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Comissão Científica:

Editorial – Inaugural Issue of ARBIO

It is with great enthusiasm that we present the inaugural issue of **ARBIO – Applied Research in Biosciences**, the scientific journal of the School of Biosciences of Elvas (ESBE).

The creation of ARBIO reflects our commitment to promoting scientific knowledge and fostering the dissemination of high-quality research in the fields of Biosciences. As a developing project, this journal aims to establish itself as a dynamic platform for scientific communication, encouraging the exchange of knowledge among researchers, academics, professionals, and students from diverse disciplines and institutions.

Although ARBIO emerged within the academic environment of ESBE, its scope extends far beyond our institution. The journal welcomes contributions from the national and international scientific community, providing an inclusive forum for the publication of original research articles, review papers, and other scientific contributions that advance knowledge and innovation.

ARBIO is dedicated to the dissemination of scientific work in the thematic areas represented at ESBE, including Veterinary Sciences and Veterinary Nursing, Agronomy, Sports Sciences, and Education and Cross-Cutting Topics. These areas encompass a wide range of subjects, from animal health, welfare, reproduction, and public health, to sustainable agriculture, natural resource management, climate change adaptation, sports performance, exercise physiology, pedagogy, ethics, scientific communication, and societal and environmental impacts.

In a world facing increasingly complex challenges, scientific progress depends on interdisciplinary collaboration and the effective dissemination of knowledge. ARBIO seeks to contribute to this mission by promoting rigorous, innovative, and socially relevant research capable of generating positive impacts on society and the environment.

We would like to express our sincere gratitude to the authors who entrusted us with their work, to the reviewers whose expertise and dedication ensure the scientific quality of the journal, and to the Editorial and Scientific Boards whose commitment has made this first issue possible.

As we launch this inaugural edition, we invite researchers, educators, professionals, and students to join us in building a vibrant scientific community around ARBIO. We hope this journal will become a valuable reference for applied research in biosciences and a catalyst for scientific collaboration across disciplines and borders.

Welcome to the first issue of ARBIO.

The Editorial Team

Editorial – Número Inaugural da ARBIO

É com enorme satisfação que apresentamos o primeiro número da **ARBIO – Applied Research in Biosciences / Investigação Aplicada em Biociências**, a revista científica da Escola Superior de Biociências de Elvas (ESBE).

A criação da ARBIO reflete o compromisso da ESBE com a promoção do conhecimento científico e com a divulgação de investigação de elevada qualidade nas diversas áreas das Biociências. Este é um projeto em desenvolvimento que pretendemos consolidar através da participação ativa de investigadores, docentes, profissionais e estudantes, contribuindo para uma maior visibilidade da produção científica e para o fortalecimento da cultura de investigação.

Embora a ARBIO tenha nascido no contexto académico da ESBE, a sua vocação é ampla e inclusiva. A revista está aberta à comunidade científica nacional e internacional, acolhendo trabalhos originais, artigos de revisão e outras contribuições científicas que promovam o avanço do conhecimento e da inovação.

A missão da ARBIO é divulgar investigação aplicada nas áreas científicas lecionadas na ESBE, nomeadamente Ciências Veterinárias e Enfermagem Veterinária, Agronomia, Ciências do Desporto e Educação e Temáticas Transversais. Estas áreas abrangem temas tão diversos como a saúde e o bem-estar animal, a reprodução e produção animal, a saúde pública, a agricultura sustentável, a gestão dos recursos naturais, a adaptação às alterações climáticas, o rendimento desportivo, a fisiologia do exercício, a pedagogia, a ética, a divulgação científica e o impacto social e ambiental da atividade humana.

Num contexto global marcado por desafios cada vez mais complexos, acreditamos que a ciência deve assumir um papel central na procura de soluções sustentáveis e inovadoras. A ARBIO pretende contribuir para esse objetivo, promovendo investigação rigorosa, interdisciplinar e relevante para a sociedade, incentivando simultaneamente a partilha de conhecimento entre diferentes áreas científicas.

Expressamos o nosso sincero agradecimento a todos os autores que contribuíram para este número inaugural, aos revisores pelo seu rigor e dedicação, e aos membros dos Conselhos Editorial e Científico, cujo empenho foi fundamental para a concretização deste projeto.

Ao iniciarmos esta nova etapa, convidamos toda a comunidade científica a participar no crescimento e consolidação da ARBIO. Esperamos que esta revista se afirme como uma referência na divulgação da investigação aplicada em Biociências e como um espaço privilegiado para o intercâmbio de ideias, a inovação e a colaboração científica.

Sejam bem-vindos ao primeiro número da ARBIO.

A Equipa Editorial

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Análise comparativa de tratamentos no enraizamento de clones de *Cannabis sativa* L.

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Summary

Cannabis sativa L. has gained increasing global prominence for medicinal and therapeutic purposes, and in Portugal it is an expanding crop. Due to the need to produce new plants through macropropagation, a cultivar with a low rooting rate was selected to evaluate rooting performance under different propagation conditions. A comparative analysis involving eight treatments was conducted to determine which treatment achieved the highest rooting success. The treatments were based on three variables: clone size, light intensity from the fifth day of rooting onwards, and the electrical conductivity of the fertigation solution. Throughout the trial, thirty-three clones were propagated for each of the eight treatments in their respective substrate trays. Measurements were taken from each clone at two different time points, and the emergence of new leaves was also monitored. Root development was assessed by counting the number of visible roots on three separate occasions to obtain more reliable results. Each of the eight treatments was replicated three times, with treatments randomly distributed within the propagation room to minimise experimental bias. Following data collection, statistical analyses were performed to determine the effects of the different treatments. Comparison with the control treatment allowed the identification of the factors that most strongly influenced clone rooting success and subsequent plant development.

Keywords: *Cannabis sativa* L; Rooting; Macropropagation; Substrate; Variety.

Resumo

A *Cannabis sativa* L. tem vindo a ganhar destaque para fins medicinais e terapêuticos em termos globais, e no nosso país é uma cultura que se encontra em expansão. Com a necessidade de gerar novas plantas a partir do processo de macropropagação foi decidido escolher uma variedade com baixa taxa de enraizamento e assim através de uma análise comparativa, utilizando 8 tratamentos, procurou-se verificar qual o tratamento com melhor taxa de enraizamento. Estes 8 tratamentos englobaram três pressupostos diferentes: o tamanho do clone, a intensidade de luz a partir do quinto dia de enraizamento e a condutividade elétrica, utilizado na fertirrega. Ao longo do ensaio foi realizada a propagação dos trinta e três clones em cada um dos oito tratamentos no respetivo tabuleiro de substrato e foram retiradas medições de cada clone em dois momentos distintos, bem como observado o aparecimento de novas folhas. A contagem do número de raízes observadas realizou-se em três dias distintos de modo a obter um resultado mais concreto. Estes oito tratamentos foram feitos com três repetições de modo a obter um resultado o mais fiável possível colocando os tratamentos de forma aleatória no interior da sala, e após a recolha de todos estes dados fazer a sua análise e obter uma conclusão. Após analisar os resultados e retirar as conclusões de todos os tratamentos em comparação com o nosso testemunho, é possível definir qual ou quais os pressupostos que influencia no enraizamento dos clones bem como no seu desenvolvimento.

