and qualitative ARFI elastography of testicular disorders in dogs was easily implemented and this study provides important data and baseline reference values for the use of this technique in Veterinary obstetrics and in the diagnosis of the main testicular disorders of animals.

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ARTIGO 3

Ultrasonography methods for predicting malignancy in canine mammary tumors

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RESEARCH ARTICLE

Ultrasonography methods for predicting malignancy in canine mammary tumors

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Abstract

The aim of this study was to evaluate and compare the efficacy of B-mode, Doppler, contrast-enhanced ultrasonography (CEUS), and Acoustic Radiation Force Impulse (ARFI) elastography in predicting malignancy in canine mammary masses. This was a prospective cohort study from 2014 to 2016, which included 153 bitches with one or more mammary masses. A total of 300 masses were evaluated by ultrasonography (B-mode, Doppler, CEUS, and ARFI) and subsequently classified as benign or malignant by histopathology. Each ultrasound parameters studied were compared between benign and malignant masses by Chi-square or Student tests and differences were considered significant when $P < 0.01$. For the variables that proved significant differences were estimated the cut-off point, sensitivity, specificity, accuracy, and area under curve (AUC) by receiver-operating characteristic curve (ROC) analysis in a logistic regression model using histopathological classification as reference, to assess and compare diagnostic performance of each technique. Out of 300 mammary masses evaluated 246 were classified as malignant and 54 as benign. B-mode measurements showed sensitivity 67.9%, and specificity 67.6% as malignancy predictors on canine mammary masses; Doppler indexes systolic (>21.2 m/s) and diastolic velocity (>4.8 m/s) sensitivity 79.2% and specificity 70.8%; CEUS wash-out time (<80.5 s) sensitivity 80.2% and specificity 16.7%; and ARFI elastography shear velocity (SWV > 2.57 m/s) sensitivity 94.7% and specificity 97.2% In conclusion B-mode and Doppler ultrasound evaluations may assist in malignancy prediction of canine mammary masses with moderate sensitivity and specificity, already the SWV was an great accurate predictor. Therefore, ARFI elastography exam inclusion in veterinary clinic oncology and research is highly recommended, since it allows fast, non-invasive, and complication-free malignancy prediction of canine mammary masses.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Mammary tumors are one of the most important disorders in women and bitches, with high morbidity and mortality, and similar biological behavior in both species [1,2]. Ultrasonography has become an important tool in neoplasm evaluation and, when combined with mammography, can aid in the diagnosis, differentiation, and prediction of malignancy in mammary tumors in human medicine [3,4,5].

Several reports have demonstrated the applicability and limitations of B-mode ultrasonography [6,7,8,9,10,11,12], Doppler [8,10,13,14,15], elastography [3,4,16,17,18,19], and contrast-enhanced ultrasonography (CEUS) [20,21,22,23] in the evaluation of breast cancer in humans and canines. However, no report has yet compared the efficacy of these ultrasonography techniques in predicting malignancy of mammary tumors.

It has been suggested that B-mode, Doppler, contrast-enhanced ultrasound, and elastography can aid in the diagnosis of malignancy in breast tumors, non-invasive diagnostics techniques of easy and fast execution, enabling immediate results to the medical and veterinary; nevertheless, these techniques are believed to have different diagnostic efficacy. Thus, the aim of this study was to evaluate and compare the efficacy of B-mode, Doppler, contrast-enhanced ultrasonography, and Acoustic Radiation Force Impulse (ARFI) elastography in predicting malignancy in canine mammary tumors.

Materials and methods

This study was approved by the Ethics Committee in the Use of Animals of the School of Agrarian Sciences and Veterinary Medicine, UNESP–Universidade Estadual Paulista, Jaboticabal-SP, Brazil (protocol No 023705/12).

Experimental design

A prospective cohort study developed between 2014 and 2016 included 153 bitches with one or more mammary masses that were brought by pet owners for care to the "Governador Laudo Natel" Veterinary Hospital, UNESP-Univ Estadual Paulista, Jaboticabal-SP, Brazil. Pet owners signed a consent form for their animal inclusion in this experiment. All animals and a total of 300 masses were evaluated by ultrasonography and subsequently classified as benign or malignant by histopathology [24,25].

Ultrasonography exam

Ultrasonography (US) was performed by a single experienced veterinary sonographer prior to mastectomy and histological identification of tumor type, using a 9.0 MHz linear transducer and ACUSON S2000® equipment (Siemens, Munich, Germany). Each mammary mass was evaluated using the different ultrasonographic methods (B-mode, Doppler, ARFI elastography, and CEUS) in the order described below.

B-mode ultrasonography

The mammary masses were evaluated by conventional ultrasonography according to: echotexture (homogenous or heterogeneous), echogenicity in relation to the adjacent and normal mammary tissue (hypo, hyperechoic, or mixed with solid or liquid components), contours/margins (defined or undefined), invasiveness (present or absent), and other findings (presence of cystic, anechoic, and hyperechoic areas or acoustic shadowing). Additionally, the length (cm), width (cm), and width/length ratio in longitudinal section and height (cm), width (cm), and width /height ratio in transverse sections were obtained.

Doppler ultrasonography

Doppler color flow imaging enabled the visualization (present or absent) and localization (peripheral, central, or diffuse) of tumoral vascularization and the identification of the type of vessel (Perinodular—vessels around mass parenchyma, mosaic—random vascular points into the parenchyma, or network) present in the tumors.

In tumoral vascularization analysis by spectral Doppler, the angle between the Doppler beam and the vessel's long axis did not exceed 60°. Color gain was adjusted to reduce excessive color noise when blood flow was too slow. A 2–4 mm gate (depending on the diameter of the vessel) with apertures was positioned at the center of the vessel to measure the flow's spectral trace, spectral curve, and vascular indexes; which were obtained automatically following software identification of the ultrasonic scanner for each waveform. A minimum of three subsequent waves was used in the evaluation. The parameters studied were: systolic velocity (SV, cm/s), diastolic velocity (DV, cm/s), resistive index (RI = (Vmax–Vmin)/Vmax), characteristic (arterial or turbulent), and pattern (high, intermediate, or low resistivity) of blood flow [10].

Contrast-enhanced ultrasonography (CEUS)

CEUS was performed using contrast-specific software (CADENCE®, Siemens, Munich, Germany) with secondary harmonic imaging and inverted pulse technique. After delineation of the mass area, the probe was held steadily and the adjustable parameters such as depth, gain, mechanical index (0.07–1.1; interval constant between different tumors), and focal zones were optimized and maintained. The contrast agent (SonoVue®, Bracco, Milan, Italy) was immediately administered as an intravenous bolus (0.1 mL, followed by 5 mL saline flush) via a catheter in the cephalic vein. Video clips were obtained for five minutes following bolus injection of contrast and recorded in the internal storage system for each mass assessed.

Microbubble perfusion and the dynamic enhancement of the image of each lesion were subsequently analyzed based on the presence or absence of contrast in the tumoral mass; perfusion time through wash-in time (WI seconds), time to enhancement peak (TP s), and washout time (WO s); and enhancement characteristics: 1) enhancement level relative to surrounding normal mammary tissue (hyper, iso, or hypo enhancement), 2) pattern (centripetal, centrifugal, or diffuse), 3) localization (central, peripheral, or diffuse), 4) internal homogeneity (homogeneous or heterogeneous), and 5) perfusion type (discreet, moderate, or increased) [21].

ARFI elastography

Qualitative and quantitative analysis were performed using the VTIQ method of ARFI (virtual touch tissue imaging quantification, 2D-SWE technique) [3,19]. Qualitative ARFI resulted in greyscale images (elastogram) that were evaluated according to deformability (deformable or not deformable), whitish tones (bluish areas—less rigid) corresponded to more elastic tissues (soft) and darker tones (reddened areas, rigid not deformable tissues) to more rigid tissues (hard). Additionally, the quality of the examination was evaluated using the display device: homogeneous and greenish images to indicate high quality of the technique; and yellowish and heterogeneous images to indicate low quality of the technique. Quantitative evaluation consisted of a software function that determined shear wave velocity once the calliper was positioned on the mass parenchyma. Six measurements of different areas in each tissue randomly selected were used to determine the mass mean shear wave velocity (SWV m/s).

Histopathological classification

Following ultrasonography evaluation, the animals were referred to the Department of Veterinary Clinics and Surgery for mastectomy. Samples of the mammary masses were collected for histopathology analysis and their macroscopic appearance evaluated. Multiple tissue fragments were fixed in 10% phosphate buffer formaldehyde solution (pH 7.4) and routinely processed for histopathology analysis prior to paraffin embedding. Tissue sections (5 μm) were mounted onto glass slides and stained with Haematoxylin and Eosin (HE).

The neoplasms were analyzed by single and experience pathologist under light microscopy and histologically classified as benign or malignant according to the criteria recommended by the World Health Organization [25]. Posterior classification and staging were made in accordance with the Consensus for diagnosis, prognosis, and treatment of Canine Mammary Tumors [24].

Statistical analysis

Statistical analysis was performed using the software R, version 3.3.0 (R® foundation for statistical computing, Austria). Qualitative ultrasound variables were compared between benign and malignant masses by Chi-square test, quantitative variables by Student test and differences were considered significant when P-value < 0.01. For ultrasonography parameters that showed significance, the cut-off point, sensitivity, specificity, accuracy, and area under curve (AUC) were calculated using histopathological classification as a reference for receiver-operating characteristic curve (ROC) analysis in a logistic regression model aimed at assessing and comparing the diagnostic performance of each technique.

Results

Out of the 300 mammary masses evaluated, 246 (82%) were histopathologically classified as malignant and 54 (18%) as benign and histopathological classification is detailed in Table 1. Ultrasonographic evaluation was performed without difficulties, intercurrences, or side effects. US findings are summarized in Tables 2 and 3, diagnostic performance variables in Table 4, and comparative receiver-operating characteristic curves in Fig 1.

B-mode ultrasonography

The B-mode US variables echotexture, contours/margins, invasiveness, echogenicity, findings, mass width/length ratio in longitudinal section and thickness in transverse section were not significantly ($P>0.01$) correlated to malignancy. Already, mass length and width in longitudinal section, and width and width/height ratio in transverse section were significantly ($P<0.001$) greater in malignant tumors and these B-mode variables showed a mean sensitivity 67.9%, specificity 67.6%, accuracy 67.5% and AUC 69.5% as malignancy predictors on canine mammary masses (Tables 1–3).

Doppler ultrasonography

Color flow Doppler imaging revealed that malignant tumors showed higher proportion ($P<0.01$) of vascularization and intermediate resistivity pattern. This technique resulted on a mean sensitivity 86.0%, specificity 47.9% and accuracy 81.5% as malignancy predictors on canine mammary masses. In turn, Doppler spectral vascular indexes SV and DV were greater ($P<0.01$) in malignant tumors and showed a mean sensitivity 79.2%, specificity 70.8%, accuracy 71.6% and AUC 73.0% as malignancy predictors on canine mammary masses. Another

Table 1. Histopathological classification of canine mammary tumors [24,25].

Classification	Type	Diagnosis	Number
Malignant	Carcinomas	Carcinoma in a mixed tumor	129
		Ductal carcinoma *in situ*	3
		Lobular carcinoma *in situ*	11
		Papillary carcinoma	27
		Tubular carcinoma	30
		Solid carcinoma	18
		Complex carcinoma	7
	Special type carcinomas	Malignant adenomyoepithelioma	8
		Secretory carcinoma	2
		Micropapillary carcinoma	5
		Anaplastic carcinoma	1
		Squamous cell carcinoma	2
		Inflammatory carcinoma	3
Total Malignant			**246**
Benign	Epithelial hyperplasia	Ductal hyperplasia	4
		Lobular Hyperplasia	7
	Benign neoplasm	Adenoma	5
		Fibroadenoma	1
		Benign mixed tumor	37
Total Benign			**54**

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blood flow characteristics and RI did not show significant (P>0.01) correlation with malignancy (Tables 1–3, Fig 2).

Contrast-enhanced ultrasonography

Contrast-enhanced ultrasonography enabled the evaluation of capillarization (macro and microcirculation) of the mammary tumors (Fig 3). However, none of the CEUS parameters evaluated showed significant (P>0.01) correlation to mammary mass malignancy. However, ROC analysis were applied for WO (P = 0.065) for comparative diagnostic performance study of US methods. WO lowers than 80.5 s showed sensitivity 80.2%, specificity 16.7%, accuracy 77.4% and AUC 74.0%.

ARFI elastography

Tissue deformability was found to be proportionally higher (P<0.01) in malignant masses. Mostly red (dark) masses on the elastogram image (not deformable) were indicative of malignancy with sensitivity 75.6%, specificity 66.7%, and accuracy 74.5%. In turn, quantitative elastography enabled the mammary masses SWV determination, which was significantly (P<0.01) higher in malignant tumors. The ROC analysis indicated that an SWV > 2.57 m/s shown to be the best (P <0.01) malignancy predictive tool of canine mammary masses (Fig 1), with sensitivity 94.7%, specificity 97.2%, accuracy 95.0% and AUC 98.5% (Tables 1–3, Figs 4 and 5).

Discussion

Ultrasonography evaluation of malignancy in mammary tumors in bitches showed variable efficacy in relation to standard methods of malignancy diagnosis. B-mode variables and vascular indexes evaluated by Doppler enabled the prediction of malignancy with moderate

Table 2. Rate of qualitative variables evaluated by different ultrasonography methods (B-mode, Doppler, contrast-enhanced ultrasonography and ARFI elastography) in malignant and benign canine mammary tumors.

Variables	Parameter	Benign	Malignant	P-value
B-Mode ultrasonography				
Echotexture	Homogenous (%)	31	50	1.0000
	Heterogeneous (%)	69	31	
Echogenicity	Hypoechoic (%)	50	30	0.0450
	Hyperechoic (%)	0	2	
	Mixed (%)	50	68	
Contours or margins	Defined (%)	97	99	0.4210
	Undefined (%)	3	1	
Invasiveness	Present (%)	0	0	1.0000
	Absent (%)	100	100	
Doppler ultrasonography				
Vascularization	Present (%)	86	67	0.0065*
	Absent (%)	14	33	
Localization	Peripheral (%)	52	75	0.1210
	Central (%)	8	4	
	Diffuse (%)	40	21	
Vessel type	Perinodular (%)	31	17	0.0318
	Mosaic (%)	31	58	
	Network (%)	38	25	
Characteristics	Arterial (%)	95	100	0.0535
	Turbulent (%)	5	0	
Patterns	High resistivity (%)	50	25	<0.0001*
	Intermediate (%)	14	63	
	Low (%)	36	12	
Contrast-enhanced ultrasonography				
Enhancement level	Hyperenhancement (%)	15	0	0.1083
	Hypoenhancement (%)	34	80	
	Isoenhancement (%)	51	20	
Pattern	Centripetal (%)	10	20	0.2149
	Centrifugal (%)	34	60	
	Diffuse (%)	56	20	
Localization	Central (%)	5	0	0.7242
	Peripheral (%)	54	40	
	Diffuse (%)	41	60	
Homogeneity	Homogeneous (%)	2	0	1.0000
	Heterogeneous (%)	98	100	
Perfusion type	Discreet (%)	15	0	0.1083
	Moderate (%)	34	80	
	Increased (%)	51	20	
ARFI Elastography				
Deformability	Deformable (%)	24	66	<0.0001*
	Not Deformable (%)	76	34	

*Difference considered significant (Chi-square test).

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sensitivity, specificity and accuracy, CEUS evaluation showed high sensitivity but low specificity, while stiffness evaluation by ARFI elastography resulted in an exceptionally effective technique for malignancy prediction in canine mammary masses.

Table 3. Mean ± SD of quantitative variables evaluated by different ultrasonography methods (B-mode, Doppler, contrast-enhanced ultrasonography and ARFI elastography) in malignant and benign canine mammary tumors.

Variables	Parameter	Benign	Malignant	P-value
		B-Mode ultrasonography		
Measures	Longitudinal width (cm)	0.78 ± 0.90	1.18 ± 1.07	0.0006*
	Longitudinal length (cm)	1.64 ± 1.26	2.48 ± 1.63	0.0002*
	Width/length ratio	0.48 ± 0.37	0.45 ± 0.15	0.3681
	Transverse height (cm)	1.54 ± 1.15	1.97 ± 1.54	0.1595
	Transverse width (cm)	0.72 ± 0.76	1.43 ± 1.23	0.0001*
	Width /height ratio	0.48 ± 0.28	0.97 ± 0.93	0.0004*
		Doppler ultrasonography		
Vascular indexes	Systolic velocity (cm/s)	18 ± 11	37 ± 27	<0.0001*
	Diastolic velocity (cm/s)	5.1 ± 3.1	8.8 ± 8.7	0.0099*
	Resistive index	0.71 ± 0.1	0.76 ± 0.1	0.0240
		Contrast-enhanced ultrasonography		
Perfusion times	Wash-in time (s)	13 ± 7.6	9.1 ± 5	0.1583
	Wash-out time (s)	20 ± 7.2	15 ± 5.7	0.0650
	Time to peak (s)	81 ± 17	64 ± 22	0.0819
		ARFI Elastography		
Shear wave velocity	SWV (m/s)	1.5 ± 0.73	5.8 ± 2.4	<0.0001*

cm: centimeters; s: seconds; m: meters; SD: standard deviation

*difference considered significant (Student test).

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Based on the results from this study, quantitative ARFI elastography proved to be the best method of ultrasonographic prediction of malignancy in mammary masses. VTIQ ARFI elastography enables the quantitative evaluation of tissues stiffness, resulting in shear wave velocity

Table 4. Predictive performance variables (%) of different ultrasonography methods in determining malignancy in canine mammary tumors using histopathological classification as a reference.

Parameters	Cut-off	Sensitivity	Specificity	Accuracy	AUC
		B-Mode ultrasonography			
Longitudinal width (cm)	>1.28	70.76	61.76	69.63	68.10
Longitudinal length (cm)	>0.52	71.19	61.76	70.00	69.3
Transverse width (cm)	>0.66	67.84	73.53	68.58	72.10
Width/height ratio	>0.49	61.95	73.53	61.92	68.50
		Doppler ultrasonography			
Systolic velocity (cm/s)	>21.2	77.83	79.17	77.54	78.70
Diastolic velocity (cm/s)	>4.8	67.92	62.50	65.68	67.30
Presence of vascularization	N/A	86.18	33.33	79.43	N/A
Intermediate resistivity	N/A	85.85	62.50	83.47	N/A
		Contrast-enhanced ultrasonography*			
Wash-out time (s)	<80.5	80.15	16.67	77.37	74.00
		ARFI Elastography			
Shear wave velocity (m/s)	>2.57	94.72	97.22	95.04	98.50
Deformable tissues	N/A	75.61	66.67	74.47	N/A

AUC: area under the curve; N/A: data not available; cm: centimeters; s: seconds; m: meters

*even without significant difference, the wash-out time of contrast-enhanced ultrasonography was analyzed for compared US techniques.

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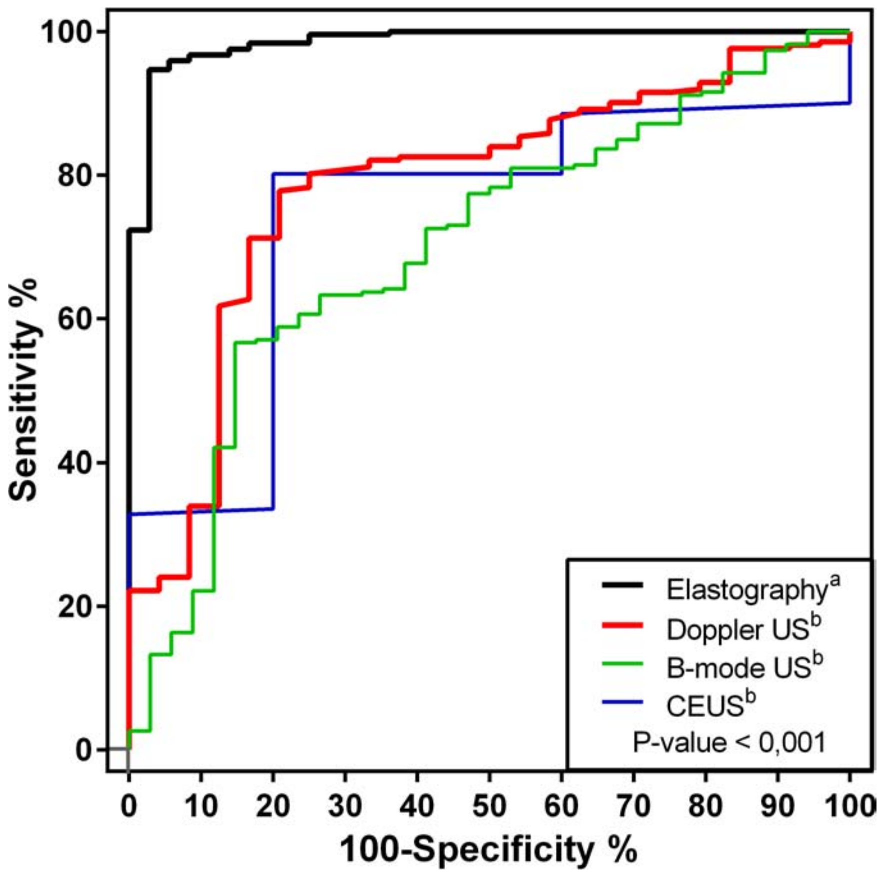

Fig 1. Receiver-operating characteristic curve comparing predictive sensitivity (%) and specificity (100-%) of different ultrasonography methods in determining malignancy in canine mammary masses using histopathological classification as a reference. Different letters indicate significant difference.

