UNIVERSIDADE FEDERAL DE UBERLÂNDIA
PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA E PARASITOLOGIA APLICADAS

GUSTAVO CAVINATO HERRERA

ALTERAÇÕES ECOCARDIOGRÁFICAS DE CÃES SUBMETIDOS A PROTOCOLOS QUIMIOTERÁPICOS COM DOXORRUBICINA

UBERLÂNDIA
2025

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA E PARASITOLOGIA APLICADAS

GUSTAVO CAVINATO HERRERA

ALTERAÇÕES ECOCARDIOGRÁFICAS DE CÃES SUBMETIDOS A PROTOCOLOS QUIMIOTERÁPICOS COM DOXORRUBICINA

Tese apresentada ao Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas da Universidade Federal de Uberlândia, como parte dos requisitos para a obtenção do título de Doutor em Imunologia e Parasitologia Aplicadas.

Orientador: Prof. Dr. Marcelo José Barbosa Silva

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

H565 Herrera, Gustavo Cavinato, 1978-
2025 Alterações ecocardiográficas de cães submetidos a protocolos quimioterápicos com doxorrubicina [recurso eletrônico] : Cardiotoxicidade associada a quimioterapia / Gustavo Cavinato Herrera. - 2025.

Orientador: Marcelo José Barbosa Silva.
Tese (Doutorado) - Universidade Federal de Uberlândia, Pós-graduação em Imunologia e Parasitologia Aplicadas.
Modo de acesso: Internet.
DOI http://doi.org/10.14393/ufu.te.2025.523
Inclui bibliografia.
Inclui ilustrações.

1. Imunologia. I. Silva, Marcelo José Barbosa,1976-, (Orient.). II. Universidade Federal de Uberlândia. Pós-graduação em Imunologia e Parasitologia Aplicadas. III. Título.

CDU: 612.017

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Imunologia e Parasitologia Aplicada
Av. Amazonas, s/n, Bloco 4C, Sala 4C218 - Bairro Umuarama, Uberlândia-MG, CEP 38400-902
Telefone: (34) 3225-8672 - www.imunoparasito.ufu.br - coipa@ufu.br

ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Imunologia e Parasitologia Aplicadas				
Defesa de:	Tese de Doutorado, número 174 do PPGIPA				
Data:	Vinte e seis de setembro de dois mil e vinte e cinco	Hora de início:	14 h	Hora de encerramento:	16 h 33 m
Matrícula do Discente:	12023IPA007				
Nome do Discente:	Gustavo Cavinato Herrera				
Título do Trabalho:	Alterações ecocardiográficas de cães submetidos a protocolos quimioterápicos				
Área de concentração:	Imunologia e Parasitologia Aplicadas				
Linha de pesquisa:	Imunobiologia dos processos alérgicos, autoimunes e tumorais				
Projeto de Pesquisa de vinculação:	Avaliação ecodopplercardiográfica de cães submetidos a protocolos quimioterápicos				

Reuniu-se no dia vinte e seis de setembro de dois mil e vinte e cinco, de forma remota, na Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Imunologia e Parasitologia Aplicadas, assim composta: Professores(as) Doutores(as): Christine Souza Martins - FAV/UnB; Thaisa Reis dos Santos - Uniube; Matias Pablo Juan Szabó - UFU; Marcelo Emílio Beletti - UFU e Marcelo José Barbosa Silva - UFU, presidente e orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da mesa, Dr(a). Marcelo José Barbosa Silva, apresentou a Comissão Examinadora e o(a) candidato(a), agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovado(a).

Esta defesa faz parte dos requisitos necessários à obtenção do título de Doutor.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

Documento assinado eletronicamente por **Marcelo José Barbosa Silva**, **Professor(a) do Magistério Superior**, em 26/09/2025, às 16:35, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Marcelo Emílio Beletti**, **Professor(a) do Magistério Superior**, em 26/09/2025, às 16:38, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Matias Pablo Juan Szabo**, **Professor(a) do Magistério Superior**, em 26/09/2025, às 16:39, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Thaisa Reis dos Santos**, **Usuário Externo**, em 26/09/2025, às 17:11, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Christine Souza Martins**, **Usuário Externo**, em 02/10/2025, às 10:51, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **6648924** e o código CRC **F65439A9**.

Referência: Processo nº 23117.061388/2025-70 SEI nº 6648924

Resumo

A doxorrubicina é um fármaco utilizado com frequência em protocolos quimioterápicos na medicina veterinária tanto por sua eficácia quanto pelo custo acessível. Seus efeitos cardiotóxicos são conhecidos e, portanto, a busca por uma utilização segura desta droga se faz necessária. Realizamos uma revisão sistemática e meta-análise com o objetivo avaliar os efeitos cardiotóxicos do fármaco na fração de ejeção de cães em protocolos de doxorrubicina. Treze artigos demonstraram alterações cardíacas na ecocardiografia com diferentes vias de administração (intravenosa e intracoronária). A meta-análise mostrou que este fármaco diminuiu a função cardíaca com uma redução média de 21,24% na fração de ejeção (FE). Uma pesquisa foi feita com 33 animais atendidos no Hospital Veterinário da Universidade Federal de Uberlândia e em clínicas conveniadas, submetidos a diferentes protocolos quimioterápicos. Os exames foram realizados em quatro momentos (T0 a T3), mensurando parâmetros como fração de ejeção, pelo método de Teicholz (FET) e Simpson (FES), fração de encurtamento (FS) e dimensões do ventrículo esquerdo na diástole e sístole. Utilizou-se modelo linear de efeitos mistos para análise estatística, considerando o tempo, uso de doxorrubicina, presença de cardiopatia prévia e suas interações. Os resultados demonstraram reduções estatisticamente significativas nos valores dos parâmetros ecocardiográficos em cães tratados com doxorrubicina, com ou sem associação a outros quimioterápicos. Essas reduções mantiveram-se dentro dos limites fisiológicos de referência para a espécie, não sendo observado disfunção cardíaca clínica. A degeneração mixomatosa crônica da valva mitral não influenciou o aparecimento de cardiotoxicidade. A idade dos cães influenciou a magnitude das alterações, com maior susceptibilidade observada em animais adultos. Os achados indicam que a cardiotoxicidade da doxorrubicina tem comportamento cumulativo e pode ser detectada precocemente por meio de monitoramento ecocardiográfico, com atenção aos pacientes que apresentarem tendência de queda da função cardíaca, ainda que esses valores permaneçam dentro dos limites de normalidade. Conclui-se que a doxorrubicina é segura quando utilizada sob acompanhamento cardiovascular, sendo recomendável a vigilância seriada de parâmetros ecocardiográficos, mesmo na ausência de sinais clínicos evidentes de cardiopatia.

Palavras-Chave: cães, doxorrubicina, ecocardiograma

Abstract

Doxorubicin is a chemotherapeutic drug widely used in veterinary medicine due to its efficacy and affordability. Its cardiotoxic effects are well recognized, making it essential to seek strategies for its safe use. We conducted a systematic review and meta-analysis to evaluate the effects of this drug on the ejection fraction (EF) of dogs undergoing doxorubicin-based protocols. Thirteen studies reported echocardiographic alterations regardless of the route of administration (intravenous or intracoronary). The meta-analysis revealed an average EF reduction of 21.24%, underscoring its adverse impact on cardiac function. Additionally, a clinical study was conducted with 33 dogs treated at the Veterinary Hospital of the Federal University of Uberlândia and affiliated clinics, who were subjected to various chemotherapy protocols. The animals were evaluated at four time points (T0 to T3), with measurements of ejection fraction using the Teicholz (EFT) and Simpson (EFS) methods, fractional shortening (FS), and left ventricular dimensions in diastole and systole. Statistical analysis was performed using a linear mixed-effects model, considering time, doxorubicin use, pre-existing heart disease, and their interactions. The results demonstrated statistically significant reductions in echocardiographic parameters in dogs treated with doxorubicin, either alone or in combination with other chemotherapeutic agents. Despite these changes, values remained within the physiological reference range for the species, and no clinical signs of cardiac dysfunction were observed. Chronic myxomatous mitral valve disease did not influence the occurrence of cardiotoxicity. Age, however, was a relevant factor, with adult dogs showing greater susceptibility. These findings suggest that doxorubicin cardiotoxicity exhibits a cumulative behavior and can be detected early through echocardiographic monitoring, particularly in patients exhibiting a trend toward declining cardiac function, even when values remain within normal limits. We conclude that doxorubicin is safe when used under cardiovascular monitoring, and serial surveillance of echocardiographic parameters is recommended, even in the absence of clinical signs of heart disease.

Keywords: dogs; doxorubicin; echocardiography

Conteúdo

Lista de Figuras

Lista de Tabelas

Agradecimentos

Agradeço em primeiro lugar a Deus, fonte de vida, sabedoria e inspiração. Ao meu orientador. Prof. Dr. Marcelo José Barbosa Silva por ter aberto as portas do laboratório para o desenvolvimento deste trabalho. Obrigado por todo conhecimento compartilhado e pela verdadeira orientação durante todas as fases de execução. Sua dedicação e comprometimento foram inspiradores. Agradeço à minha esposa Keni, pelo incentivo, suporte nos momentos difíceis e por sempre acreditar em mim. Você é o amor da minha vida! Aos meus filhos, Giovanna, Davi e Samuel que me impulsionam a buscar forças e sempre seguir em frente para dar o melhor que posso a vocês! Aos meus pais Nivaldo (em memória) e Neta, que proporcionaram o que eu precisava para que tudo pudesse se iniciar, desde a escola até a graduação.

1 Fundamentação teórica

1.1 Neoplasia em cães

A prevalência de pacientes oncológicos na medicina veterinária vem aumentando de forma considerável, fato este associado ao aumento da expectativa de vida dos animais, à melhora na alimentação e ao avanço da capacidade diagnóstica, relacionada ao desenvolvimento tecnológico dos exames complementares e à maior especialização dos profissionais (De Nardi, A. B. et al., 2002).

As neoplasias, caracterizadas pelo crescimento e multiplicação desordenados das células, podem ser classificadas em benignas e malignas. Tumores benignos geralmente permanecem localizados, não invadindo tecidos circundantes, enquanto os tumores malignos têm a capacidade de infiltrar tecidos adjacentes, vasos sanguíneos e linfonodos, requerendo, portanto, procedimentos e tratamentos mais complexos (Yadav, P. et al., 2025).

Diferentes tipos de neoplasias acometem os animais, e fatores como sexo, idade e raça podem auxiliar no diagnóstico. Pacientes idosos são mais predispostos, principalmente acima de dez anos. Fêmeas apresentam maior predisposição, sendo os tumores mamários os mais comuns. Dentre os órgãos mais afetados estão a pele e o subcutâneo, sendo o mastocitoma o mais frequente. A neoplasia sistêmica mais comum é o linfossarcoma, e o osteossarcoma acomete principalmente animais de maior porte (De Nardi, A. B. et al., 2002).

Uma variedade de técnicas e tecnologias está disponível para diagnóstico preciso e conduta terapêutica assertiva para os diferentes tipos de neoplasias caninas. Dentre as principais, destacam-se os exames laboratoriais e de imagem por sua sensibilidade e acurácia. Ecografia, tomografia e radiografia estão entre as técnicas de imagem mais utilizadas. Nos exames laboratoriais a citologia, histopatologia, PCR, imunohistoquímica e exames de sangue de rotina são empregados com frequência (Yadav, P. et al., 2025).

1.2 Quimioterapia em cães

A quimioterapia é utilizada na rotina de pacientes veterinários, sendo as drogas citotóxicas empregadas tanto para a remissão completa de neoplasias sistêmicas, como o linfoma, quanto para redução tumoral e aumento da expectativa e qualidade de vida dos animais com cânceres metastáticos, como o osteossarcoma (MacDonald, V., 2009).

A associação de duas ou três drogas citostáticas compõe a maioria dos protocolos quimioterápicos em medicina veterinária. Nas neoplasias mamárias em cadelas, a ciclofosfamida, a doxorrubicina e o 5-fluorouracil são os fármacos mais utilizados (Cirillo, J. V., 2008). Em tumores hemolinfáticos e no tumor venéreo transmissível (TVT), o sulfato de vincristina é amplamente empregado, sendo associado à ciclofosfamida e prednisolona no primeiro caso (Faro, A. M., 2008).

As drogas citostáticas caracterizam-se por apresentar o menor índice terapêutico em relação a outros fármacos, gerando, assim, inúmeros efeitos colaterais. Dentre os efeitos gerais podem ocorrer supressão da medula óssea, alterações gastroentéricas e alopecia. Alguns fármacos apresentam efeitos específicos, como a doxorrubicina, que é cardiotóxica; a cisplatina, nefrotóxica; e a ciclofosfamida, que causa irritação na parede da vesícula urinária (MacDonald, V., 2009).

Os diversos efeitos colaterais que podem acometer os pacientes oncológicos são fatores que limitam o seu uso e, consequentemente, reduzem o efeito do tratamento. Pacientes neutropênicos podem ter o tratamento interrompido ou necessitar da redução em 20% da dose do fármaco, o que pode diminuir em até 50% a sua eficácia (MacDonald, V., 2009). A mesma autora relata alterações gastroentéricas relacionadas ao uso da cisplatina, fazendo-se necessário, nesses casos, o uso de antieméticos antes da administração do fármaco, evitando-se assim quadros severos de desidratação. Portanto, o acompanhamento desses pacientes durante os protocolos quimioterápicos faz-se necessário para avaliação da presença e intensidade dos efeitos colaterais.

1.3 Doxorrubicina

A doxorrubicina pertence à classe das antraciclinas, sendo um antibiótico antitumoral utilizado no tratamento de neoplasias em humanos e animais. Linfoma, osteossarcoma e carcinomas são patologias para as quais esse fármaco pode ser prescrito, podendo ser administrado isoladamente ou em associação com outras drogas (Hallman et al., 2019).

A cardiotoxicidade da doxorrubicina já foi relatada em diversas espécies. Em humanos, esse efeito é dose-dependente, sendo recomendada, em alguns casos, a descontinuidade do tratamento ou até mesmo sua contraindicação (Costa, F. S., 2008). A doxorrubicina tem potencial para causar lesão no miocárdio, levando à necrose da musculatura cardíaca com consequente perda da função sistólica e dilatação, alterações essas observadas por meio do ecodopplercardiograma.

As alterações histológicas causadas pela doxorrubicina incluem degeneração vacuolar citoplasmática, miocitólise, atrofia de miócitos e fibrose. O reflexo dessas alterações

é a dilatação atrioventricular esquerda, gerando insuficiência cardíaca congestiva, arritmias e morte súbita (Silva, C. E. V., 2005). Os efeitos cardiotóxicos relacionados a outros fármacos quimioterápicos não foram descritos em medicina veterinária.

O efeito cumulativo da doxorrubicina tem papel importante tanto no aparecimento quanto na intensidade das lesões cardíacas. A forma de administração (bolus ou infusão contínua) e a via (endovenosa ou intracardíaca), assim como a associação com outros fármacos, também podem potencializar seus efeitos na musculatura cardíaca (Alvares-Cardona, J. e Lenihan, D. J., 2019).

Os efeitos cardiotóxicos da doxorrubicina não impedem seu uso e, portanto, ela continua sendo uma opção para o tratamento de cães com câncer. Aqueles que defendem seu uso justificam-se pela efetividade contra as células neoplásicas e pela possibilidade de monitoramento cardiológico dos pacientes durante o protocolo (Hallman, B. E. et al., 2018).

1.4 Ecodopplercardiograma

O ecocardiograma é um método de diagnóstico por imagem não invasivo, capaz de mensurar o diâmetro das câmaras cardíacas, a espessura da musculatura e das valvas, além de avaliar aspectos hemodinâmicos e as funções sistólica e diastólica (Boon, 2011). Esse exame é amplamente utilizado em medicina veterinária, sendo descritos valores de referência para diversas faixas de peso e raças de cães.

Por meio da avaliação ecocardiográfica, o médico-veterinário é capaz de detectar e quantificar alterações cardíacas, instituir terapêutica apropriada e, diante de cardiopatias, determinar o prognóstico do paciente. As medidas obtidas em modo M e B, utilizadas em exames de rotina, permitem dimensionar o diâmetro, a área e o volume das câmaras cardíacas (Visser, L. C. et al., 2019).

A avaliação da fração de encurtamento e de ejeção tem sido utilizada para o acompanhamento de pacientes submetidos a protocolos quimioterápicos com doxorrubicina, mas essa abordagem é considerada insensível para detectar pequenas alterações, especialmente aquelas que ocorrem de forma rápida e podem ser irreversíveis (Gallay-Lepoutre, J. et al., 2016).

Novas técnicas têm surgido com o objetivo de melhorar a acurácia na detecção de alterações sistólicas. Dentre elas, destaca-se o Doppler tecidual, que quantifica a velocidade intramiocárdica, mostrando-se superior à avaliação convencional. Outra técnica é o *speckle tracking*, que quantifica a função sistólica por meio da interação entre o feixe de ultrassom e as fibras do miocárdio, avaliando a taxa de deformação

dos diferentes segmentos miocárdicos (Mantovani et al., 2015).