Palavras-chave: *Cannabis sativa* L.; Enraizamento; Macropropagação; Substrato; Variedade

Introdução

Nos dias de hoje é bastante comum fazer-se referência à cannabis medicinal e associar-se esta planta ao seu potencial médico. No entanto, nem sempre foi assim. A cannabis foi descoberta na China, há cerca de 12000 anos e era recorrentemente plantada pelas suas características psicoativas. Com o passar dos séculos esta planta começou a ser usada por pessoas em todo o mundo, tanto pelas suas características psicoativas, como pelas suas características medicinais (Crocq, 2020). Atualmente, a cannabis é bastante utilizada em tratamentos para diferentes tipos de doença (Hoch et al., 2024).

À medida que a ciência foi evoluindo, também o conhecimento sobre as particularidades medicinais desta planta foi progredindo. Sabe-se, atualmente, que o ∆9-tetrahidrocanabinol (THC) e o canabidiol (CBD) tem várias propriedades farmacológicas já comprovadas, contudo, estas propriedades não são encontradas em grandes quantidades na planta cannabis (Ioannidis, 2020).

Devido a este facto, a procura por variedades de plantas de cannabis que apresentam características como valores elevados de THC e CBD têm decorrido a um ritmo acelerado, culminado na descoberta de variedades de cannabis com concentrações substanciais de THC e CBD (Ioannidis, 2020). Conforme estes estudos foram sendo realizados, a descoberta destas variedades de cannabis com as concentrações procuradas começaram a ser utilizadas na forma de clones. Este método de propagação destacou a importância do enraizamento da planta, e, consequentemente o tempo de desenvolvimento das primeiras folhas. O tipo de substratos usados para o enraizamento também tem vindo a ganhar popularidade, uma vez que se verificou a ligação entre o tipo de substrato e o crescimento saudável da planta (Ioannidis, 2020).

Neste contexto, também é importante salientar a relevância da escolha de variáveis como a condutividade elétrica da solução de hidratação, a intensidade luminosa, e o tamanho inicial dos clones no efeito direto em processos fisiológicos que influenciam o enraizamento, o número de folhas e o desenvolvimento inicial das plantas. Por isso, um estudo detalhado destes parâmetros permite compreender de que forma estes fatores contribuem para o sucesso da propagação vegetativa, possibilitando a otimização de protocolos e a obtenção de resultados mais consistentes e eficientes.

Objetivos

O presente trabalho teve como objetivo principal avaliar e comparar o desempenho de oito tratamentos distintos no enraizamento de clones da cultivar HB155, variedade que apresenta boas concentrações de THC e CBD embora com baixas taxas de enraizamento e resultados heterogéneos. Em particular, pretendeu-se:

- Avaliar o efeito do tamanho inicial dos clones (7,5 cm versus 10 cm) no processo de enraizamento.
- Analisar a influência da condutividade elétrica (1,5 versus 2,5 mS/cm) da solução de hidratação inicial.
- Estudar o impacto da intensidade luminosa aplicada a partir do quinto dia (80,68 densidade de fluxo de fotões fotossintéticos (PPFD) versus 124,8 PPFD).
- Quantificar o número de raízes, o desenvolvimento foliar e o crescimento em altura sob cada tratamento.
- Identificar qual ou quais tratamentos promovem maior sucesso de enraizamento, comparativamente ao protocolo testemunho (tratamento 6).
- Propor recomendações técnicas visando melhorar a eficiência da propagação da cultivar HB155.

Materiais e Métodos

Local de Estudo

O ensaio foi conduzido nas instalações de propagação da empresa localizada na Herdade das Barrocas. A exploração opera sob regulamentação para produção de *Cannabis sativa* L. com fins exclusivamente medicinais, seguindo os princípios das *Good Agricultural and Collection Practices* (GACP).

As atividades decorreram entre 17 de outubro e 4 de novembro de 2025, período durante o qual foram monitorizadas todas as fases do processo: seleção das plantas-mãe, preparação dos clones, aplicação dos tratamentos, enraizamento, manutenção ambiental e recolha de dados.

Caracterização das Instalações de Propagação

A sala de propagação onde decorreu o estudo é uma estrutura de ambiente controlado, equipada com sistemas de:

- Iluminação díodo emissor de luz (LED) de espectro otimizado para propagação;
- Controlo termo-higrométrico automatizado;
- Tabuleiros de propagação com células individuais;
- Sistemas de irrigação manual e de nebulização;
- Fluxo de ar regulado, garantindo ventilação adequada e renovação atmosférica constante.

A temperatura média mantida durante o ensaio variou entre 24 e 26 ºC, com humidade relativa inicial dentro das campânulas entre 85% a 99%, reduzida gradualmente à

medida que se intensificou a fase de enraizamento. Ficando o tabuleiro sem campânula, a humidade da sala varia entre 65-75%.

Material Vegetal

O material vegetal consistiu em estacas clonais obtidas de plantas-mãe da cultivar HB155, geneticamente estáveis e mantidas em condições sanitárias rigorosas. As plantas-mãe, previamente higienizadas e podadas para fins de uniformização, apresentavam elevado vigor vegetativo, garantindo que as estacas fossem retiradas de tecidos jovens, fisiologicamente ativos e aptos à formação de raízes adventícias. As estacas utilizadas, foram retiradas de 12 plantas mãe todas elas com o mesmo fenótipo.

Cada estaca foi colhida da extremidade apical, com orientação para cortes limpos, precisos e efetuados com lâminas esterilizadas entre plantas.

Preparação dos Clones

Após a colheita das estacas, procedeu-se a:

- Remoção parcial das folhas basais com auxílio da tesoura evitando excesso de superfície transpiratória;
- Imersão da zona basal em solução de hidratação, com pH a 5,9 e transferência das estacas para o laboratório de propagação;
- Corte diagonal da base da estaca, ampliando a área disponível para formação de raízes adventícias, e de seguida o corte da ponta das folhas;
- De modo a promover o enraizamento utilizou-se previamente à colocação dos clones em substrato o Rhizopon AA 0.5% numa concentração de 10-25 mg / clone;
- Inserção imediata no substrato previamente humedecido.

Substrato Utilizado

O substrato utilizado para o ensaio visou facilitar a indução radicular. Recorreu-se ao uso de diversos Jiffy®preforma. Os Jiffy®preforma são fornecidos em tabuleiros de 66 alvéolos, porém utilizaram-se 33 alvéolos por tratamento devido ao número elevado de tratamentos para a área de ensaio disponível.

- Turfa (30%), garantindo retenção hídrica adequada;
- Fibra de coco (70%), responsável por arejamento e estrutura física;
- pH (entre 4.7 e 5.3) estabilizado e baixa condutividade elétrica.

As células de propagação apresentaram elevada capacidade de drenagem, reduzindo o risco de saturação e asfixia radicular.

Descrição dos Tratamentos

Foram testadas oito combinações experimentais, resultantes da interação de três fatores principais:

1. Tamanho inicial dos clones

- 7,5 cm
- 10 cm

2. Condutividade elétrica da solução de hidratação

- 1,5 mS/cm
- 2,5 mS/cm

3. Intensidade luminosa aplicada a partir do 5.º dia

- 80,68 PPFD
- 124,8 PPFD

Cada tratamento incluiu 33 clones, totalizando 99 indivíduos, distribuídos por 3 repetições de 11 clones (Tabela 1).

O tratamento 6 foi considerado testemunho, por corresponder ao protocolo habitualmente utilizado pela empresa.