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(waves that return from target tissues) estimation. This modern tool showed the highest diagnostic efficacy in differentiating malignant and benign mammary masses, corroborating with recent reports [3,4,18,19,26,27,28,29] and the only reliable parameter. Cut-off values above 2.57 m/s showed an impressive 95% diagnostic accuracy and 98% AUC, with adequate sensitivity and specificity values. The cut-off values in this study were lower than those from previous reports; however, with greater sensitivity and specificity for women. In human medicine, some authors [19,26,30,31,32] have reported cut-off values ranging from 2.9–6.4 m/s, which have been associated to 76–91% sensitivity and 80–95% specificity, close values to the observed in the present study and which corroborate the effectiveness of the ARFI technique applied in evaluation of mammary tumors in canines.

The elastogram characteristics obtained in this study were adequate in the diagnosis of malignancy and similar to those previously described in benign mammary lesions in women [4,16,17,18] and bitches [3], with whitish tones (less rigid) in benign and darker tones (rigid not deformable tissues) in the malignant masses. The greater stiffness observed in malignant tumors is a consequence of the stromal reaction induced by the mammary carcinoma, which is associated with increased levels of collagen [3].

B-mode ultrasonography showed low efficacy in the differentiation of mammary tumors and findings such as invasiveness, irregular contours, acoustic shadowing, and echotexture were not indicative of malignancy; in disagreement with some reports that have considered

Fig 2. B-mode and Doppler ultrasonographic image of a canine mammary tumor—Solid carcinoma in female dog. B-mode image of mammary tumor (arrows) with presence of intratumoral calcification (cl) (A). Spectral Doppler ultrasonographic image of mass (arrows) with tumoral neovascularization and waveforms highlighting blood flow with indicated values of malignancy (high resistance and values on the right hand side) (B).

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these characteristics to be indicative of malignant tumors [6,7,8,9,11]. Corroborating the results from this study, B-mode ultrasonography has been considered to be a technique with low specificity (e.g. compared to mammography) when used as an isolated method of evaluation [14,33]. Additionally, this low specificity can be justified by clinical and biological profile [34,35] and histopathological variability of each tumoral type [10], ie, the morphological and structural heterogeneity of benign and malignant tumor in humans and canines, besides the presence of nonspecific characteristics theses masses, makes it difficult the differentiation of tumor types by the B-mode image.

Malignant tumors are often larger that benign ones due to parenchyma alterations (e.g. secondary tissue lesion such as edema, necrosis, calcification, and hemorrhage) that produce liquid and solid components at echogenicity evaluation [10,12,36,37]. These assertions corroborate with the larger size (longitudinal length and width, and transverse width and width/height ratio) observed in the malignant masses. Transverse width/height ratios in breast cancer (Japan Society of Ultrasonics in Medicine) [38] and cut-off values greater than 0.7 have been described [12] as malignancy predictor tools, with 56.3% sensitivity and 92.9% specificity, similarly to the results obtained in the present study.

Color and Spectral Doppler (vascular index and tracing characteristics) have been shown to be important ultrasonography techniques in the differentiation of malignant and benign tumors [10,13,15,39,40]. High values of SV and DV observed in the present study have been described as satisfactory indicators of malignancy in mammary masses in humans

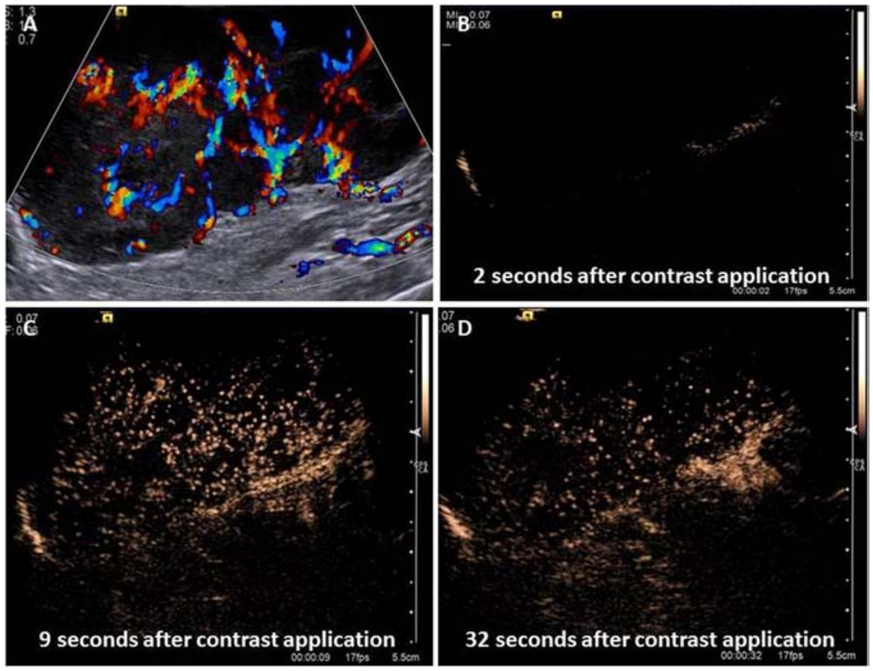

Fig 3. Contrast-enhanced ultrasonography (CEUS) image of a canine mammary tumor—Solid carcinoma in female dog. Color Doppler in mammary neoplasm (A) highlighting the presence of neovascularization in the tumor. The contrasted technique was applied to evaluate capillarization characteristics of the tumor: (B)—absence of contrast, (C) peak enhancement and diffuse enhancement, and (D) contrast wash-out. Time acquisition after contrast medium injection is located at the bottom of the right side of the images (B, C and D).

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[13,39,40,41,42] and animals [10]. Furthermore, the findings from this study on high/low resistivity patterns are suggestive of malignancy, as previously described [8,14,15], and may be correlated to the presence of tortuous vascular networks in malignant tumors [14] and demonstrate the influence of neoplastic vascular organization (neovascularization characteristics) in tumoral nutrition [10,14]. It is important to note that diastolic velocity and blood flow patterns had not yet been described in the literature as predictors of malignancy in mammary tumors.

Contrast-enhanced ultrasonography proved ineffective in the differentiation of mammary tumors; however, it proved useful in the identification of tumoral macro and microcapillarization. These findings differ from most reports on CEUS diagnostic efficacy in the characterization of mammary masses in humans [20,21,22,23], which have suggested this technique to be an acceptable predictor of malignancy. These divergent results may be due to the limited samples of benign neoplasm in this study and/or the differences in imaging methods. However, despite the low diagnostic efficiency, these results provide novel values for CEUS in canine mammary masses. A high degree of contrast enhancement has been considered as an indicative of benignity [21] and was proportionally higher in the benign masses analyzed in the present study. This increase in intensity in benign masses has been correlated to inflammation and fibroadenomas, probably due to inadequate intratumoral angiogenesis and, consequently, insufficient capillary network to support tissue development [43].

Conclusions

In conclusion B-mode and Doppler ultrasound evaluations may assist in malignancy prediction of canine mammary masses with moderate sensitivity and specificity, already the SWV

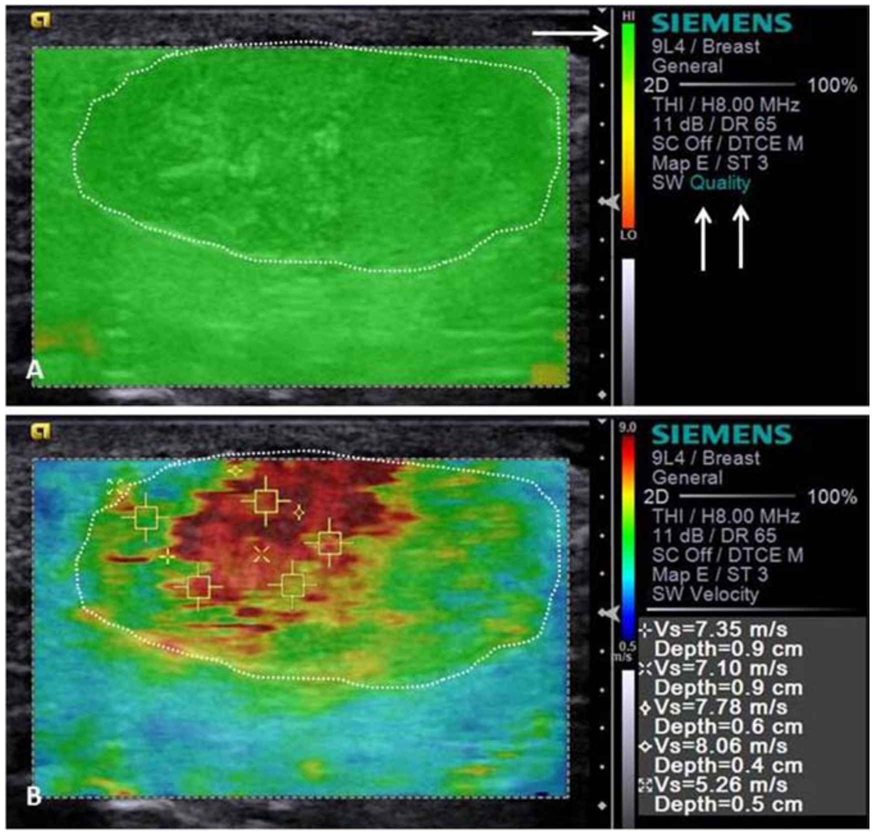

Fig 4. Acoustic radiation force impulse (ARFI) elastography image showing stiffness characteristics in a canine mammary tumor—Carcinoma in a mixed tumor in female dog. High quality image map (arrows) of VTIQ shows a homogeneous green picture of the lesion (white dotted line delimiting the neoplasm) (A). In VTIQ shear wave velocity mode (B), SWV values in the lesion (white dotted line delimiting the neoplasm) were measured and repeated five times. In elastogram (VTIQ qualitative—B), the image of the neoplasm (arrows) is heterogenous and not deformable, with rigid tissue (reddened areas in the central region) and with soft tissue (greenish areas in the peripheral region).

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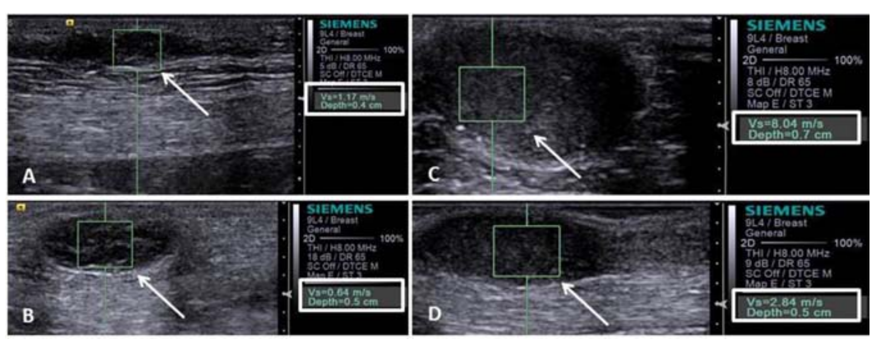

Fig 5. Image of the acoustic radiation force impulse (ARFI) elastography, which shows the values of shear velocity (right bottom corner) of images in the mammary tumors (arrows): A)–mixed benign tumor—shear velocity of 1.17 m/s; B) mixed benign tumor—shear velocity of 0.64 m/s; C) carcinoma in a mixed tumor (grade III)—shear velocity of 8.04 m/s; and D) carcinoma in a mixed tumor (grade II)—shear velocity of 2.84 m/s.

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was an great accurate predictor. Therefore, ARFI elastography exam inclusion in veterinary clinic oncology and research is highly recommended, since it allows fast, non-invasive, and complication-free malignancy prediction of canine mammary masses.

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ARTIGO 4

Accuracy of four ultrasonography techniques in predicting histopathological classification of canine mammary carcinomas

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WILEY

Accuracy of four ultrasonography techniques in predicting histopathological classification of canine mammary carcinomas

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Abstract

Due to the importance of presurgical, noninvasive, and accurate diagnostic tools in mammary carcinoma characterization, this prospective secondary observational cohort study was designed to evaluate and compare the diagnostic accuracy of B-mode, Doppler, contrast enhancement ultrasonography, or acoustic radiation force impulse–elastography in identifying mammary carcinomas types with high degree of malignancy. A total of 246 mammary carcinomas from 141 female dogs were analyzed using B-mode, Doppler, contrast enhancement ultrasonography, and acoustic radiation force impulse ultrasonography prior to their histopathological classification according to types (simple, complex, or special) and grade (I, II, or III). Qualitative and quantitative variables were compared between carcinoma types and grades by Fisher's or analysis of variance. Diagnostic performance was estimated by receiver-operating characteristic analysis, using histopathological classification as a reference. Deformability (acoustic radiation force impulse) had a diagnostic specificity of 100% and sensitivity of 12% in identifying special carcinomas. A width:length ratio greater than 0.53 can be suggestive of special carcinoma, with 80% sensitivity and 76% specificity. Contrast wash-in and peak enhancement times lower than 7.5 and 13.5 s, respectively, were indicative of complex carcinoma at 62% sensitivity and 60% specificity. Contrast wash-in, peak enhancement, and wash-out times greater than 6.5, 12.5, and 64.5 s, respectively; were indicative of grade II and III carcinoma at 68% sensitivity and 62% specificity. In conclusion, B-mode ultrasonography, contrast enhancement ultrasonography, and acoustic radiation force impulse–elastography enabled the identification of some of the characteristics of high-grade mammary carcinoma types and grades in female dogs with limited accuracy. The findings from this study may contribute to oncology research and clinical management canine patients.

KEYWORDS
canine, contrast-enhanced ultrasound, Doppler, elastography, tumors

1 | INTRODUCTION

Mammary tumors in female dogs are of great clinical relevance due to their high prevalence and mortality rate, which varies with the histopathological classification and graduation of the tumor.[1,2] The clinical–surgical approach and prognosis may vary with tumor types and grade; therefore, the use of noninvasive presurgical accurate diagnostic techniques that enable the differentiation of these tumors is a clinical strategy with great diagnostic, prognostic, and therapeutic value in patients with malignant mammary neoplasms.[3]

Ultrasound examination of mammary masses has been recently considered an important clinical tool in the identification of malignant neoplasms in female dogs. It has been suggested that B-mode ultrasonography could predict tumoral malignancy through heterogeneous echotexture; however, the accuracy of the reports was unclear. Mammary mass depth : width ratio obtained by B-mode ultrasonography resulted in 56.3% sensitivity and 92.9% specificity in predicting malignancy. Similarly, Doppler mode ultrasonography reported 65% sensitivity and 100% specificity in predicting malignancy. Acoustic radiation force impulse elastography was considered a promising technique in determining tumor malignancy. On the other hand, contrast-enhanced ultrasonography enables the evaluation of capillarization evaluation but not malignancy differentiation.[2,4–6]

A recent study involving 153 female dogs and 300 mammary masses evaluated and compared the efficacy of the ultrasound methods currently available, B-mode, Doppler, contrast enhanced ultrasonography, and elastography–acoustic radiation force impulse in predicting tumor malignancy. B-mode measurements showed 67.9% sensitivity and 67.6% specificity; Doppler, 79.2% sensitivity and 70.8% specificity; contrast enhancement ultrasonography, 80.2% sensitivity and 16.7% specificity; and acoustic radiation force impulse shear wave velocities, 94.7% sensitivity and 97.2% specificity.[7]

To date, no study has attempted to correlate ultrasound examination and the histopathological type and/or grade of canine mammary carcinoma. Due to the importance of these diagnostic approaches in improving the prognosis and treatment of female dogs affected by mammary tumors, it was hypothesized that B-mode, Doppler, contrast enhancement ultrasonography, and elastography–acoustic radiation force impulse ultrasound evaluation can assist in noninvasive presurgical and identification of high-grade canine mammary carcinomas and, thus, provide immediate results for a more specific

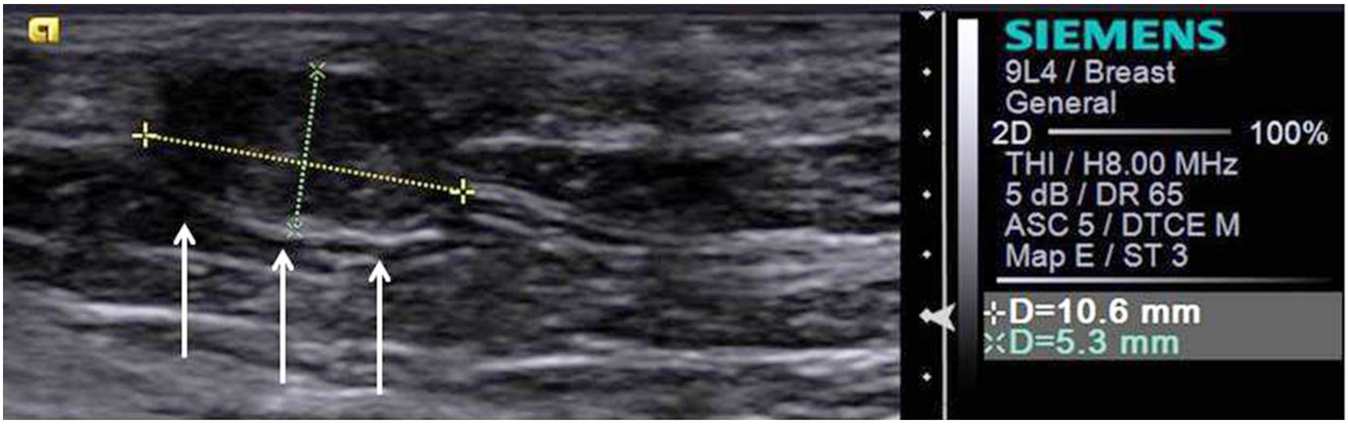

FIGURE 1 B-mode ultrasonography image of malignant adenomyoepithelioma (special mammary carcinoma) in a female dog (arrows). Longitudinal section of the mammary lesions for length and width measurements [Color figure can be viewed at wileyonlinelibrary.com]

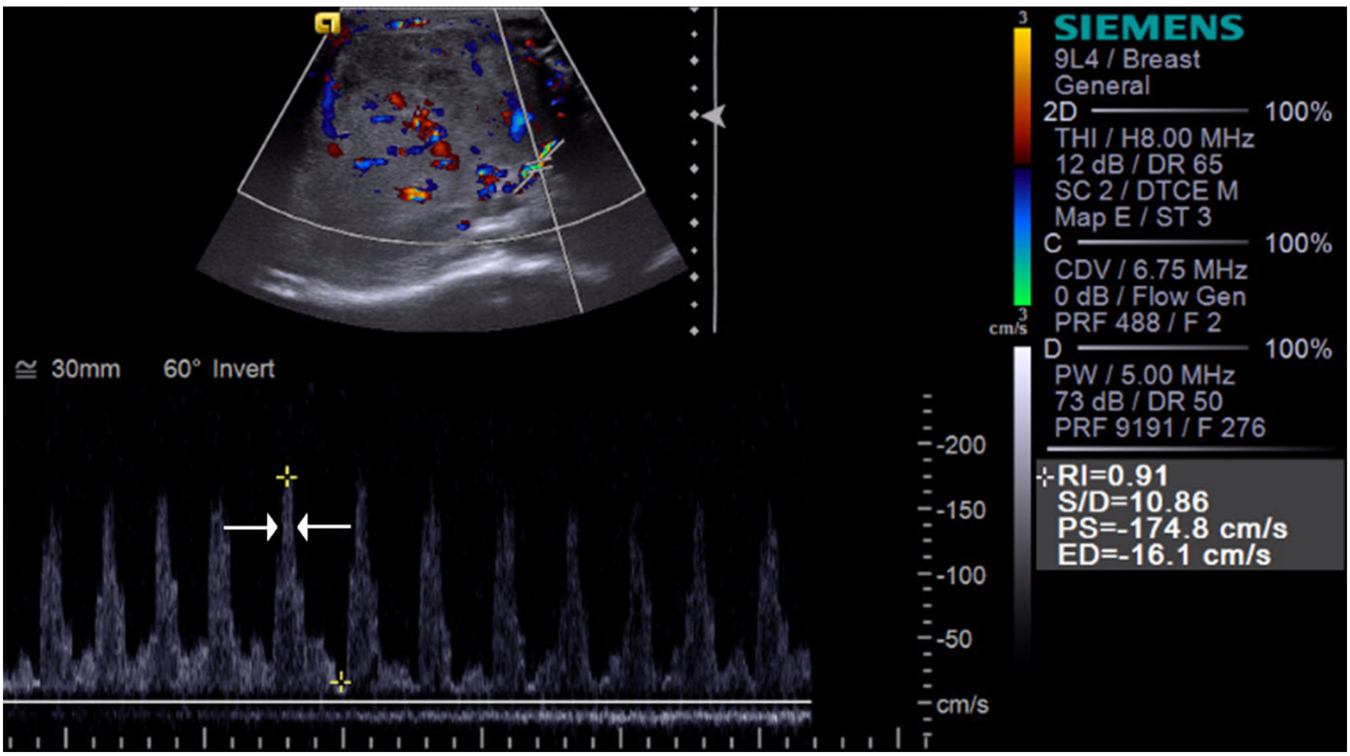

FIGURE 2 Doppler ultrasonography image of mammary carcinoma (grade I) in a mixed tumor in a female dog. Spectral Doppler ultrasonography image of mass with tumoral neovascularization and waveforms (broken arrow) blood flow of malignant lesion [Color figure can be viewed at wileyonlinelibrary.com]

clinical–surgical intervention. Thus, the aim of this study was to evaluate and compare the diagnostic accuracy of these ultrasound methods in the identification of mammary carcinoma types with a high degree of malignancy in female dogs with naturally occurring mammary neoplasms.