2 Objetivos

2.1 Objetivo geral

Avaliar através do ecodopplercardiograma cães submetidos a quimioterapia, provenientes do setor de clínica e cirurgia oncológica (SECCON) do Hospital Veterinário da Universidade Federal de Uberlândia, realizando exames pré e pós medicação, obtendo assim informações sobre estas drogas no sistema cardiovascular.

2.2 Objetivos específicos

- Avaliar periodicamente de pacientes submetidos à quimioterapia;
- Avaliar os efeitos deletérios na função sistólica e diâmetro das câmaras cardíacas das drogas utilizadas na quimioterapia com doxorrubicina.
- Verificar se a presença de doença cardíaca prévia foi fator agravante à cardiotoxicidade;
- Comparar os dados obtidos com a revisão sistemática realizada durante o período de coleta de dados.

Capítulo 1

Artigo publicado

3 Capítulo 1: artigo publicado

Veterinary Medicine and Science

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Dogs

Impact of Doxorubicin on Cardiac Function in Dogs: Ejection Fraction Changes and Heart Failure Risk

Gustavo Cavinato Herrera[1] | Luiz Ricardo Soldi[1] | Leandro Machado Oliveira[2] | Luiz Renato Paranhos[3] | Marcelo José Barbosa Silva[1]

[1]Institute of Biomedical Science, Federal University of Uberlândia—UFU, Uberlândia, Minas Gerais, Brasil | [2]Oral Sciences/Periodontics at Universidade Federal de Santa Maria, Santa Maria, Brasil | [3]School of Dentistry, Federal University of Uberlândia—UFU, Uberlândia, Minas Gerais, Brasil

Correspondence: Gustavo Cavinato Herrera (gustavocherrera@ufu.br)

Received: 27 November 2024 | **Accepted:** 27 June 2025

Funding: The authors received no specific funding for this work.

Keywords: doxorubicin | echocardiography | ejection fraction

ABSTRACT

Doxorubicin is an antitumor antibiotic. It is often used in veterinary medicine to treat and extend the lives of dogs with cancer. A cardiotoxic side effect can lead to heart failure and treatment discontinuation. This systematic review and meta-analysis aimed to evaluate the drug's cardiotoxic effects on the ejection fraction (EF) of dogs in doxorubicin protocols. The search was done in eight databases, with a total of 3587 articles screened, resulting in fifteen eligible articles included. A report on the included studies' methods and results was done. It also assessed the risk of bias. Thirteen articles demonstrated cardiac changes in echocardiography with different routes of administration (intravenous and intracoronary). The intracoronary route was more toxic, and in all six studies performed, there was heart failure. The intravenous route caused heart failure in six of the nine studies. A meta-analysis showed this drug worsened heart disease. It included four studies where it significantly lowered the EF. Overall, the intervention produced a mean reduction of 21.24% in EF. This review shows doxorubicin's impact on cardiac function. It reveals the need for careful monitoring of each patient.

1 | Introduction

The prevalence of cancer in veterinary medicine has been increasing considerably, a fact associated with the increase in life expectancy of animals, improvements in nutrition and increased diagnostic capacity due to the technological development of complementary exams and greater specialization of professionals (Nardi et al. 2002). Chemotherapy is used in the routine of veterinary cancer patients, with cytotoxic drugs used for the goal of complete remission of systemic neoplasms, such as lymphoma, reduction of tumours and increase in life expectancy and quality of life of animals with metastatic cancers (Macdonald 2009).

Malignant neoplasms such as lymphoma, osteosarcoma, and carcinomas respond to doxorubicin treatment, an antitumor antibiotic widely used in veterinary medicine (Hallman et al. 2019). This drug generates the formation of free radicals that can damage the cell membrane and DNA, altering their function (Pereira Neto et al. 2006). The cardiotoxicity of doxorubicin has been reported in several species, including humans. This effect has been described as dose-dependent, with treatment being recommended in some cases or contraindicated in others (Costa et al. 2008), because doxorubicin has the potential to cause myocardial injury, leading to necrosis of the cardiac muscle with consequent loss of systolic function and dilation of the

chambers, with these changes being observed through Doppler echocardiography.

Echocardiography is a non-invasive imaging diagnostic method capable of measuring the diameter of the heart chambers, the thickness of the muscles and valves, and evaluating haemodynamic aspects and systolic and diastolic functions (Boon 2011). This test is widely used in veterinary medicine, and reference values for the various weight ranges and breeds of dogs have been described. Symptomatic cardiotoxicity in dogs treated with doxorubicin is not a consensus, and the dose used, the residual effect, and the patient's condition are factors that influence the presence of these signs. Thus, this systematic review aims to analyse the experimental studies that treated dogs with doxorubicin and identify the main echocardiographic changes observed in the studies.

2 | Methods

2.1 | Registration and Protocol

The project was initially registered with the Open Science Framework (OSF) (registration available at: https://osf.io/esa4k/) to promote transparency and reproducibility of the study. This systematic review followed the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) (Page et al. 2021) and was conducted following the Joanna Briggs Institute Manual (JBI) (JBI Manual for Evidence Synthesis).

2.2 | Question and Eligibility Criteria

This systematic review was conducted to answer the question: 'What are the echocardiographic alterations found in dogs undergoing doxorubicin treatment?', following the PVO (population, variables and outcomes) structuring strategy. The strategy developed consists of (1) population: dogs; (2) variable: doxorubicin treatment and (3) outcomes: echocardiographic parameters.

2.2.1 | Inclusion Criteria

Studies that performed doxorubicin treatment in dogs were included. This inclusion considered studies that administered doxorubicin in isolation, allowing direct evaluation of its effects. Among these studies, those that presented echocardiographic evaluation before, during and after treatment were included, allowing a longitudinal study of each case. Both prospective randomized and non-randomized studies were considered. No date restriction was applied to this systematic review and only articles in language of Anglo-Saxon or Latin origin were included.

2.2.2 | Exclusion Criteria

Review articles, editorials, letters and opinion pieces were excluded. Studies lacking echocardiographic data before, during, and after treatment were also excluded, as these data are essential to assess doxorubicin's cardiotoxicity accurately. Pre-existing heart conditions could affect results, and the cumulative effect of doxorubicin is a major contributor to cardiotoxicity. Finally, studies without a control group in tests involving other drug combinations and articles in languages other than Latin or Anglo-Saxon were excluded.

2.3 | Sources of Information, Research, and Selection of Studies

Electronic searches were performed in the following databases: BMC, Embase, Lilacs, Livivo, Medline (PubMed) and the VHL Regional Portal. For the search in the gray literature, Google Scholar and Open Access Theses and Dissertations (OATD) were used.

Search strategies with Boolean markers 'AND' and 'OR' were performed, respecting the syntax rules of each database (Table 1). The results obtained were exported to EndNote WebTM software (Clarivate Analytics, Philadelphia, USA), in which duplicates were automatically removed. The remaining results were exported to the online tool Rayyan QCRI (Qatar Computing Research Institute, Doha, Qatar) for the study screening phase.

Two reviewers systematically analysed all selected articles in three phases. In the first phase, two eligibility reviewers (GCH and LRS) methodically analysed the titles, followed by the abstracts of the studies. Disagreements between examiners were analysed, and the decision was made by a third examiner (MJBS). In the first phase, articles unrelated to the topic, studies with other species, and abstracts that did not meet the eligibility criteria were excluded. In the third phase, full texts of the preliminary eligible studies were obtained and evaluated. Articles that did not present the echocardiographic results before, during and after treatment with doxorubicin were excluded in this phase as well.

2.4 | Assessment of Sponsorship Status

To assess the risk of bias in the studies included in this review, the sources of sponsorship were investigated, as studies sponsored by pharmaceutical companies may be favourable to the product or drug compared to other sponsoring sources. To classify the sponsorship status, the following definition was used: unclear: articles that did not contain sponsorship information, making it impossible to identify whether they were sponsored; not sponsored: articles in which the authors declare that they do not have financial support; and sponsored: articles that contained statements by the authors of financial support from pharmaceutical companies.

2.5 | Risk of Bias of the Studies

Using the JBI Systematic Reviews tool for randomized clinical trials (RCT) and uncontrolled clinical trials (UCT) (JBI Manual for Evidence Synthesis), the risk of bias in each article was assessed. Each question evaluated was classified as 'yes' when there was no bias for the question, 'no' if the study was biased for the question, 'unclear' when the information was not sufficient to answer that question and 'not applicable' when the question was not appropriate for that study.

TABLE 1 | Strategies for databases search.

Database	Search strategy
BMC veterinary	'Dogs' OR 'Canine' AND 'doxorrubicin' OR 'anthracycline' AND 'cardiotoxicity' OR 'cardiac disease' OR 'cardiomiopathy' OR 'left ventricular function' AND 'Echocardiogram' OR 'Echodopplercardiogram'
Embase	('Dogs'/exp OR 'dogs' OR 'canine'/exp OR 'canine') AND ('chemotherapy'/exp OR 'chemotherapy' OR 'doxorrubicin' OR 'adriamycin'/exp OR 'adriamycin' OR 'anthracycline'/exp OR 'anthracycline') AND ('echocardiogram'/exp OR 'echocardiogram' OR 'echodopplercardiogram')
Lilacs	('Dogs' OR 'canine') AND ('doxorrubicin' OR 'anthracycline') AND ('cardiotoxicity' OR 'cardiac disease' OR 'cardiomiopathy' OR 'left ventricular function')
Livivo	'Dogs' OR 'canine' AND 'doxorrubicin' OR 'anthracycline' AND 'cardiotoxicity' OR 'cardiac disease' OR 'cardiomiopathy' OR "left ventricular function' AND 'echocardiogram' OR 'echodopplercardiogram' dogs
Medline-PubMed	'Dogs' OR 'canine' AND 'doxorrubicin' OR 'anthracycline' AND 'cardiotoxicity' OR 'cardiac disease' OR 'cardiomiopathy' OR 'left ventricular function' AND 'echocardiogram' OR 'echodopplercardiogram' OR 'echodopplercardiography'
Portal regional da BVS	('Dogs' OR 'canine' AND 'doxorrubicin' OR 'anthracycline') AND ('cardiac disease')
Gray literature	
Google scholar	'Dogs' OR 'canine' AND 'doxorrubicin' OR 'anthracycline' AND 'cardiotoxicity' OR 'cardiac disease' OR 'cardiomiopathy' OR 'Left ventricular function' AND 'echocardiogram' OR 'echodopplercardiogram'
OATD	(['Dogs' OR 'Canine'] AND ['doxorrubicin' OR 'anthracycline'] AND [cardiomiopathy])

Abbreviation: OATD, Open Access Theses and Dissertations.

2.6 | Data Analysis

Data were summarized in evidence tables to determine study characteristics. Differences in EF were analysed using a pair-wise meta-analysis format. The results for FS were not suitable for meta-analysis due to significant methodological heterogeneity. When change scores were not reported, they were calculated by subtracting the final from baseline scores and estimating the change in standard deviation (SD) using the formula $SD_{delta} = \sqrt{[(SD_{baseline})^2 + (SD_{final})^2 - (2 \times r \times SD_{baseline} \times SD_{final})]}$, in which we assumed $r = 0.5$ (Higgins et al. 2019). The mean difference (MD) was defined along with 95% confidence intervals (CI) as a summary estimate. All tests were two-tailed, with a significance level set at 0.05—except Cochran's Q test (significance level at 0.1) which was used to assess the presence of heterogeneity and quantified it using the I^2 statistic, with values of 75%–100% indicating high heterogeneity. High heterogeneity was anticipated; therefore, a random-effects model with the DerSimonian and Laird variance estimator was used (DerSimonian and Laird 1986). Prediction intervals for pooled MDs were estimated to provide a range of expected effects (Borenstein et al. 2017). Heterogeneity was explored through subgroup analyses according to the cumulative dose. All analyses were performed using Stata, version 14.0 (Stata Corporation).

As fewer than 10 studies were included in this meta-analysis, formal quantification of publication bias and assessment of heterogeneity using meta-regression were not performed.

3 | Results

3.1 | Study Selection

An electronic search across eight databases, including gray literature, generated a total of 3587 results. After removing 150 duplicates—144 electronically and 11 manually—3364 articles were excluded on the basis of the title review. Following a thorough evaluation of the abstracts, an additional 34 articles were eliminated, leaving 37 articles for qualitative assessment. Ultimately, 15 studies met the established eligibility criteria and were included in this systematic review (Figure 1) (Styles et al. 1983; Unverferth et al. 1985; Hanai et al. 1996; Vaynblat et al. 1997, 2002; Shah et al. 1997; Toyoda et al. 1998; Christiansen et al. 2002; Eya et al. 2005; Alves de Souza and Camacho 2006; Sousa et al. 2014; Tater et al. 2017; Beaumier et al. 2020; Matsuura et al. 2021; Surachetpong et al. 2016) (Figure 2).

3.2 | Characteristics of the Included Studies

The articles were published between 1983 and 2023 and conducted in six different countries: five in the United States of America, three in Germany, two in Japan, two in Brazil, one in Canada, and one in Thailand. According to the experimental design, nine studies were randomized and seven were non-randomized. In the 15 eligible studies, 189 dogs participated in the research.

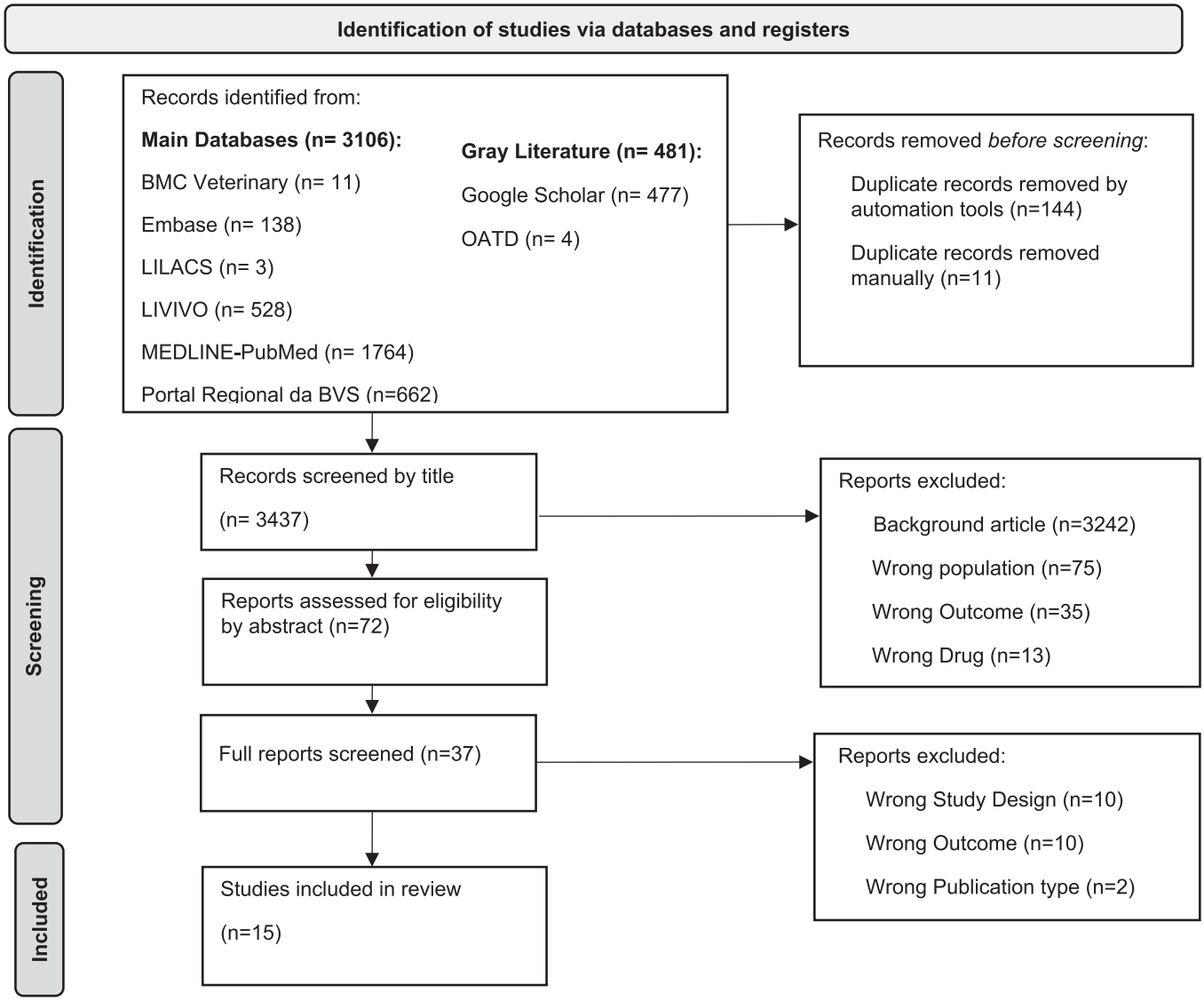

FIGURE 1 | Database search and record selection flow diagram. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources. OATD, Open Access Theses and Dissertations. *Source:* Page et al. (2021). https://doi.org/10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/.