Tabela 1 Descrição dos diferentes tratamentos utilizados

Tratamento	Tamanho do clone	Condutividade elétrica da solução de hidratação	Intensidade luminosa aplicada a partir do 5.º dia
1	7,5	1,5	80.68 PPFD
2	7,5	2,5	80.68 PPFD
3	7,5	1,5	124.8 PPFD
4	7,5	2,5	124.8 PPFD
5	10	1,5	80.68 PPFD
6	10	2,5	80.68 PPFD
7	10	1,5	124.8 PPFD
8	10	2,5	124.8 PPFD

Condições Ambientais Durante o Ensaio

Durante o ensaio os tabuleiros ficaram acondicionados dentro de tabuleiro cobertos por uma campânula.

A manutenção ambiental seguiu um protocolo em três fases:

Fase I – indução (dias 0–5)

- Altos níveis de humidade relativa dentro da campânula (85–99%).
- Nebulização suave para evitar murchamento inicial.
- Iluminação moderada, ajustada para evitar fotoinibição.
- Mudança de campânula diariamente, a partir do segundo dia.

Fase II – iniciação e expressão (dias 5–12)

- Abertura dos buracos da campânula entre 15 a 20 minutos diariamente, de modo a promover a resistência fisiológica.
- Inicio do enraizamento no interior do substrato.
- Ajuste da intensidade luminosa conforme tratamento.

Fase III – aclimatação (dias 12–18)

- Remoção das campânulas durante o máximo de tempo suportado pelos clones.
- São visíveis as raízes que circundam o substrato.
- Maior exposição luminosa.
- Redução significativa da humidade.
- Preparação para transplante.

Os valores ambientais foram monitorizados diariamente e ajustados para garantir estabilidade.

Avaliações e Parâmetros Medidos

Os parâmetros avaliados foram selecionados com base na literatura relativa à propagação vegetativa de *Cannabis sativa L*. e na experiência operacional da unidade produtiva.

a) Número de raízes por clone

- Registado nos dias 12, 15 e 18 após a plantação;
- A observação ocorreu por inspeção visual cuidadosa, removendo ligeiramente o substrato sem danificar as raízes.

b) Número de folhas por clone

- Contabilizado nos dias 15 e 18;
- Permitiu avaliar vigor vegetativo durante o enraizamento.

c) Crescimento em altura

- Medido nos dias 0 e 12;
- Registado com régua milimétrica, desde a base do caule ao ápice.

d) Taxa de sobrevivência

- Registada diariamente ao longo do ensaio.

Tratamento Estatístico dos Dados

Os dados recolhidos foram organizados em base matricial para posterior análise comparativa. Embora este estudo tenha carácter aplicado e exploratório, foram considerados os seguintes procedimentos:

- Comparação direta entre médias dos tratamentos;
- Identificação de tendências de resposta;
- Discussão qualitativa suportada por valores médios e amplitude de variação.

Para análise estatística foram realizadas análises univariada e multivariada para detetar se existiram diferenças significativas nos diferentes tratamentos e dias de amostragem. As análises estatísticas dos dados foram realizadas utilizando o software PRIMER v6 (Clarke e Warwick, 2001) com o software complementar PERMANOVA (Anderson et al., 2008). A análise PERMANOVA foi conduzida em uma matriz de similaridade de Bray-Curtis (Clarke e Green, 1988). A hipótese nula foi rejeitada a um nível de significância <0.05 (se o número de permutações fosse menor que 150, uma permutação de Monte Carlo *p* será usada). Sempre que foram detetadas interações significativas nos efeitos dos fatores (Tratamento e Tempo), estas foram examinadas usando comparações a posteriori (par a par), usando 9999 permutações sob um modelo reduzido.

Cronograma Experimental

O ensaio decorreu ao longo de 18 dias, organizados da seguinte forma:

- Dia 0 – colheita das estacas, preparação e plantação;
- Dias 1–5 – fase de aclimatação;
- Dia 5 – aplicação diferenciada da intensidade luminosa;
- Dias 12, 15 e 18 – avaliações sucessivas do desenvolvimento radicular;
- Dia 18 – última avaliação e encerramento do ensaio.

Resultados

O tratamento 2 apresentou maior percentagem no número de clones com formação de folhas após 15 e 18 dias de ensaio (37,4 e 68,7%, respetivamente). Os tratamentos 1, 5 e 6, após 18 dias, apresentaram um crescimento de 60% em número de folhas quando comparado ao tratamento 2 (Tabela 2). Os restantes tratamentos apresentaram um crescimento de 42% após 18 dias de ensaio.

Tabela 1 Percentagem do número de clones com formação de folhas após 15 e 18 dias em cada um dos tratamentos (1 – 8).

Tratamentos	Percentagem de clones com folhas (%)	
	15 dias	18 dias
1	29,3	61,6
2	37,4	68,7
3	18,2	36,4
4	16,2	46,5
5	32,3	62,6
6	31,3	60,6
7	19,2	32,3
8	23,2	41,4

Após 18 dias de ensaio, três tratamentos apresentaram percentagens superiores a 70% no número de clones com formação de raízes: os tratamentos 1, 2 e 5 (74,7%, 79,8% e 74,7%, respetivamente). Nos restantes tratamentos, a percentagem de clones com formação de raízes foi inferior a 70%, sendo o valor mais baixo observado no tratamento 7 (Tabela 3).

Tabela 2 Percentagem do número de clones com formação de raízes após 12, 15 e 18 dias em cada um dos tratamentos (1 – 8).

Tratamentos	**Percentagem de raízes (%)**		
	12 dias	15 dias	18 dias
1	15,2	32,3	74,7
2	23,2	39,4	79,8
3	8,1	22,2	52,5
4	8,1	19,2	59,6
5	20,2	35,4	74,7
6	18,2	32,3	69,7
7	5,1	19,2	40,4
8	10,1	23,2	47,5

No tratamento 2 a média ± erro padrão (SE) no número de raízes após 12 dias foi de 2.34 ± 0.68 e após 15 dias foi de 7.09 ± 1.34. Após 18 dias de ensaio, o tratamento 2 apresentou maior média no número de raízes 15.59 ± 1.76. O tratamento 6 apresentou valores médios de 2,52 ± 0,89 aos 12 dias e 7,50 ± 1,72 aos 15 dias, atingindo 14,85 ± 2,07 raízes aos 18 dias. No tratamento 5, a média de raízes após 12 dias foi de 1.56 ± 0.59 e após 15 dias foi de 5.30 ± 1.20. Após 18 dias de ensaio o tratamento 5 destacou-se com uma média de número de raízes de 14.17 ± 1.62 (Figura 1).

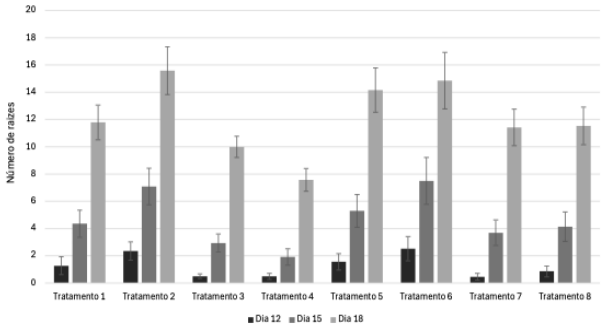

Figura 1 Média ± erro padrão (se) do número de raízes após 12, 15 e 18 dias de ensaio em cada um dos tratamentos (1, 2, 3, 4, 5, 6, 7 e 8).

Apesar destas diferenças nas médias do número de raízes entre os tratamentos após 18 dias de ensaio, a análise de permutação PERMANOVA aplicada aos tratamentos somente revelou valores significativamente maiores entre todos os tratamentos em comparação ao tratamento 4 ($p < 0,05$). As restantes comparações entre os tratamentos não apresentaram diferenças significativas ($p > 0,05$) (Tabela 4).