2 | METHODS

This study was a prospective secondary observational cohort design and was in keeping with the guidelines set out by the Control of Animal Experimentation. The primary study was conducted during the period of 2014–2016 and approved by the Ethics Committee in the Use of Animals of the Universidade Estadual Paulista, Jaboticabal-São Paulo, Brazil (protocol 023705/12). Free and informed consent was obtained from the owners prior to the animals being included in the primary study. For this secondary study, the data from physical and ultrasound examinations prior to mastectomy and histopathological classification of the canine mammary masses diagnosed as carcinomas in the primary study were retrieved and reanalyzed.[7] A total of 246 mammary carcinomas were analyzed. The tumor clinical characteristics evaluated were as follows: location, growth time, palpation consistency, and surface area.

As part of the inclusion criteria for this secondary study, ultrasonography for each included dog had been carried out by a single experienced sonographer (15 years), using virtual touch system, contrast agent technology, and a linear array 4–18 MHz transducer (Acuson S2000TM, eSie TouchTM, and CadenceTM; Siemens, Munich, Germany) according to the methodology previously described.[7] The variables analyzed by B-mode ultrasonography were echotexture (homogenous or heterogeneous); contours/margins (defined or undefined); invasiveness (present or absent); length (cm), width (cm), and width:length ratio in longitudinal sections; and height (cm), width (cm), and width:height ratio in transverse sections (Figure 1). The variables analyzed by Doppler mode ultrasonography were vascular visualization by color flow (present or absent), systolic velocity (cm/s), diastolic velocity (cm/s), and resistive index (Figure 2). The variables analyzed by contrast enhancement ultrasonography were contrast wash-in time (s), time to peak enhancement (s), and washout time (s) (Figure 3); whole those by acoustic radiation force impulse elastography were qualitative deformability (deformable or not deformable) and mass mean shear wave velocity (m/s) (Figure 4).

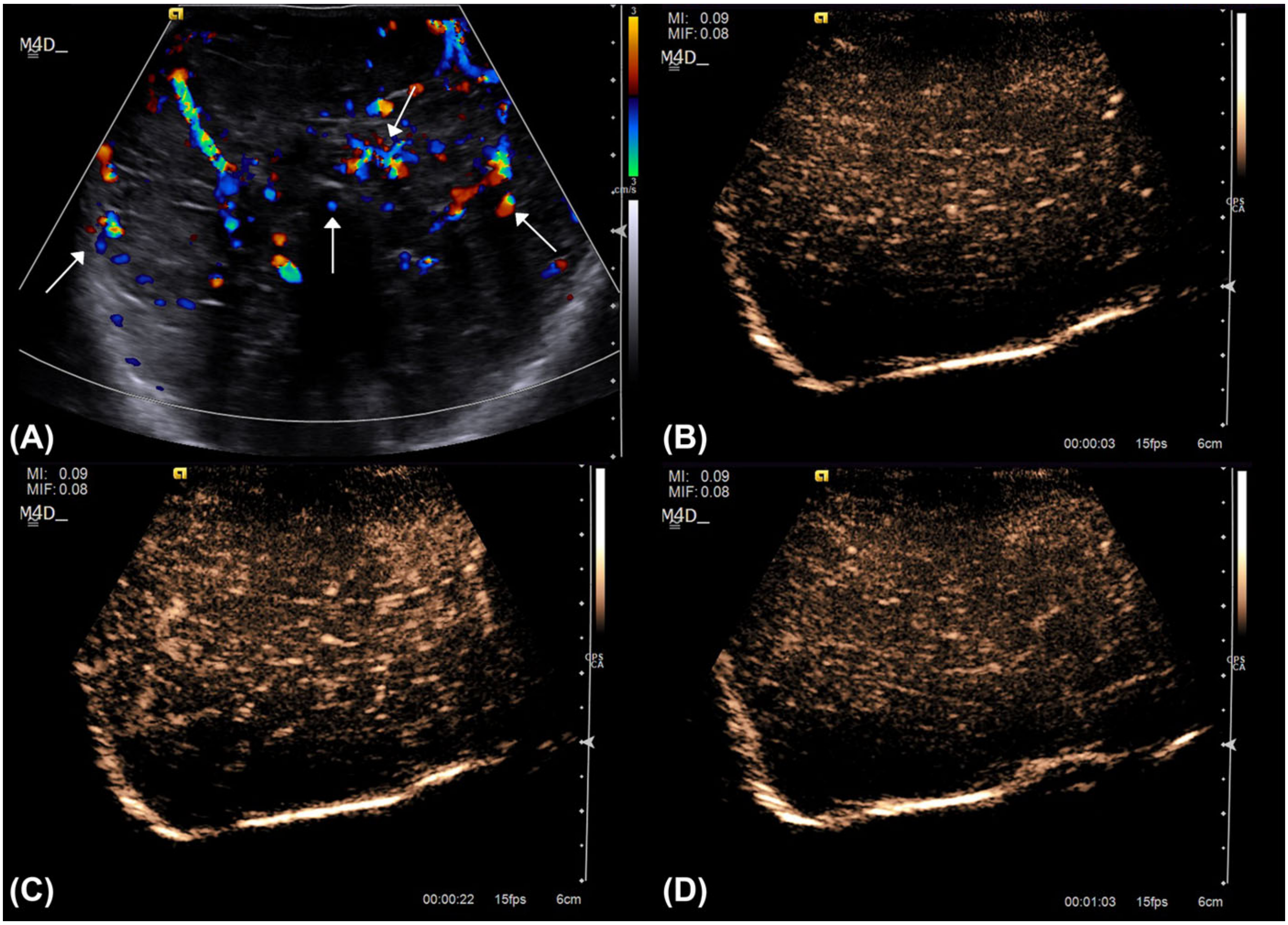

FIGURE 3 Contrast-enhanced ultrasonography image of spindle cell carcinoma in a female dog. A, Neovascularization (arrows) in mammary tumor by color Doppler ultrasonography. Contrast-enhanced ultrasonography: B, absence of contrast in tumor, C, peak enhancement, and D, washout phase [Color figure can be viewed at wileyonlinelibrary.com]

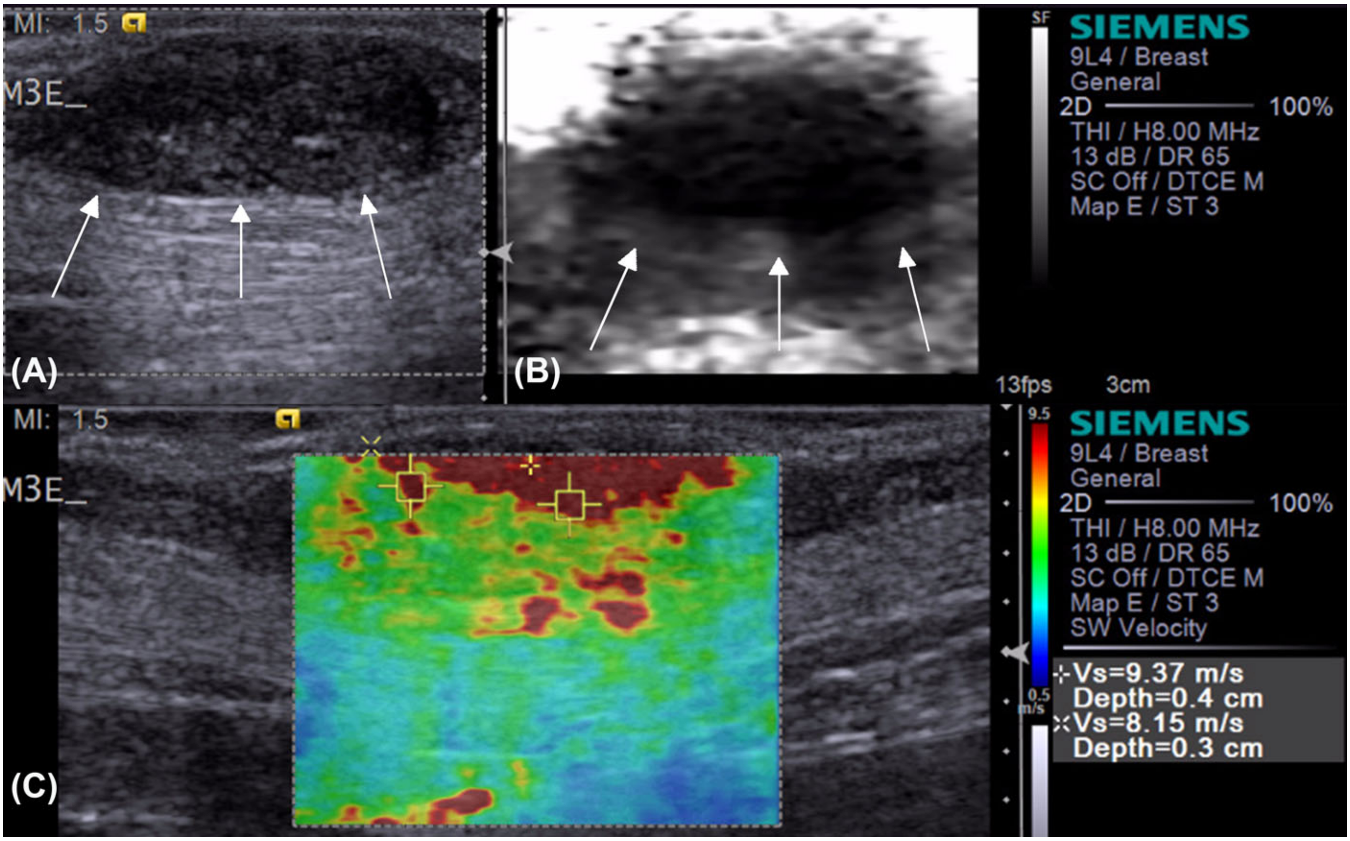

FIGURE 4 Acoustic radiation force impulse elastography image showing stiffness characteristics in malignant adenomyoepithelioma in a female dog. Image of the qualitative acoustic radiation force impulse: A, B-mode ultrasonography of mammary tumor (arrows) and B, elastogram revealing the characteristics of the stiffness of the lesion: not deformable and homogeneous with hard areas (black). C, Image of quantitative acoustic radiation force impulse in mammary tumor showing the presence of calipers in the parenchyma to record shear velocity [Color figure can be viewed at wileyonlinelibrary.com]

Histopathological diagnosis and classification for each of the carcinomas were based on interpretations by a single experienced pathologist (25 years) following the methodology previously described.[7] Carcinomas were classified into simple, complex, or special types and grade I, II, or III according to literature.[7–9]

Statistical tests were selected and performed by one author (R.A.R.U). Qualitative ultrasound variables were compared between carcinoma types and grades using Fisher's exact test. Quantitative variables were analyzed by analysis of variance once normal distribution of model residues and homoscedasticity of variances were confirmed by the Shapiro–Wilk and Bartlett tests, respectively. Cutoff point, sensitivity, specificity, accuracy, and area under curve were calculated for significantly ($P < 0.05$) different ultrasonography parameters, using histopathological classification as a reference for receiver-operating characteristic curve analysis in a logistic regression model. Statistical analysis was performed using R software version 3.3.0 (R^{TM} Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

Of the 246 masses diagnosed as carcinomas (Table 1), 102 (42%) were classified as simple carcinomas, 136 (55%) as complex carcinomas, and eight (3%) as special carcinomas. A total of 96 masses were classified as grade I (39%), 105 as grade II (43%), and 45 as grade III (18%) carcinomas.

The right mammary chain was affected in 114 cases (46%) and the left in 132 cases (54%); with seven (3%) carcinomas were located in the first, 38 (14%) in the second, 54 (22%) in the third, 72 (30%) in the fourth, and 75 (31%) in the fifth mammary glands. Owners reported that 204 (83%) neoplasms grew rapidly (in less than 6 months) and 42 (17%) grew slowly (in more than 6 months). Clinical examination revealed firm and soft consistency in 234 (95%) and 12 (5%) of tumors, respectively; with regular surface being observed in 157 tumors (64%) and irregular in 89 (36%).

The results obtained for the qualitative variables evaluated are summarized in Tables 2 and 3. The deformability evaluated by acoustic radiation force impulse–elastography was significantly different ($P = 0.0142$) between carcinoma types, with special carcinomas being proportionally less deformable than the rest. Thus, nondeformable acoustic radiation force impulse–elastography findings indicate, with 12% sensitivity, 100% specificity, and 75% accuracy, that mammary neoplasm to be a special type carcinoma. All other qualitative parameters did not vary between carcinoma types or grades ($P > 0.05$).

The quantitative results of B-mode, Doppler, contrast enhancement ultrasonography, and acoustic radiation force impulse–elastography

TABLE 1 Histopathological classifications and grades of the canine mammary carcinomas evaluated[8]

Carcinoma type	Diagnosis	Grade	Number
Simple	Tubular carcinoma	I	11
		II	17
		III	2
	Papillary carcinoma	I	5
		II	16
		III	6
	Solid carcinoma	I	3
		II	6
		III	9
	Lobular carcinoma in situ	II	8
	Lobular carcinoma in situ with microinvasion	II	3
	Micropapillary carcinoma	III	5
	Ductal carcinoma in situ	II	3
	Inflammatory carcinoma	II	3
	Squamous cell carcinoma	III	2
	Secretory carcinoma	II	2
	Anaplastic carcinoma	II	1
Complex	Carcinoma in a mixed tumor	I	77
		II	46
		III	6
	Complex carcinoma	III	7
Special	Malignant adenomyoepithelioma	III	6
	Spindle cell carcinoma	III	2

evaluation, in which carcinomas are classified by type and grade, are summarized in Tables 4 and 5. Only width:length ratio measured by B-mode ultrasonography and perfusion times by contrast enhancement ultrasonography were significantly ($P < 0.05$) different between carcinoma types or grades.

The width:length ratio was significantly higher ($P = 0.012$) in special type carcinomas, resulting in an identification cohort value greater than 0.53, with 80% sensitivity, 76% specificity, 78% accuracy, and 80% area under the curve.

Contrast wash-in and peak enhancement times were significantly higher ($P = 0.0205$ and 0.0361, respectively) in simple and special type carcinomas. Therefore, cohort values lower than 7.5 s for wash-in times and lower than 13.5 s to peak enhancement were indicative of complex type carcinoma; with mean sensitivity of 62%, specificity of 60%, accuracy of 53%, and area under the curve of 62%. Similarly, contrast wash-in, peak enhancement, and washout times were significantly lower ($P = 0.0065$, 0.0109, and 0.0242; respectively) in the grade I carcinomas. Thus, cohort values greater than 6.5 s for wash-in, 12.5 s for peak, and 64.5 s for washout times were indicatives of grades II and III carcinoma; with mean sensitivity of 68%, specificity of 62%, accuracy of 63%, and area under the curve of 66%.

4 | DISCUSSION

The mammary ultrasound exam by B-mode ultrasonography, contrast enhancement ultrasonography, and acoustic radiation force impulse–elastography enabled the identification of high-grade canine mammary carcinoma types with limited accuracy. Thus, these ultrasound techniques may assist in the presurgical identification of special and complex type carcinomas and grade II and III carcinomas with moderate efficacy.

Echotexture, margin, and invasiveness evaluation by B-mode ultrasonography did not allow differentiation of canine mammary carcinomas, probably due to the high variability in the morphostructural characteristics of each tumor type and consequently limiting the applicability of this method, in agreement with studies in women with breast carcinomas.[11,12]

The large size of mammary masses has been described as a poor prognosis factor in female dogs, especially when associated with regional abnormalities of the lymphoid tissue, rapid growth, and/or ulceration.[3,10,13,14] In literature, the use of B-mode ultrasonography to analyze 30 malignant, eight atypical, and 11 benign mammary lesions in female dogs concluded that the larger masses were correlated to high-grade malignancy and worse prognosis.[15] However, in the present study, only width:length ratio greater than 0.53 obtained by B-mode ultrasonography enabled the identification of special type carcinomas with acceptable accuracy. These findings are in agreement with previous reports, which have identified this ratio to be an indicator of malignancy in mammary tumors in women and female dogs.[6,7,16] Thus, this parameter can be used as a simple and economical tool in the diagnosis of mammary neoplasms in female dogs.

Color and spectral Doppler variables in this study did not provide any data that could be used in the identification of high-grade mammary carcinomas. All the tumors evaluated showed signs of neovascularization and high vascular indexes, both of which are reported indicators of malignancy.[2,7,17] Although it was expected that the high-grade carcinomas would induce greater vascular development to ensure their growth, these changes were neither detected by Doppler ultrasonography nor were the tissue alterations that occurred within these tumors, such as central necrosis, which enables aggressive behavior without the need for an exacerbated blood supply.[11,18]

Contrast-enhanced ultrasonography, which enables the noninvasive evaluation of tissue perfusion, identified complex carcinomas with moderate accuracy, due to the shorter periods of contrast wash-in and peak enhancement times observed in this type of tumor. These findings corroborated previous reports, which correlated lower perfusion times with more severe types of breast tumors in women.[19,20] On the other hand, an increase in perfusion times (wash-in, peak enhancement, and washout times) enabled the detection of grade II and III carcinomas, with moderate accuracy. These results contradicted reports on breast cancer in women.[19,20] However, it is possible that tumors with complex behaviors expand differently in the female dog due to the anatomical characteristics and larger mammary glands when compared to women.[8,21] Furthermore, this different configuration could possibly lead to the slower perfusion observed in high-grade carcinomas.

TABLE 2 Qualitative parameters of canine mammary carcinomas according to B-mode and Doppler ultrasonography, and acoustic radiation force impulse elastography

Parameter	Findings	Carcinoma type: Simple ($n = 102$)	Complex ($n = 136$)	Special ($n = 8$)	*P*-value
B-Mode ultrasonography					
Echotexture	Homogenous (%)	30	32	13	0.6002
	Heterogeneous (%)	70	68	88	
Contours or margins	Defined (%)	98	100	87	0.1950
	Undefined (%)	2	0	13	
Invasiveness	Present (%)	0	0	0	1.000
	Absent (%)	100	100	100	
Doppler ultrasonography					
Vascularization	Present (%)	100	100	100	1.000
	Absent (%)	0	0	0	
ARFI elastography					
Deformability	Deformable (%)	18	31	0	0.0142*
	Not deformable (%)	82	69	100	

Notes. ARFI, acoustic radiation force impulse.
*Significantly different by the Fisher exact test ($P < 0.05$).

TABLE 3 Qualitative parameters of canine mammary carcinomas according to B-mode and Doppler ultrasonography, and acoustic radiation force impulse elastography

Parameter	Findings	Carcinoma grade: I ($n = 96$)	II ($n = 105$)	III ($n = 45$)	*P*-value
B-Mode ultrasonography					
Echotexture	Homogenous (%)	34	32	20	0.2117
	Heterogeneous (%)	66	68	80	
Contours or margins	Defined (%)	100	100	98	0.1829
	Undefined (%)	0	0	2	
Invasiveness	Present (%)	0	0	0	1.0000
	Absent (%)	100	100	100	
Doppler ultrasonography					
Vascularization	Present (%)	100	100	100	1.0000
	Absent (%)	0	0	0	
ARFI elastography					
Deformability	Deformable (%)	26	29	11	0.0659
	Not deformable (%)	74	71	89	

Notes. ARFI, acoustic radiation force impulse.

The use of acoustic radiation force impulse–elastography enables qualitative (deformable or nondeformable) and quantitative (shear wave velocity) evaluation. The qualitative evaluation in this study revealed that 84% of carcinomas had nondeformable characteristics, in agreement with previous studies that have considered this as malignancy factor.[7] Although 100% of the special carcinomas evaluated were classified as nondeformable at 100% specificity, the sensitivity of 12% does not enable this parameter to be considered a test with diagnostic value in the identification of special carcinomas. Contrary to expectations, quantitative evaluation did not result in significant differences between carcinoma types or grades. Thus, the carcinoma masses analyzed presented high velocity (6.0 ± 2.4 m/s) correlated with low elasticity and rigid consistency, as previously described.[7,22,23] Even though special and complex carcinomas showed greater rigidity in the qualitative and quantitative evaluation, the diagnostic technique is not sensitive enough for differentiation of these tumor types. The fast development of increasingly accurate elastographic techniques may enable this differentiation in the near future.