Two different doxorubicin route applications were assessed: intravenous (nine studies) and intracoronary (six studies). A dose of 1.5 mg/kg was used in one study using the intravenous route; in the other eight studies, the dose was 1 mg/kg (30 mg/m^2). The dose administered intracoronarily ranged from 0.25 mg/kg (three studies), 0.7 mg/kg (two studies) and 10 mg/animal (mean of 0.30 mg/kg) (one study). Applications were administered weekly or at 3-week intervals, with treatment duration ranging from 3 to 12 applications.

3.3 | Individual Study Results

Of the 15 articles eligible for this systematic review, 13 reported cardiac changes after doxorubicin administration. Decreased function was the main change found, with the shortening fraction (FS) and ejection fraction (EF) as variables, representing impaired cardiac muscle contractility, reported in 100% of the studies with echocardiographic changes. The increase in the internal diameter of the heart, described in 10 articles, appears to be another important variable. The intracoronary route for administration of doxorubicin was shown to be more toxic, and in all six studies performed, there was cardiac dilation with decreased function. The intravenous route caused heart failure in six of the nine studies. The main echocardiographic findings are shown in Table 2.

3.4 | Meta-Analysis

The meta-analysis included four studies, with a total of 79 animals. Overall, the intervention produced a mean reduction of 21.24% in EF (95% CI: 13.63%, 28.85%; I^2 = 83.3%). When stratifying the analysis according to the cumulative dose, the mean reductions in EF levels were similar; however, heterogeneity in the 3.5 mg/kg subgroup was important (I^2 = 90.5%). We expect that in some 95% of intervals of all populations, the true MD falls in the approximate range of −12.52% to 55.01% (Figure 2).

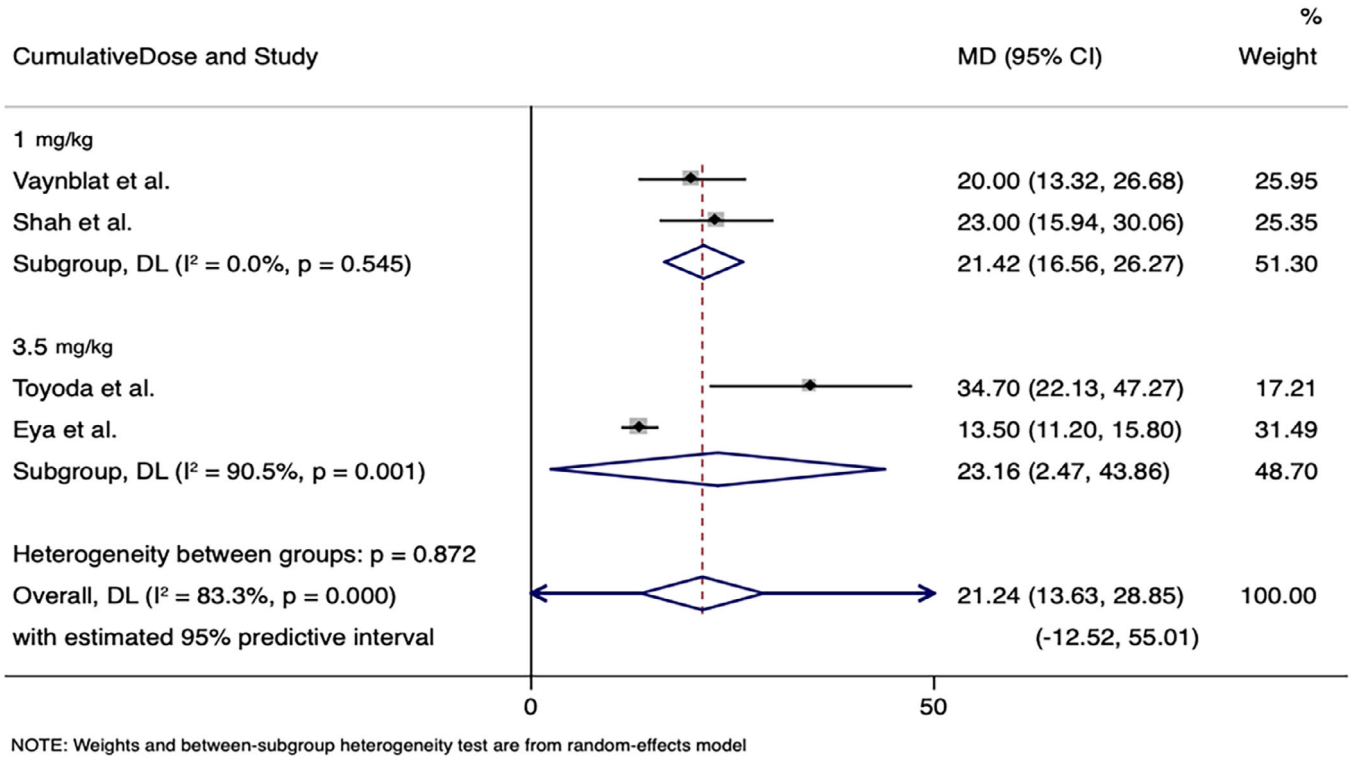

FIGURE 2 | Meta-analysis of the included studies. CI, confidence intervals; MD, mean difference.

3.5 | Risk of Bias for Each Study

Information on the risk of bias is shown in Table 3. The articles were classified as RCT and quasi-experimental studies. Overall, most studies presented a low risk of bias, one study a high risk, and six a moderate risk.

3.6 | Assessment of the Sponsorship Status of the Studies

Table 4 presents the results of the assessment of the sources of sponsorship of the studies included in this review. In eight articles, the sources of sponsorship were research institutes; in the remaining seven studies, clear information on the sponsorship status was not declared. No studies declared sponsorship by pharmaceutical companies.

4 | Discussion

Doxorubicin, an anthracycline widely used in antineoplastic treatment in dogs, is known for its cardiotoxic effect, causing cellular damage that leads to cardiac dilation and loss of function (Surachetpong et al. 2016). Haemodynamic echocardiography is safe and can detect cardiac injury caused by cardiotoxic medications (Hanton et al. 2008). EF, which is characterized by the percentage of blood ejected by the left ventricle in each cardiac cycle, is an echocardiographic parameter used to measure myocardial function and was shown to be reduced in 11 of the 15 studies evaluated. This finding corroborates the importance of its evaluation in patients undergoing chemotherapy protocols with this drug. Doxorubicin is known to cause several forms of cardiac injury, including myocardial fibrosis, atrophy, and myocyte lysis (Mauldin et al. 1992). This damage leads to stiffening of the cardiac muscle fibre, reducing its capacity and number. This decrease in both the functionality and quantity of heart muscle cells diminishes contractility, which is often measured by a decrease in BF. which was observed in eight of the 15 studies analysed in this review. This finding of reduced BF was consistent across eight of the 15 studies analysed in this review.

Antineoplastic antibiotics act on cell DNA, inhibiting its replication, and their main drugs are doxorubicin, mitoxantrone, actinomycin, bleomycin and epirubicin. Due to differences in price and effectiveness, there is a need for studies that evaluate and compare these drugs. Mitoxantrone, for example, has a mechanism of action and effectiveness like doxorubicin albeit with milder side effects when compared (Franco et al. 2019). One study compared the cytotoxic effects of mitoxantrone with doxorubicin, with the former presenting no evidence of cardiac changes while the latter did, though gastrointestinal effects and myelosuppression were observed with mitoxantrone (Henderson et al. 1989). Its use in veterinary medicine is still limited due to its high cost, which can be up to four times higher when compared to doxorubicin. Being an available alternative, more studies with this drug must be carried out, making it a valid option, especially for patients with previous heart disease.

In the included studies that used the intracoronary route, all dogs developed heart failure, as expected, as the objective was to evaluate this outcome (Vaynblat et al. 1997, 2002; Shah et al. 1997; Toyoda et al. 1998; Christiansen et al. 2002; Eya et al. 2005). The greater toxicity can be explained by the higher concentration of the drug in the heart muscle as the application is made directly through the implantation of specific devices. The use

TABLE 2 | Characteristics of eligible studies.

References	Sample size	DOXO administration	Dose	Echocardiographic findings	EF		FS		Total cumulative dose
Styles et al. (1983)	9	Intra venous infusions	1 mg/kg	↓function	↓24%	75–57	—	—	12 mg/kg
Unverferth et al. (1985)	38	Intra venous infusions	1 mg/kg	↓function, ↑internal diameter	—	—	↓38%	—	12 mg/kg
Hanai et al. (1996)	12	Intra venous infusions	1.5 mg/kg	↓function	—	—	↓65%	50—16	10.5 mg/kg
Vaynblat et al. (1997, 2002)	10	Intracoronary	0.25 mg/kg	↓function, ↑internal diameter	↓33%	60—40	—	—	1 mg/kg
Shah et al. (1997)	20	Intracoronary	0.25 mg/kg	↓function, ↑internal diameter	↓39%	60—37	—	—	1 mg/kg
Toyoda et al. (1998)	9	Intracoronary	0.7 mg/kg	↓function, ↑internal diameter	↓43%	71–36.3	↓40%	36.5–21.7	3.5 mg/kg
Christiansen et al. (2002)	6	Intracoronary	10 mg/animal	↓function, ↑internal diameter	↓50%	61.1–30.5	↓33%	41.7–27.9	40 mg/animal
Vaynblat et al.	17	Intracoronary	0.25 mg/kg	↓function, ↑internal diameter	↓25%		—	—	1 mg/kg
Eya et al. (2005)	7	Intracoronary	0.7 mg/kg	↓function, ↑internal diameter	↓37%	35.5–22	—	—	3.5 mg/kg
Alves de Souza and Camacho (2006)	13	Intra venous infusions	30 mg/m^2	↓function, ↑internal diameter	↓45%	66–36	↓55%	36–17	240 mg/m^2
Sousa et al. (2014)	7	Intra venous infusions	30 mg/m^2	↓function, ↑internal diameter	↓36.5%	66.7–42	↓47.5%	36.1–19.1	210 mg/m^2
Surachetpong et al. (2016)	12	Intra venous infusions	30 mg/m^2	↓function	↓13%	62.21–54.74	↓30.5%	36.06–25.27	120 mg/m^2
Tater et al. (2017)	14	Intra venous infusions	30 mg/m^2	No echocardiographic changes	—	—	—		120 mg/m^2
Beaumier et al. (2020)	9	Intra venous infusions	30 mg/m^2	↓function, ↑internal diameter	↓25.7%	70.74–60.67	↓14.3%	38.91–28.9	120 mg/m^2
Matsuura et al. (2021)	6	Intra venous infusions	30 mg/m^2	No echocardiographic changes	(40–34) p 0.13	—	(78–70) p 0.15	—	180 mg/m^2

Abbreviation: EF, ejection fraction.

TABLE 3 | Risk of bias.

Authors, (year)	Type of study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	% of Yes	Risk of bias
Styles et al. (1983)	RCT	—	—	✓	—	—	—	✓	✓	✓	✓	✓	✓	✓	61.53	Moderate
Unverferth et al. (1985)	RCT	✓	—	✓	—	—	—	✓	✓	✓	✓	✓	✓	✓	69.23	Moderate
Hanai et al. (1996)	RCT	—	—	✓	—	—	—	✓	✓	✓	✓	✓	✓	✓	61.53	Moderate
Vaynblat et al. (1997)	RCT	—	✓	✓	—	—	—	✓	✓	✓	✓	✓	✓	✓	69.23	Moderate
Shah et al. (1997)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low
Toyoda et al. (1998)	RCT	U	—	✓	—	—		✓	✓	✓	✓	✓	✓	✓	61.53	Moderate
Christiansen et al. (2002)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low
Vaynblat et al. (2002)	RCT	✓	—	✓	—	—	—	✓	✓	✓	✓	✓	✓	✓	69.23	Moderate
Eya et al. (2005)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low
Alves de Souza and Camacho (2006)	RCT	✓	—	✓	—	—	✓	✓	✓	✓	✓	✓	✓	✓	76.92	Low
Sousa et al. (2014)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low
Surachetpong et al. (2016)	RCT	—	—	—	—	—	—	—	✓	✓	✓	✓	✓	✓	46.15	High
Tater et al. (2017)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low
Beaumier et al. (2020)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low
Matsuura et al. (2021)	Quasi-experimental	✓	✓	—	—	✓	✓	✓	✓	✓	N/A	N/A	N/A	N/A	77.77	Low

Note: ✓—yes; —No; U—unclear; randomized studies: 'Q1. Was true randomization used for assignment of participants to treatment groups? Q2. Was allocation to treatment groups concealed? Q3. Were treatment groups similar at the baseline? Q4. Were participants blind to treatment assignments? Q5. Were those delivering treatment blind to treatment assignment? Q6. Were outcomes assessors blind to treatment assignment? Q7. Were treatment groups treated identically other than the intervention of interest? Q8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed? Q9. Were participants analysed in the groups to which they were randomized? Q10. Were outcomes measured in the same way for treatment groups? Q11. Were outcomes measured in a reliable way? Q12. Was appropriate statistical analysis used? Q13. Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?'; Quasi experimental studies—(Q1) 'is it clear in the study what is the cause' and what is the 'effect' (i.e., there is no confusion about which variable comes first)?' (Q2) 'Were the participants included in any comparisons similar?' (Q3) 'Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?' (Q4) 'Was there a control group?' (Q5) 'Were there multiple measurements of the outcome both pre and post the intervention/exposure?' (Q6) 'Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?' (Q7) 'Were the outcomes of participants included in any comparisons measured in the same way?' (Q.8) 'Were outcomes measured in a reliable way?' (Q9) 'Was appropriate statistical analysis used?'.
Abbreviations: √, yes; —, no; U, unclear; N/A, not applicable, RCT: randomized control study.

TABLE 4 | Sponsorship status of the studies.

References	Sponsorship status	Sponsorship
Styles et al. (1983)	Sponsored	Canadian Cancer Society Alberta Cancer Board, Edmonton, Alberta
Unverferth et al. (1985)	Unclear	
Hanai et al. (1996)	Unclear	
Vaynblat et al. (1997, 2002)	Sponsored	Laboratories of Columbus, OH Gore & Associates Inc, of Flagstaff, AZ
Shah et al. (1997)	Sponsored	The Foundation for Surgical Education and Investigation Inc., SUNY-HSC at Brooklyn The Maimonides Research and Development Foundation Adria Laboratories, Columbus, Ohio, for graciously providing our laboratory with doxorubicin
Toyoda et al. (1998)	Unclear	
Christiansen et al. (2002)	Unclear	
Vaynblat et al. (1997, 2002)	Sponsored	The Foundation for Surgical Education and Investigation Inc., SUNY-HSC at Brooklyn The Maimonides Research and Development Foundation Adria Laboratories, Columbus, Ohio, for graciously providing our laboratory with doxorubicin
Eya et al. (2005)	Unclear	
Alves de Souza and Camacho (2006)	Sponsored	Fundação de Amparo à Pesquisa do Estado de São Paulo Royal Canin
Sousa et al. (2014)	Unclear	
Surachetpong et al. (2016)	Sponsored	TRF Grant for New Researcher, the Thailand Research Fund
Tater et al. (2017)	Unclear	
Beaumier et al. (2020)	Sponsored	Tufts University Companion Health Fund Barkley Fund and Shipley Foundation
Matsuura et al. (2021)	Sponsored	Japan Society for the Promotion of Science (JSPS)

of the intravenous (IV) route carried out for antineoplastic treatment also presented cardiotoxic potential, with doses above 30 mg/m^2 not being recommended, nor cumulative doses above 250 mg/m^2, which increases the incidence of heart failure (Pereira Neto et al. 2006). In six articles included in this review, the intravenous was the route for doxorubicin administration, and in four studies, there was heart disease. In one study, cumulative doses of 90 mg/m^2 and an interval between applications of 3 weeks were sufficient to demonstrate cardiotoxicity (Alves de Souza and Camacho 2006), demonstrating that, even though the intracoronary route is more cardiotoxic, the therapeutic doses used for antineoplastic treatment (IV) may be sufficient to generate heart disease.

The four studies evaluated in this meta-analysis showed a decrease in EF and, consequently, a decrease in cardiac function (Vaynblat et al. 1997, 2002; Shah et al. 1997; Toyoda et al. 1998; Eya et al. 2005). The degree of decreased function was similar even when comparing different cumulative doses (1.0 and 3.5). In a meta-analysis study carried out by Jeyaprakash et al., the average reduction for EF was 5.4%. This meta-analysis included various anthracyclines, such as doxorubicin and epirubicin, highlighting the cardiotoxic potential of this class of drugs and the importance of individual assessment of each patient before including them in oncological treatments, as pre-existing heart disease can favour the appearance of heart damage, and this drug is contraindicated for individuals with severe heart disease.