Tabela 3 Detalhes do teste PERMANOVA de 1 fator "Tratamento" (T) (1 nível, fixo), para a variável número de raízes após 18 dias de ensaio. Os valores a negrito destacam efeitos e interações significativas (p < 0,05).

Comparações	t	Permutações	P(MC)
T1, T2	1,132	9914	0,2571
T1, T3	0,37487	9907	0,7401
T1, T4	2,1865	9917	**0,0273**
T1, T5	1,0517	9905	0,2955
T1, T6	0,654	9913	0,5292
T1, T7	9,5226E-2	9930	0,9711
T1, T8	1,8995E-2	9910	0,9998
T2, T3	1,1429	9927	0,2549
T2, T4	3,0338	9905	**0,0018**
T2, T5	0,28367	9923	0,8401
T2, T6	0,42845	9922	0,7126
T2, T7	0,84545	9940	0,3945
T2, T8	1,0242	9936	0,3105
T3, T4	2,4754	9910	**0,0148**
T3, T5	1,0674	9921	0,2942
T3, T6	0,77325	9924	0,4428
T3, T7	0,31947	9880	0,7703
T3, T8	0,35132	9921	0,7619
T4, T5	3,2354	9921	**0,0015**
T4, T6	2,5674	9913	**0,0123**
T4, T7	2,0469	9904	**0,0449**
T4, T8	2,0785	9925	**0,0376**
T5, T6	0,37833	9929	0,7443
T5, T7	0,78861	9931	0,4472
T5, T8	0,96359	9932	0,3349
T6, T7	0,47845	9922	0,6543
T6, T8	0,59957	9916	0,582
T7, T8	8,3911E-2	9913	0,9782

Após 12 dias de tratamento, o tratamento 6 apresentou maior média ± erro padrão (SE), 0.60 ± 0.07 no crescimento do caule, seguido do tratamento 2 com 0.53 ± 0.06, tratamento 3 com 0.51 ± 0.09, tratamento 4 com 0.37 ± 0.05, tratamento 7 com 0.36 ± 0.04, tratamento 5 com 0.35 ± 0.04, tratamento 1 com 0.34 ± 0.03 e tratamento 8 com 0.34 ± 0.04 (Figura 2).

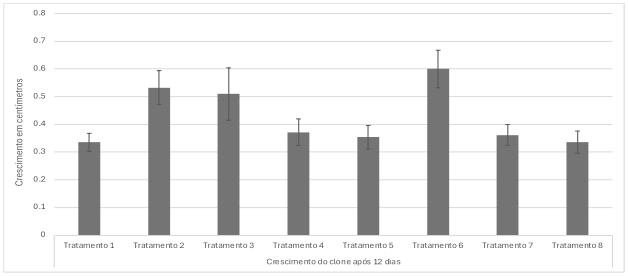

Figura 2 Média ± erro padrão (SE) do crescimento do caule após 12 dias de ensaio em cada um dos tratamentos (1, 2, 3, 4, 5, 6, 7 e 8).

A análise de permutação PERMANOVA aplicada aos tratamentos não revelou diferenças significativas entre os tratamentos 6, 2 e 3 em termos de crescimento do caule. No entanto, o crescimento dos caules no tratamento 6 foi significativamente mais elevado em comparação a todos os outros tratamentos (p < 0,05) após 12 dias de ensaios. Para além disso, a análise de permutação PERMANOVA revelou que o crescimento do caule no tratamento 2 não apresentou diferenças significativas em comparação ao tratamento 7 (p > 0,05), e o tratamento 3 não apresentou diferenças significativas (p > 0,05) em comparação aos restantes tratamentos (Tabela 5).

Tabela 4 Detalhes do teste PERMANOVA de 1 fator "Tratamento" (1 nível, fixo), para a variável crescimento do caule após 12 dias de ensaio. Os valores a negrito destacam efeitos e interações significativas (p < 0,05).

Comparações	t	Permutações	P(MC)
T1, T2	2,6559	9938	**0,0058**
T1, T3	1,0846	9897	0,2859
T1, T4	0,42207	9798	0,7326
T1, T5	0,57513	9919	0,5993
T1, T6	3,2048	9933	**0,0009**
T1, T7	0,83902	9037	0,4056
T1, T8	0,28115	9620	0,8521
T2, T3	1,3094	9924	0,1857
T2, T4	1,9356	9936	**0,0485**
T2, T5	2,9146	9950	**0,0031**
T2, T6	0,77911	9939	0,4671
T2, T7	1,4812	9862	0,1328
T2, T8	2,5376	9928	**0,0107**
T3, T4	0,68621	9880	0,5274
T3, T5	1,1055	9862	0,2718
T3, T6	1,6794	9933	0,0843
T3, T7	0,76951	9680	0,4641
T3, T8	1,0003	9864	0,3293
T4, T5	0,75079	9858	0,4669
T4, T6	2,4516	9936	**0,011**
T4, T7	0,47905	8856	0,6825
T4, T8	0,5388	9706	0,635
T5, T6	3,3566	9941	**0,0008**
T5, T7	1,179	9546	0,2397
T5, T8	0,25151	9828	0,8819
T6, T7	2,0318	9907	**0,041**
T6, T8	3,0014	9940	**0,0024**
T7, T8	0,97087	7742	0,3353

Discussão

Os resultados obtidos demonstraram que o tratamento 2 apresentou os melhores resultados em termos de percentagem de enraizamento, formação de folhas e número médio de raízes.

Referentemente à influência do tamanho das estacas no enraizamento, verificámos que os clones com 10 cm (tratamento 6) apresentaram uma maior percentagem de crescimento do caule, nomeadamente o tratamento 6 diferenciou-se pela obtenção dos melhores resultados entre os 8 tratamentos no requisito do crescimento do caule, seguido do tratamento 2, com clones com um tamanho de 7.5 cm. Estes resultados podem dever-se a dois fatores, primeiro, ao fator de as maiores estacas possuírem tecidos onde existe uma maior acumulação de hidratos de carbono, que são bastante importantes para a primeira fase do enraizamento, e segundo ao fator de estacas mais compridas serem mais espessas no caule e por conseguinte, terem maior capacidade para suportar variações climáticas (Deron, 2018; Hesami, 2023). Estudos realizados em espécies propagadas (como eucaliptos híbridos) por estacas demostram que o comprimento da estaca, influencia diretamente a formação/número de raízes e o desenvolvimento de novas raízes. Em eucaliptos híbridos, estacas com cerca de 8–10 cm de comprimento apresentaram uma maior percentagem de enraizamento, número de raízes e crescimento vegetativo do que estacas muito pequenas (mais ou menos 5 cm), o que pode sugerir que há um intervalo ótimo de comprimento que tem como consequência o melhor desenvolvimento radicular (Naidu, 2009). Os resultados obtidos no presente estudo corroboram estas observações onde se verificou que, aos 18 dias, os clones submetidos ao tratamento 6 apresentaram um crescimento do caule superior em relação aos clones do tratamento 2. Este resultado sugere que as condições associadas ao tratamento 6, proporcionaram um melhor desempenho no desenvolvimento inicial das plantas. Assim, os dados obtidos reforçam a ideia de que a otimização do comprimento das estacas constitui um fator determinante para o sucesso do enraizamento e para o subsequente crescimento vegetativo.