The current study has some limitations that must be addressed. Although the sample size was adequate (n = 246), the prevalence of special carcinomas was very low (n = 6, 3.3%), which could have compromised the sensitivity of the techniques analyzed in the identification of this type of carcinoma. Mean tumoral size was small ((2.6 ± 1.7) × (1.6 ± 1.2) cm), which may have made ultrasonography

TABLE 4 Mean ± standard deviation of quantitative variables of canine mammary carcinomas according to B-mode and Doppler ultrasonography, contrast enhanced ultrasonography, and acoustic radiation force impulse elastography

Variables	Carcinoma type: Simple ($n = 102$)	Complex ($n = 136$)	Special ($n = 8$)	*P*-value
B-Mode ultrasonography				
Longitudinal width (cm)	2.52 ± 1.60	2.41 ± 1.64	3.58 ± 2.19	0.3790
Longitudinal length (cm)	1.12 ± 0,94	1.19 ± 1.14	2.09 ± 1.35	0.2892
Width:length ratio	0.43 ± 0.14[a]	0.47 ± 0.15[a]	0.56 ± 0.07[b]	0.0117*
Transverse height (cm)	1.82 ± 1.30	2.04 ± 1.67	3.17 ± 1.65	0.1522
Transverse width (cm)	1.42 ± 1.20	1.41 ± 1.25	2.06 ± 1.30	0.4890
Width:height ratio	0.48 ± 0.28	0.90 ± 0.81	0.60 ± 0.15	0.9034
Volume (cm^3)	10.23 ± 24.46	14.40 ± 38.99	28.70 ± 26.40	0.3750
Doppler ultrasonography				
Systolic velocity (cm/s)	38.02 ± 26.30	34.45 ± 24.44	68.30 ± 45.30	0.2259
Diastolic velocity (cm/s)	8.78 ± 6.01	7.67 ± 6.00	24.90 ± 30.80	0.1088
Resistive index	0.75 ± 0.10	0.77 ± 0.10	0.74 ± 0.19	0.3903
Contrast-enhanced ultrasonography				
Wash-in time (s)	9.64 ± 4.79[a]	8.07 ± 4.99[b]	11.88 ± 5.08[a]	0.0205*
Time to peak (s)	15.25 ± 5.69[a]	13.79 ± 5.60[b]	18,25 ± 5.01[a]	0.0361*
Wash-out time (s)	62.42 ± 21.88	64.38 ± 22.59	76.25 ± 25.87	0.2739
ARFI elastography				
SWV (m/s)	5.53 ± 2.23	5.88 ± 2.57	6.59 ± 2.46	0.3163

Notes. Different letters (a and b) on the same row indicate significant difference between carcinoma types by the Tukey test ($P < 0.05$). ARFI, acoustic radiation force impulse; CEUS, contrast-enhanced ultrasonography; SD, standard deviation; SWV, shear wave velocity.
*Significant difference by analysis of variance ($P < 0.05$).

TABLE 5 Mean ± standard deviation of quantitative variables of canine mammary carcinomas according to B-mode and Doppler ultrasonography, contrast-enhanced ultrasonography, and acoustic radiation force impulse elastography

Variables	Carcinoma grade: I ($n = 96$)	II ($n = 105$)	III ($n = 45$)	*P*-value
B-Mode ultrasonography				
Longitudinal width (cm)	1.04 ± 0.94	1.17 ± 1.08	1.56 ± 1.25	0.2595
Longitudinal length (cm)	1.04 ± 0.94	1.17 ± 1.08	1.56 ± 1.25	0.1219
Width:length ratio	0.44 ± 0.15	0.45 ± 0.14	0.50 ± 0.15	0.0940
Transverse height (cm)	2.00 ± 1.70	1.83 ± 1.34	2.29 ± 1.57	0.4798
Transverse width (cm)	1.30 ± 1.04	1.44 ± 1.36	1.70 ± 1.29	0.2646
Width:height ratio	0.96 ± 0.92	0.97 ± 0.94	0.95 ± 0.93	0.6242
Volume (cm^3)	9.63 ± 28.78	13.59 ± 37.66	19.47 ± 32.50	0.2475
Doppler ultrasonography				
Systolic velocity (cm/s)	36.45 ± 29.34	33.81 ± 19.20	46.44 ± 33.69	0.2356
Diastolic velocity (cm/s)	7.63 ± 5.89	8.21 ± 6.38	12.53 ± 15.38	0.1163
Resistive index	0.77 ± 0.10	0.76 ± 0.10	0.74 ± 0.12	0.3903
Contrast-enhanced ultrasonography				
Wash-in time (s)	7.50 ± 4.21[a]	10.12 ± 5.34[b]	9.85 ± 4.82[b]	0.0065*
Time to peak (s)	13.13 ± 5.16[a]	15.40 ± 5.95[b]	16.62 ± 5.38[b]	0.0109*
Washout time (s)	61.52 ± 19.62[a]	71.31 ± 22.59[b]	75.00 ± 24.67[b]	0.0242*
ARFI elastography				
SWV (m/s)	6.06 ± 2.49	5.51 ± 2.40	5.69 ± 2.38	0.3497

Notes. Different letters (a and b) on the same row indicate significant difference between carcinoma types by the Tukey test ($P < 0.05$). ARFI, acoustic radiation force impulse; CEUS, contrast-enhanced ultrasonography; SD, standard deviation, SWV, shear wave velocity.
*Significant difference by Analysis of variance ($P < 0.05$).

evaluation difficult. Lastly, contrast enhancement ultrasonography and acoustic radiation force impulse elastography techniques are currently only available at research centers and university hospitals, temporarily limiting the clinical application of these exams.

In conclusion, mammary ultrasonography evaluation by B-mode, contrast enhancement ultrasonography, and acoustic radiation force impulse–elastography enabled the grade and some characteristics of high-grade mammary carcinomas in this sample of female dogs to be identified with limited accuracy. A width:length ratio greater than 0.53 enabled the identification of special type carcinomas with 80% sensitivity and 76% specificity. Contrast wash-in and peak enhancement times lower than 7.5 and 13.5 s, respectively, were indicatives of a complex type carcinoma with 62% sensitivity and 60% specificity. Contrast wash-in, peak enhancement, and washout times greater than 6.5, 12.5, and 64.5 s, respectively; were indicatives of grade II and III carcinoma with 68% sensitivity and 62% specificity. This study provides relevant information that may contribute to the research and clinical management of oncological canine patients.

LIST OF AUTHOR CONTRIBUTIONS

Category 1

- **(a)** Conception and Design: Feliciano MAR, Ramirez RAU, Carvalho CF
- **(b)** Acquisition of Data: Feliciano MAR, Ramirez RAU, Maronezi MC, Maciel GS, Avante ML, Senhorello ILS, Mucédola T, Gasser B, Carvalho CF, Vicente WRR
- **(c)** Analysis and Interpretation of Data: Feliciano MAR, Ramirez RAU

Category 2

- **(a)** Drafting the Article: Feliciano MAR, Ramirez RAU
- **(b)** Revising Article for Intellectual Content: Feliciano MAR, Ramirez RAU, Maronezi MC, Maciel GS, Avante ML, Senhorello ILS, Mucédola T, Gasser B, Carvalho CF, Vicente WRR

Category 3

- **(a)** Final Approval of the Completed Article: Feliciano MAR, Ramirez RAU, Maronezi MC, Maciel GS, Avante ML, Senhorello ILS, Mucédola T, Gasser B, Carvalho CF, Vicente WRR

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ARTIGO 5

Ultrasonography for lymph nodes metastasis identification in bitches with mammary neoplasms

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Ultrasonography for lymph nodes metastasis identification in bitches with mammary neoplasms

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The aim of this study was to evaluate and compare the diagnostic accuracy of B-mode, Doppler ultrasonography and Acoustic Radiation Force Impulse (ARFI) elastography in the identification of axillary and inguinal lymph nodes metastasis in bitches with mammary neoplasms. The axillary (n = 96) and inguinal (n = 100) lymph nodes of 100 bitches were evaluated using B-Mode, Colour Doppler and ARFI-elastography. After this evaluation, mastectomy and lymph nodes excision were performed and these structures were histologically classified as free, reactive or metastatic. Ultrasonographic parameters were compared by Chi-Square or ANOVA tests and if they are significant, discriminative power analysis according to histopathological classification was performed (ROC analysis). The ARFI-elastography shear wave velocity (SWV) enabled metastasis identification in inguinal (sensitivity 95% specificity 87%) and axillary lymph nodes (sensitivity 100% specificity 94%). While B-Mode ultrasound Short/Long axis ratio evaluation of inguinal and axillary lymph nodes only resulted in a sensitivity around of 71% and specificity of 55%. In conclusion, B-Mode ultrasonography may contribute to diagnosis of metastasis in axillary and inguinal lymph nodes of bitches affected by mammary neoplasm with limited accuracy, while SWV evaluation proved to be an excellent diagnosis tool, which allows differentiation between free, reactive and tumour metastatic lymph nodes.

The mammary neoplasms in bitches and women exhibit similar biological behavior[1–3], its metastatic capacity can reach 50% and it is the main cause of mortality[4–6]. Metastasis occur mainly to axillary and inguinal lymph nodes in bitches[7] and to axillary in women[8], being an unfavourable prognosis factor for any of these patients[9,10]. Thus, the clinical evaluation of these structures becomes essential for adequate tumour staging, selection of therapeutic management, planning surgical margins and issuing a precise prognosis[10,11]. Clinical, radiologic, ultrasonographic and cytological examination may aid in the presumption of lymph nodes metastases, although they are not accurate in detection of micro metastasis and neoplastic cells clusters[12].

Therefore, definitive diagnosis of lymph nodes metastasis is based on histopathological examination, which requires surgical excision[13] a procedure with highly complications risk, mainly due to proximity of large vessels and nervous plexus to the axillary lymph node. These risks limit this technique application, even though it is advocated by the "Consensus for the diagnosis, prognosis and treatment of canine mammary neoplasms" as essential for patient diagnosis and prognosis[10,11,14]. The clinical and ultrasonographic examination of the regional lymph nodes are useful in the attempt to identify metastasis in a non-invasive and risk-free way. However, the few available analyses are considered limited because they use features such as increased size, central distribution of vascular flow and elevation of vascular indices as suggestive of metastasis without indicating the sensitivity and specificity, which is necessary to imply these techniques as diagnostic methods[13,15–17].

With the advancement of ultrasonography, elastography has emerged as a method that allows a non-invasive evaluation of tissue elasticity and has become an efficient tool for identification of malignant mammary lesions

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Mammary gland (site)	Number of neoplasms
1st Cranial thoracic	15
2nd Cranial thoracic	29
3rd Cranial abdominal	81
4th Caudal Abdominal	85
5th Inguinal	32
Total neoplasms	242

Table 1. Mammary glands bearing neoplasms.

in humans and animals[18,19]. Recently, clinical studies have evaluated the accuracy of this technique to identify metastasis in axillary lymph nodes of women with breast cancer and presented extremely promising results[20–22]. However, elastography studies were not found in canines.

Considering the requirement of non-invasive, accurate and risk-free identification of regional lymph nodes metastasis in bitches added to the promising results found by previous elastography studies in women, this study aims to evaluate and compare the accuracy of B-mode, Doppler and ARFI-elastography techniques for diagnosis of axillary and inguinal lymph nodes metastasis in bitches with mammary neoplasms.

Results

A total of 100 bitches with 242 breast lesions (44 different neoplastic types) were included in this study. The Table 1 presents the site of the mammary glands bearing the neoplasms. Of these patients, 100 inguinal and 96 axillary lymph nodes were surgically collected and histologically analysed (four axillary lymph nodes were not collected due to impossibility of surgical identification). In this study, only one axillary lymph node was identified in the 96 bitches studied (all animals), two inguinal lymph nodes in 6 bitches and one inguinal lymph node in the remaining 94 bitches. The histopathological results of multiple inguinal lymph nodes were same in all 6 cases.

From 100 inguinal lymph nodes, 30% (30) were histopathological classified as free, 51% (51) as reactive, and 19% (19) as metastatic. Out of the 96 axillary lymph nodes, 35% (33) were classified as free, 56% (54) as reactive, and 9% (9) as metastatic. Excision of the axillary lymph nodes did not result in major postoperative complications, only 5 large animals presented transient subcutaneous oedema (seroma), probably due to the deeper location of this structure that warrants greater manipulation than in small and medium-sized animals.

Ultrasound evaluation was performed without difficulties, intercurrences or side effects. The qualitative and quantitative findings of inguinal lymph nodes evaluation are summarized in the Table 2, and those of the axillary lymph nodes in the Table 3.

The B-Mode and Doppler ultrasound parameters were similar ($P > 0.05$) between the histopathological classification (Figs 1 and 2).

At the ARFI-elastography, the qualitative evaluation revealed that lymph nodes with metastasis presented less deformability ($P < 0.01$) than normal lymph nodes. In the quantitative elastographic evaluation, SWV was greater ($P < 0.01$) in lymph nodes with metastasis than in reactive, and in these greater ($P < 0.01$) than in normal (Fig. 3).

The results of the discriminative power analysis for identification of inguinal and axillary lymph nodes metastatic, reactive or altered (metastatic + reactive) are shown in the Table 4, and the graphical representation of the comparative study of the ROC analysis, which allowed the identification of the lymph nodes SWV as the most applicable variable for distinguish between metastatic, reactive or altered ($P < 0.01$) is presented in Fig. 4.

Discussion

B-mode ultrasound Short/Long axis ratio evaluation of axillary and inguinal lymph nodes in bitches with mammary neoplasms allowed identifying metastasis with moderate accuracy. The evaluation of the SWV by ARFI-elastography proved to be a technique with exceptional accuracy in these structures, while the vascular evaluation by Colour Doppler did not present any parameter that would allow determining metastasis in these organs.

The SWV was significantly higher in metastatic and reactive tissues compared to free tissues, with a general discriminative accuracy to identify altered lymphatic tissues around 90%, proving to be a suitable, accurate and non-invasive method for both altered differentiation and malignancy diagnosis in axillary and inguinal lymph nodes of bitches with mammary neoplasms. This increase in metastatic lymph nodes stiffness corroborates the results reported in women by Tamaki *et al.*[21], who described a SWV cohort value >1.44 m/s, with sensitivity of 83% and specificity of 70% for metastasis detection in axillary lymph nodes. Our results indicate a great SWV cohort value >2.5 m/s, with great sensitivity of 95% and specificity of 87%, for axillary lymph nodes metastasis identification.

The findings obtained in the qualitative ARFI- elastography evaluation were similar to those described by Choi *et al.*[23] in women metastatic lymph nodes, who described greater rigidity in these structures (areas with reddish tones) than in reactive ones (areas with greenish tones) and free ones (areas with bluish tones). Our results also corroborate with Seiler *et al.*[24], that observed that malignant tissues present higher scores of tissue stiffness through compression elastography and computational analysis of the images in different lymph nodes of 51 canine and feline patients. Regarding the qualitative evaluation of deformation and homogeneity, no differences were observed among the tissues studied, since these may exhibit similar parenchymal variations[10].

Parameter		Free	Reactive	Metastatic	P-value
B-Mode ultrasonography					
Shape	Elongated	80% (24)	31% (16)	22% (4)	0,6104
	Rounded	20% (6)	69% (35)	78% (15)	
Echotexture	Homogeneous	97% (29)	91% (46)	67% (13)	0,5017
	Heterogeneous	3% (1)	9% (5)	33% (6)	
	Mixed	0% (0)	0% (0)	0% (0)	
Echogenicity	Hypoechoic	97% (29)	98% (50)	89% (17)	0,2865
	Hyperechoic	3% (1)	2% (1)	0% (0)	
	Mixed	0% (0)	0% (0)	11% (2)	
Short/Long axis ratio		0,38 ± 0,10	0,37 ± 0,11	0,34 ± 0,10	0,4286
Doppler ultrasonography					
Vascularization	Present	50% (15)	58% (30)	60% (11)	0,1307
	Absent	50% (15)	42% (21)	40% (8)	
Localization	Peripheral	100% (30)	98% (50)	78% (15)	0,1537
	Central	0% (0)	2% (1)	22% (4)	
ARFI-Elastography					
Deformability	Hard	10% (3)[a]	15% (8)[a]	67% (13)[b]	0,0004*
	Soft	90% (27)	85% (43)	33% (6)	
Pattern	Homogeneous	97% (29)[a]	96% (49)[a]	44% (8)[b]	0,0009*
	Heterogeneous	3% (1)	4% (2)	56% (11)	
SWV (m/s)		1,91 ± 0,44[a]	2,29 ± 0,19[b]	2,99 ± 0,64[c]	0,0001*
Depth (cm)		1,05 ± 0,40	1,00 ± 0,38	1,10 ± 0,30	0,6075

Table 2. Qualitative variables in percentage of cases; and Mean ± SD of quantitative variables evaluated by different ultrasonography methods (B-mode, Doppler and ARFI-elastography) in inguinal lymph nodes of bitches with mammary neoplasms. cm: centimetres; s: seconds; m: meters; SD: standard deviation; *Considered statistically significant, where different letters indicate significance between the histopathological diagnoses ($p < 0.05$).

Parameter		Free	Reactive	Metastatic	P-value
B-Mode ultrasonography					
Shape	Elongated	45% (15)	31% (17)	22% (2)	0,1161
	Rounded	55% (18)	69% (37)	78% (7)	
Echotexture	Homogeneous	82% (27)	91% (49)	67% (6)	0,0949
	Heterogeneous	18% (6)	9% (5)	33% (3)	
	Mixed	0 (0)	0% (0)	0% (0)	
Echogenicity	Hypoechoic	97% (32)	98% (53)	89% (8)	0,1981
	Hyperechoic	3% (1)	2% (1)	0% (0)	
	Mixed	0% (0)	0% (0)	11% (1)	
Short/Long axis ratio		0,47 ± 0,13	0,49 ± 0,12	0,53 ± 0,16	0,4724
Doppler ultrasonography					
Vascularization	Present	10% (3)	28% (15)	22% (2)	0,3154
	Absent	90% (30)	72% (39)	78% (7)	
Localization	Peripheral	91% (30)	98% (53)	78% (7)	0,3858
	Central	9% (3)	2% (1)	22% (2)	
ARFI-Elastography					
Deformability	Hard	6% (2)[a]	15% (8)[b]	67% (6)[b]	0,0005*
	Soft	94% (31)	85% (46)	33% (3)	
Pattern (%)	Homogeneous	100% (33)[a]	96% (52)[a]	44% (4)[b]	0,0001*
	Heterogeneous	0% (0)	4% (2)	56% (5)	
SWV (m/s)		1,87 ± 0,27[a]	2,30 ± 0,35[b]	3,02 ± 0,50[c]	0,0001*
Depth (cm)		1,19 ± 0,36	1,34 ± 0,39	1,37 ± 0,52	0,1989

Table 3. Qualitative variables in percentage of cases; and Mean ± SD of quantitative variables evaluated by different ultrasonography methods (B-mode, Doppler and ARFI-elastography) in axillary lymph nodes of bitches with mammary lesions. cm: centimetres; s: seconds; m: meters; SD: standard deviation; *Considered statistically significant, where different letters indicate significance between the histopathological diagnoses ($p < 0.05$).

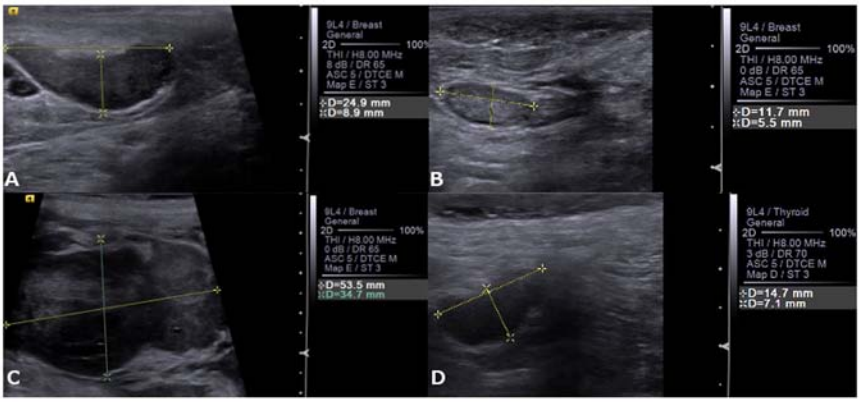

Figure 1. B-Mode ultrasound images of a canine loco regional lymph nodes. (**A**) inguinal lymph node with metastasis note the elongated shape. (**B**) normal inguinal lymph node. (**C**) axillary lymph node with metastasis note the rounded shape. (**D**) normal axillary lymph node.

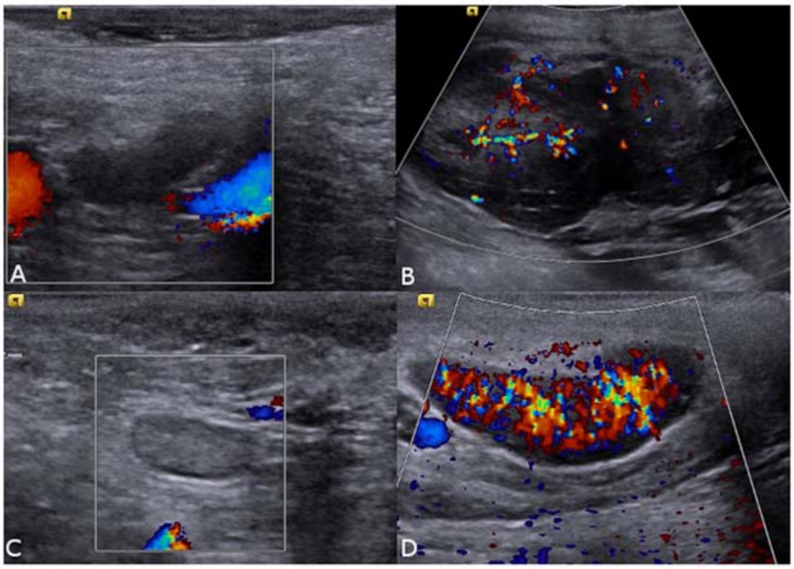

Figure 2. Colour Doppler ultrasound images of canine loco regional lymph nodes. (**A**) Normal axillary lymph node with absence of vascularization. (**B**) Axillary lymph node with metastasis and presence of neovascularization, (**C**) normal inguinal lymph node with absence of vascularization and (**D**) inguinal lymph node with metastasis and presence of neovascularization.

The qualitative and quantitative increase in tissue stiffness observed in reactive and metastatic lymph nodes is probably due to: abnormal cell proliferation, differentiation in areas with micro calcification and abnormal tissues deposition in the malignant tissue stroma[17,25].