The studies carried out by Tater et al. (2017) and Matsuura et al. (2021) evaluated patients undergoing cancer treatment and healthy patients, respectively. Their finding did not prove significant echocardiographic changes as a decrease in EF and FS, even in different research conditions. These data reinforce that doxorubicin is still a viable treatment option for canine cancer patients, given its already proven effectiveness and low cost for those responsible. Additionally, the use of cardioprotectors in human patients reduces the harmful effects, by reducing the oxidative stress of cardiac cells through the removal of free radicals. The administration of rosuvastatin, for example, significantly reduced the cardiotoxic effect of doxorubicin (Kettana et al. 2024) proving to be an option for further studies in dogs.

The included studies were classified as randomized and quasi-experimental according to the model adopted. The differences between the models resulted from different approaches such as the use of animals from routine veterinary oncology care,

evaluation of the cardioprotective effect of drugs applied in conjunction with doxorubicin, and studies that aimed to generate heart failure for different subsequent treatments. These differences in study design resulted in inconsistencies across the studies, such as a lack of standardized groups or even the absence of a control group in some cases. This lack of standardization posed challenges for pairing studies and conducting meaningful statistical analyses.

A key limitation of this systematic review is the small sample size in most of the included studies, except for Unverferth et al. (1985), which evaluated 38 animals. Furthermore, the use of varying echocardiographic parameters across the studies hindered statistical comparisons.

In conclusion, future research should prioritize larger sample sizes and standardize echocardiographic variables to facilitate a more robust evaluation of doxorubicin-induced heart failure. Despite its established cardiotoxicity, doxorubicin remains a valuable antineoplastic drug in canine cancer treatment due to its efficacy. This review confirms the impact of doxorubicin on cardiac function, revealing the critical importance of comprehensive monitoring, including pre-, intra- and post-chemotherapy assessments, along with individualized risk evaluation for each patient. Additionally, the use of modern anthracyclines may offer a less cardiotoxic alternative, enhancing the safety of future treatments of canine cancer patients.

Author Contributions

Gustavo Cavinato Herrera: conceptualization, methodology, formal analysis, writing – original draft, writing – review and editing, visualization. **Luiz Ricardo Soldi**: conceptualization, methodology, formal analysis, writing – original draft, writing – review and editing, visualization. **Leandro Machado Oliveira**: methodology, formal analysis, data curation. **Luiz Renato Paranhos**: methodology, writing – review and editing. **Marcelo José Barbosa Silva**: conceptualization, methodology, writing – review and editing, project administration, supervision. All authors contributed to the article and approved the submitted version.

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70497.

References

Aromataris, E., C. Lockwood, K. Porritt, B. Pilla, Z. Jordan, editors. 2024. *JBI Manual for Evidence Synthesis*. JBI. https://synthesismanual.jbi.global.

Alves de Souza, R. C., and A. A Camacho. 2006. "Neurohormonal, Hemodynamic, and Electrocardiographic Evaluations of Healthy Dogs Receiving Long-Term Administration of Doxorubicin." *American Journal of Veterinary Research* 67, no. 8: 1319–1325. https://doi.org/10.2460/AJVR.67.8.1319.

Beaumier, A., S. R. Robinson, N. Robinson, et al. 2020. "Extracellular Vesicular MicroRNAs as Potential Biomarker for Early Detection of Doxorubicin-Induced Cardiotoxicity." *Journal of Veterinary Internal Medicine* 34, no. 3: 1260–1271. https://doi.org/10.1111/JVIM.15762.

Boon, J. 2011. "Veterinary Ehocardiography. Evaluation of Size, Function, and Hemodynamics str. 105–196." In *Veterinary Echocardiography 2 Ed West Sussex*, 2nd Ed. 632. Wiley-Blackwell. https://www.wiley.com/en-no/Veterinary+Echocardiography%2C+2nd+Edition-p-9780813823850.

Borenstein, M., J. P. T. Higgins, L. V. Hedges, and H. R Rothstein. 2017. "Basics of Meta-Analysis: I^2 is Not an Absolute Measure of Heterogeneity." *Research Synthesis Methods* 8, no. 1: 5–18. https://doi.org/10.1002/jrsm.1230.

Christiansen, S., K. Redmann, H. H. Scheld, et al. 2002. "Adriamycin-Induced Cardiomyopathy in the Dog—An Appropriate Model for Research on Partial Left Ventriculectomy?" *Journal of Heart and Lung Transplantation* 21, no. 7: 783–790. https://doi.org/10.1016/S1053-2498(02)00402-3.

Costa, F. S., A. Q. A. de Sobrinho, T. Roesler, and D. C Oliveira. 2008. "Avaliação da Função Miocárdica em cães e Gatos Pela Técnica de Doppler Tecidual Durante Quimioterapia com Doxorrubicina." *Pubvet* 2, no. 21: Art#235.

DerSimonian, R., and N Laird. 1986. "Meta-Analysis in Clinical Trials." *Controlled Clinical Trials* 7, no. 3: 177–188. https://doi.org/10.1016/0197-2456(86)90046-2.

Eya, K., E. Tuzun, J. Conger, et al. 2005. "Effect of Pump Flow Mode of Novel Left Ventricular Assist Device Upon End Organ Perfusion in DOGS WITH DOXORUBICIN INDUCED HEART FAILURE." *Asaio Journal* 51, no. 1: 41–49. https://doi.org/10.1097/01.MAT.0000150510.03339.AD.

Franco, N., E. BortolottoDegregori, M. N. F. de, L. G. Teixeira, R. M. P. da, and E. A Contesini. 2019. "Utilização de Antibióticos Quimioterápicos na Oncológica de Pequenos Animais: Revisão." *Pubvet* 13, no. 02: 1–8. https://doi.org/10.31533/PUBVET.V13N2A279.1-8.

Hallman, B. E., M. L. Hauck, L. E. Williams, P. R. Hess, and S. E Suter. 2019. "Incidence and Risk Factors Associated With Development of Clinical Cardiotoxicity in Dogs Receiving Doxorubicin." *Journal of Veterinary Internal Medicine* 33, no. 2: 783–791. https://doi.org/10.1111/JVIM.15414.

Hanai, K., K. Takaba, S. Manabe, M. Nakano, A. Kohda, and M Matsuo. 1996. "Evaluation of Cardiac Function by Echocardiography in Dogs Treated With Doxorubicin." *Journal of Toxicological Sciences* 21, no. 1: 1–10. https://doi.org/10.2131/JTS.21.1.

Hanton, G., V. Eder, G. Rochefort, P. Bonnet, and J. M Hyvelin. 2008. "Echocardiography, a Non-Invasive Method for the Assessment of Cardiac Function and Morphology in Preclinical Drug Toxicology and Safety Pharmacology." *Expert Opinion on Drug Metabolism & Toxicology* 4, no. 6: 681–696. https://doi.org/10.1517/17425255.4.6.681.

Henderson, I. C., J. C. Allegra, T. Woodcock, et al. 1989. "Randomized Clinical Trial Comparing Mitoxantrone With Doxorubicin in Previously Treated Patients With Metastatic Breast Cancer." *Journal of Clinical Oncology* 7, no. 5: 560–571. https://doi.org/10.1200/JCO.1989.7.5.560.

Higgins, J. P. T., J. Thomas, J. Chandler, M. Cumpston, T. Li, and M. J Page. 2019. *Cochrane Handbook for Systematic Reviews of Intervention*. Edited by V. A. Welch. Cochrane.

Kettana, K. M., S. M. ElHaggar, M. A. Alm El-Din, and D. R ElAfify. 2024. "Possible Protective Effect of Rosuvastatin in Chemotherapy-Induced Cardiotoxicity in HER2 Positive Breast Cancer Patients: A Randomized Controlled Trial." *Medical Oncology* 41, no. 8: 196. https://doi.org/10.1007/S12032-024-02426-1.

Macdonald, V. 2009. "Chemotherapy: Managing Side Effects and Safe Handling." *Canadian Veterinary Journal* 50, no. 6: 665–668. https://pmc.ncbi.nlm.nih.gov/articles/PMC2684058/.

Matsuura, K., K. Shiraishi, A. S. Mandour, et al. 2021. "The Utility of Intraventricular Pressure Gradient for Early Detection of Chemotherapy-Induced Subclinical Cardiac Dysfunction in Dogs." *Animals (Basel)* 11, no. 4: 1122. https://doi.org/10.3390/ANI11041122.

Mauldin, G. E., P. R. Fox, A. K. Patnaik, B. R. Bond, S. C. Mooney, and R. E Matus. 1992. "Doxorubicin-Induced Cardiotoxicosis. Clinical Features in 32 Dogs." *Journal of Veterinary Internal Medicine* 6, no. 2: 82–88. https://doi.org/10.1111/J.1939-1676.1992.TB03156.X.

Nardi, A. B. D. E., S. Rodaski, R. S. Sousa, et al. 2002. "Prevalência de Neoplasias e Modalidades de Tratamentos em Cães, Atendidos no Hospital Veterinário da Universidade Federal do Paraná." *Archives of Veterinary Science* 7, no. 2: 15–26. https://doi.org/10.5380/AVS.V7I2.3977.

Page, M. J., J. E. McKenzie, P. M. Bossuyt, et al. 2021. "The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews." *BMJ* 372: n71. https://doi.org/10.1136/BMJ.N71.

Pereira Neto, G. B., J. N. B. Andrade, M. G. Sousa, and A. A Camacho. 2006. "Holter Electrocardiography in Dogs Showing Doxorubicin-Induced Dilated Cardiomyopathy." *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 58, no. 6: 1037–1042. https://doi.org/10.1590/S0102-09352006000600010.

Shah, H. R., M. Vaynblat, G. Ramdev, J. N. Cunningham, and M Chiavarelli. 1997. "Experimental Cardiomyopathy as a Model of Chronic Heart Failure." *Journal of Investigative Surgery* 10, no. 6: 387–396. https://doi.org/10.3109/08941939709099603.

Sousa, M. G., D. Paulino-Junior, J. P. E. Pascon, G. B. Pereira-Neto, R. Carareto, and A. A Camacho. 2014. "Assesment of the TEI Index of Myocardial Performance in Dogs With Doxorubicin-Induced Cardiomiopathy." *Archivos de Medicina Veterinaria* 46, no. 1: 63–68. https://doi.org/10.4067/S0301-732X2014000100009.

Styles, C. B., A. A. Noujaim, B. I. Jugdutt, et al. 1983. "Effect of Doxorubicin on [ω-I-131]Heptadecanoic Acid Myocardial Scintigraphy and Echocardiography in Dogs." *Journal of Nuclear Medicine* 24, no. 11: 1012–1018. https://jnm.snmjournals.org/content/24/11/1012.

Surachetpong, S. D., P. Teewasutrakul, and A Rungsipipat. 2016. "Serial Measurements of Cardiac Troponin I (cTnI) in Dogs Treated With Doxorubicin." *Japanese Journal of Veterinary Research* 64, no. 4: 221–233.

Tater, G., N. Eberle, S. Hungerbuehler, et al. 2017. "Assessment of Cardiac Troponin I (cTnI) and Tissue Velocity Imaging (TVI) in 14 Dogs With Malignant Lymphoma Undergoing Chemotherapy Treatment With Doxorubicin." *Veterinary and Comparative Oncology* 15, no. 1: 55–64. https://doi.org/10.1111/VCO.12135.

Toyoda, Y., M. Okada, and M. A Kashem. 1998. "A canine Model of Dilated Cardiomyopathy Induced by Repetitive Intracoronary Doxorubicin Administration." *Journal of Thoracic and Cardiovascular Surgery* 115, no. 6: 1367–1373. https://doi.org/10.1016/S0022-5223(98)70221-1.

Unverferth, D. V., C. V. Leier, S. P. Balcerzak, and R. L Hamlin. 1985. "Usefulness of a Free Radical Scavenger in Preventing Doxorubicin-Induced Heart Failure in Dogs." *American Journal of Cardiology* 56, no. 1: 157–161. https://doi.org/10.1016/0002-9149(85)90585-5.

Vaynblat, M., M. Chiavarelli, H. R. Shah, et al. 1997. "Cardiac Binding in Experimental Heart Failure." *Annals of Thoracic Surgery* 64, no. 1: 81–85. https://doi.org/10.1016/S0003-4975(97)00349-4.

Vaynblat, M., H. R. Shah, D. Bhaskaran, et al. 2002. "Simultaneous Angiotensin Converting Enzyme Inhibition Moderates Ventricular Dysfunction Caused by Doxorubicin." *European Journal of Heart Failure* 4, no. 5: 583–586. https://doi.org/10.1016/S1388-9842(02)00091-0.

Capítulo 2

Manuscrito para submissão

4 Capítulo 2: manuscrito para submissão

TÍTULO: Echocardiographic changes in dogs undergoing chemotherapy protocols with doxorubicin

4.1 Introduction

Echocardiography is a widely used, non-invasive diagnostic tool in veterinary medicine, offering the potential for early detection of cardiac abnormalities. Among its applications is the monitoring of cardiotoxic effects induced by chemotherapy (Boon, 2011). Doxorubicin, a commonly used antitumor drug in both human and veterinary medicine, has a well-documented cardiotoxic impact, particularly with cumulative dosing. Despite its known risks, doxorubicin remains a cornerstone in cancer treatment due to its high efficacy and accessibility.

However, current echocardiographic assessments in veterinary settings often rely on routine parameters, which may not be sensitive enough to detect subtle or early myocardial changes by assessing the cumulative impact on cardiac function in canine patients.

This research addresses a critical gap in the monitoring of canine oncology patients, aiming to improve diagnostic sensitivity for cardiotoxicity.

The prevalence of oncologic patients in veterinary medicine has been increasing considerably, as supported by research studies (De Nardi et al., 2002). This trend is associated with the increased life expectancy of animals, improved nutrition, and enhanced diagnostic capabilities, which are related to technological advancements in complementary tests and greater specialization among professionals (Adams, V.J. et al., 2010; Repetti, C.S.F et al., 2023; Simon, B.T. and Steagall, P.V., 2017). Chemotherapy is routinely used in veterinary patients, with cytotoxic drugs employed to achieve complete remission of systemic neoplasms, such as lymphoma, reduce tumor size, and increase life expectancy and quality of life in animals with metastatic cancers, including osteosarcoma (MacDonald, V., 2009). Most chemotherapy protocols in veterinary medicine consist of a combination of two or three cytostatic drugs. For example, in canine mammary tumors, cyclophosphamide, doxorubicin, and 5-fluorouracil are the most used drugs (Cirillo, J.V., 2008). Vincristine sulfate is widely used in hemolymphatic tumors and transmissible venereal tumors, often in combination with cyclophosphamide and prednisolone in the former case (Faro, A.M. et al., 2008). Doxorubicin, an antitumor antibiotic from the anthracycline class, is used to treat neoplasms in both humans and

animals. This drug may be prescribed alone or in combination with other medications for conditions such as lymphoma, osteosarcoma, and carcinomas (Hamer, A.S. et al., 1991; Cirillo, J.V., 2008; Hallman et al., 2019).

The cardiotoxicity of doxorubicin has been reported in several species. In humans, this effect has been described as dose-dependent, and in some cases, discontinuation or even contraindication of treatment is recommended (Costa, F.S., 2008). Doxorubicin causes myocardial injury leading to cardiac muscle necrosis, systolic dysfunction, and dilation, which can be observed through echocardiography.

Histological changes caused by doxorubicin include cytoplasmic vacuolar degeneration, myocyte lysis, myocyte atrophy, and fibrosis. These changes can result in left atrial and ventricular dilation, leading to congestive heart failure, arrhythmias, and sudden death. (Silva, C.E.V., 2005). Cardiotoxic effects related to other chemotherapeutic agents have not been widely reported in veterinary medicine.

The cumulative effect of doxorubicin plays an important role in both the onset and severity of cardiac lesions. The administration method (bolus or continuous infusion), the route (intravenous or intracardiac), and its combination with other drugs may also potentiate its effects on cardiac muscle (Alvares-Cardona, J. and Lenihan, D. J., 2019).

Despite its cardiotoxic effects, doxorubicin remains a treatment option for canine cancer patients. Advocates for its use cite its effectiveness against cancer cells and the ability to monitor each patient via cardiac exams during the administration protocol (Hallman, B.E. et al., 2018), in addition to its affordable cost for less fortunate owners.

Echocardiography is a non-invasive imaging method that measures chamber diameters, muscle and valve thickness, and evaluates hemodynamics, as well as systolic and diastolic function (Boon, 2011). This examination is extensively used in veterinary medicine, and reference values are provided for diverse weight categories and dog breeds.

Using echocardiography, the veterinarian can identify and measure cardiac abnormalities, facilitate suitable treatments, and assess prognosis in cases of heart disease. Routine M-mode and B-mode measurements help estimate chamber sizes, areas, and volumes (Visser, L.C. et al., 2019). Shortening fraction and ejection fraction assessments have been used to monitor patients undergoing chemotherapy with doxorubicin. However, this approach is seen as insensitive for detecting small changes, particularly when damage occurs rapidly and may be irreversible (Gallay-Lepoutre, J. et al., 2016).

This study aimed to assess whether echocardiography can effectively detect and quantify the cardiotoxic effects of doxorubicin, alone or in combination with other chemotherapeutics. The dogs came from the Oncology Clinic and Surgery Department

(SECCON) at the Veterinary Hospital of the Federal University of Uberlândia.