Contabilizando o impacto da condutividade elétrica na fase inicial da hidratação, reportamos que uma condutividade de 1,5 teve um menor efeito no crescimento dos clones, das raízes e das folhas, apenas com um Ec de 2,5 mS/cm, como aplicado no tratamento 2 e 6, apresentou benefícios para a hidratação. É também importante destacar que o tratamento com Ec 2,5 (tratamento 2 com clones de 7.5 cm) apresentou maior formação de folhas em comparação aos tratamentos com Ec 1,5 (como tratamento 1 com clones com 7.5 cm e 5 com clones com o tamanho de 10 cm). Ou seja, em termos de avaliação de diferentes parâmetros, nomeadamente para o crescimento do caule e das raízes, percebeu-se que um Ec de 2,5 influência positivamente o crescimento do caule e

das raízes, como observado no tratamento 2 e 6 cujo crescimento tanto dos clones como das raízes foram superiores. Estes resultados poderão indicar que em clones com tamanho reduzido, consequentemente com reserva interna e uma área para realizar a fotossíntese reduzida, poderão influenciar na passagem pelo stress osmótico quando os valores de condutividades são elevados (Dilena et al., 2025, Xu, 2021). Contudo, clones maiores tem maior possibilidade de ultrapassar este stress devido a terem uma maior capacidade metabólica. Nomeadamente, Janssen (2021) descreveu que uma concentração moderada de nutrientes, geralmente entre 1,6 – 2,4 mS/cm, favorece o desenvolvimento radicular e foliar de estacas, demonstrando que existe um efeito dependente do tipo de cultivar e das condições de cultivo. Verificou-se que estes resultados apresentam uma dualidade de critérios e que consequentemente a Ec não deve ser tida em conta como apenas um fator independente, mas como um fator acoplado a outros fatores já aqui descritos.

No que diz respeito ao efeito da intensidade luminosa, percebemos que este foi um dos fatores mais divergente entre os tratamentos, mais especificamente, a aplicação de 124,8 PPFDF (tratamento 3, 4, 7 e 8) e 80,68 PPFD (tratamento 1,2, 5 e 6). Estudos indicam que elevada intensidade luminosa pode aumentar a necessidade da planta por água e energia, o que pode culminar em stress hídrico e reduzir eficiência de enraizamento.

Estes estudos poderão justificar o porquê de os tratamentos submetidos à aplicação de 124,8 PPFD resultarem em percentagens menores de clones com folhas ou raízes em comparação com 80,68 PPFD aplicados ao tratamento 2 e 6 (Xu, 2021).

Baseando-nos nestes fatores, compreende-se que os resultados indicam que o sucesso do enraizamento depende de mais do que um fator e da interação entre eles, sendo que a agregação mais efetiva foi entre os clones com 10 cm, uma intensidade de 80,68 PPFD e Ec moderada de 2,5 mS/cm. Colocamos a hipótese de esta ser a melhor conjugação devido a um clone maior ter reservas mais alargadas, à intensidade luminosa influenciar positivamente a rápida reposição energética e à Ec correta fornecer os nutrientes essenciais sem causar stress aos clones.

Dos oito tratamentos estudados, o tratamento dois foi o que obteve um melhor resultado, seguido do tratamento seis que é o tratamento utilizado na empresa e concluímos que o procedimento usado é eficiente, mas pode ser sempre melhorado através das combinações mencionadas no decorrer do estudo para promover uma taxa de enraizamento superior.

Os resultados obtidos podem ser transacionados para uma produção de maior escala, uma vez que são relevantes para produção da Cannabis medicinal .

Apesar deste estudo apresentar resultados bastante interessantes, é necessário considerar certas limitações, como a ausência de uma análise estatística inferencial, o uso de uma só cultivar e limite temporal de apenas 18 dias. No futuro recomendamos que se façam análises mais especificas às raízes formadas, que se acompanhe o desenvolvimento dos clones nas diferentes fases e que se realizem novos teste com diferentes espetros luminosos e fotoperíodos.

Conclusões

O ensaio realizado permitiu fazer-se a comparação entre os oito tratamentos no enraizamento de clones da cultivar HB155 de *Cannabis sativa L.* A variedade em questão já era referenciada como contendo baixas taxas de enraizamento durante a propagação vegetativa. A análise dos resultados, juntamente com a informação recolhida na presente pesquisa bibliográfica, permitiu identificar fatores críticos que influenciam a taxa de enraizamento e opções concretas para a melhoria do protocolo de propagação utilizado neste caso na empresa.

As principais conclusões retiradas foram que o tamanho inicial da estaca (10 cm) foi o fator que teve o maior impacto positivo, tendo influenciado significativamente o maior crescimento do caule. Já as estacas com (7,5 cm) promoverão o maior crescimento de número de raízes, desenvolvimento foliar, bem como o crescimento em altura. As estacas com tamanho mais curto (7,5 cm) apresentaram menor robustez fisiológica e mais sensibilidade a fatores ambientais.

A intensidade de luz (80,68 PPFD), que foi utilizada a partir do quinto dia do ensaio demonstrou ser uma mais-valia para acelerar o metabolismo fotossintético dos clones, para promover a atividade meristemática e fazer com que os desenvolvimentos das raízes adventícias aconteçam de forma mais rápida e eficiente.

A condutividade elétrica da solução de fertirrega (1,5 vs 2,5 mS/cm) teve impacto dependendo do tamanho inicial da estaca, sendo que o Ec de 2,5 apresentou vantagens tanto para os clones de 10 cm, como para os clones de 7,5 cm.

Após uma análise completa dos resultados, o sucesso do enraizamento depende da interação entre os fatores avaliados, não sendo possível otimizar apenas uma variável isolada.

O testemunho, neste caso o (Tratamento 6) é o protocolo definido pela Herdade das Barrocas, e apresentou um desempenho intermédio, o que demonstra que é eficaz, apesar de não ser a combinação mais eficiente entre os tratamentos estudados.

Pela interpretação dos resultados a combinação com maior sucesso em termos de enraizamento da cultivar HB155, seria estacas de 7,5 cm, intensidade de luz de 80,68 PPFD

a partir do quinto dia e a condutividade elétrica moderada a elevada (1,5-2,5 mS/cm).

O presente estudo parece sugerir que o tratamento 2 apresenta uma melhor capacidade de enraizamento quando comparado com os outros tratamentos realizados. No entanto, é importante referir que novos estudos devem ser realizados, por exemplo através do recurso a outras condições, de modo a comprovar estatisticamente que o tratamento 2 é de facto melhor que o tratamento 6. Se no futuro aplicarmos o conhecimento adquirido no presente ensaio, poderá aumentar a taxa de sucesso na propagação da cultivar HB155, em termos de tempo para o enraizamento, ter um lote mais uniforme e por consequência ter um produto final de qualidade.

Através deste estudo é possível, alterar e melhorar o processo de clonagem da cultivar HB155, deixando em aberto ainda inúmeras combinações que podem ser exploradas como por exemplo outras combinações de fito reguladores, diferentes condições ambientais bem como a utilização de outras técnicas de propagação.

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The Role of High-Intensity Interval Training in Cardiovascular Prevention and Rehabilitation: A Review

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Summary

In the last decade, high-intensity interval training (HIIT) has been the subject of many studies as an efficient intervention for improving various metabolic and cardiovascular markers. This integrative review examines in detail the impacts of HIIT on hypertension, cardiac rehabilitation, VO_2max, cardiometabolic risk, coronary heart disease/heart failure, and type 2 diabetes, gathering evidence from 40 studies published from 2015 to 2025. The results indicate that HIIT decreases blood pressure, significantly increases VO_2max, improves glycemic control, reduces arterial deficiency, and improves functional capacity in cardiac patients. We can conclude that when properly recommended, high-intensity interval training (HIIT) may be an important part of cardiovascular prevention and rehabilitation programs. It seems to be an effective exercise technique for improving numerous cardiovascular and metabolic outcomes.