It is important to emphasize that results obtained with ARFI-elastography exam of sentinel lymph nodes can guide the adequate therapeutic management, tumour staging and prognosis formulation in patients affected by mammary neoplasms. It helps to determine the necessity of the axillary lymph node excision and, consequently, the extension of the surgical procedure, which may limit postoperative complications, common in this procedure[21,26]. Thus, it is expected that clinical application of this technique may not only reduce complications rates by saving the patient from unnecessary trauma when the lymph node is not affected, but also to adjust therapy and prognosis in patients with metastasis[21].

On the other hand, the B-mode evaluation allowed determination that Short/Long axis ratio of inguinal and axillary lymph nodes were decreased and increased respectively when these tissues had metastasis, with a general discriminative accuracy around 60% for identification of altered lymph nodes. This result correlates with Choi *et al.*[27] and Muramoto *et al.*[25], that evaluated axillary lymph nodes of women and inguinal lymph nodes of bitches with mammary neoplasms, respectively. According to Chammas *et al.*[28], inflamed lymphoid structures tend to increase their size proportionally in all planes whereas neoplastic lymph nodes grow disproportionately, losing their original shape, like that observed in the present study.

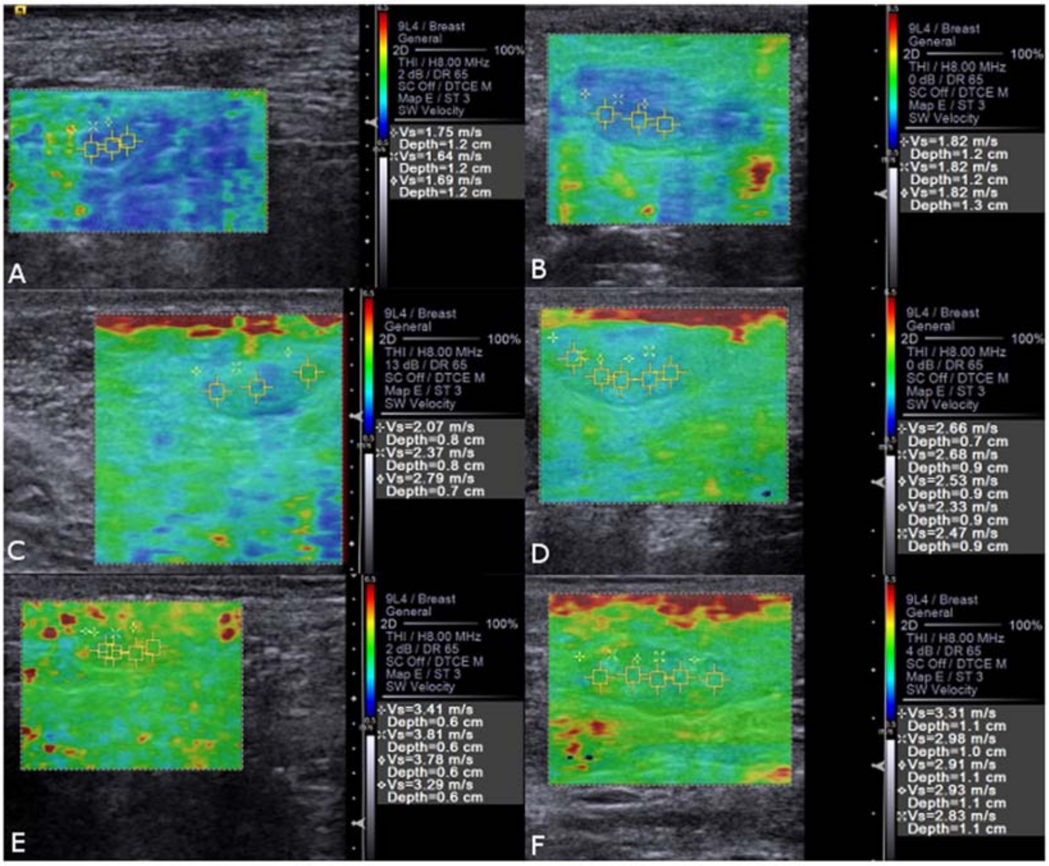

Figure 3. Qualitative and quantitative ARFI-elastography images of (**A**) normal axillary lymph node, (**B**) normal inguinal lymph node with homogeneous pattern and SWV in m/s. (**C**) Reactive axillary lymph node, (**D**) reactive inguinal lymph node with heterogeneous pattern and SWV in m/s. (**E**) Axillary lymph node with metastasis, (**F**) inguinal lymph node with metastasis with heterogeneous pattern and SWV in m/s.

Parameter	Diagnosis	Cut-off point	Sens%	Spec%	Accur%	AUC%
Inguinal lymph nodes						
Short/Long axis ratio	Altered	<0,41	76	47	67	59
	Metastatic	<0,38	63	41	45	56
Vascularization	Altered	Peripheral	22	97	44	59
	Metastatic	Peripheral	26	86	75	55
SWV (m/s)	Reactive	>2,1	92	83	89	82
	Metastatic	>2,5	95	87	90	92
	Altered	>2,1	94	83	90	85
Deformability	Metastatic	Hard	53	83	58	87
Pattern	Metastatic	Heterog	32	94	46	82
Axillary lymph nodes						
Short/Long axis ratio	Altered	>0,45	67	52	62	58
	Metastatic	>0,51	78	62	64	67
Vascularization	Altered	Peripheral	5	90	34	52
	Metastatic	Peripheral	18	95	86	60
SWV (m/s)	Reactive	>2,0	93	81	89	89
	Metastatic	>2,4	100	94	90	99
	Altered	>2,0	94	81	90	91
Deformability	Metastatic	Hard	67	80	86	69
Pattern	Metastatic	Heterog	56	98	94	86

Table 4. Diagnostic performance variables (%) of different ultrasound methods to predict metastasis, reactivity or alterations (metastasis + reactivity) in inguinal and axillary lymph nodes of bitches with mammary neoplasms. CV: Cohort Value; Sens: Sensitivity; Spec: Specificity; Accur: Accuracy; AUC: area under curve; SWV: Share Wave Velocity; cm: centimetres; s: seconds; m: meters; Heterog: Heterogeneous.

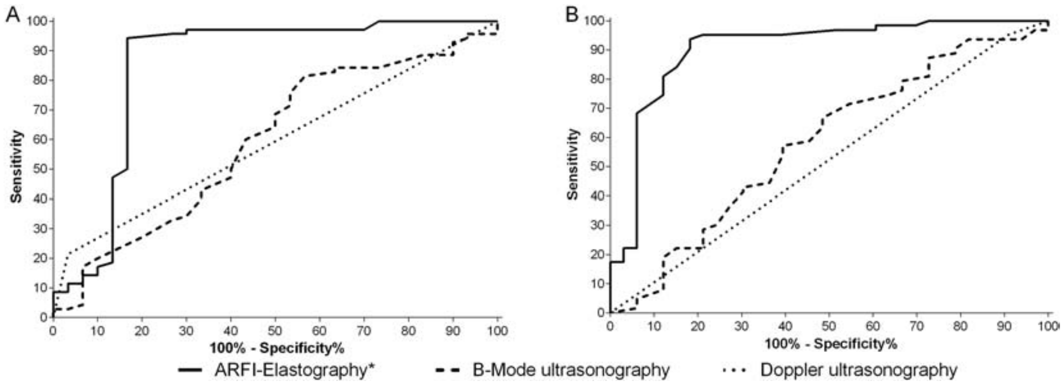

Figure 4. ROC (Receiving Operating Characteristic) curves that compare the predictive sensitivity % and 100% – specificity % of different ultrasound methods for determination of metastasis in (**A**) inguinal and (**B**) axillary lymph nodes of bitches affected by mammary neoplasms using the histopathological classification as reference. * indicate significant difference (P < 0.01).

The lymph nodes short/long axis ratio evaluation demonstrate a moderated discriminative power to identify alterations studied in the regional lymph nodes, according to studies carried out in women by Vassallo *et al.*[29] and bitches by Nyman *et al.*[15] and Muramoto *et al.*[25]. These authors verified that this ratio increases in tissues with metastasis, while Muramoto *et al.*[25] commented that no reference standards can be established for this relation due to the great variability of lymph nodes studied (high standard deviations). These controversial observations highlight the importance of this study regarding the use of modern sonographic techniques trying to improve the accuracy of metastasis diagnosis in sentinel lymph nodes.

The B-mode qualitative parameters (echogenicity and echotexture) did not demonstrate effectiveness to differentiate lymphoid tissue with metastasis nor reactive, corroborating with the previous reports[15,17,25]. The presence of necrotic, liquefaction or haemorrhagic areas, or areas that intersect normal and with tumour alterations tissues, micro calcifications and gross calcifications[13,28] promote a tissue variability in the lymph nodes, which results in visualization of heterogeneous echotexture in all tissues evaluated[17,25]. Regarding echogenicity, variations can be observed in tissues with or without metastasis in women, which establish relation between reduction of echogenicity and increase in cellularity, observed in lesions as basic as hyperplasia[30].

An unexpected result was that the vascularization detection and classification by colour Doppler did not allowed the differentiation of altered lymphoid tissues. This result may be correlated with the fact that variations in the vascularization of neoplastic and non-neoplastic tissues are due to inflammatory factors, observed in sentinel tissues with any inflammatory reaction which promotes detectable fluxes in normal and reactive lymph nodes and even less evident vascularization in ischemic foci derivate from metastatic lymph nodes necrosis[17,25].

In conclusion, from the results of this study, B-mode ultrasonography can contribute to the diagnosis of metastasis in the axillary and inguinal lymph nodes of bitches affected by mammary neoplasms with limited accuracy. In contrast, the ARFI-elastography evaluation of SWV of these lymph nodes proved to be an excellent diagnostic technique, with high accuracy to differentiate reactive, metastatic or altered lymph nodes. It is recommended the inclusion of ARFI-elastography in the evaluation of the regional lymph nodes of oncologic patients because it is a rapid, non-invasive, complication-free, high sensitivity, specificity and accuracy technique capable of assist in the clinical/surgical management of patients affected by neoplasms.

Methods

This study followed the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA). Prospective clinical study, approved by the Ethics Committee in the Use of Animals of the São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil (protocol n° 9.950/16), developed between March 2016 and August 2017. All bitches attend due to the presence of mammary lesions at the Institutional Veterinary Hospital, during this period were included after approval by free and informed consent by their tutors.

Single experienced sonographer (5 years) examined the axillary and inguinal lymph nodes with a 9.0 MHz linear transducer and Acuson S2000® equipment (Siemens, Munich, Germany), by the sonographic methods: B-mode, colour Doppler and ARFI-elastography.

At B-mode, the size (length and height) of the lymph nodes was measurement to calculate the short/long axis ratio, the shape was classified subjectively as elongated when one side was evidently larger than the other or rounded when the sides were apparently similar, the contours as regular or irregular, the echotexture as homogeneous or heterogeneous and the echogenicity classified according to adjacent tissues as hypoechoic, hyperechoic or mixed.

By Colour Doppler was studied the presence of vascularization and classified its localization as peripheral (apparently normal) or central (apparently neovascularization) using these image parameters: wall filter 3 Hz; pulse repetition 1099 Hz; gain 0db; peak hold 1 sec; colour sensitivity 4; persistence 2 level; mechanical index 0.9; and thermal index 1.8. Using a region of interest (ROI) that includes the lymph node parenchyma and at least 30% of adjacent tissue.

The ARFI-elastography was performed using the Virtual Touch Tissue Imaging Quantification (VTIQ) software[18]. This assembles a colour chart map known as elastogram, which assesses the qualitative deformability characteristic, where bluish tones correspond to deformable tissues (soft) and reddish to non-deformable tissues (hard). The elastogram was also classified as homogeneous when the image presented the same tone throughout the lymph node or as heterogeneous when presented different shades. Immediately thereafter, for quantitative evaluation, at least three ROI were selected by placing the 25 mm^2 pre-defined calliper over the elastogram trying to cover the largest area of lymph nodes and include peripheral and central regions. The ultrasound software automatically provides the shear wave velocity (SWV m/s), of each of these ROI and the mean SWV was calculate for each lymph node.

After ultrasound examination, the patients were submitted to unilateral radical mastectomy and ipsilateral axillary and inguinal lymph nodes excision. To confirm that the axillar lymph node extracted was the same one examined, some illegibility criteria were adopted: (1) the ultrasound exam and the excision were performed on the same day; (2) the patent blue injection was made immediately previous to surgical approach to confirm the detection and excision of the sentinel lymph node; and (3) the measurements and morphological aspects obtained in the ultrasound were compared with the dimensions and macroscopic morphological aspects at surgery excision. And in relation to the inguinal lymph node, it is noteworthy that the entire mammary chain and inguinal lymph nodes are removed and sent as a block for histopathological analysis, so if there were more than one inguinal lymph node it would be confirmed by the pathologist. The lymph nodes and mammary tissues were preserved in 10% formalin, routinely processed and examined under a light microscope by a single experienced pathologist (15 years) who determines the neoplasms diagnosis and classifies the lymph nodes as free (absence of histopathological changes), reactive (inflammatory alterations) or metastatic (micro metastasis or neoplastic cells clusters) following the recommendation of Cassali *et al.*[10,21].

Statistical analysis was performed using the software R, version 3.3.0 (R® foundation for statistical computing, Austria). Qualitative variables were compared between histopathological classifications by Chi-square test, quantitative variables by ANOVA test and differences were considered significant when P-value <0.05. For ultrasonography parameters that showed significance, the cut-off point, sensitivity, specificity, accuracy, and area under curve (AUC) were calculated using histopathological classifications as a reference for receiver-operating characteristic curve (ROC) analysis in a logistic regression model aimed at assessing and comparing the diagnostic performance of each technique.

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Author Contributions

P.S., R.A.R.U., W.R.R.V. and M.A.R.F.: Conception and Design; P.S., P.S., M.C.M., B.G., L.P., I.R.H.G.: Acquisition of Data; P.S., R.A.R.U., M.C.M., B.G., L.P., I.R.H.G. and M.A.R.F.: Analysis and Interpretation of Data; P.S., R.A.R.U., B.G., I.R.H.G., M.C.M. and and M.A.R.F.: Drafting the Article; R.A.R.U., W.R.R.V. and M.A.R.F.: All authors Final Approval of the Completed Article.

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ARTIGO 6

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Malignancy prediction of cutaneous and subcutaneous neoplasms in canines using B-mode ultrasonography, Doppler, and ARFI elastography

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Abstract

Background: Cutaneous and subcutaneous neoplasms are highly prevalent in dogs, ranging from benign to highly aggressive and metastatic lesions. The diagnosis is obtained through histopathology, however it is an invasive technique that may take a long time to obtain the result, delaying the beginning of the adequate treatment. Thus, there is a need for non-invasive tests that can help in the early diagnosis of this type of cancer. The aim of this study was to verify the accuracy of B-mode ultrasonography, Doppler, and ARFI elastography to predict malignancy in cutaneous and subcutaneous canine neoplasms. In addition, we aim to propose an ultrasonography evaluation protocol and perform the neoplasms characterization using these three proposed techniques.

Results: Twenty-one types of specific neoplasm were diagnosed, and using B-mode, we verified the association between heterogeneous echotexture, invasiveness, presence of hyperechoic spots, and cavity areas with malignancy. An increased pulsatility was verified in malignant neoplasms using Doppler (cut-off value > 0.93). When using the elastography, malignancy was associated with non-deformable tissues and shear wave velocity > 3.52 m/s. Evaluation protocols were proposed associating 4, 5, 6, or 7 malignancy predictive characteristics, and characterization was done for all tumors with at least two cases.

Conclusions: We concluded that ultrasonography methods are promising and effective in predicting malignancy in these types of tumors, and the association of methods can increase the specificity of the results.

Keywords: Oncology, Ultrasonography, Cancer, Canine

Background

The cutaneous and subcutaneous neoplasms are frequently observed in the canine species, originating from different cell types, and may present different biological behaviors from benign to highly aggressive and metastatic lesions [1, 2]. These lesions, especially the malignant ones, can promote significant alterations and can cause pain, inflammation, infections, hemodynamic changes, and when metastatic can compromise the function of other organs, which can lead to death [3, 4]. Fast and accurate diagnosis is essential to establish adequate therapy favoring the prognosis and survival of patients [5, 6].

The final diagnosis is obtained via histopathologic analysis, whose samples were collected by incisional or

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excisional biopsies. Both biopsy techniques are invasive and require anesthesia for the patients [7]. In many cases, there is a delay in obtaining the results, that may slow down the therapeutic approach, which is essential to increase patient survival [8].

The cytopathological evaluation can be used to provide faster results besides being more cost-efficient. However, cytopathology is an invasive technique that cannot promote the final diagnosis, grade some tumor types, have a considerable rate of inconclusive results, and its diagnostic accuracy is variable [9–11]. When considering the limitations of this type of evaluation, fast and noninvasive techniques are required to aid in early diagnosis and therapeutic management.

In human medicine, the ultrasonography technique has been used since the 1990s in skin cancer studies, and it already has applicability in malignancy prediction and differentiation between some tumor types, such as squamous cells and basal cell carcinomas [12, 13]. In veterinary medicine, specifically in the canine species, B-mode, Doppler, and ARFI elastography have already shown promise in malignancy prediction of breast tumors, where malignant neoplasms presented increased dimension and higher systolic and diastolic vascular velocities, as well as high shear velocity values (> 2.57 m/s) [14].

As for cutaneous and subcutaneous canine neoplasms, a few studies have demonstrated the applicability of ultrasonographic methods on tumor diagnosis and differentiation. A preliminary study found that predominantly hypoechoic, heterogeneous neoplasms with signs of invasiveness in adjacent tissues were more prone to malignancy [15]. On Doppler, it has been verified that cutaneous mast cell tumors have lower resistivity indices than soft tissues sarcomas and benign lesions [16]. Malignant neoplasms demonstrated greater stiffness on elastography when compared to benign tissues. However, the assessment was only qualitative, without the obtention of quantitative values of this stiffness [17].

Based on the possibility of skin tumors malignancy prediction in canines using ultrasound techniques, this study aimed to evaluate cutaneous and subcutaneous neoplasms using B-mode, Doppler, and ARFI elastography, to determine the accuracy of ultrasonography methods, suggest an evaluation protocol for these neoplasms, and perform the ultrasonographic characterization of the specific tumor types included.

Results

Histopathologic results

A total of 130 cutaneous and subcutaneous neoplasms (98 malignant and 32 benign) were evaluated, resulting in 21 histopathologic classifications (Table 1). The most prevalent malignant neoplasms in this study were the high-grade cutaneous mast cell tumors (18.46%). In comparison, the most prevalent benign neoplasms were lipomas (13.07%).

Table 1 Histopathologic classification, malignancy, and number of cutaneous and subcutaneous neoplasms evaluated by ultrasound

Classification	Malignancy	n
Adenocarcinoma	Malignant	1
Sebaceous adenoma	Benign	6
Basal cell carcionoma	Malignant	2
Squamous cell carcinoma	Malignant	15
Mixed carcinoma	Malignant	2
Apocrine cystadenoma	Benign	2
Fibrosarcoma	Malignant	4
Cavernous hemangioma	Benign	6
Hemangiopericytoma	Malignant	1
Hemangiosarcoma	Malignant	5
Cutaneous lymphoma	Malignant	13
Lipoma	Benign	17
Infiltrative lipoma	Benign	1
High-grade cutaneous mast cell tumor	Malignant	24
Low-grade cutaneous mast cell tumor	Malignant	10
Combined subcutaneous mast cell tumor	Malignant	1
Infiltrative subcutaneous mast cell tumor	Malignant	2
Amelanotic melanoma	Malignant	9
Grade II soft tissue sarcoma	Malignant	7
Grade III soft tissue sarcoma	Malignant	1
Grade II perivascular sheath tumor	Malignant	1
Total	**–**	**130**

B-mode ultrasonography

In B-mode, measurements of length (3.02 ± 2.85 cm), width (2.58 ± 2.18 cm), and height (1.79 ± 1.71 cm) were not associated with tumor malignancy, as well as echogenicity, capsule, and echotexture pattern (smooth or rough) (Table 2). It was found that echotexture ($P = 0.007$), invasiveness in adjacent tissues ($P = 0.002$), hyperechogenic spots ($P = 0.031$), and cavitary areas ($P = 0.001$) were shown to be predictive characteristics of malignancy. This way, heterogeneous neoplasms with signs of invasiveness, presence of hyperechogenic spots, and cavitary areas were more likely to be malignant (Fig. 1). The predictive values of sensitivity, specificity, accuracy, PPV and NPV are shown in Table 2, and the ultrasonographic characterization of neoplasms is shown in Table 3.