4.2 Methodology

Patient selection and echocardiographic procedures

The data for this study were obtained from animals treated at the Oncology Clinic and Surgery Department (SECCON) of the Veterinary Hospital of the Federal University of Uberlândia (HV–UFU), and from patients treated at private veterinary clinics in the city of Uberlândia, MG, in accordance with a partnership agreement. The patients undergoing chemotherapy followed the protocol below. An echocardiographic exam was performed using two-dimensional, M-mode, pulsed Doppler (PW), continuous Doppler (CW), and color flow mapping (CFM), as recommended by the Echocardiography Committee of the Specialty of Cardiology – American College of Veterinary Internal Medicine (Thomas et al., 1993) and the American Society of Echocardiography, with modifications proposed by Boon (2011) and Chetboul (2002). The animals were positioned in lateral recumbency and manually restrained without sedation or anesthesia.

A total of 33 animals referred to SECCON and specialized oncology clinics with an indication for chemotherapy were included in this study with prior authorization from their guardian and signed informed consent forms. Patients eligible for chemotherapy - i.e., those without echocardiographic abnormalities (systolic or diastolic dysfunction) or hematologic alterations—were selected. These patients were divided into three groups: No doxo – patients undergoing chemotherapy protocols without doxorubicin; With Doxo – patients undergoing protocols exclusively with doxorubicin; Associations – patients undergoing protocols with doxorubicin combined with other chemotherapy agents.

These animals were evaluated before (T0) and during the chemotherapy protocol, always after each session (T1, T2, and T3). The deleterious effects associated with different degrees of drug cardiotoxicity were assessed, with systolic function assessed by echocardiography. The parameters evaluated were: Ejection fraction by the Teicholz (FET) and Simpson (FES) methods, shortening fraction (FS), left ventricular diameter in diastole and systole.

All echocardiographic exams were performed by a single operator, using a General Electric (GE) Logiq F6 ultrasound system equipped with sector transducers with frequencies between 3 and 7 MHz.

This study was submitted to and approved by the Ethics Committee for the Use of Animals of UFU (CEUA/UFU), under protocol number 054/20.

Statistical Analysis

Comparative analysis among chemotherapy protocols

To compare diastolic and systolic diameters across the three chemotherapy protocols, the **Kruskal-Wallis test** was used, followed by **Dunn's test** with Bonferroni-adjusted pairwise comparisons. The Kruskal-Wallis test is a non-parametric method suitable for comparing three or more independent groups when data do not meet normality assumptions. Kendall's coefficient of concordance ($\hat{W}$) was used to assess effect size, indicating the strength of agreement among group rankings.

For shortening fraction (FS) and ejection fraction (FES and FET), **one-way ANOVA** and **Bonferroni-adjusted post-hoc tests** were employed. ANOVA is appropriate for comparing means among three or more groups with normal distribution and homogeneity of variances. Effect size was measured using partial eta-squared (η_p^2), which quantifies the proportion of total variability attributable to the factor under study.

Influence of Age on Echocardiographic Parameters

Patients were categorized into two age groups: young dogs (2–7 years) and elderly dogs (over 8 years). The **Mann-Whitney U test**, a non-parametric test for comparing two independent groups, was used to analyze diastolic and systolic diameters. The effect size, measured by the biserial rank correlation ($\hat{r}_{\text{biserial}}$).

The **Student's t-test** for independent samples was used to compare shortening fraction (FS) and ejection fraction (FES and FET) between age groups. Effect size was calculated using Hedges' g, a robust measure for small sample sizes.

Repeated measures analysis with linear mixed model

To analyze the progression of FES (Simpson's ejection fraction) values over time in the group without Doxorubicin, a Linear Mixed-Effects Model (LMM) was used. This type of model is appropriate for longitudinal data with repeated measurements per subject, allowing the modeling of intra-subject correlation.

The model was specified using the `lme()` function from the `nlme` package in R, with Time as a fixed effect and Individual as a random intercept (`random = ~1 | Individual`), using the Maximum Likelihood method (`method = "ML"`).

After fitting the model, the following assumptions were verified:

- Overall model fit: using the `check_model()` function, residual behavior and overall model adequacy were evaluated.
- Residual autocorrelation: the `check_autocorrelation()` function was used to check for serial dependence of residuals.
- Heteroscedasticity: the `check_heteroscedasticity()` function evaluated the homogeneity of residual variance, with graphical inspection support.
- Residual normality: the Shapiro-Wilk test (`shapiro.test()`) was applied to normalized residuals (`resid(type = ‘‘normalized’’)`) to assess the assumption of normality.

These steps ensured the inferential validity of the fitted model, conferring greater robustness to the statistical conclusions.

Models with interaction between time and pre-existing heart disease

In addition to analyzing FES in the group without Doxorubicin, linear mixed models were fitted to investigate the effect of the interaction between time and the presence of pre-existing cardiac alterations (`doenca_previa`) on various echocardiographic parameters: ventricular cavity diameter in diastole and systole, shortening fraction (FS), ejection fraction by Teichholz method (FET), and Simpson method (FES).

The `doenca_previa` variable was created based on echocardiographic criteria observed at baseline (time zero), with animals considered “altered” if they showed thickening or prolapse of the mitral valve or mitral regurgitation of minimal degree or greater.

The fixed effect of Time represents the average evolution of the parameter throughout chemotherapy sessions, while the fixed effect of `doenca_previa` assesses initial differences between groups. The Time $\times$ `doenca_previa` interaction tests whether the trajectory over time differs between groups with and without prior cardiac alterations.

Models were fitted separately for the following groups:

- Doxorubicin-only protocol
- Doxorubicin + combination protocol

The `lme()` function from the `nlme` package was used, specifying random intercepts per individual and maximum likelihood estimation (`method = ‘‘ML’’`), allowing model comparison with and without interaction as needed.

The significance analysis of the Time $\times$ `doenca_previa` interaction tested whether the progression of echocardiographic parameters differed between dogs with or without pre-existing heart disease within each therapeutic protocol.

Main adjusted models included:

- `cavdiast_doxo_modelo_interacao`: Diastolic Cavity in the Doxorubicin group.
- `cavdiast_assocdoxo_modelo_interacao`: Diastolic Cavity in the Doxorubicin + combinations group.

Equivalent models were performed for other parameters (FET, FES, FS, Systolic Cavity), following the same structure and modeling logic.

These models enabled a more comprehensive examination of the hypothesis that pre-existing cardiac alterations could impact the cardiac response to doxorubicin throughout treatment.

Animals with pre-existing disease were considered those presenting some degree of mitral valve insufficiency or degeneration, provided that no alterations in systolic or diastolic function were observed.

4.3 Results

Descriptive data

The study population's baseline characteristics are presented in Table 1. The majority of the dogs are of undefined breed (SRD), accounting for 45% of the sample, followed by breeds such as Yorkshire and ShihTzu, each representing 9.1%. The population is predominantly female (82%), with a median age of 10.0 years (interquartile range: 7.0, 12.0). The median weight of the animals is 12 kg (interquartile range: 7, 21). Regarding protocols, 52% of participants are in the "Associations"group, 36% in the "Doxo"group, and 12% in the "No doxo"group.

The Table 2 presents baseline characteristics of patients by treatment group: *No doxo* ($N = 4$), *Doxo* ($N = 12$), and *Associations* ($N = 17$). The median age was highest in the *No doxo* group (12.5 years) and lowest in the *Doxo* group (8.0 years). Weight (kg) varied across groups, with the *Doxo* group showing the highest median weight (17 kg). Differences in age and weight between groups were assessed using the Kruskal-Wallis test, with no statistically significant differences observed (age: $p = 0.067$, weight: $p = 0.2$).

Table 1: Baseline characteristics of the study population

Characteristic	N = 33
Breed	
Mixed-breed	15 (45%)
Yorkshire	3 (9.1%)
Shihtzu	3 (9.1%)
Pug	2 (6.1%)
Poodle	2 (6.1%)
Pitbull	2 (6.1%)
Maltes	2 (6.1%)
Pinscher	1 (3.0%)
Golden Retriever	1 (3.0%)
Chowchow	1 (3.0%)
Border Collie	1 (3.0%)
Age (years)	10.0 (7.0, 12.0)
Gender	
Female	27 (82%)
Male	6 (18%)
Weight (Kg)	12 (7, 21)
Protocol	
No doxo	4 (12%)
Doxo	12 (36%)
Associations	17 (52%)

[1] n (%); Median (Q1, Q3)

Table 2: Patient characteristics by treatment group and associations.

	No doxo ($N = 4^{\dagger}$)	**Doxo** ($N = 12^{\dagger}$)	**Assoc.** ($N = 17^{\dagger}$)	**p-value**[2]
Age (years)	12.5 (10.5, 13.5)	8.0 (5.0, 10.0)	11.0 (8.0, 12.0)	0.067
Weight (kg)	5 (4, 13)	17 (8, 22)	10 (6, 20)	0.2

[†] Median (Q1, Q3) [2] Kruskal-Wallis rank sum test

Doxorubicin-based protocols are associated with cardiac dilation and reduced systolic function

The parameters were obtained in M-mode and B-mode through a proper parasternal transverse cut using the Logiq F6 GE device, with frequencies ranging from 3 to 7 MHz. These parameters correspond to the diastolic and systolic diameters of the heart under three different chemotherapy protocols: without doxorubicin (*No doxo*), with doxorubicin (*Doxo*), and with doxorubicin associated with other drugs (*Associations*).

In the graphs related to the diastolic diameter (Figure 1A) and systolic diameter (Figure 1B), it is observed that the chemotherapy protocol did not influence diastolic diameter ($\chi^2(2) = 4.81, p = 0.09$). However, the systolic diameter of patients treated with doxorubicin was higher than that of untreated patients ($p = 0.03$).

The effect size, measured by Kendall's coefficient of concordance ($\hat{W}$), indicated a weak agreement among the rankings of the groups for both diastolic ($\hat{W} = 0.15$) and systolic ($\hat{W} = 0.21$) diameters, suggesting a inconsistent trend in the differences observed across the protocols.

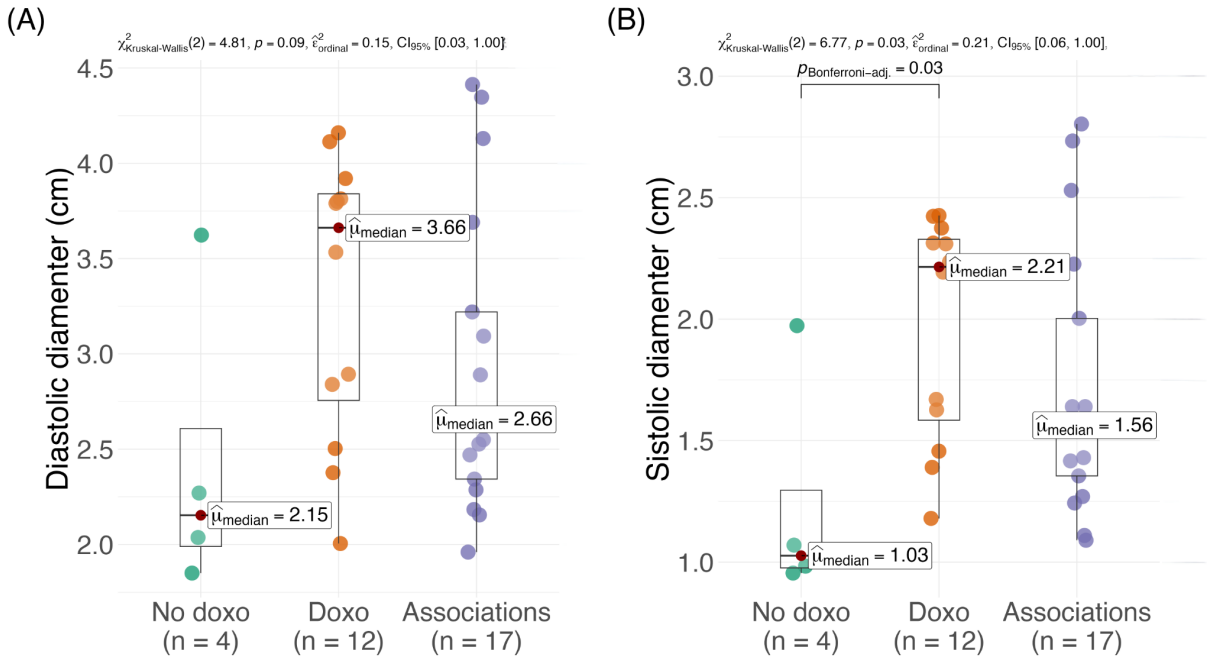

Figure 1: Comparison of cavity diameters across different chemotherapy protocols. Comparison of diastolic (A) and systolic (B) diameters of the left ventricular cavity in dogs undergoing different chemotherapy protocols: without doxorubicin (*No doxo*), with doxorubicin (*Doxo*), and with doxorubicin associated with other drugs (*Associations*). The boxplots show the median, interquartile range, and individual data points for each group. Statistical analysis was performed using the Kruskal-Wallis test followed by the Dunn test with Bonferroni-adjusted pairwise comparisons. Significant differences are indicated by p-values above the brackets. Kendall's coefficient of concordance ($\hat{W}$) indicated a weak effect size for both diastolic ($\hat{W} = 0.09$) and systolic ($\hat{W} = 0.21$) diameters, reflecting a consistent trend in the differences observed across the protocols.

Significant differences were not observed in the shortening fraction (FS), with a weak effect size ($F(2, 29) = 1.57, p = 0.23, \eta_p^2 = 0.03$) (Figure 2A). The ejection fraction, calculated by the Simpson method (FES), showed no statistically significant differences among the groups ($F(2, 30) = 1.78, p = 0.19, \eta_p^2 = 0.04$), suggesting that the proposed chemotherapy protocol did not significantly impact this variable (Figure 2B). However, the ejection fraction calculated by the Teicholz method (FET) was significantly higher in the group that did not receive doxorubicin compared to the group that received doxorubicin ($p = 6.76 \times 10^{-3}$) and group that used doxorubicin combined with other drugs ($p = 9.01 \times 10^{-3}$) (Figure 2C). Despite a significant difference, the effect size was low, suggesting a small clinical impact ($\eta_p^2 = 0.24$).

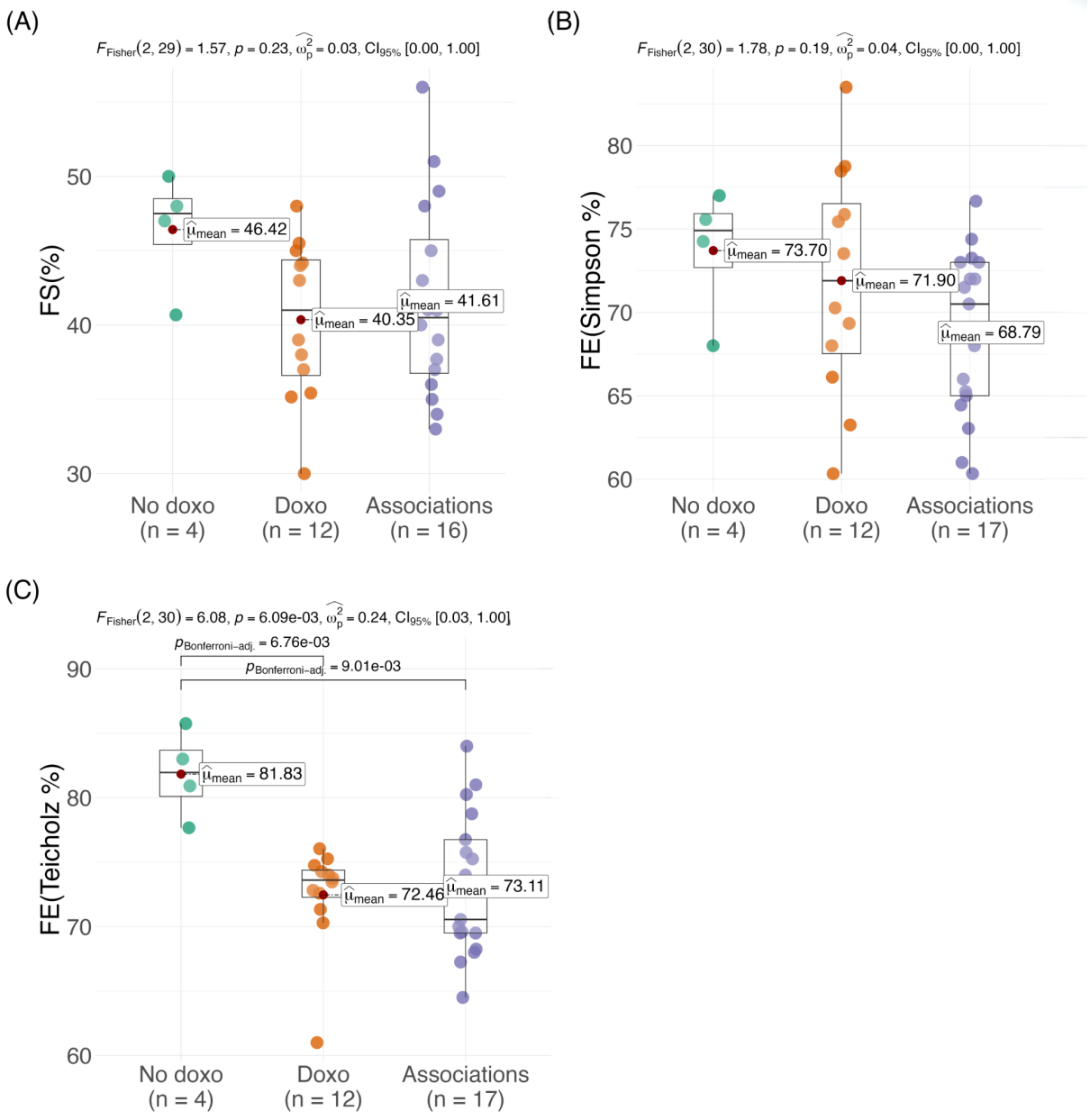

Figure 2: Comparison of cardiac functional parameters across different chemotherapy protocols. (A) Shortening fraction (FS), (B) Ejection fraction by Simpson method (FES), and (C) Ejection fraction by Teicholz method (FET) in dogs undergoing different chemotherapy protocols: without doxorubicin (*No doxo*), with doxorubicin (*Doxo*), and with doxorubicin associated with other drugs (*Associations*). The boxplots show the median, interquartile range, and individual data points for each group. Statistical analysis was performed using ANOVA with Bonferroni-adjusted pairwise comparisons. Significant differences are indicated by p-values above the brackets. The effect sizes, measured by partial eta-squared (η_p^2), were 0.23 for FS, 0.04 for FES, and 0.24 for FET, indicating small effects.