Keywords: HIIT; cardiovascular diseases; health; programs.

Resumo

Na última década, o treino intervalado de alta intensidade (HIIT) tem sido objeto de numerosos estudos enquanto intervenção eficaz para a melhoria de diversos marcadores metabólicos e cardiovasculares. Esta revisão integrativa analisa detalhadamente os impactos do HIIT na hipertensão arterial, na reabilitação cardíaca, no VO_2máx, no risco cardiometabólico, na doença arterial coronária/insuficiência cardíaca e na diabetes mellitus tipo 2, reunindo evidência proveniente de 40 estudos publicados entre 2015 e 2025. Os resultados indicam que o HIIT reduz a pressão arterial, aumenta significativamente o VO_2máx, melhora o controlo glicémico, reduz a rigidez arterial e melhora a capacidade funcional em doentes cardíacos. Conclui-se que, quando devidamente prescrito, o treino intervalado de alta intensidade (HIIT) pode constituir uma componente importante dos programas de prevenção e reabilitação cardiovascular. Este método de exercício revela-se eficaz na melhoria de diversos parâmetros cardiovasculares e metabólicos.

Palavras-chave: HIIT; doenças cardiovasculares; saúde; programas.

Introduction

High-intensity interval training (HIIT) has emerged as one of the most effective approaches to improving cardiovascular and metabolic health. Its structure, characterized by short periods of intense exercise alternating with active recovery phases, provides distinct physiological stimuli that result in more effective adaptations than those observed in moderate-intensity continuous training (MICT), and recent reviews highlight the impact of HIIT on reducing blood pressure, increasing cardiorespiratory capacity, and glycemic control (Ko et al., 2025; Reljic, 2025).

This review integrates scientific evidence from 40 articles published between 2015 and 2025, focusing on the various relevant domains in which HIIT training can contribute to health and to the prevention and treatment of cardiovascular diseases.

Methods

An integrative narrative review was conducted, including several articles from systematic reviews, meta-analyses, clinical trials, physiological studies, and narrative reviews. The articles were organized according to different cardiovascular domains and pathologies, such as hypertension, cardiac rehabilitation, VO_2max, cardiometabolic risk, coronary artery disease, heart failure, and type 2 diabetes, in the following databases: Google Scholar, Journal of Science and Medicine in Sport, Clinical Nutrition ESPEN, Br J Sports Med, European Review for Medical and Pharmacological Sciences, SciEP, MDPI, Journal of Physical Education & Health, European Heart Journal, and Frontiers.

Studies published between January 2015 and March 2025 were included in the literature search, which was carried out between February and March 2025. "High-intensity interval training," "HIIT," "cardiovascular disease," "hypertension," "cardiac rehabilitation," "VO_2max," "heart failure," and "type 2 diabetes" were among the search phrases used. Studies that assessed how HIIT affected functional, metabolic, or cardiovascular outcomes in clinical or healthy populations were considered. Excluded were editorials, conference abstracts, duplicate records, and research unrelated to cardiovascular health. The review contained forty studies that satisfied the eligibility requirements.

Results

HIIT and High Blood Pressure

Hypertension is one of the main modifiable risk factors for cardiovascular disease. High-intensity interval training (HIIT) has shown consistent results in reducing blood pressure.

A meta-analysis by (Romero-Vera et al., 2024) revealed considerable decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) after the implementation of HIIT training programs. Adeloye et al. (2024) confirm these results, highlighting that HIIT training can be especially beneficial for people with resistant hypertension.

(Way et al., 2019) demonstrated that HIIT reduces blood pressure over 24 hours, measured by ambulatory blood pressure monitoring (ABPM), more significantly than moderate-intensity continuous training (MICT). The mechanisms involved include decreased arterial stiffness, reduced sympathetic nervous system activity, increased baroreceptor reflex sensitivity, decreased oxidative stress, and improved autonomic nervous system function (D'Almeida et al., 2025; Grässler et al., 2021).

Table 1. Effects of HIIT on Arterial Hypertension

Domain	Main Results	Evidence
Systolic Blood Pressure	Significant reduction after 4-12 weeks of HIIT	(Adeloye et al., 2024; Romero-Vera et al., 2024)
Diastolic Blood Pressure	Consistent reduction, greater than MICT	(Romero-Vera et al., 2024; Way et al., 2019)
24-hour Blood Pressure	Greater reduction than MICT	(Ko et al., 2025; Way et al., 2019)

HIIT in Cardiac Rehabilitation

One of the areas where high-intensity interval training (HIIT) has demonstrated the greatest effectiveness is cardiac rehabilitation. Functional capacity, assessed by peak VO_2, is one of the main indicators of cardiovascular mortality.

Yue et al., (2022) demonstrated that HIIT considerably improves peak VO_2 in patients with coronary artery disease, often surpassing moderate-intensity continuous training (MICT).

Wu & Wang, (2026) found that HIIT increases cardiac efficiency in patients with heart failure, encompassing both heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF).

Regarding the safety of training, Turnbull et al., (2025) demonstrated that HIIT programs performed at home are safe and effective.

Table 2. Effects of HIIT in Cardiac Rehabilitation

Domain	Main Results	Evidence
VO_2peak	↑ greater than MICT	(Wu & Wang, 2026; Yue et al., 2022)
Funcional Capacity	Significant ↑ functional capacity in CAD and HF	(Okamura et al., 2024; Wu & Wang, 2026)
Quality of Life	↑ quality of life in multiple domains	(Turnbull et al., 2025; Yue et al., 2022)

Note: MICT - moderate-intensity continuous training; CAD - coronary artery disease; HF - heart failure

HIIT and VO_2Max.

One of the most relevant indicators of cardiovascular health and longevity is VO_2max. de Oliveira-Nunes et al., (2021) demonstrated that HIIT training results in superior increases in VO_2max compared to MICT. Men et al., (2025) confirmed that HIIT training is one of the most efficient approaches to increase maximum oxygen uptake.

Table 3. Effects of HIIT on VO_2max.

Mechanism	Description	Evidence
Maximum cardiac output	↑ stroke volume	(Wu & Wang, 2026; Yue et al., 2022)
Ventilatory efficiency	↓ ventilatory cost	(Jovanović et al., 2024; Yang et al., 2025)
Adaptations in young people	↑ cardiorespiratory fitness	(Jovanović et al., 2024; Poon et al., 2024)

HIIT and Cardiometabolic Risk

High-intensity interval training (HIIT) has demonstrated significant results in reducing cardiometabolic risk. Viderman et al., (2025) concluded that HIIT improves triglyceride levels, blood glucose, insulin resistance, and systemic inflammation. Batacan et al., (2017) and Cadenas-Sanchez et al., (2024) highlight that HIIT is effective for people with metabolic syndrome and obesity.