Doppler

The absence of vascularization on Doppler was verified in 59 neoplasms, while 39 had mild vascularization,

Table 2 Results of association between the mode-B ultrasonographic characterization of malignant cutaneous and subcutaneous canine neoplasms and their predictive values (sensibility, specificity, accuracy, positive predictive value, and negative predictive value) for those with $P<0.05$

Characteristic	*P*-value	Se (%)	Sp (%)	Ac (%)	PPV (%)	NPV (%)
Length	0.780	–	–	–	–	–
Width	0.795	–	–	–	–	–
Height	0.619	–	–	–	–	–
Echogenicity	0.059	–	–	–	–	–
Echotexture	0.007	96.87	18.75	93.04	78.15	66.66
Echotexture pattern	0.915	–	–	–	–	–
Invasiveness	0.002	70.83	59.37	68.14	83.95	40.42
Capsule	0.099	–	–	–	–	–
Hyperechoic spots	0.031	50.00	71.87	55.38	84.48	31.94
Cavity areas	0.001	69.38	62.50	67.69	85.00	40.00

Se sensitivity; *Sp* specificity; *Ac* accuracy; *PPV* positive predictive value; *NPV* negative predictive value

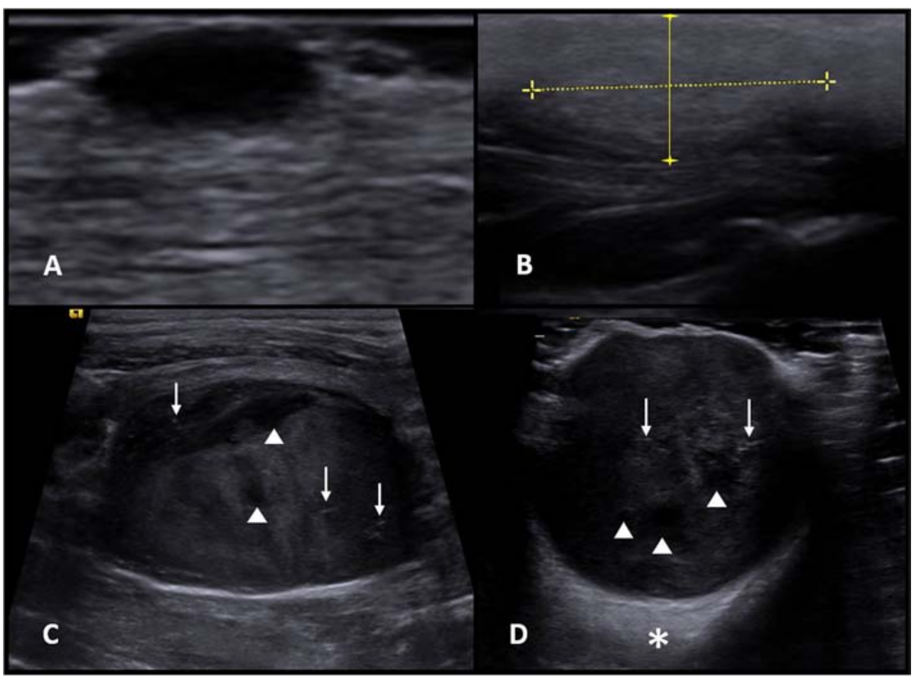

Fig. 1 B-mode ultrasound images in longitudinal section of canine cutaneous neoplasms. **a** Apocrine cystadenoma - predominantly hypoechoic, homogeneous, non-encapsulated, noninvasive, lacking hyperechogenic spots and cavitary areas; **b** lipoma - predominantly hyperechogenic, homogeneous, non-encapsulated, noninvasive, with absence of hyperechogenic points and cavitary areas; **c** Low-grade mast cell tumor - predominantly hypoechogenic, heterogeneous, partially encapsulated, with signs of invasiveness in adjacent tissues, presence of hyperechogenic spots (arrows), and cavitary areas (arrowheads); **d** high-grade mast cell tumor - predominantly hypoechogenic, heterogeneous, encapsulated, with signs of invasiveness in adjacent tissues, presence of hyperechogenic spots (arrows), and cavitary areas (arrowheads). Note reactivity of adjacent musculature (asterisk)

21 moderate, and 11 intense. Although an association between vascularization intensity and malignancy was not observed (Table 4), only one benign neoplasm (infiltrative lipoma) presented intense vascularization. There were also no associations between tumor malignancy, location, and vascularization pattern.

Identification of arterial flow using pulsed Doppler was only possible in 51 neoplasms. Of these, 42 were

Table 3 B-mode ultrasonographic characterization (echogenicity, echotexture, echotexture pattern, invasiveness, capsule, hyperechogenic spots, and cavitary areas) of cutaneous and subcutaneous canine neoplasms for tumor types that presented two or more cases

Histopathological classification (n)	Echogenicity	Echotexture	Echotexture pattern	Invasiveness	Capsule	Hyperechogenic spots	Cavitary areas
Sebaceous adenoma (6)	Hypoechogenic 83.3% Hyperechogenic 16.7%	Homogeneous16.7% Heterogeneous 83.3%	Smooth 33.3% Gross 66.7%	Invasive 50% Noninvasive 50%	Present 16.7% Absent 83.3%	Present 50% Absent 50%	Present 50% Absent 50%
Basal cell carcinoma (2)	Hypoechogenic 50% Hyperechogenic 50%	Homogeneous 0% Heterogeneous 100%	Smooth 0% Gross 100%	Invasive 100% Noninvasive 0%	Present 50% Absent 50%	Present 100% Absent 0%	Present 100% Absent 0%
Squamous cell carcinoma (15)	Hypoechogenic 53.3% Hyperechogenic 46.7%	Homogeneous 0% Heterogeneous 100%	Smooth 60% Gross 40%	Invasive 66.7% Noninvasive 33.3%	Present 6.7% Absent 93.3%	Present 40% Absent 60%	Present 60% Absent 40%
Mixed carcinoma (2)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 0% Heterogeneous 100%	Smooth 0% Gross 100%	Invasive 100% Noninvasive 0%	Present 0% Absent 100%	Present 0% Absent 100%	Present 0% Absent 100%
Apocrine cystadenoma (2)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 50% Heterogeneous 50%	Smooth 50% Gross 50%	Invasive 50% Noninvasive 50%	Present 0% Absent 100%	Present 0% Absent 100%	Present 50% Absent 50%
Fibrosarcoma (4)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 0% Heterogeneous 100%	Smooth 25% Gross 75%	Invasive 75% Noninvasive 25%	Present 25% Absent 75%	Present 50% Absent 50%	Present 70% Absent 30%
Cavernous hemangioma (6)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 33.3% Heterogeneous 66.7%	Smooth 83.3% Gross 16.7%	Invasive 16.7% Noninvasive 83.3%	Present 33.3% Absent 67.7%	Present 33.3% Absent 67.7%	Present 50% Absent 50%
Hemangiosarcoma (5)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 0% Heterogeneous 100%	Smooth 80% Gross 20%	Invasive 20% Noninvasive 80%	Present 0% Absent 100%	Present 20% Absent 80%	Present 100% Absent 0%
Cutaneous lymphoma (13)	Hypoechogenic 84.6% Hyperechogenic 15.4%	Homogeneous 0% Heterogeneous 100%	Smooth 61.5% Gross 38.5%	Invasive 100% Noninvasive 0%	Present 15.4% Absent 84.6%	Present 15.4% Absent 84.6%	Present 46.2% Absent 53.8%
Lipoma (17)	Hypoechogenic 41.2% Hyperechogenic 58.8%	Homogeneous 11.8% Heterogeneous 88.2%	Smooth 41.2% Gross 58.8%	Invasive 41.2% Noninvasive 58.8%	Present 0% Absent 100%	Present 17.6% Absent 82.4%	Present 83.5% Absent 16.5%
High-grade cutaneous mast cell tumor (24)	Hypoechogenic 79.2% Hyperechogenic 20.8%	Homogeneous 4.2% Heterogeneous 95.8%	Smooth 45.8% Gross 54.2%	Invasive 79.2% Noninvasive 20.8%	Present 29.2% Absent 70.8%	Present 58.3% Absent 41.7%	Present 83.3% Absent 16.7%
Low-grade cutaneous mast cell tumor (10)	Hypoechogenic 90% Hyperechogenic 10%	Homogeneous 0% Heterogeneous 100%	Smooth 20% Gross 80%	Invasive 80% Noninvasive 20%	Present 20% Absent 80%	Present 90% Absent 10%	Present 90% Absent 10%
Infiltrative subcutaneous mast cell tumor (2)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 0% Heterogeneous 100%	Smooth 0% Gross 100%	Invasive 100% Noninvasive 0%	Present 100% Absent 0%	Present 0% Absent 100%	Present 0% Absent 100%
Amelanotic melanoma (9)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 0% Heterogeneous 100%	Smooth 33.3% Gross 66.7%	Invasive 50% Noninvasive 50%	Present 16.7% Absent 83.3%	Present 44.4% Absent 55.6%	Present 55.6% Absent 44.4%

Table 3 (continued)

Histopathological classification (n)	Echogenicity	Echotexture	Echotexture pattern	Invasiveness	Capsule	Hyperechogenic spots	Cavitary areas
Grade II soft tissue sarcoma (7)	Hypoechogenic 100% Hyperechogenic 0%	Homogeneous 0% Heterogeneous 100%	Smooth 100% Gross 0%	Invasive 100% Noninvasive 0%	Present 100% Absent 0%	Present 100% Absent 0%	Present 100% Absent 0%

Table 4 Results of the association between the characteristics observed by color Doppler and pulsed Doppler with malignant cutaneous and subcutaneous canine neoplasms and their predictive values (cut-off value, sensibility, specificity, and area under the curve) for those with $P<0.05$

Characteristic	*P*-value	Cut-off value	Se (%)	Sp (%)	AUC (%)
Intensity	0.211	–	–	–	–
Location	0.617	–	–	–	–
Patter	0.171	–	–	–	–
Systolic peak	0.635	–	–	–	–
Diastolic velocity	0.971	–	–	–	–
Resistivity index	0.071	–	–	–	–
Pulsatility index	0.015	>0.93	90.50	55.60	75.70

Se sensitivity; *Sp* specificity; *AUC* area under the curve

malignant (82.35%) and only nine benign (17.65%). It was found that the peak values of systolic velocity, diastolic velocity, and resistivity index were not predictive of malignancy using the pulsed Doppler. However, the pulsatility index proved to be significant in differentiating between malignant and benign neoplasms ($P=0.015$), with a cut-off value >0.93 as indicative of malignancy, with 90.5% sensitivity, 55.6% specificity, and 75.7% accuracy (Fig. 2). The Doppler ultrasonographic characterization of cutaneous neoplasms is shown in Table 5.

ARFI Elastography

Both qualitative and quantitative assessments were shown to be significant in predicting malignancy (Table 6). Regarding deformability, it was observed that 11 benign and nine malignant neoplasms were classified as deformable, while 21 benign and 89 malignant were non-deformable. Deformability was shown to be predictive of tumor malignancy with 90.2% sensitivity, 35.48% specificity, 87.09% accuracy, 81.3% PPV, and 55% NPV.

In the quantitative elastography study, greater rigidity was observed in malignant (3.72 ± 1.94 m/s) compared to benign neoplasms (3.21 ± 1.86 m/s); consequently, SWV above 3.52 m/s was indicative of malignancy (Fig. 3), with 54.1% sensitivity, 68.7% specificity, and AUC of 62.7%. Among the benign neoplasms, adenomas had high rigidity (4.12 ± 2.06), hence the most rigid adenoma had a mean SWV of 8.3 m/s. Of the malignant neoplasms, the most rigid were soft tissue sarcomas (4.11 ± 1.81 m/s) and mast cell tumors (3.76 ± 1.92 m/s), however, the largest SWV observed was in a squamous cell carcinoma (9.1 m/s). The characterization of tumor stiffness by ARFI elastography is shown in Table 7.

Association of malignancy predictive characteristics

All characteristics that presented significant results in malignancy prediction were considered for the association between the different types of ultrasound techniques. Thus, seven tumor characteristics were considered: heterogeneous, invasive, presence of hyperechogenic points, presence of cavitary areas, PI above 0.93, non-deformable, and above 3.52 m/s SWV.

It was observed that 85 neoplasms had at least four malignancy predictive characteristics (Table 8). Seventy-two (84.7%) neoplasms were indeed malignant, and only 13 (15.3%) were benign. Five or more malignancy predictive characteristics were found in sixty neoplasms, where 53 (88.3%) were malignant and seven (11.7%) were benign. Forty-five neoplasms had at least six characteristics, where 41 (87.2%) were malignant, and four (12.8%) were benign. When considering all seven malignancy predictive characteristics, 16 neoplasms were computed, where 14 (87.5%) were malignant, and only two (12.5%) were benign.

Discussion

This study provides important information regarding the diagnosis and classification of cutaneous and subcutaneous canine neoplasms, as it was possible to establish malignancy predictive characteristics by all techniques used (B-mode, Doppler, and ARFI elastography). In addition, it was possible to determine an ultrasound examination protocol that could contribute to lesions diagnosis and prognosis and provide individual ultrasound characteristics for each studied tumor type. Because it is a complementary method, its characteristics are highly sensitive and have a positive predictive value. These results were obtained in all three ultrasound techniques that were performed.

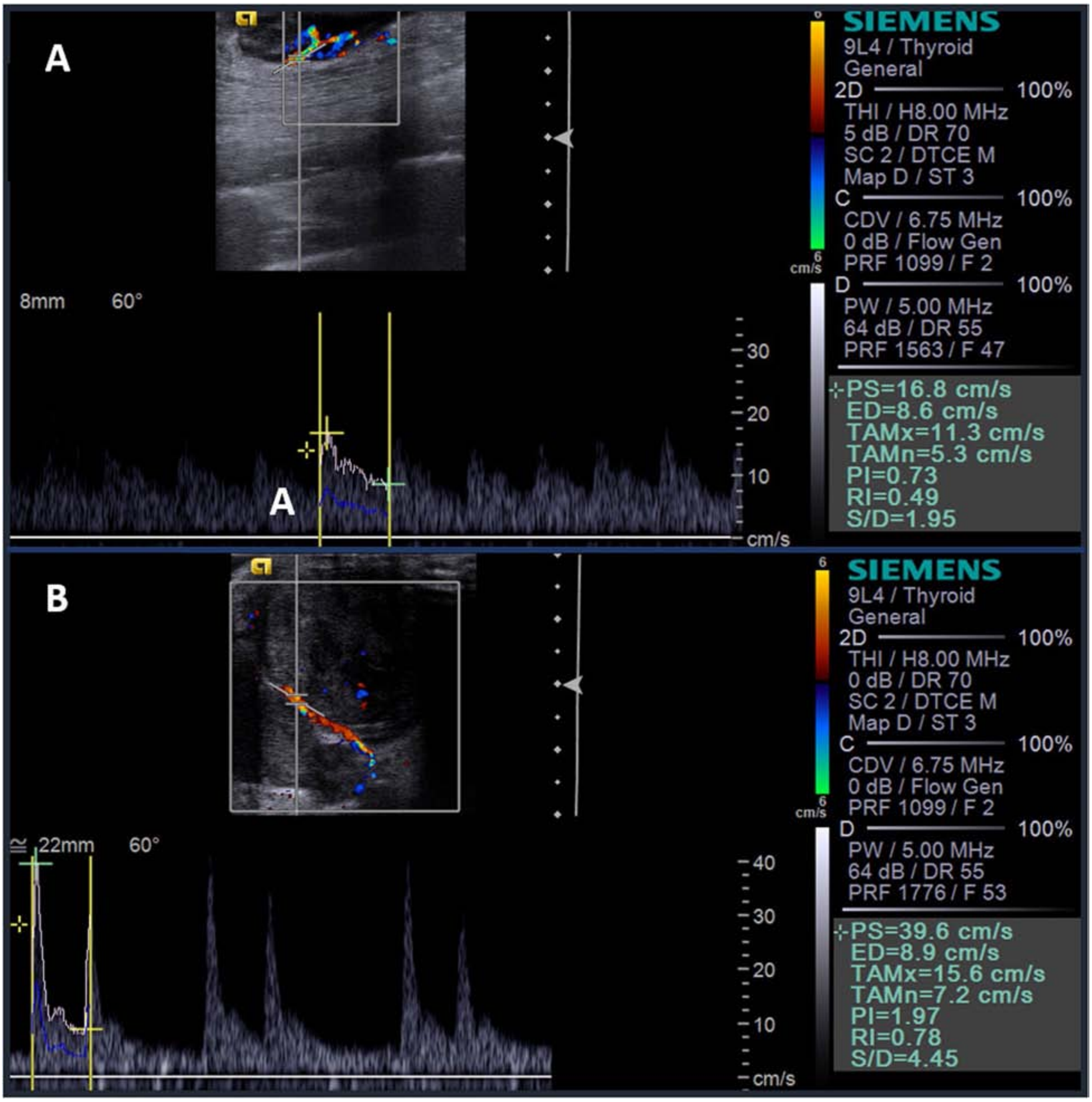

Fig. 2 Ultrasonographic images of canine cutaneous neoplasms obtained by pulsed Doppler, showing spectral tracings and Doppler velocimetry indices calculations. **a** Benign neoplasm (cavernous hemangioma) with 0.73 (not indicative of malignancy) pulsatility index (PI); **b** Malignant neoplasm (grade II soft tissue sarcoma) presenting 1.97 PI (indicating malignancy)

Given the high number of cutaneous and subcutaneous neoplasm types, it should be considered that they have different structural components and biological behaviors. They can range from benign to highly aggressive and metastatic lesions [18], which justifies the moderate results observed. The authors would like to emphasize the importance of studies regarding specific cancer types, as the present study results differed from previous canine mammary tumors findings. In another study, with breast tumors, different characteristics and predictive values of malignancy were found [14].

There were no associations between malignancy and tumor measurements in this study, which can be explained by the fact that neoplasms were diagnosed at different stages. There were no associations with

Table 5 Ultrasonographic characterization by Doppler (intensity, location and vascularization pattern, systolic peak, diastolic velocity, resistivity index, and pulsatility index) of cutaneous and subcutaneous canine neoplasms for tumor types that presented two or more cases

Histopathological classification (n)	Intensity	Location	Pattern	SP (cm/s) (mean ± SD)	DV (cm/s) (mean ± SD)	RI (mean ± SD)	PI (mean ± SD)
Sebaceous adenoma (6)	Absent 33.3% Discrete 16.7% Moderate 50% Intense 0%	Central 0% Peripheral 0% Diffuse 100%	Perinodular 0% Mosaic 25% Network 75%	8.4 ± 2.21	2.8 ± 1.22	0.67 ± 0.12	2.03 ± 1.14
Basal cell carcinoma (2)	Absent 0% Discrete 100% Moderate 0% Intense 0%	Central 0% Peripheral 0% Diffuse 100%	Perinodular 0% Mosaic 0% Network 100%	16.73 ± 29.18	4.45 ± 5.88	0.7 ± 0.11	5.0 ± 9.32
Squamous cell carcinoma (15)	Absent 80% Discrete 13.3% Moderate 0% Intense 6.7%	Central 0% Peripheral 0% Diffuse 100%	Perinodular 0% Mosaic 66.7% Network 33.3%	10.15 ± 8.55	5.5 ± 5.65	0.52 ± 0.15	1.52 ± 0.91
Mixed carcinoma (2)	Absent 100% Discrete 0% Moderate 0% Intense 0%	NA	NA	NA	NA	NA	NA
Apocrine cystadenoma (2)	Absent 50% Discrete 50% Moderate 0% Intense 0%	Central 0% Peripheral 100% Diffuse 0%	Perinodular 100% Mosaic 0% Network 0%	NA	NA	NA	NA
Fibrosarcoma (4)	Absent 75% Discrete 25% Moderate 0% Intense 0%	Central 0% Peripheral 50% Diffuse 50%	Perinodular 0% Mosaic 100% Network 0%	NA	NA	NA	NA
Cavernous hemangioma (6)	Absent 50% Discrete 50% Moderate Intense	Central 0% Peripheral 33.3% Diffuse 66.7%	Perinodular 0% Mosaic 100% Network 0%	5.85 ± 1.77	2.35 ± 0.35	0.58 ± 0.06	1.06 ± 0.17
Hemangiosarcoma (5)	Absent 80% Discrete 20% Moderate 0% Intense 0%	Central 0% Peripheral 0% Diffuse 100%	Perinodular 0% Mosaic 100% Network 0%	9.2*	3.4*	0.63*	4.14*
Cutaneous lymphoma (13)	Absent 38.5% Discrete 53.8% Moderate 7.7% Intense 0%	Central 0% Peripheral 62.5% Diffuse 37.5%	Perinodular 62.5% Mosaic 37.5% Network 0%	64.7 ± 40.91	18.8 ± 17.16	0.73 ± 0.18	2.18 ± 1.35
Lipoma (17)	Absent 70.6% Discrete 23.5% Moderate 5.9% Intense 0%	Central 20% Peripheral 20% Diffuse 60%	Perinodular 0% Mosaic 40% Network 60%	18.03 ± 5.71	8.67 ± 1.24	0.49 ± 1.74	0.82 ± 0.58
High-grade cutaneous mast cell tumor (24)	Absent 4.2% Discrete 33.3% Moderate 41.7% Intense 20.8%	Central 13% Peripheral 8.7% Diffuse 78.3%	Perinodular 4.4% Mosaic 39.1% Network 56.5%	17.07 ± 23.89	5.02 ± 5.18	0.66 ± 0.12	3.87 ± 7.2
Low-grade cutaneous mast cell tumor (10)	Absent 40% Discrete 40% Moderate 20% Intense 0%	Central 16.7% Peripheral 33.3% Diffuse 50%	Perinodular 0% Mosaic 66.7% Network 33.3%	28.76 ± 40.17	4.35 ± 5.56	0.67 ± 0.1	4.47 ± 8.83
Infiltrative subcutaneous mast cell tumor (2)	Absent 0% Discrete 0% Moderate 100% Intense 0%	Central 100% Peripheral 0% Diffuse 0%	Perinodular 0% Mosaic 0% Network 100%	2.31 ± 1.12	5.0 ± 4.21	0.78 ± 0.1	4.11 ± 2.32
Amelanotic melanoma (9)	Absent 33.3% Discrete 16.7% Moderate 0% Intense 50%	Central 0% Peripheral 0% Diffuse 100%	Perinodular 0% Mosaic 25% Network 75%	18.55 ± 27.51	4.89 ± 5.81	0.69 ± 0.11	4.29 ± 7.89
Grade II soft tissue sarcoma (7)	Absent 0% Discrete 0% Moderate 50% Intense 50%	Central 0% Peripheral 0% Diffuse 100%	Perinodular 0% Mosaic 0% Network 100%	8.98 ± 6.56	3.37 ± 2.59	0.66 ± 0.1	4.57 ± 8.44