Age influences ventricular dimensions and systolic function in oncologic dogs

For evaluation of age on the echocardiographic parameters, the patients were classified as young dogs (2–7 years) and elderly dogs (over 8 years). The diastolic diameter of the cavity was higher in adult dogs than in elderly dogs ($W = 193.00, p = 0.01$) (Figure 3A). The same pattern was observed for the diastolic diameter of the cavity ($W = 198.00, p = 7.44 \times 10^{-3}$) (Figure 3B). The effect size was moderated for diastolic $\hat{r}_{\text{biserial}} = 0.53$ and systolic $\hat{r}_{\text{biserial}} = 0.57$ dimensions, indicating moderate clinical importance. These results suggest that age has a significant impact on diastolic and systolic diameters, with elderly dogs exhibiting smaller cavity sizes compared to adult dogs. This indicates that age-related changes in cardiac structure are clinically relevant in oncologic patients, although the influence of the chemotherapy protocol should also be considered.

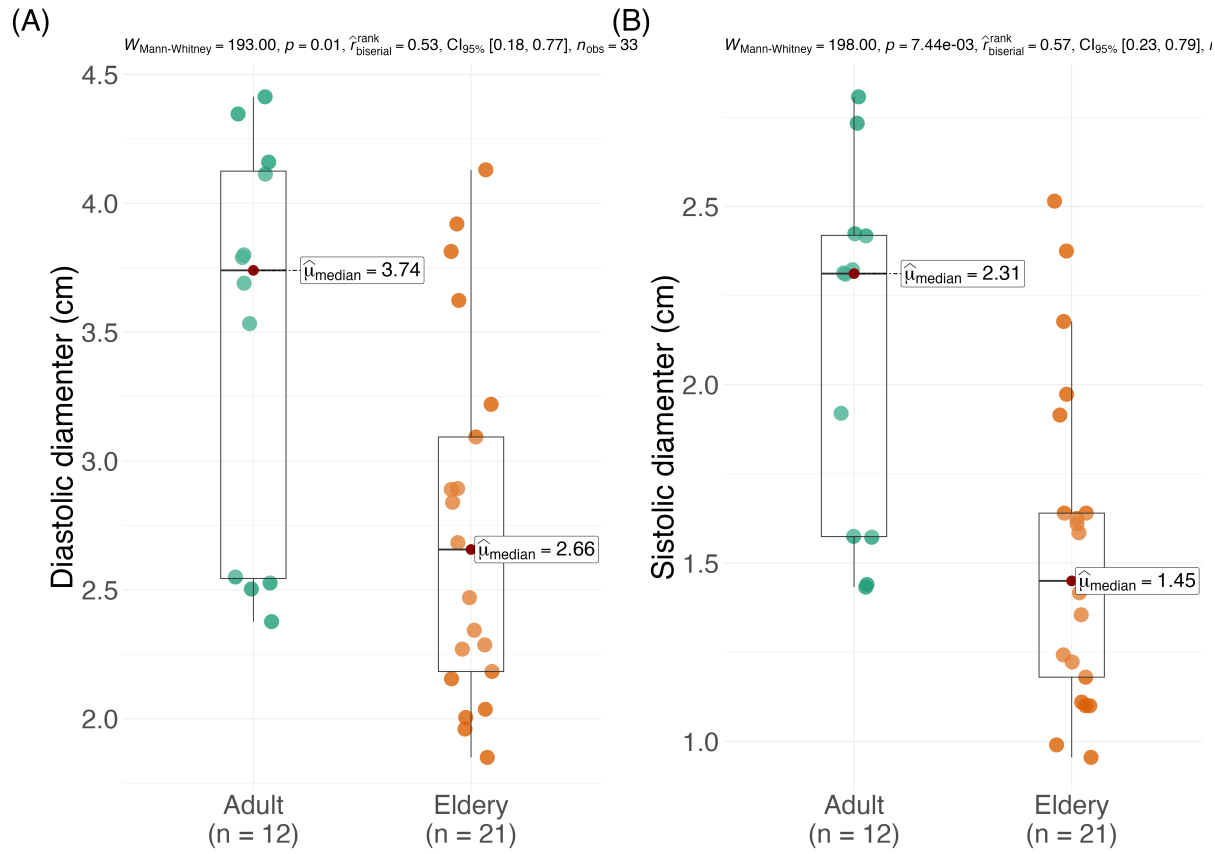

Figure 3: Comparison of structural parameters between young (2–7 years) and elderly (over 8 years) dogs. (A) Diastolic diameter and (B) systolic diameter. The boxplots show the median, interquartile range, and individual data points for each group. Statistical analysis was performed using the Mann-Whitney U test, revealing statistically significant differences in both diastolic and systolic diameters. The moderate effect sizes $\hat{r}_{\text{biserial}} = 0.53$ for diastolic diameter and $\hat{r}_{\text{biserial}} = 0.55$ for systolic diameter) further support the clinical relevance of these findings.

The parameters for shortening fraction (FS), ejection fraction by Simpson method (FES), and ejection fraction by the Teicholz method (FET) were compared between young (2–7 years) and elderly (over 8 years) dogs. FS was higher in adult dogs compared to elderly dogs ($t(30) = 1.95, p = 0.06$). The result did not reach conventional statistical significance, but the effect size was large ($g = 0.75, 95\%CI[-0.02, 1.50]$) (Figure 4A). However, FES did not show a statistically significant difference between adult and elderly dogs. ($t(31) = 0.94, p = 0.35$), and the effect size was small ($g = 0.35, 95\%CI[-0.38, 1.08]$) (Figure 4B). FET was significantly higher in eldery dogs compared to adult dogs ($t(-3.58) = -4.86, p = 1.15 \times 10^{-3}$), with a large effect size ($g = -1.29, 95\%CI[-2.04, -0.52]$) (Figure 4C).

Although FES did not reach statistical significance, the small effect size suggests that age-related differences in this parameter, if present, are likely minimal and may not be clinically meaningful. The significant differences in FS and FET, combined with their large effect sizes, suggest that aging may impact certain aspects of cardiac function in oncologic patients, particularly those related to systolic performance.

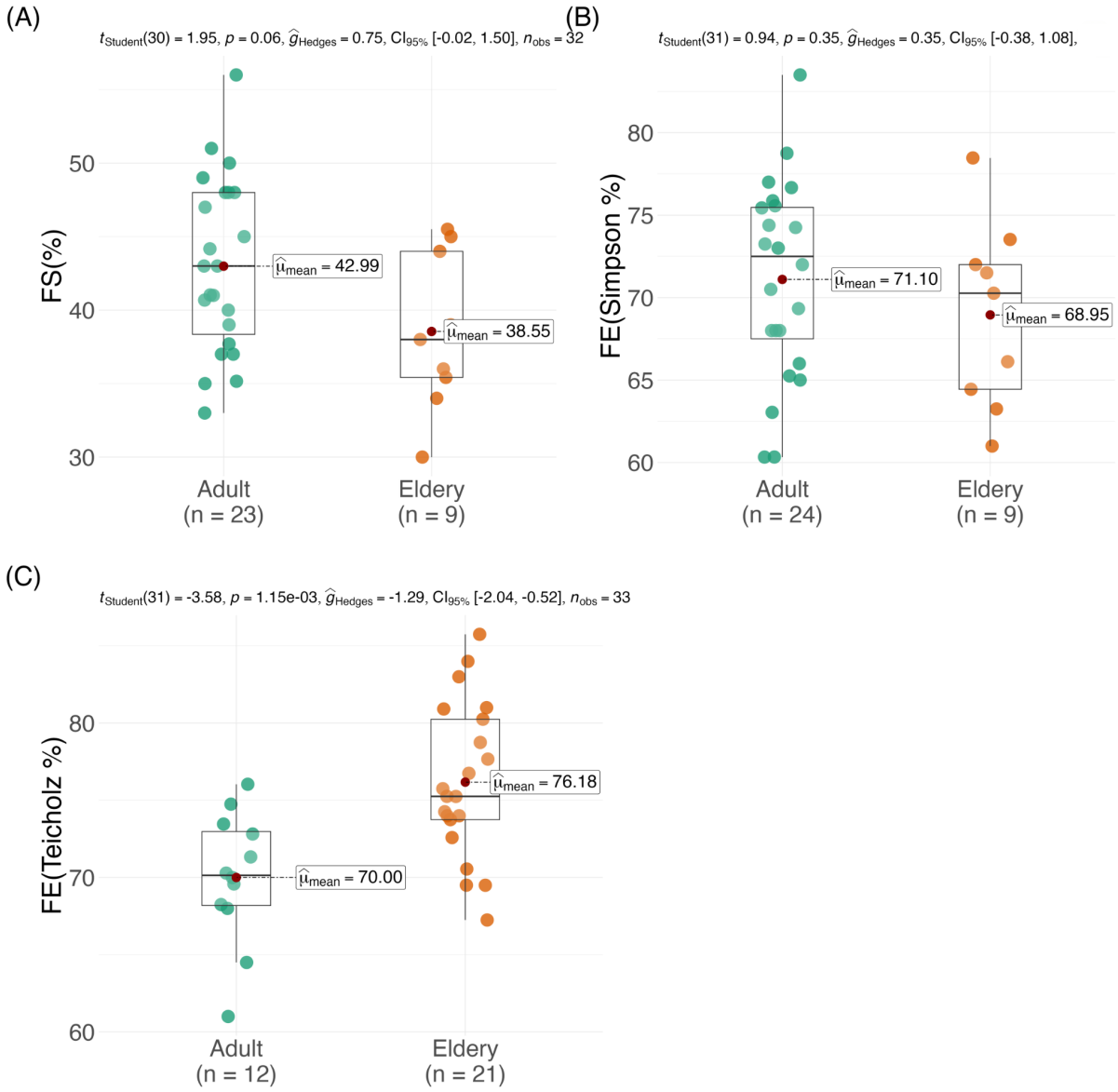

Figure 4: Comparison of cardiac functional parameters between young (2–7 years) and elderly (over 8 years) dogs. (A) Shortening fraction (FS), (B) Ejection fraction by the Simpson method (FES), and (C) Ejection fraction by the Teicholz method (FET). The boxplots show the median, interquartile range, and individual data points for each group. Statistical analysis was performed using Student's t-test. Significant differences are indicated by p-values, and effect sizes are reported as Hedges' g. FS and FET showed large effect sizes ($g = 0.75$ and $g = -1.29$, respectively), while FES did not reach statistical significance and had a small effect size ($g = 0.35$).

Longitudinal decline in systolic function and ventricular remodeling with doxorubicin exposure

A linear mixed-effects model (LME) was used to evaluate the effects of doxorubicin over time and across treatment groups on five cardiac function parameters: EF

(Simpson), EF (Teicholz), FS, and systolic and diastolic ventricular cavity dimensions.

Reduction in EF (Simpson) at T3 in the doxorubicin group

A significant reduction in EF (Simpson) was observed at timepoint T3 in the group treated with doxorubicin compared to T0. This reduction ($\Delta = -6.58$, $\mathrm{SD} = 2.7$, $p = 0.02$) was not seen in the group without doxorubicin or in the doxorubicin with combinations group, where the results did not reach statistical significance. For other parameters, such as EF (Teicholz) and FS, the changes from T0 were not statistically significant, although numerical reductions were noted at earlier timepoints (Table 3).

Table 3: Comparative analysis of the parameters EF (Simpson). EF (Teichholz). and FS for each protocol between time points T0–T3.

	FES			FET			FS		
	No doxo	Doxo	Assoc.	No doxo	Doxo	Assoc.	No doxo	Doxo	Assoc.
T0	–	–	–	–	–	–	–	–	–
T1	-4.78 [5.1] p = 0.39	-2.74 [2.2] p = 0.24	-1.33 [2.2] p = 0.55	4.79 [3.6] p = 0.23	-0.3 [2.1] p = 0.86	-0.29 [1.9] p = 0.88	6.08 [4.1] p = 0.19	-0.37 [1.8] p = 0.84	2.51[2.5] p = 0.33
T2	-5.45 [5.5] p = 0.37	-3.34 [2.4] p = 0.18	-3.00 [2.3] p = 0.21	2.83 [3.9] p = 0.50	1.28 [2.2] p = 0.57	-3.56 [2.1] p = 0.09	2.86 [4.4] p = 0.54	0.86 [1.9] p = 0.66	0.65 [2.6] p = 0.81
T3	9.22 [8.1] p = 0.31	-6.58 [2.7] **p = 0.02**	-3.63 [2.5] p = 0.16	1.65 [4.5] p = 0.73	0.80 [2.5] p = 0.75	0.09 [2.2] p = 0.97	3.20 [5.1] p = 0.55	0.38 [2.2] p = 0.86	2.50 [2.9] p = 0.39
Num.obs.#	12	41	59	13	41	59	13	41	59

Note: The value represents the difference in means comparing each respective time point to T0. The value in brackets is the standard deviation of the difference in means. The p-value refers to the statistical significance of the comparison. A result was considered statistically significant if $p < 0.05$. # Number of observations in each treatment group.

Late systolic cavity reduction and a trend toward diastolic decrease in the doxorubicin group

The systolic cavity dimension significantly decreased at T3 in animals treated with doxorubicin ($\Delta = 0.22$, $\mathrm{SD} = 0.1$; $p = 0.05$), suggesting late-stage systolic remodeling. The diastolic cavity also showed a tendency toward reduction at the same time point

($p = 0.10$), although this difference was not statistically significant. These findings indicate a structural impact of doxorubicin over time (Table 4).

Table 4: Comparative analysis of the parameters systolic cavity and diastolic cavity for each protocol at all time points.

	Sistolic cavitiy			Diastolic cavity		
	No doxo	Doxo	Associations	No doxo	Doxo	Associations
T0	–	–	–	–	–	–
T1	-0.05 [0.2]	0.03 [0.1]	0.06 [0.1]	0.09 [0.2]	-0.02 [0.1]	0.09 [0.1]
	p = 0.78	p = 0.75	p = 0.38	p = 0.71	p = 0.82	p = 0.22
T2	-0.11 [0.2]	-0.07 [0.1]	0.07 [0.1]	-0.13 [0.3]	-0.09 [0.1]	0.01 [0.1]
	p = 0.57	p = 0.44	p = 0.38	p = 0.64	p = 0.32	p = 0.95
T3	0.01 [0.2]	-0.22 [0.1]	0.05 [0.1]	0.05 [0.3]	-0.18 [0.1]	0.06 [0.1]
	p = 0.97	**p = 0.05**	p = 0.59	p = 0.88	p = 0.10	p = 0.48
Num.obs.#	13	41	59	13	41	59

Note: The value represents the difference in means comparing each respective time point to T0. The value in brackets is the standard deviation of the difference in means. The p-value refers to the statistical significance of the comparison. A result was considered statistically significant if $p < 0.05$. # Number of observations in each treatment group.

Doxorubicin and combination protocols reduce EF (Simpson) compared to controls at T3

EF (Simpson) was significantly lower at T3 in both the doxorubicin group ($\Delta = 18.74$, $\mathrm{SD} = 8.5$; $p = 0.04$) and the doxorubicin with combinations group (Δ = 18.39, SD = 8.3; p = 0.04) compared to the group without doxorubicin. This points to a strong treatment-related impairment in systolic function after multiple exposures (Table 5).