Table 4. Effects of HIIT on Cardiometabolic Risk

Marker	HIIT Effect	Evidence
Triglycerides	Significant ↓	(Batacan et al., 2017; Viderman et al., 2025)
Insulin resistance	Increased sensitivity	(Cadenas-Sanchez et al., 2024; Viderman et al., 2025)
Visceral fat	Marked ↓	(Batacan et al., 2017; Viderman et al., 2025)
Lipid profile	Increased HDL, Decreased TG	(Batacan et al., 2017; Viderman et al., 2025)

Note: HDL – High-Density Cholesterol; TG – Triglycerides

HIIT in Coronary Artery Disease and Heart Failure

High-intensity interval training (HIIT) has been the subject of numerous studies in patients with coronary artery disease (CAD) and heart failure (HF). Okamura et al., (2024) demonstrated improvements in functional capacity and coronary perfusion.

Ko et al., (2025) highlight reductions in arterial stiffness and advances in autonomic function.

HIIT in Type 2 Diabetes

High-intensity interval training (HIIT) has demonstrated particularly significant benefits in people with type 2 diabetes (T2DM).

Feng et al., (2024) demonstrated improvements in HbA1c, blood glucose, and HOMA-IR levels. Al-Mhanna et al., (2025) showed that HIIT is more effective than moderate-intensity continuous training (MICT) in people with “diabesity”.

Table 5. Effects of HIIT on Coronary Artery Disease and Heart Failure

Domain	Results	Evidence
Autonomic function	↑ HRV	(Grässler et al., 2021; Ko et al., 2025)
Arterial stiffness	↓ PWV, ↓ AIx	(Jiao et al., 2025; Ko et al., 2025)
Coronary perfusion	↑ vasodilation	(Ko et al., 2025; Okamura et al., 2024)
Functional capacity	↑ peak VO_2	(Wu & Wang, 2026; Yue et al., 2022)
Quality of life	↑ significant	(Okamura et al., 2024; Turnbull et al., 2025)

Note: HRV - Heart rate variability; PWV - Pulse wave velocity; AIx - Augmentation index

Table 6. Effects of HIIT on Type 2 Diabetes

Marker	Effect of HIIT	Evidence
Fasting Blood Glucose	Consistent ↓	(Al-Mhanna et al., 2025; Feng et al., 2024)
Insulin Sensitivity	↑ GLUT4	(Al-Mhanna et al., 2025; Viderman et al., 2025)
Visceral Fat	Marked ↓	(Cadenas-Sanchez et al., 2024; Viderman et al., 2025)

Discussion

The evidence synthesized in this integrative review indicates that high-intensity interval training (HIIT) is a promising exercise-based strategy for improving several clinically relevant cardiovascular and metabolic outcomes (Atakan et al., 2021). Across the six domains examined, the most consistent benefits were observed in cardiorespiratory fitness, particularly VO_2max or peak VO_2, blood pressure regulation, arterial stiffness, glycemic control, and functional capacity in patients with established cardiovascular disease. These outcomes are clinically meaningful because reduced cardiorespiratory fitness, hypertension, vascular dysfunction, and impaired glucose metabolism are strongly associated with increased cardiovascular morbidity and mortality.

Regarding hypertension, the available evidence suggests that HIIT can promote reductions in systolic and diastolic blood pressure, including improvements in 24-hour ambulatory blood pressure (Costa et al., 2018). These effects may be explained by improvements in endothelial function, reduced arterial stiffness, enhanced autonomic regulation, and lower oxidative stress. Nevertheless, the magnitude of the response is likely to depend on baseline blood pressure, medication use, training duration, exercise intensity, and the degree of clinical supervision. Therefore, although HIIT may be useful in hypertensive populations, particularly when individualized, it should not be presented as uniformly superior for all patients or clinical contexts (Taylor et al. 2019).

In cardiac rehabilitation, HIIT appears to be especially relevant because of its capacity to improve peak VO_2 and functional capacity in patients with coronary artery disease and heart failure (Wang et al., 2022). Improvements in aerobic capacity are particularly important in these populations, as exercise tolerance is closely related to functional independence, symptom burden, quality of life, and prognosis. Compared with moderate-intensity continuous training (MICT), HIIT may provide a greater physiological stimulus for improving aerobic fitness in selected and clinically stable patients (Taylor et al., 2019). However, the choice between HIIT and MICT should be based on clinical status, exercise tolerance, patient preference, adherence potential, comorbidities, and safety considerations rather than on a universal assumption of superiority.

The findings concerning cardiometabolic risk and type 2 diabetes further support the potential value of HIIT in populations with elevated cardiovascular risk. Improvements in glycemic control, insulin sensitivity, visceral adiposity, triglycerides, and inflammatory markers indicate that HIIT may contribute to the management of metabolic dysfunction frequently associated with cardiovascular disease (Campbell et al., 2019). These adaptations may result from enhanced skeletal muscle glucose uptake, improved mitochondrial function, increased peripheral insulin sensitivity, and reductions in adiposity. Nevertheless, the clinical relevance of these changes should be interpreted considering differences in participant characteristics, intervention protocols, dietary control, and follow-up duration across studies.

Another important finding is the potential effect of HIIT on vascular and autonomic function in patients with coronary artery disease and heart failure. Reductions in arterial stiffness and improvements in heart rate variability and coronary perfusion suggest that HIIT may influence not only

exercise capacity but also mechanisms involved in cardiovascular regulation (Liu et al., 2022). However, these mechanistic outcomes remain less consistently assessed than VO_2max or blood pressure, and further controlled studies are necessary to determine whether these physiological improvements translate into long-term reductions in cardiovascular events, hospital admissions, or mortality.

From a clinical perspective, the findings of this review support the inclusion of HIIT as one possible component of cardiovascular prevention and rehabilitation programs. Its implementation should, however, be preceded by appropriate clinical screening and individualized exercise prescription. Training intensity, interval duration, recovery periods, progression, supervision requirements, and monitoring strategies should be adapted according to disease severity, functional capacity, treatment status, and patient experience (Coates et al., 2023). This is particularly relevant for individuals with heart failure, uncontrolled hypertension, multiple comorbidities, or reduced exercise tolerance.

Furthermore, the evidence in this review may have been impacted by language limitations and publishing bias. Several limitations should be considered when interpreting the findings of this review. First, the integrative narrative design includes different types of evidence, such as systematic reviews, meta-analyses, clinical trials, physiological studies, and narrative reviews, which limit direct comparison across studies. Second, substantial heterogeneity is likely to exist regarding HIIT protocols, intervention duration, outcome measures, clinical populations, and comparator groups. Third, the methodological description does not indicate the use of a formal risk-of-bias assessment or a structured quality appraisal of the included evidence. Consequently, although the overall findings are encouraging, conclusions regarding the superiority of HIIT over MICT should remain cautious and outcome specific.

Future research should focus on standardized HIIT protocols, longer follow-up periods, clinically relevant endpoints, and direct comparisons between supervised, home-based, and hybrid interventions. Additional studies are also needed to identify which patients benefit most from HIIT and to determine the optimal balance between effectiveness, safety, feasibility, and long-term adherence in different cardiovascular populations.

Conclusion

The evidence examined in this integrative review suggests that high-intensity interval training (HIIT) can be an effective exercise strategy for improving cardiorespiratory fitness, blood pressure, vascular function, glycemic control, and functional capacity in individuals with cardiovascular disease or increased cardiometabolic risk. The most consistent evidence appears to concern improvements in VO_2max or peak VO_2, an outcome of particular clinical relevance in cardiovascular prevention and rehabilitation.

HIIT may offer additional benefits compared with moderate-intensity continuous training for selected outcomes and appropriately screened patients; however, its use should be guided by individualized prescription, clinical supervision when indicated, progressive adaptation, and careful monitoring of symptoms and exercise tolerance. Therefore, HIIT should be considered a valuable option within comprehensive cardiovascular prevention and rehabilitation programs, rather than a universally superior intervention for all individuals.