*Only one neoplasm with the arterial flow; *SP* systolic peak; *DV* diastolic velocity; *RI* resistivity index; *PI* pulsatility index; *SD* Standard deviation; *NA* not applicable

Table 6 Results of the association between ARFI electrographic findings and malignancy of canine cutaneous and subcutaneous neoplasms

Characteristic	*P*-value	Se (%)	Sp (%)	Ac (%)	PPV (%)	NPV (%)	AUC (%)
Deformability	< 0.001	90.2	35.48	87.09	81.3	55.00	–
SWV	0.024	54.1	68.7	–	–	–	62.7

SWV shear wave velocity; *Se* sensitivity; *Sp* specificity; *Ac* accuracy; *PPV* positive predictive value; *NPV* negative predictive value; *AUC* area under the curve

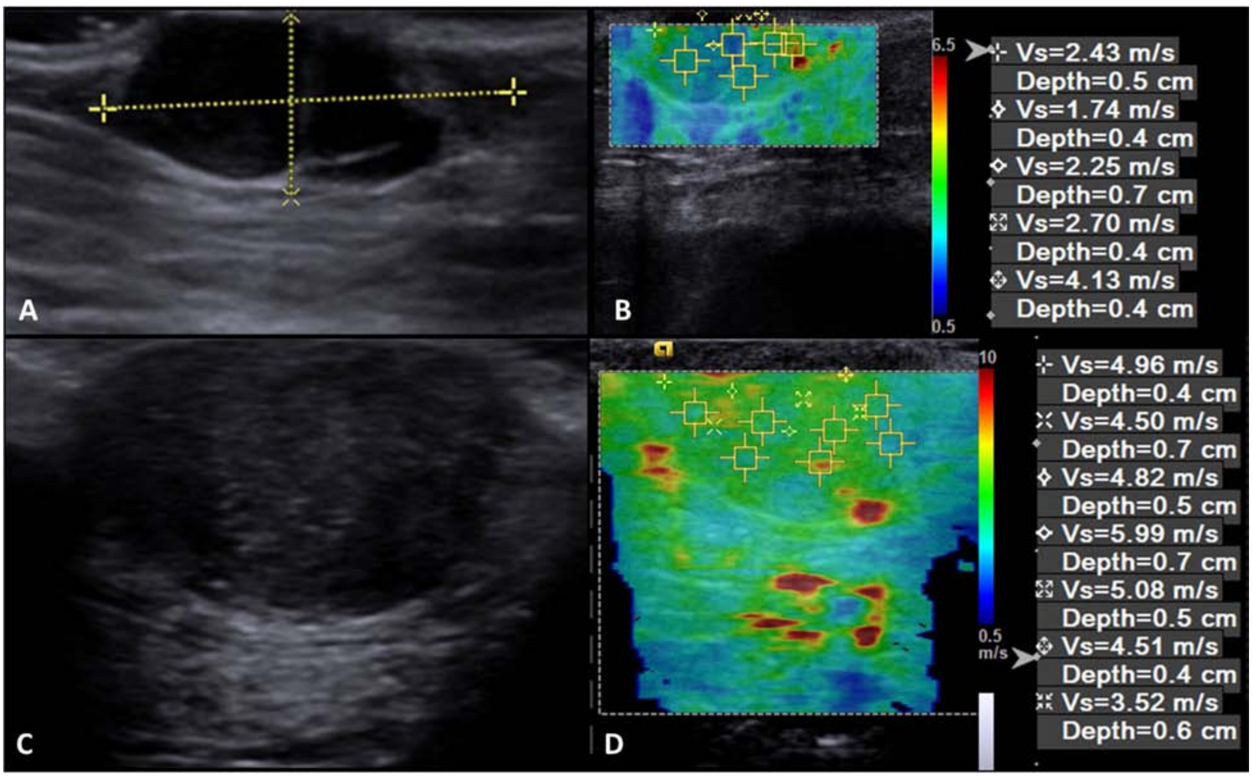

Fig. 3 B-mode **(a, c)** and ARFI elastography **(b, d)** ultrasound images of canine cutaneous neoplasms. In **(a)** and **(b)**, hemangioma – elastogram image showing predominance of blue colors (less rigid) and with mean shear wave velocity (SWV) of 2.65 m/s (not indicative of malignancy); In **(c)** and **(d)**, high-grade cutaneous mast cell tumor – elastogram showing a predominance of green and yellow colors (intermediate stiffness) and with an average SWV of 4.77 m/s (indicating malignancy)

echogenicity, which may be related to the different pathological processes involved, such as active inflammation or tissue necrosis in different tumor types [19]. A preliminary study involving 42 cutaneous neoplasms showed an association between malignancy and hypoechogenicity [15]. A greater number of neoplasms and specific types of skin cancer that were included in the present study may explain the discrepancy between the two studies.

The heterogeneous echotexture indicative of malignancy seen in cutaneous and subcutaneous tumors is explained by the different structural components, such as the presence of cavitary areas, points of fibrosis, or microcalcifications. The association between heterogeneous echotexture and malignancy was already demonstrated in previous studies with different types of neoplasms (cutaneous and mammary) in both humans and animals [13, 15–17, 20].

It was possible to identify the signs of invasiveness in adjacent tissues because of their reactivity or the difficult tumors delimitation and then associate it with malignancy. This association is justified because malignant neoplasms tend to be more aggressive and invasive than benign ones, even requiring a greater safety margin when surgically removed [21].

On Doppler, it was not verified any qualitative characteristic with malignancy. It is known that tumor growth, both in malignant and benign lesions, is dependent on the blood supply [22]. Therefore, it is reasonable the fact that no significant results were obtained in neoplasm differentiation through

Table 7 Ultrasonographic characterization by ARFI elastography (deformability and shear wave velocity – SWV) of cutaneous and subcutaneous canine neoplasms for tumor types that presented two or more cases

Histopathological classification (n)	Deformability	SWV (m/s) (mean ± SD)
Sebaceous adenoma (6)	Deformable 16.7% Non-deformable 83.3%	3.82 ± 1.92
Basal cell carcinoma (2)	Deformable 0% Non-deformable 100%	3.87 ± 1.84
Squamous cell carcinoma (15)	Deformable 0% Non-deformable 100%	3.82 ± 1.96
Mixed carcinoma (2)	Deformable 0% Non-deformable 100%	2.46 ± 0.19
Apocrine cystadenoma (2)	Deformable 0% Non-deformable 100%	3.72 ± 1.81
Fibrosarcoma (4)	Deformable 0% Non-deformable 100%	3.89 ± 1.88
Cavernous hemangioma (6)	Deformable 0% Non-deformable 100%	3.87 ± 1.79
Hemangiosarcoma (5)	Deformable 20% Non-deformable 80%	2.9 ± 1.97
Cutaneous lymphoma (13)	Deformable 0% Non-deformable 100%	3.6 ± 1.97
Lipoma (17)	Deformable 64.7% Non-deformable 35.3%	3.83 ± 1.84
High-grade cutaneous mast cell tumor (24)	Deformable 4.2% Non-deformable 95.3%	3.76 ± 1.92
Low-grade cutaneous mast cell tumor (10)	Deformable 0% Non-deformable 100%	3.91 ± 1.79
Infiltrative subcutaneous mast cell tumor (2)	Deformable 0% Non-deformable 100%	3.96 ± 1.83
Amelanotic melanoma (9)	Deformable 0% Non-deformable 100%	3.72 ± 1.83
Grade II soft tissue sarcoma (7)	Deformable 0% Non-deformable 100%	3.67 ± 1.8

Table 8 Descriptive and predictive values of ultrasound assessment protocols, associating malignancy predictive characteristics of cutaneous and subcutaneous canine neoplasms verified by B-mode ultrasonography, Doppler, and ARFI elastography

Predictive characteristics	Total (n)	Malignant (n)	Benign (n)	*P*-value	Se (%)	Sp (%)	Ac (%)	PPV (%)	NPV (%)
Four or more	85	72	13	0.001	73.46	59.37	70	84.7	42.22
Five or more	60	53	7	0.002	54.08	78.12	60	88.33	35.71
Six or more	45	41	4	0.002	41.83	87.5	53	91.11	32.94
All seven	16	14	2	0.230	–	–	–	–	–

n total number; *Se* sensitivity; *Sp* specificity; *Ac* accuracy; *PPV* positive pr edictive value; *NPV* negative predictive value

these characteristics even though other researchers showed associations with malignancy in other tumor types, such as breast cancer in women and canine mast cell tumors [23, 24].

Even though no vascularization points were observed in some tumors by color Doppler, the lack of vascularization should not be ruled out. It is known that the color Doppler technique has limitations at microvascular level and tissue perfusion, requiring other methods for diagnostic complementation, such as contrasted ultrasound [14]. Nevertheless, this technique was not available and could not be tested in the present study. This Doppler technique limitation contributed to the impossibility of evaluating all neoplasms by pulsed Doppler, with the Doppler velocimetry indices being calculated for only a portion of those who presented vascularization in color Doppler.

The lack of association between RI, systolic peak, and diastolic velocity with malignancy could be because it was only possible to identify the arterial flow in 9 benign neoplasms, predominantly in malignant lesions (82.35% of cases). However, a PI increase in malignant neoplasms was verified. The increase in this index has already been associated with malignancy in other types of lesions, such as ovarian and thyroid tumors in humans and metastases in canine lymph nodes. These may be related to the compressive effect tumor, the angiogenesis process, and the presence of arteriovenous shunts, which promote turbulent flows with high perfusion rates [25–27].

In the same way, as B-mode observed heterogeneity, the increased rigidity observed in malignant neoplasms can also be explained by tissue components they may present. In a previous study, greater stiffness was found in malignant mammary tumors in female dogs compared to benign ones, and this increase in stiffness was justified by the presence of areas of fibrosis, microcalcifications, and even collagen deposition [14].

The study of the rigidity of skin neoplasms in dogs has already been carried out qualitatively and semiquantitatively (through scores) through elastography, with greater rigidity being observed in malignant tissues, however no real quantitative values of the shear wave velocity were obtained. Only subjective analysis [17]. On the other hand, this study provides more detailed information regarding neoplasms stiffness since it was possible to verify that an SWV greater than 3.52 m/s was indicative of malignancy. In addition, the elastography method used (ARFI method) allows more reliable results that are easy to perform, with greater reproducibility and less interobserver variability than sonoelastography [28].

Some benign neoplasms, such as adenomas, showed high tissue stiffness, justified by the accumulation of keratin and predominantly lymphoplasmacytic inflammatory infiltrate [29], that cause rigidity alterations in the keratinocytes and extracellular matrix [30, 31].

Because ultrasonography is a complementary exam and should not be used alone to diagnose neoplasms, in this study, we demonstrate the importance of the association between the findings of the different techniques performed. These have been already described for evaluating breast tumors in women, where an increase in accuracy was found when elastography and Doppler findings were associated [24]. In our study, as we increased the number of malignancy predictive characteristics, there was a decrease in the number of false positives, increase in protocol specificity, and positive predictive value.

Among the study's limitations, it should be considered that some tumor types had a low experimental number, and as noted in this discussion and we had some values discrepancies (e.g., adenomas), which may be responsible for the low specificity and accuracy values that were observed.

Conclusions

Findings from this study indicate that ultrasonography has good applicability in the malignancy prediction of cutaneous and subcutaneous canine neoplasms through different techniques, so that heterogeneous, invasive neoplasms, with the presence of hyperechogenic points and cavitary areas, with PI greater than 0.93, non-deformable and with SWV greater than 3.52 m/s were more prone to malignancy. This study presents quick and noninvasive results and can be used as a complementary method for this diagnosis. Furthermore, we found that the assessment protocol by associating the findings of different ultrasound techniques allows for greater reliability in diagnosing malignancy in this type of cancer, increasing the specificity according to the greater number of predictive characteristics presented by the neoplasm.

Methods

Experimental design

This study was carried out according to the ARRIVE guidelines 2.0 (2020). Prospective data collection was conducted between September 2019 and June 2021. Sixty-six dogs of different breeds and ages (9.45 ± 2.58 years) from the hospital routine presented cutaneous or subcutaneous neoplasms were enrolled in the study. The Veterinary Oncology sector previously evaluated all patients.

Ultrasound evaluation

Trichotomy of the tumor region was done with up to two centimeters of the peritumoral region. In order to maintain the patient's comfort during the examination and without sedation or anesthesia, patients were positioned in decubitus according to the anatomical location of the neoplasms. ACUSSON S2000™ equipment (Siemens®, Munich, Germany) was used for all the techniques performed, with a linear transducer and frequency ranging from 8 to 10Mhz. In addition, an ultrasonographic conductive gel was used throughout the examination.

B-mode ultrasound

The transducer was positioned in the central superficial region of the neoplasms, adjusting the focus, gain, and depth as needed. After adjusting the device, the nodules and masses were measured in longitudinal (length and height) and transversal (width) sections. The characteristics of echogenicity (hypoechogenic or hyperechogenic), echotexture (homogeneous or heterogeneous), echotexture pattern (coarse or smooth), invasiveness in adjacent tissues (presence or absence), capsule (presence or absence), cavitary areas (presence or absence), and hyperechogenic points (presence or absence) were evaluated.

Doppler

The color Doppler function was activated to identify neovascularization, and the pulse repetition frequency (PRF) was adjusted to 977 Hz. When necessary, changes were made to the pre-established PRF. Tumor neovascularization was characterized according to its intensity (absent, mild, moderate, or intense), location (central, peripheral, or diffuse), and pattern (perinodular, mosaic, or network).

The pulsed wave Doppler was activated and used only for those neoplasms that presented vascularization at color Doppler examination. At this stage, the PRF used in the qualitative assessment was maintained, and the caliper was adjusted to cover 2/3 of the vessel's caliber, and using an angulation towards the vessel when necessary, respecting the limit of 60° degrees. At least three spectral traces were obtained [14] to get the peak values of systolic velocity (m/s), diastolic velocity (m/s), resistivity index (RI), and pulsatility index (PI).

ARFI Elastography

The elastographic evaluation was performed using the VTIQ method (virtual touch tissue imaging quantification, 2D-SWE technique). Color elastograms were performed in the qualitative study. Where blue colors represented more elastic areas, green and yellow represented intermediate stiffness, and red corresponded to more rigid areas. Based on the color pattern, neoplasms were classified according to their deformability (deformable or non-deformable). The same elastograms were used for quantitative analysis, and at least three areas of interest (ROIs) were selected. The number of ROIs varied according to the size of the neoplasm, that is, the larger the structure, the more ROIs were measured. The choice of these areas was made to cover both the most rigid and most elastic regions, aiming to obtain a more reliable total representative value. Those areas were randomly chosen to obtain the mean shear wave velocity (SWV - m/s), quantified by the VTIQ software, and using total stiffness as a representative value [14].

Histopathological evaluation

After ultrasound examinations, clinical care was continued, and biopsies (incisional or excisional) were performed to obtain the definitive diagnosis. Patients were individually anesthetized, and surgical protocols were defined under the recommendation of the responsible veterinarian. These tumor samples were fixed in 10% formalin and sent to the veterinary pathology laboratory within the university, where histological cuts were performed to make slides stained with hematoxylin and eosin and, in cases of mast cell tumors, with toluidine blue. After histopathological diagnosis, neoplasms were classified as benign or malignant, as established by the World Health Organization (WHO).

Statistical analysis

All data were analyzed using the SPSS Statistics 20 package (IBM®, New York, United States), and a significance level of 95% was used for all tests ($P < 0.05$). Echogenicity, echotexture, texture pattern, invasiveness, capsule, hyperechogenic spots, cavitary areas, and deformability were associated with malignancy using the Chi-square test, and sensitivity, specificity, accuracy, and positive (PPV) and negative (NPV) predictive values were calculated. Logistic regression was performed to differentiate malignancy according to the intensity, location, and pattern of vascularization. The other characteristics were submitted to the Kolmogorov-Smirnov normality test. The Mann-Whitney test was performed to analyze length, width, height, systolic peak, diastolic velocity, and pulsatility index. While for the resistivity index and SWV, a t-test was performed for independent samples. A ROC curve was used to obtain the cut-off point, sensitivity, specificity, and area under the curve for significant results.

Afterward, the variables with significant results were selected, and a descriptive analysis of the association between the different ultrasound techniques was performed. Furthermore, they were grouped into four groups: 1) presence of at least four predictive malignancy characteristics; 2) at least five characteristics; 3) at least six characteristics; 4) seven characteristics. Thus, the chi-square test verified an association with malignancy, and the values of sensitivity, specificity, accuracy, PPV, and NPV were calculated. Additionally, descriptive analysis was performed and expressed in percentages of the qualitative ultrasonographic characteristics and the mean and standard deviation of the quantitative characteristics for each tumor type included in this study, except for single cases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-021-03118-y.

Additional file 1.

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Authors' contributions
ICKC, MARF, RARU and ABN planned the experimental design. ICKC and RKC performed the ultrasound exams. ICKC and RARU performed a statistical analysis. ICKC and EMB performed the writing and translation of the manuscript. MARF and ABN reviewed the writing of the manuscript. All authors approved the final version of the article.

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Availability of data and materials
The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Animal Care and Use Committee of Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, Brazil (Protocol 010047/19) and the owners formally agreed, through signing a term of responsibility, to enroll their animals in this study. All methods were performed in accordance with National Animal Testing Control Board (CONCEA).

Consent for publication
Not applicable.

Competing interests
The authors declare having no competing interests.

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Accuracy of B-mode ultrasound and ARFI elastography in predicting malignancy of canine splenic lesions

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Accuracy of B-mode ultrasound and ARFI elastography in predicting malignancy of canine splenic lesions

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The objective was to evaluate the accuracy of B-mode ultrasonography and ARFI elastography in detecting malignancy in canine splenic lesions. Thirty-seven spleens with abnormalities (16 benign and 21 malignant) from dogs of different breeds and ages were evaluated. Echogenicity, echotexture, organ length and height were evaluated using B-mode. By ARFI elastography, tissue stiffness was evaluated qualitatively (elastogram) and quantitatively (measuring the shear wave velocity—SWV). Lesions were classified as diffuse, focal or multifocal (cranial, medial or caudal portion) and comparisons of the SWV between the injured and non-injured areas were performed. In the B-mode, no features were associated to malignancy ($P > 0.05$). In the elastogram, 35 spleens were non-deformable and 2 deformable, having no association with malignancy. The greater SWV was observed in malignant lesions (3.4 ± 0.6 m/s), followed by areas free from alterations (2.1 ± 0.3 m/s) and benign lesions (1.7 ± 0.5 m/s), with difference between groups ($P < 0.0001$). It was found that a SWV > 2.6 m/s indicates malignancy of canine splenic lesions (sensitivity of 95%, specificity of 100%, PPV of 100%, NPV of 94% and accuracy of 97%), concluding that ARFI elastography is a promising technique for differentiating malignancy in these lesions.

Splenic tumors have clinical relevance in the clinical routine of small animals, presenting a malignancy prevalence of 58% when patients have masses or nodules larger than 1 cm[1]. These animals may show nonspecific clinical signs such as weakness, anorexia, and lethargy[2]. Among the most diagnosed malignant and benign alterations in the spleen are hemangiosarcoma with an occurrence of 44.1% and nodular lymphoid hyperplasia affecting 20.1% of the animals[3]. The prognosis varies from favorable to reserved depending on the diagnosis of the splenic lesion[4], which can be performed using ultrasound-guided fine-needle aspirate cytology or histopathological analysis of material collected by biopsies or splenectomy[5–7].

In medicine, accurate and rapid diagnosis is essential for patient prognosis and therapy[8]. Ultrasonography is a sensitive technique to detect subtle alterations or abnormalities that affect the splenic parenchyma in small animals[7], as it allows evaluating the organ in terms of size, contours, echogenicity, echotexture and identifying focal or diffuse lesions[9]. It is difficult to differentiate between benign and malignant processes using B-mode, as malignant and benign splenic tumors may show similar patterns of echogenicity and echotexture while tumors with the same histopathological result may show different echo patterns[10].

Other ultrasound techniques such as Doppler and contrast-enhanced ultrasound (CEUS) have been studied as adjuvants in the differentiation of splenic lesions. CEUS showed no significant difference between malignant and benign parenchymal lesions in dogs[11] as well as there was similarity in color Doppler and power Doppler between these lesions[12]. Thus, both methodologies, so far, cannot be used as predictors of malignancy in dog spleens.

Elastography provides complementary information to conventional ultrasound examinations, adding stiffness assessment as another measurable property. Among its modalities, the Acoustic Radiation Force Impulse (ARFI) is able to provide quantitative and qualitative information by generating shear waves[13]. ARFI has gained

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Histopathological diagnosis	Classification	n	%
Splenic hemangiosarcoma	Malignant	10	27.0
Splenic hematoma	Benign	8	21.7
Complex lymphoid follicular hyperplasia	Benign	4	10.8
Lymphocytic lymphoma	Malignant	4	10.8
follicular lymphoma	Malignant	3	8.1
Epithelioid hemangiosarcoma	Malignant	2	5.4
Severe atrophy of white and red pulp	Benign	1	2.7
Splenosis	Benign	1	2.7
Splenic fibrosarcoma	Malignant	1	2.7
Splenic hemangioma	Benign	1	2.7
Extramedullary hematopoiesis	Benign	1	2.7
Poorly differentiated splenic sarcoma	Malignant	1	2.7
Total	–	37	100

Table 1. Histopathological diagnosis and classification of splenic lesions in dogs previously submitted to the ARFI elastography evaluation. *n* number.