EF (Teicholz) and FS are reduced at T1 and T2 in treated groups

Marked reductions in EF (Teicholz) and FS were observed in both the doxorubicin and combination groups at early time points (T1 and T2). For EF (Teicholz) at T1, the reduction was significant in the doxorubicin group (Δ = 12.82, SD = 4.1; p < 0.01) and in the doxorubicin with combinations group (Δ = 10.85, SD = 3.9; p < 0.01). At T2, the values remained suppressed in the doxorubicin group (= 9.52, SD = 4.0; p = 0.02) and the doxorubicin with combinations group (Δ = 12.24, SD = 3.8; p < 0.01) (Table 2).

For FS, T1 values showed reductions in the doxorubicin group (= 12.51, SD = 3.6; p < 0.01) and in the doxorubicin with combinations group (Δ = 10.71, SD = 3.5; p < 0.01).

Table 5: Comparative analysis of the parameters EF (Simpson), EF (Teichholz), and FS between treatment protocols at time points T1 to T3.

	FES			FET			FS		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
No doxo	–	–	–	–	–	–	–	–	–
Doxo	0.56	-0.29	-18.74	-12.82	-9.52	-9.97	-12.51	-8.22	-9.57
	[3.9]	[5.7]	[8.5]	[4.1]	[4.0]	[5.7]	[3.6]	[3.3]	[4.7]
	p=0.89	p=0.96	**p=0.04**	**p<0.01**	**p=0.02**	p=0.10	**p<0.01**	**p=0.02**	p=0.06
Associations	-1.99	-3.20	-18.39	-10.85	-12.24	-8.91	-10.71	-9.43	-8.57
	[3.8]	[5.5]	[8.3]	[3.9]	[3.8]	[5.4]	[3.5]	[3.2]	[4.5]
	p=0.60	p=0.57	**p=0.04**	**p<0.01**	**p<0.01**	p=0.12	**p<0.01**	**p<0.01**	p=0.08
Num.obs.#	33	27	19	33	27	20	33	27	20

Note: Non-highlighted values represent the difference in means comparing the doxorubicin-treated group with the group that did not receive doxorubicin. The value in brackets is the standard deviation of the difference in means. The p-value refers to the statistical significance of the comparison. A result was considered statistically significant if $p < 0.05$. # Number of observations at each time point.

At T2, FS was still reduced for doxorubicin (Δ = 8.22, SD = 3.3; p = 0.02) and for the doxorubicin with combinations group (Δ = 9.43, SD = 3.2; p < 0.01) (Table 3).

Although EF and FS values remained lower at T3, the differences were no longer statistically significant for EF (Teicholz) when doxorubin was used alone (= 9.97, SD = 5.7; p = 0.10) or associated with other drugs (Δ = 8.91, SD = 5.4; p = 0.12. The same pattern was observed for FS when doxorubicin was used alone (Δ = 9.57, SD = 4.7; p = 0.06) or associated with other drugs (Δ = 8.57, SD = 4.5; p = 0.08). However, still suggests a persistent trend toward functional decline (Table 3).

Early increase in systolic cavity volume with doxorubicin at T1

The systolic cavity dimension was significantly increased at T1 in the doxorubicin group compared to group without doxorubicin (Δ = 0.7, SD = 0.3; p = 0.03), suggesting early ventricular dilation in response to treatment. A similar trend was observed at T2 (p = 0.07), while no significant differences were found in diastolic cavity measurements across groups at any timepoint (Table 6).

FS reduced at T2 in the combination group

The only statistically significant result in the adjusted multivariate model was a reduction in FS at T2 in the doxorubicin with combinations group (β = 6.63, SD = 3.0;

Table 6: Comparative analysis of the parameters Systolic Cavity and Diastolic Cavity between treatment protocols at time points T1 to T3.

	Systolic cavity			Diastolic cavity		
	T1	T2	T3	T1	T2	T3
No doxo	–	–	–	–	–	–
Doxo	0.7	0.6	0.5	0.7	0.8	0.7
	[0.3]	[0.3]	[0.4]	[0.4]	[0.4]	[0.5]
	p=0.03	p=0.07	p=0.20	p=0.10	p=0.11	p=0.19
Associations	0.4	0.5	0.6	0.4	0.4	0.7
	[0.3]	[0.3]	[0.4]	[0.4]	[0.4]	[0.5]
	p=0.13	p=0.16	p=0.12	p=0.34	p=0.37	p=0.17
Num.obs.#	33	27	20	33	27	20

Note: The value represents the mean difference between the treated groups and the control group without doxorubicin. Values in brackets indicate the standard deviation. The p-value refers to the statistical significance of the comparison. A result was considered statistically significant if $p < 0.05$. # Number of observations at each time point.

p = 0.03). All other comparisons, including EF (Simpson and Teicholz) and the effect of pre-existing disease, did not show statistically significant changes in the adjusted models (table 7).

T3 Interaction Between Pre-Existing Disease and Systolic Volume Confirms Structural Impact

A significant interaction between T3 and pre-existing disease was found in the systolic cavity model (β = 0.53, SD = 0.2; p = 0.02), suggesting that animals with underlying conditions may exhibit increased susceptibility to structural changes late in treatment. Additionally, the doxorubicin group alone showed a significant reduction in systolic cavity size at T3 (β = 0.60, SD = 0.2; p < 0.01), reinforcing the contraction-related effects of treatment. No significant findings were observed for the diastolic cavity or at earlier timepoints. (table 8).

In summary, doxorubicin, especially when combined with other drugs, caused progressive reductions in EF and FS and led to structural remodeling of the left ventricle, particularly evident at T3. Pre-existing cardiac disease had a limited impact, except for one significant interaction affecting systolic volume at the end of the observation period.

Table 7: Analysis of linear models for Simpson EF (FES). Teichholz EF (FET). and FS according to time. use of doxorubicin. presence of pre-existing disease. and their interactions.

	FES		FET		FS	
	No doxo	Assoc.	No doxo	Assoc.	No doxo	Assoc.
T1	-3.28	-0.68	-1.81	-2.54	-1.66	-2.00
	[3.6]	[3.2]	[3.3]	[2.8]	[2.9]	[3.6]
	p=0.38	p=0.83	p=0.59	p=0.38	p=0.58	p=0.59
T2	-5.76	-1.84	-1.58	-6.63	-1.71	-3.09
	[3.9]	[3.4]	[3.5]	[3.0]	[3.1]	[3.8]
	p=0.16	p=0.59	p=0.66	**p=0.03**	p=0.59	p=0.42
T3	-7.66	-5.59	3.72	-1.38	2.44	-1.01
	[5.1]	[3.8]	[4.5]	[3.3]	[4.0]	[4.2]
	p=0.15	p=0.15	p=0.42	p=0.68	p=0.55	p=0.81
pre-existing disease	-4.43	2.84	-2.27	0.41	-2.62	-4.60
	[4.9]	[3.6]	[3.6]	[3.6]	[3.1]	[4.0]
	p=0.38	p=0.45	p=0.54	p=0.91	p=0.41	p=0.26
T1 × pre-existing disease	0.91	-1.22	2.44	4.24	2.21	8.51
	[4.8]	[4.4]	[4.3]	[3.9]	[3.8]	[5.0]
	p=0.85	p=0.78	p=0.58	p=0.28	p=0.57	p=0.10
T2 × pre-existing disease	4.08	-2.19	4.83	6.10	4.35	7.23
	[5.1]	[4.7]	[4.6]	[4.2]	[4.1]	[5.3]
	p=0.43	p=0.65	p=0.30	p=0.15	p=0.29	p=0.18
T3 × pre-existing disease	1.91	3.54	-3.67	2.81	-2.45	6.73
	[6.2]	[5.1]	[5.5]	[4.5]	[4.8]	[5.7]
	p=0.76	p=0.49	p=0.51	p=0.54	p=0.62	p=0.25
Num.obs.#	41	59	41	59	41	59

Note: Values represent the coefficients of the interaction model, with standard deviations in brackets. p-values $p < 0.05$ are shown in bold. # Number of observations in each protocol.

4.4 Discussion

This study evaluated 33 dogs receiving three different chemotherapy protocols: one with doxorubicin alone, another combining doxorubicin with one or more chemotherapeutic agents, and a third without doxorubicin. Animals were monitored with echocardiographic exams prior to each doxorubicin dose. The administered dose was 30 mg/m², reaching a total cumulative dose of 90 mg/m². None of the animals exhibited any clinical signs of heart failure.

An increase in the left ventricular internal diameter in both diastole and systole has been described by other authors, even when therapeutic doses were used and cumulative doses started at 90 mg/m² (Souza, M.G. et al., 2013; Hallman, B.E. et

Table 8: Analysis of linear models for left ventricular cavity dimensions (systolic and diastolic) according to time. use of doxorubicin. presence of pre-existing disease. and their interactions.

	Diastolic cavity		Diastolic cavity	
	Doxo	Associations	Doxo	Associations
T1	0.04 [0.1]	0.11 [0.1]	-0.13 [0.1]	0.10 [0.1]
	p=0.76	p=0.31	p=0.36	p=0.37
T2	0.00 [0.1]	0.11 [0.1]	-0.08 [0.2]	0.03 [0.1]
	p=0.98	p=0.35	p=0.61	p=0.82
T3	-0.60 [0.2]	0.17 [0.1]	-0.33 [0.2]	0.20 [0.1]
	p<0.01	p=0.19	p=0.10	p=0.13
pre-existing disease	-0.27 [0.3]	-0.26 [0.3]	-0.59 [0.4]	-0.37 [0.4]
	p=0.35	p=0.38	p=0.20	p=0.36
T1 × pre-existing disease	-0.02 [0.2]	-0.08 [0.2]	0.19 [0.2]	-0.01 [0.2]
	p=0.92	p=0.57	p=0.31	p=0.94
T2 × pre-existing disease	-0.13 [0.2]	-0.07 [0.2]	-0.02 [0.2]	-0.04 [0.2]
	p=0.46	p=0.64	p=0.90	p=0.79
T3 × pre-existing disease	0.53 [0.2]	-0.23 [0.2]	0.22 [0.2]	-0.25 [0.2]
	p=0.02	p=0.19	p=0.36	p=0.15
Num.obs.#	41	59	41	59

Note: Values represent the coefficients of the interaction model, with standard deviations in brackets. p-values $p < 0.05$ are highlighted in bold. # Number of observations in each protocol.

al., 2018). Our results corroborate these studies, with an increase in sistolic diameter observed in animals receiving doxorubicin. Ejection fraction, a primary measure for assessing left ventricular systolic function, can be evaluated using two different methods: Teichholz (EF_T) and Simpson (EF_S). EF_S tends to be the more sensitive method as it measures the left ventricular area while considering variations in its shape (Kosaraju, A. et al., 2023). Both FS (shortening fraction) and EF_T decreased in the group of patients that received doxorubicin, either alone or in combination with other drugs, even though the values remained within the normal range for the species and weight. Gallay-Lopoutre, J. and collaborators did not find changes in FS in their study; however, despite FS values staying within reference limits, our study showed a reduction in this parameter in animals that received doxorubicin. It is worth noting that this reduction in EF_T and FS was more significant in adult dogs compared to elderly ones. This can be attributed to the greater bioavailability of the drug in adult animals, which have a greater metabolization capacity, leading to more severe lesions in cardiomyocytes. . Therefore, age should be considered a relevant variable when evaluating cardiotoxicity, especially when interpreting reductions in parameters such as EF_T and FS.

As previously described in humans and dogs, anthracycline-induced cardiotoxicity is dose-dependent, with the cumulative effect of this drug being the main cause. Cumula-

tive doses from 122 to 265 mg/m² and, in rarer cases, from 90 mg/m² can cause heart failure (Mauldin, G.E. et al., 1992; Souza, M.G. et al., 2013; Hallman, B.E. et al., 2018). At a cumulative dose of 90 mg/m² (T3 timepoint), a decrease in EF_S was observed in animals treated with doxorubicin alone. The isolated use of this drug is generally associated with shorter application intervals compared to its use in combination with other drugs, as combination protocols tend to involve longer cycles, which may explain the tendency for this parameter to decrease in this group (Gallay-Lopoutre, J. et al., 2016).

Decreases in EF_T and EF_S were observed when analyzing data over time (T0, T1, T2, and T3), with significant drops at T1 and T2 and borderline values at T3 in both the doxorubicin-alone and combination groups, supporting studies that show the cumulative effect of this drug. Hallman et al. (2018) observed clinical cardiotoxicity in dogs after the fourth dose of doxorubicin, with most of these animals showing a trend toward systolic function decline in earlier exams. Additionally, the systolic cavity showed a significant increase at T1.

The decline in values indicative of cardiac function (EF_S, EF_T, and FS) is associated with three main factors: oxidative stress, inflammation, and mitochondrial dysfunction. Through these mechanisms, triggered by the administration of doxorubicin, various programmed cell death processes, including apoptosis, pyroptosis, necroptosis, autophagy, and ferroptosis, are initiated, ultimately leading to heart failure. (Lighua, C. et al., 2024; Yanzhao, L. et al., 2024).

Overall, the results indicate that doxorubicin, either alone or in combination, promotes significant changes in ventricular function, particularly in ejection fraction and systolic cavity size. The most pronounced effects were observed from T1 onwards, with worsening at T2 and T3, suggesting a progressive impact of drug-induced cardiotoxicity. Mitral valve disease is the most common cardiopathy in dogs, mainly affecting adult and elderly dogs, the same age group in which neoplasms are more frequent. We evaluated whether pre-existing valvular disease influenced the echocardiographic parameters of dogs treated with doxorubicin. No significant changes were found when comparing the presence or absence of mitral valve disease, which is consistent with the results of Hallman, B. et al., 2018. , who, in their evaluation of risk factors for cardiotoxicity associated with doxorubicin, found that valve disease did not negatively influence the results.

The use of anthracyclines in humans is common, and monitoring of their cardiotoxic effects involves echocardiography in addition to sensitive biomarkers for detecting cardiac injury. Strain longitudinal global (SLG) and two- and three-dimensional assessments are performed in the follow-up of these patients. The cardio-oncology guidelines

recommend that a decrease greater than or equal to 15% in GLS or a reduction greater than or equal to 10% in the basal rate of left ventricular ejection fraction are considered cardiac dysfunction, even without clinical signs (Hajjar, L.A. et al., 2020). In this study, the ejection fraction values measured by the Teicholz and Simpson methods did not show a reduction greater than 10%, and therefore, none of the patients evaluated presented cardiac dysfunction.

We observed a reduction in systolic parameters such as LF, TE, and EEP during doxorubicin treatment, and these values remained within the normal range established for dogs of similar species, size, and age. This raises a relevant question regarding the sensitivity of the currently used clinical reference ranges: could small changes within the “normal” range already indicate the onset of subclinical dysfunction? Previous studies suggest that percentage variations within this range may be associated with early structural changes in the myocardium and precede clinical signs of cardiotoxicity. Thus, exclusive reliance on reference ranges may not be sufficient to identify early changes induced by cardiotoxic chemotherapeutic agents, especially when patients are not followed longitudinally. Therefore, we emphasize the importance of serial and individualized monitoring of cardiac function, paying close attention to progressively decreasing trends, even if the absolute values remain within normal limits.

The use of cardioprotective agents in dogs undergoing doxorubicin protocols such as carvedilol (Vaynblat, M. et al., 2002), antioxidants (Xin, Y. et al., 2011; Pino, E.H.M. et al., 2021), and diphenhydramine (Willcox, J.L. et al., 2020) has provided little or no benefit to these patients. No cardioprotective agents were used in this study. In this context, regardless of whether cardioprotectors are used, the administration of doxorubicin should be accompanied by cardiac function monitoring in each patient to minimize the occurrence of clinical or subclinical cardiac dysfunction. Doxorubicin acts by damaging the DNA of cancer cells, disrupting cell membranes, and generating oxidative stress that triggers apoptosis and consequently leads to cell death (Thorn, C.F. et al., 2011). This enhances its anticancer effect, and combined with the low treatment cost, makes its use feasible despite its cardiotoxic risks. The limitations of this study included the small sample size, resulting from the dependence on clinical routine and patient eligibility for chemotherapy protocols; the difficulty in monitoring animals after chemotherapy sessions due to the unavailability of owners to return with their pets; and the heterogeneity of the protocols used, the intervals between administrations, and the different types of diagnosed tumors, all of which may influence the individual response to the drugs administered.

In light of this and the findings of this study, we reiterate the importance of longitudinal monitoring of echocardiographic parameters during doxorubicin use, even in combined regimens, with particular attention to the later stages of treatment. Early surveillance

strategies and individualized preventive interventions are recommended to mitigate the cardiovascular effects associated with the use of this chemotherapeutic agent.

4.5 Conclusion

The use of doxorubicin was shown to be safe in this study, as no patient developed clinical signs of cardiac dysfunction or changes that could be classified as subclinical. Monitoring the animals during treatment is essential, as the results indicate a trend of decline in echocardiographic parameters assessing systolic heart function. Strategies for early detection of declining cardiac function are necessary for the safe use of this drug.