Variable	Classification	Benign	Malignant	P-comparation	P-diagnosis
Width (cm)	NA	5.9 ± 3.1	7.4 ± 3.6	0.2430	0.2442
Length (cm)	NA	5.0 ± 2.1	5.8 ± 3.2	0.4211	0.5604
Echogenicity	Anechoic	1/16 (6%)	1/21 (4%)	0.8732	–
	Hypoechoic	9/16 (56%)	10/21 (48%)		
	Mixed	6/16 (38%)	10/21 (48%)		
Echotexture	Heterogeneous	14/16 (88%)	20/21 (95%)	0.3523	–
	Homogeneous	2/16 (12%)	1/21 (5%)		

Table 2. Association between B-mode ultrasound characteristics (height, length, echogenicity and echotexture) and malignancy of splenic lesions in dogs. *NA* not applicable.

notoriety in veterinary medicine and medicine for demonstrating applicability in differentiating malignancy in thyroid[14], cervical lymph nodes[15], pancreas[16] and mammary tumors[17].

The quantitative evaluation of ARFI elastography is performed by measuring the shear wave velocity (SWV) and, through this technique, it has already been possible to differentiate benign from malignant lesions in different types of canine neoplasms (mammary, cutaneous and subcutaneous) and obtain cut-off values for this differentiation[17,18]. Also, in patients with breast cancer, it was possible to verify an increase in SWV in metastatic lymph nodes, differentiating from normal or inflamed tissues.

Believing that there are ultrasound and stiffness differences between malignant and benign splenic lesions in dogs, the present study aimed to evaluate the accuracy of B-mode ultrasound and ARFI elastography in detecting malignancy in splenic lesions in dogs. Our first hypothesis is that malignant tumors are more rigid than benign lesions, corroborating the results already found in other tissues. The second hypothesis is that benign tumors have greater rigidity when compared to normal splenic tissue.

Results

In the present study, 37 spleens were evaluated, of which 10 (27%) had diffuse lesions, 6 (16%) lesions in the cranial region, 9 (24%) in the medial region and 12 (33%) in the caudal region. Of these lesions, 16 (43%) were classified as benign and 21 (57%) as malignant. The histopathological diagnosis of the lesions is shown in Table 1.

The size of the spleen was similar in patients with benign and malignant lesions, and the height and length of the organ were not indicative of malignancy. Findings of echogenicity and echotexture were also similar between malignant and benign splenic alterations (Table 2).

In the qualitative elastography evaluation, the spleen was classified as non-deformable in 35 (95%) and as deformable in 2 (5%) of the 37 examinations performed, however this classification is not related to the malignancy of the lesions ($P = 0.465$). Furthermore, there was a homogeneous stiffness pattern in 14 (88%) and heterogeneous in 2 (12%) of the 16 organs with benign lesions; in the 21 malignant lesions, the pattern was homogeneous in 16 (76%) and heterogeneous in 5 (24%). As with deformability, tissue stiffness homogeneity pattern was also not associated with malignancy ($P = 0.2596$).

The quantitative ARFI assessment of the different regions of the spleen was similar ($P = 0.8856$) between the cranial, caudal and medial portions and, in this way, the averages of each of these regions were calculated and used for the subsequent analysis, which were considered similar to each other in this general analysis (cranial 2.6 ± 0.8 m/s; medial 2.6 ± 0.9 m/s; caudal 2.5 ± 0.8 m/s; $P = 0.9705$).

Variable	Benign	Malignant	P-Value	CP	Se%	Sp%	PPV%	NPV%	Acc%	AUC
SWV cranial	2.0 ± 0.5	3.0 ± 0.8	< 0.0001	> 2.6	71.4	93.8	93.8	71.4	81.1	0.8899
SWV Medial	1.9 ± 0.3	3.1 ± 0.8	< 0.0001	> 2.3	85.7	93.8	95.0	88.2	91.9	0.9077
SWV Caudal	2.0 ± 0.5	3.0 ± 0.7	< 0.0001	> 2.5	76.2	93.8	94.1	75.0	83.8	0.8661
SWV of the lesion and medial in diffuse	1.7 ± 0.5	3.4 ± 0.5	< 0.0001	> 2.6	95.2	100	100	94.1	97.3	0.9911
Standard deviation of SWV	0.3 ± 0.2	0.3 ± 0.2	0.4799	NA	NA	NA	NA	NA	NA	NA
Evaluation depth	2.1 ± 0.5	2.1 ± 0.6	0.8707	NA	NA	NA	NA	NA	NA	NA

Table 3. Mean and standard deviation of shear wave velocity (SWV) for predicting malignancy in splenic lesions of dogs. *NA* not applicable, *CP* cutoff point, *Se* sensibility, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value.

Comparing the SWV from the lesion-free regions (2.1 ± 0.3 m/s) and the regions that presented focal or multifocal benign lesions (1.7 ± 0.5 m/s), it was observed that the free regions have a higher SWV (P = 0.0036). Focal or multifocal malignant lesions, on the other hand, had a SWV (3.4 ± 0.6 m/s) significantly greater than benign lesions and lesion-free areas (P < 0.0001).

In the analysis of the ability to predict malignancy through elastography, the anatomical regions in the organs that presented diffuse lesions were compared, and the medial region had the largest area under the curve (AUC = 0.9077) when compared to the cranial region (AUC = 0.8899) and the caudal region (AUC = 0.8661). For this reason, the SWV value of the medial area was used as the region of interest for the diagnosis of diffuse lesions.

Table 3 contains the mean values of the comparative descriptive statistical analyzes and the results of the analysis of the ROC curves to differentiate malignant lesions in the studied spleens. When comparing the SWV of the localized lesion regions and the medial region of diffuse lesions, it was possible to observe that in the benign lesions (1.7 ± 0.5 m/s) this variable is significantly lower (P < 0.0001) when compared to malignant lesions (3.4 ± 0.5 m/s), the discriminative power analysis indicated that a SWV > 2.6 m/s in focal lesions or in the medial region in diffuse lesions, indicates malignancy of the splenic lesion with a sensitivity of 95%, specificity of 100%, positive predictive value of 100%, negative predictive value of 94%, and an accuracy of 97%. The Fig. 1 illustrates the ARFI elastographic assessment in a benign (splenic hematoma) and a malignant (lymphoma) lesion. The standard deviation of these SWVs, as well as the depth of assessment, were similar in the different types of lesions and without any predictive power.

Discussion

The present study provided important information regarding the prediction of malignancy in splenic lesions of dogs using ARFI elastography. Malignant lesions presented greater tissue stiffness when compared to benign lesions, with a shear velocity (SWV) greater than 2.6 m/s, with a diagnostic accuracy of 97%. These results are important to determine a more adequate treatment and prognosis, as well as enabling a more agile therapeutic approach, in addition to these data being able to direct studies to other species.

The spleen is a reticuloendothelial organ[20] and, due to its functional and anatomical characteristics, it is prone to develop neoplastic and non-neoplastic lesions[21]. Changes in the splenic parenchyma can be detected by conventional ultrasound[10], however, this technique has low accuracy for differentiating malignancy from splenic changes[22]. In our study, there was no difference between the B-mode findings of malignant and benign lesions, which makes this method inconclusive, requiring more accurate techniques, such as cytology or histopathology or magnetic resonance[23].

Ultrasound-guided aspiration puncture is considered invasive and complementary in splenic lesions, but reliable results for the diagnosis of abnormalities are often not obtained[7]. In contrast, histopathological examination is considered the gold standard for diagnosing these conditions as it is capable of providing accurate results[24] and, therefore, it was the method of choice for diagnosing splenic lesions in this study. However, the collection of material for histopathology is more invasive and, in many cases, the delay in obtaining the diagnosis can retard the initiation of adequate therapy and, consequently, worsen the patient's prognosis[24].

ARFI elastography proved to be a non-invasive technique capable of differentiating malignancy in different types of splenic lesions, with excellent predictive values and higher accuracy than other tests such as magnetic resonance which has 94% accuracy for differentiation between malignant and benign splenic lesions in dogs[23,25].

Even if no association of qualitative characteristics (deformity and homogeneity) with malignancy of splenic lesions was observed, it is known that these assessments are evaluator-dependent[26] and, consequently, are subject to different interpretations. However, in this study, it was possible to establish a significant difference in the shear velocity values of malignant and benign lesions, being an effective method to complement the study in B-mode, highly predictive of malignancy and that does not depend on the observer or the observations made and this so that it was possible to establish a cutoff value for the determination of malignancy (> 2.6 m/s).

The applicability of ARFI elastography has grown exponentially in recent years in medicine and veterinary medicine, so that this diagnostic modality can be used, in humans, as a predictor of malignancy in lymph node lesions[15,27], in thyroid[28] and splenomegaly[29,30]. Through this technique, it was already possible to verify that, in cases of breast cancer in bitches, metastatic lymph nodes had a higher shear wave velocity than healthy lymph nodes[31], which is a promising technique in veterinary oncology.

In a previous study[29], it was possible to verify differences in stiffness between spleens of healthy human patients, with hepatoportal, myeloproliferative and infectious diseases, with the control group having the lowest

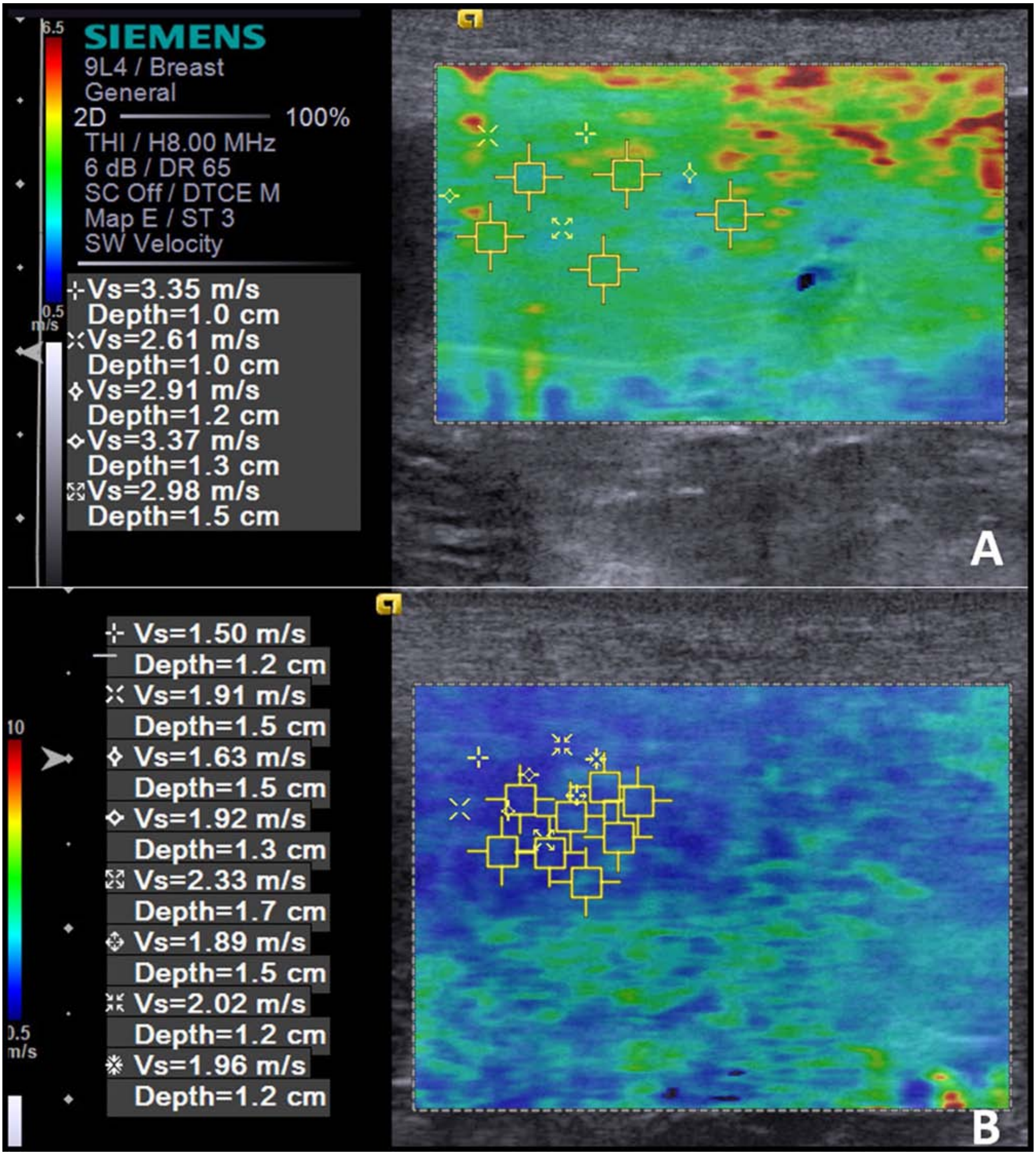

Figure 1. Ultrasonographic image of the spleen of two canine patients, using ARFI elastography (Virtual Touch Tissue Imaging and Quantification Elastography method). (**A**) Spleen with diffuse malignant alteration (multicentric lymphoma) presenting a mean shear velocity of 3.04 m/s; (**B**) Spleen with benign focal alteration (splenic hematoma) presenting an average shear velocity of 1.89 m/s.

shear velocity, while the group with hepatoportal diseases had higher values for this measurement. This result was replicated[30], demonstrating that the technique, in addition to being highly accurate for differentiating pathological processes, has good reproducibility of results.

Even though studies demonstrating the applicability of ARFI elastography in the study of splenic lesions in veterinary medicine have not been published so far, it has already been possible to differentiate malignancy between different mammary tumors of bitches, where malignant lesions were more rigid by ARFI elastography, with a sensitivity of 94.7% and a specificity of 97.2%[17].

The increased stiffness observed in malignant splenic lesions in this study may be correlated with different pathological processes, such as type III collagen deposition, areas of fibrosis or microcalcifications[17], while benign lesions, such as for example hematomas/clots, are basically formed by red blood cells and fibrin[32], causing a decrease in this stiffness, making it even less rigid than in healthy tissue and free from injury.

The use of non-invasive tests without risk to patients and that allow for a differential diagnosis between malignant and benign lesions is of vital importance for animals and humans. Our results demonstrated that ARFI is useful in differentiating malignant and benign lesions in the spleen regardless of their location.

In conclusion, in this preliminary study the ARFI elastography made it possible to differentiate malignant and benign lesions in the spleen of dogs, and it was established that lesions with a shear wave velocity greater than 2.6 m/s are more likely to be malignant, with excellent predictive values. Thus, this imaging technique proved to be superior to conventional ultrasonography, which did not show satisfactory results for this differentiation.

Methods

Ethical aspects. All methods were performed in accordance with the relevant guidelines and regulations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and were approved by the Ethics Committee in the Use of Animals of the São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil (protocol number 014899/19) and follows the recommendations in the ARRIVE guidelines. Thirty-seven dogs of different breeds and ages were included, from the clinical routine of the Institution, during the years 2019 and 2020. For patient selection, the presence of splenic lesions identified prior to the B-mode exam was considered as an inclusion criterion.

Ultrasound and elastographic evaluation. For the B-mode and elastographic examinations, a wide trichotomy of the abdominal region was performed and then the animals were positioned in dorsal and/or lateral decubitus. All exams were performed by the same experienced operator, without knowledge of the animal's clinical history and using an AcusonS2000® (Siemens®, Munich, Germany) ultrasonographic device with convex and linear multifrequency transducers (4.0–9.0 MHz).

In B-mode, the spleen was evaluated in transverse and longitudinal sections along its entire length, aiming to perform a complete tissue scan to identify the presence of lesions. Splenic alterations were classified as focal when there was a nodule in the splenic parenchyma, multifocal when there was more than one delimited area of lesion, or diffuse when there was change in the entire splenic parenchyma. Regarding ultrasonographic features, echogenicity (anechoic, hypoechoic, hyperechoic or mixed), echotexture (homogeneous or heterogeneous), contours/borders (regular or irregular) and organ size were evaluated, in addition to the size of focal lesions (length, width and length/width ratio). In diffuse lesions, the types (multiple circumscribed or disseminated alteration), quantity (when quantifiable) and size of the lesions (length, width and length/width ratio) were determined.

After the B-mode evaluation, ARFI elastography (qualitative and quantitative) was performed using the Virtual Touch Tissue Imaging and Quantification Elastography method, with the same ultrasound device. Qualitative ARFI provided color images (elastogram) that were evaluated through visual deformability (deformable or non-deformable), according to the shades of color observed, with bluish areas indicating soft or deformable tissues (elastic or not very rigid) and reddish shades indicating hard or non-deformable (rigid) tissues. The software itself features image quality control in which homogeneous greenish images indicated high quality of the technique and yellowish and heterogeneous images as low-quality technique. When inappropriate images were obtained, the exam was repeated.

For quantitative ARFI evaluation, tissue elasticity was automatically measured using the shear wave velocity (SWV) of the regions of interest (ROIs) defined by the operator by placing a 2.5 mm^2 calipter on the elastogram images according to the following criteria: In spleens with one or more circumscribed lesions, at least three ROIs (those necessary to include the entire affected area) were selected from the abnormal area to obtain the mean SWV values, taking care to exclude vascular structures and cystic areas as described previously in evaluations of focal liver lesions in humans[32]. In addition, two ROIs were selected in each of the anatomical regions of the spleen (without apparent abnormalities): cranial extremity, body and caudal extremity to calculate the mean SWV of the apparently healthy tissue as already described in dogs[19].

In spleens with diffuse lesions, a minimum of 12 ROIs were included, four in each of the previously defined areas of the splenic parenchyma (cranial, body and caudal), including the largest area of tissue and superficial, middle and deep subregions; for the calculation of the mean SWV of the spleen with diffuse lesion, taking care to exclude vascular structures, areas with a cystic, necrotic or calcified appearance as described by the consensus of the society of radiologists for the evaluation of diffuse liver lesions in humans[33].

Biopsy and splenic histopathological analysis. After ultrasound evaluation, tissue samples were collected, both from normal and altered areas, from all evaluated spleens. Ultrasound-guided incisional biopsy procedures with a trucut needle or surgical excisional biopsy under general anesthesia were performed, according to the protocol defined by the veterinarian responsible for the patient. The collected fragments were fixed in a 10% formalin solution, buffered with phosphate (pH 7.4) and processed until inclusion in paraffin and the prepared slides stained with hematoxylin/eosin. All samples were evaluated by the same experienced pathologist and were classified as malignant neoplastic lesion, benign neoplastic lesion or non-neoplastic lesion; focal or diffuse for each of the categories, totaling 6 types of classification.

Data availability

Statistical analysis was performed using the R software (R® Foundation for Statistical Computing, Vienna, Austria). Initially, the normal distribution (Shapiro–Wilk test) and the homoscedasticity of the variances (Barlett test) were tested. The real or transformed variables were then compared between the different ROIs by region, between regions, between free tissues, benign and malignant lesions, regions between diffuse lesions and medial region in diffuse lesions as well as affected regions between benign and malignant diagnosed by histopathological examination by Student's t-test. Subsequently, the parameters that showed significant differences ($P \leq 0.05$) were submitted to discriminative power analysis (malignant lesions) through the Receiver Operating Characteristic Curves (ROC curve) and the cutoff point, sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under the curve (AUC) were calculated, using the logistic regression model.

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Competing interests
The authors declare no competing interests.

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CONSIDERAÇÕES FINAIS

Com o objetivo de verificar a aplicabilidade da elastografia ARFI na detecção de malignidade tecidual em neoplasias de cães: **1º artigo** – verificou-se que uso da ARFI pode ajudar na diferenciação entre neoplasias mamárias malignas e benignas em cadelas; **2º artigo** – a ARFI quantitativa e qualitativa das lesões testiculares em cães fornece importantes achados para o diagnóstico das principais afecções testiculares nesses animais; **3º artigo** – avaliações ultrassonográficas modo-B e Doppler podem auxiliar na predição de malignidade em tumores mamários de cadela com sensibilidade e especificidade moderadas, porém a velocidade de cisalhamento obtida pela ARFI se apresenta como ótimo preditor para essa finalidade; **4º artigo** – a elastografia ARFI possibilitou verificar as características de carcinomas com alto grau, associando o aumento de rigidez com o tipo especial dessas anormalidades; **5º artigo** – a avaliação ARFI de linfonodos loco-regionais mostrou-se um excelente método diagnóstico, com alta acurácia para diferenciar linfonodos reativos, metastáticos ou alterados, recomendando-se a inclusão da elastografia ARFI na avaliação dos linfonodos regionais de pacientes oncológicos; **6º artigo** – achados como tecidos não deformáveis e valores para velocidade de cisalhamento maiores que 3,52 m/s são indicativos, junto com outros dados ultrassonográficos, de que os nódulos cutâneos e subcutâneos em cães são mais propensos a malignidade; **7º artigo** – a ARFI possibilitou a diferenciação entre tumores malignos e lesões benignas no baço de cães, verificando-se que lesões com velocidade de cisalhamento maior que 2,6 m/s são mais propensas a serem malignas, com excelentes valores preditivos. Diante do exposto, a técnica ARFI tem importante aplicabilidade na detecção da malignidade nas diferentes lesões supracitadas em cães, sendo indicada sua utilização na conduta diagnóstica de pacientes caninos com alterações em glândulas mamárias, linfonodos, pele e subcutâneo, testículos e baço. Salienta-se que outros estudos já estão em andamento na avaliação de diversos tecidos e seus resultados preliminares já demonstram que a ARFI deve ser um dos métodos de imagem de escolha para avaliar de diferentes anormalidades nos animais e auxiliar na detecção de malignidade tecidual na rotina veterinária.

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