4.6 Referências

ADAMS, V.J., Evans, K.M., Sampson, J., Wood, J.L.N., 2010a. Methods and mortality results of a health survey of purebred dogs in the UK. The Journal of Small Animal Practice 51, 512–524.

https://doi.org/10.1111/j.1748-5827.2010.00974.x

Alvarez-Cardona, J., Lenihan, D.J., 2019a. Anthracycline Cardiotoxicity: It Is Possible to Teach an Old Dog Some New Tricks. Cardiology Clinics 37, 355–363.

https://doi.org/10.1016/j.ccl.2019.08.001

BOON, J. A. Evaluation of size, function and hemodynamics. In Manual of Veterinary Echocardiography. 2nd edn. Ed J. A. Boon. Blackwell Publishing, Chichester, U.K. pp 105-198

CIRILLO, J. V. *Tratamento quimioterápico das neoplasias mamárias em cadelas e gatas*. [s.d.].

COSTA, f.s., sobrinho, a.q.a., roesler, t., de oliveira, d.c., borlini, d.c., dos santos, w.g., monteiro, j.n.m. avaliação da função miocárdica em cães e gatos pela técnica de doppler tecidual durante quimioterapia com doxorrubicina, revisão de literatura . Revista pubvet, v.2, n.21, art. 235, 2008.

FARO, A. M. et al. *Avaliação hematológica em cães submetidos ao tratamento quimioterápico com sulfato de vincristina, prednisona e ciclofosfamida. Estudo experimental*. [s.d.].

GALLAY-LEPOUTRE, J.; BÉLANGER, M. C.; NADEAU, M. E. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. *Research in Veterinary Science*, v. 105, p. 153–159, abr. 2016.

https://doi.org/10.1016/j.rvsc.2016.02.001

HAJJAR L A , Costa I B S S , Lopes M A C Q , Hoff P M G , Diz M D P E , Fonseca S M R , Bittar C S , et al. Diretriz Brasileira de Cardio-oncologia – 2020. Arq. Bras. Cardiol. 2020;115(5):1006-43.

HALLMAN, B. E. et al. Incidence and risk factors associated with development of clinical cardiotoxicity in dogs receiving doxorubicin. *Journal of Veterinary Internal Medicine*, v. 33, n. 2, p. 783–791, mar. 2019.

https://doi.org/10.1111/jvim.15414

HAMMER, Ai.S., Couto, C.G., Filppi, J., Getzy, D., Shank, K., 1991. Efficacy and Toxicity of VAC Chemotherapy (Vincristine, Doxorubicin, and Cyclophosphamide) in Dogs with Hemangiosarcoma. Veterinary Internal Medicne 5, 160–166.

https://doi.org/10.1111/j.1939-1676.1991.tb00943.x

KOSARAJU, A. et al. Left Ventricular Ejection Fraction. Em: *StatPearls* [Internet]. [s.l.] StatPearls Publishing, 2023.

LI, Y.; YAN, J.; YANG, P. The mechanism and therapeutic strategies in doxorubicin-induced cardiotoxicity: Role of programmed cell death. *Cell Stress and Chaperones*, v. 29, n. 5, p. 666–680, 1 out. 2024.

https://doi.org/10.1016/j.cstres.2024.09.001

MACDONALD, V., 2009. Chemotherapy: Managing side effects and safe handling. Can Vet J 50, 665–668.

MANTOVANI, M. M. et al. Systolic cardiac function assessment by feature tracking echocardiography in dogs with myxomatous mitral valve disease. *Journal of Small Animal Practice*, v. 56, n. 6, p. 383–392, 2015.

https://doi.org/10.1111/jsap.12347

MAULDIN, G. E. et al. Doxorubicin-Induced Cardiotoxicosis: Clinical Features in 32 Dogs. *Journal of Veterinary Internal Medicine*, v. 6, n. 2, p. 82–88, mar. 1992.

https://doi.org/10.1111/j.1939-1676.1992.tb03156.x

NARDI, A. B. D. et al. *Prevalência de neoplasias e modalidades de tratamentos em cães, atendidos no Hospital Veterinário da Universidade Federal do Paraná*. Archives of Veterinary Science, 2002.

PINO, E. H. M. et al. Evaluation of cardioprotective effects of carvedilol in dogs receiving doxorubicin chemotherapy: A prospective, randomized, double-blind, placebo-controlled pilot study. *Research in Veterinary Science*, v. 135, p. 532–541, mar. 2021.

https://doi.org/10.1016/j.rvsc.2020.11.007

REPETTI, C.S.F., Rueda, J.R., Porto, C.D., Franco, R.P., Girio, R.J.S., Manhoso, F.F.R., da Costa, I.B., 2023. Palliative care for cancer patients in veterinary medicine. Vet Med (Praha) 68, 2–10.

https://doi.org/10.17221/76/2022-VETMED

SILVA, C.E.V., Camacho, A.A., 2005. Alterações ecocardiográficas em cães sob tratamento prolongado com doxorrubicina. Arq. Bras. Med. Vet. Zootec. 57, 300–306.

https://doi.org/10.1590/S0102-09352005000300004

SIMON BT, Steagall PV. The present and future of opioid analgesics in small animal practice. J Vet Pharmacol Ther. 2017 Aug;40(4):315-326. doi: 10.1111/jvp.12377. Epub 2016 Nov 30. PMID: 27900781.

https://doi.org/10.1111/jvp.12377

SONG, L. et al. Mechanisms of doxorubicin-induced cardiac inflammation and fibrosis; therapeutic targets and approaches. *Archives of Biochemistry and Biophysics*, v. 761, p. 110140, 1 nov. 2024.

https://doi.org/10.1016/j.abb.2024.110140

SOUSA, M. et al. Assesment of the TEI index of myocardial performance in dogs with doxorubicin-induced cardiomiopathy. *Archivos de Medicina Veterinaria*, v. 46, n. 1, p. 63–68, 2014.

https://doi.org/10.4067/S0301-732X2014000100009

THORN, C. F. et al. Doxorubicin pathways: pharmacodynamics and adverse effects. *Pharmacogenetics and Genomics*, v. 21, n. 7, p. 440–446, jul. 2011.

https://doi.org/10.1097/FPC.0b013e32833ffb56

VAYNBLAT, M. et al. Simultaneous angiotensin converting enzyme inhibition moderates ventricular dysfunction caused by doxorubicin. *European Journal of Heart Failure*, v. 4, n. 5, p. 583–586, out. 2002.

https://doi.org/10.1016/S1388-9842(02)00091-0

VISSER, L. C. et al. Echocardiographic quantitation of left heart size and function in 122 healthy dogs: A prospective study proposing reference intervals and assessing repeatability. *Journal of Veterinary Internal Medicine*, v. 33, n. 5, p. 1909–1920, set. 2019.

https://doi.org/10.1111/jvim.15562

WILLCOX, J. L. et al. Intramuscular Diphenhydramine Does Not Affect Acute Doxorubicin Infusion-Related Arrhythmia Number or Severity in a Prospective Crossover Study in Canine Lymphoma: A Pilot Study. *Frontiers in Veterinary Science*, v. 7, p. 368, 17 jul. 2020.

https://doi.org/10.3389/fvets.2020.00368

XIN, Y. et al. Electrocardiographic and Biochemical Evidence for the Cardioprotective Effect of Antioxidants in Acute Doxorubicin-Induced Cardiotoxicity in the Beagle Dogs. *Biological and Pharmaceutical Bulletin*, v. 34, n. 10, p. 1523–1526, 2011.

https://doi.org/10.1248/bpb.34.1523

YADAV PRIYANKA, H. B. *A Comprehensive Review of Commonly Available Anticancer Plants*. 18 maio 2025.

Apêndice

Universidade Federal de Uberlândia
Pró-Reitoria de Pesquisa e Pós-Graduação
- Comissão de Ética na Utilização de Animais (CEUA) -
Rua Ceará, S/N - Bloco 2D, sala 08 - Campus Umuarama - Uberlândia-MG
CEP 38405-315; e-mail:ceua@propp.ufu.br; www.comissoes.propp.ufu.br
Telefone: 3225-8652

TERMO DE CONSENTIMENTO DO RESPONSÁVEL PELO(S) ANIMAL(AIS)

Você está sendo convidado para participar de uma pesquisa intitulada Avaliação ecodopplercardigráfica de cães submetidos a protocolos quimioterápicos, sob responsabilidade do pesquisador Gustavo Cavinato Herrera. Este estudo foi aprovado pela Comissão de Ética no uso de Animais da Universidade Federal de Uberlândia, CEUA-UFU, CNPJ 25.648.387/0001-18, e tem por objetivos fazer a avaliação ecodopplercardiográfica de cães submetidos a quimioterapia, provenientes do setor de clínica e cirurgia oncológica (SECCON) do Hospital Veterinário da Universidade Federal de Uberlândia, realizando exames pré e pós medicação, obtendo assim informações sobre estas drogas no sistema cardiovascular.

Para participar deste estudo, o(s) animais serão submetidos ao exame ecodopplercardiográfico antes, durante e após o término do protocolo quimioterápico. O animal será colocado sobre a mesa de exame em decúbito lateral, contido pelo seu tutor, e assim será realizado o exame, não sendo necessário sedação. Todas as vezes que o animal vier para medição quimioterápica ele será submetido ao exame.

O cronograma proposto de atividades é: O exame será realizado antes do início do protocolo quimioterápico e antes de cada seção de quimioterapia. O exame será repetido 3 meses após o término do tratamento.

O exame ecodopplercardiográfico não riscos ao animal nem ao seu tutor

Este estudo possibilitará os benefícios de conhecer o grau de cardiotoxicidade dos diversos agentes antineoplásicos utilizados na medicina veterinária

Esclarecemos ainda, que sua autorização para a inclusão do(s) seu(s) animal(is) nesse estudo é voluntária. Seu(s) animal(is) poderá(ão) ser retirado(s) do estudo, a qualquer momento, sem que isso cause qualquer prejuízo a ele(s).

A confidencialidade dos seus dados pessoais será preservada.

Os membros da CEUA ou as autoridades regulatórias poderão solicitar suas informações, e nesse caso, elas serão dirigidas especificamente para fins de inspeções regulares.

O Médico Veterinário responsável pelo(s) seu(s) anima(is) será o(a) Dr(a) Gustavo Cavinato Herrera, inscrito(a) no CRMV-MG sob o número 7332. Além dele, a equipe do Pesquisador Principal também se responsabilizará pelo bem estar do(s) seu(s) animal(is) durante todo o estudo e ao final dele.

Quando for necessário, durante ou após o período do estudo, você poderá entrar em contato com o Pesquisador Principal ou com a sua equipe pelos seguintes contatos:

Tel. de emergência: 34-991037199
Endereço: Avenida dos Ferreiras 475
Telefone: 34-32257411

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, a declaração anexa.

DECLARAÇÃO DE CONSENTIMENTO

Fui devidamente esclarecido(a) sobre todos os procedimentos deste estudo, seus riscos e benefícios ao(s) animal(is) pelo(s) qual(is) sou responsável. Fui também informado que posso retirar

meu(s) animal(is) do estudo a qualquer momento. Ao assinar este Termo de Consentimento, eu _____, portador do RG _____, declaro que autorizo a participação do(s) meu(s) animal(is) identificado(s), a seguir, neste projeto.

Este documento será assinado em duas vias, sendo que uma via ficará comigo e outra com o pesquisador.

Identificação do(s) animal(is) (repetir tantas vezes quantos foram os animais)
Nome ou Número de identificação: (especificar)
Espécie: (especificar)
Raça: (especificar)

Uberlândia, ____de _____de 20__.

---------- ----------

Assinatura do Proprietário Assinatura do Pesquisador

Table 9: Resumo dos casos clínicos de cães tratados com quimioterapia.

Paciente	Raça	Idade	Sexo	Peso (Kg)	Protocolo	Data T0	Data T1	Data T2	Data T3
1	Pug	3	Macho	9,3	CFM, DOX, VIN e PRE	25/01/23	12/02/23	29/03/23	21/06/23
2	SRD	9	Fêmea	26,4	DOX	20/09/22	24/01/23		
3	SRD	2	Macho	20,5	DOX	13/07/22	08/08/22	12/03/23	03/10/22
4	SRD	7	Macho	7,9	DOX	17/03/22	04/04/22	25/04/22	06/06/22
5	Border Collie	4	Fêmea	25,6	DOX	14/06/22	28/07/22	11/10/22	
6	Pug	3	Fêmea	9,8	CFM, DOX, VIN e PRE	15/03/22	14/04/22		
7	Pinscher	9	Fêmea	4,4	CBP	17/08/22	30/11/22		
8	Pitbull	5	Fêmea	20,4	DOX	01/12/22	20/03/23	10/04/23	
9	Golden	5	Fêmea	36,2	DOX e CFM	18/02/23	11/03/23	01/04/23	27/04/23
10	Maltes	19	Fêmea	4,1	DOX, CBP e CFM	23/11/23	21/12/23		
11	Maltes	7	Fêmea	21,6	DOX e CFM	29/07/23	11/09/23	29/09/23	23/10/23
12	SRD	5	Fêmea	12,25	DOX	06/10/23	30/10/23	20/Nov	11/12/23
13	SRD	11	Fêmea	12,25	CFM, DOX, VIN e PRE	11/05/23	03/07/23	23/08/23	09/11/23
14	Pitbull	11	Macho	37,7	DOX e CBP	28/08/23	18/09/23	09/10/23	
15	Chowchow	11	Fêmea	20,1	DOX e CBP	17/11/22	30/12/22	13/02/23	28/03/23
16	Poodle	11	Fêmea	7	DOX e CBP	30/05/23	22/06/23		
17	Yorkshire	14	Fêmea	5	DOX e CBP	09/03/23	19/04/23	01/06/23	17/07/23
18	SRD	10	Fêmea	8,7	DOX	14/09/22	16/11/22	13/02/23	06/04/23
19	Shihtzu	9	Fêmea	8	DOX e CBP	01/08/23	12/09/23	13/10/23	
20	SRD	14	Fêmea	20,7	CFM	23/03/23	06/07/23	17/08/23	
21	Yorkshire	10	Fêmea	5,8	CFM, DOX, VIN e PRE	02/07/22	01/09/22	07/10/22	19/11/22
22	Shihtzu	12	Fêmea	6,8	DOX	11/10/22	28/12/23		
23	SRD	15	Fêmea	13,2	CFM, DOX, VIN e PRE	06/02/22	28/02/23	28/03/23	
24	SRD	8	Macho	34,4	DOX e CFM	14/07/22	23/08/22	15/09/22	
25	SRD	13	Fêmea	13,6	DOX e CFM	11/10/22	02/12/22	28/12/22	25/01/23
26	SRD	12	Fêmea	4,1	CBP	07/02/23	18/04/23	26/09/23	30/10/23
27	SRD	8	Fêmea	6,5	DOX	07/02/23	14/03/23	18/04/23	06/05/23
28	Yorkshire	12	Fêmea	3,4	CFM, DOX, VIN e PRE	10/12/22	21/01/23	11/03/23	24/04/23
29	Shihtzu	13	Fêmea	5,7	CBP	28/12/22	22/02/23	19/04/23	10/05/23
30	Poodle	10	Fêmea	5,2	DOX e CBP	20/06/23	07/08/23	20/09/23	01/11/23
31	SRD	10	Fêmea	14,3	DOX	06/02/23	27/02/23	03/04/23	24/04/23
32	SRD	13	Fêmea	19,3	DOX	16/02/23	14/03/23	06/04/23	03/05/23
33	SRD	8	Macho	24	DOX	19/10/22	16/11/22	22/12/22	19/01/23

Abreviações: CFM (Ciclofosfamida), **DOX** (Doxorrubicina), **VIN** (Vincristina), **PRE** (Prednisona), **CBP** (Carboplatina).

Table 10: Diagnósticos

Paciente	Diagnóstico
1	Linfoma
2	Hemangiossarcoma
3	TVT recidivante após 6 sessões de Vincristina
4	Sarcoma mesenquimal
5	Linfoma
6	Linfoma
7	Carcinoma cístico papilifero Grau II + Neo mama reicidente
8	Carcinoma Espinocelular
9	Sarcoma de tecidos moles grau II
10	Neoplasia pulmonar
11	Hemangiossarcoma esplenico
12	TVT recidivante após 6 sessões de Vincristina
13	Linfoma multicêntrico
14	Osteossarcoma mandibular
15	Carcinoma mamário em tumor misto grau III
16	Carcinoma mamário sólido grau III
17	Carcinoma mamário papilífero grau II e adenocarcinoma papilar em pulmão
18	Hemangiossarcoma
19	Carcinoma mamário em tumor misto grau I
20	Hemangiossarcoma
21	Linfoma
22	Hemangiossarcoma
23	Mastocitoma
24	Hemangiossarcoma esplenico
25	Carcinoma mamário com metastase pulmonar
26	Carcinoma mamário
27	Neoplasia esplenica
28	Linfoma multicêntrico
29	Carcinoma mamário
30	Carcinoma mamário tubular grau I e sarcoma estromal mamário
31	Adenocarcinoma intestinal
32	Hemangiossarcoma esplenico
33	Hemangiossarcom cutâneo