Although the available evidence is promising, further high-quality studies using standardized protocols, longer follow-up periods, and clinically meaningful endpoints are required to clarify the long-term effectiveness, safety, and applicability of HIIT across different cardiovascular conditions and patient profiles.

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The Impact of European Players on the NBA: A Critical Review of the Literature

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Summary

A critical review of the literature on the impact of European players in the NBA, situated within the context of sports globalization and athlete migration. Based on a systematic review of theoretical and empirical studies, organized into three main themes—globalization and migration, a structural comparison between the NBA and European basketball, and the specific role of European players—we draw on authors such as Maguire, Bourdieu, and Appadurai to interpret the NBA as the hegemonic center of a global sports field, fueled by flows of talent, capital, identity, and images. A synthesis of the analyzed studies shows that European leagues function primarily as spaces for training and exporting players (brain drain), while the NBA asserts itself as a global media product, with a closed and highly commercialized model, distinct from the European system, which is more open and fragmented. In terms of gameplay, the arrival of Europeans is associated with tactical hybridization: the NBA incorporates elements of FIBA basketball (emphasis on teamwork, control of the pace) while evolving toward a model centered on spacing and the three-point shot. Comparative studies indicate that European players in the NBA are distinguished more by physical characteristics and physical impact than by differences in decision-making. On a symbolic level, these migrations reinforce the prestige of European basketball, but accentuate tensions of loyalty between clubs, national teams, and identity.

Keywords: Basketball; NBA; European players; globalization of sports; athlete migration; game analysis; tactical hybridization; sports brain drain.

Resumo

Esta revisão crítica da literatura analisa o impacto dos jogadores europeus na NBA, enquadrando-o no contexto da globalização do desporto e da migração de atletas. Com base numa revisão sistemática de estudos teóricos e empíricos, organizada em três temas principais — globalização e migração, comparação estrutural entre a NBA e o basquetebol europeu, e o papel específico dos jogadores europeus —, recorre-se aos contributos de autores como Maguire, Bourdieu e Appadurai para interpretar a NBA como o centro hegemónico de um campo desportivo global, alimentado por fluxos de talento, capital, identidade e imagens. A síntese dos estudos analisados demonstra que as ligas europeias funcionam predominantemente como espaços de formação e exportação de jogadores (brain drain), enquanto a NBA se afirma como um produto mediático global, assente num modelo fechado e altamente comercializado, distinto do sistema europeu, mais aberto e fragmentado. Ao nível do jogo, a chegada de

jogadores europeus está associada a uma hibridização tática, traduzida na incorporação, pela NBA, de elementos característicos do basquetebol da FIBA, como a valorização do jogo coletivo e o controlo do ritmo de jogo, evoluindo simultaneamente para um modelo centrado no espaçamento ofensivo e no lançamento de três pontos. Os estudos comparativos indicam que os jogadores europeus na NBA se distinguem sobretudo pelas suas características físicas e pelo impacto físico no jogo, mais do que por diferenças na tomada de decisão. Do ponto de vista simbólico, estas migrações reforçam o prestígio do basquetebol europeu, mas acentuam igualmente tensões de lealdade entre clubes, seleções nacionais e identidades desportivas.

Palavras-chave: basquetebol; NBA; jogadores europeus; globalização do desporto; migração de atletas; análise do jogo; hibridização tática; brain drain desportivo.

Introduction

In recent years, the presence of European players in the National Basketball Association (NBA) has become the norm rather than the exception. The NBA began as an exclusively North American league and has evolved into a global arena, integrating athletes trained in various European countries who play prominent roles. This paradigm shift raises a key question: What is the impact of European players in the NBA on the tactics and performance of how basketball is played at the highest level?

To answer this question, we draw on the literature on the globalization of sport and athlete migration (Bale & Maguire, 1994; Maguire, 2011; Frick, 2009; Kahn, 2000; Poli, 2010), research on the internationalization of the NBA (Eschker et al., 2004; Elliott & Weedon, 2011; Mills & Fort, 2014), and to game analysis studies in elite basketball (Sampaio et al., 2008; Gómez et al., 2015; Zuccolotto et al., 2018). The concepts of globalization, global dynamics and sport, the circulation of talent, identity, and symbols are explored by authors such as Pierre Bourdieu and Arjun Appadurai.

The aim of this study is to compile and critically analyze the existing literature, focusing on three key areas: (i) how the globalization of sports shapes player migration; (ii) the structural differences between the NBA and European basketball; (iii) the role played by European players in the NBA, in terms of tactics, performance, and culture.

Methodology

The methodology included a literature review organized around three main themes a structured narrative technique was used in the review. The globalization of basketball, athlete migration, the internationalization of the NBA, European basketball development systems, and the performance of European players competing in North American professional basketball were the main topics of the literature search, which took place between January and March 2025.The research was conducted in scientific databases. Combinations of English and Spanish keywords, such as "NBA," "European players," "international basketball," "athlete migration," "sports globalization," "EuroLeague," "performance analysis," "competitive balance," and "tactical evolution," were used to search Dialnet, Google Scholar, Web of Science, and Scopus. Boolean operators (AND/OR) were used in the search method to find research on the performance-related, sociological, cultural, and economic aspects of European NBA involvement. Studies were chosen according to how well they addressed the goals of the review. Peer-reviewed journal papers, scholarly monographs, and empirical studies that addressed athlete migration, globalization processes, competitive dynamics, and elite basketball performance metrics were given priority. Publications that presented inadequate methodological information or had no direct bearing on the research themes were not included in the final synthesis. After initial screening, the works were analyzed and synthesized into thematic matrices studies were chosen according to how well they addressed the goals of the review. Peer-reviewed journal papers, scholarly monographs, and empirical studies that addressed athlete migration, globalization processes, competitive dynamics, and elite basketball performance metrics were given priority. Publications that presented inadequate methodological information or had no direct bearing on the research themes were not included in the final synthesis. We chose to include classic works on globalization (Bale & Maguire, 1994; Maguire, 2011; Appadurai, 1996), economic and sociological studies (Frick, 2009; Kahn, 2000; Poli, 2010), research on international players in the NBA (Eschker et al., 2004; Mills & Fort, 2014), and tactical analyses (Sampaio et al., 2008; Gómez et al., 2015; Goldsberry, 2019), among others that we considered relevant to our initial research question.

Results

Professional basketball is now a global system, and the NBA occupies the center of that network. The findings can be interpreted considering Maguire and Appadurai's analyses of globalization and sports migration. The NBA's position as a "central" market for attracting European talent confirms the center–periphery logic described by Maguire (1999), according to which certain leagues function as hegemonic hubs for the accumulation of economic, symbolic, and media capital, while others act primarily as spaces for training and exporting athletes (Bale & Maguire, 1994).

According to Bourdieu (1998), sports have become a prime arena for the accumulation of economic and symbolic capital, where practices are shaped by commercial and media interests. This dynamic reinforces inequalities and transforms the game into a global spectacle, subordinated to market demands and the logic of visibility. Thus, million-dollar contracts, television rights, and transnational sponsorships are not merely financial mechanisms, but instruments of power that redefine the sporting arena and its hierarchies.

Zhe Ye (2025) discusses the impact of globalization on the sports industry, highlighting how this phenomenon has driven economic growth, promoted the internationalization of talent, and facilitated the spread of local sports cultures. Through the case study of Melbourne, the author demonstrates that international events, such as the Australian Open, not only boost the local economy but also attract athletes and foster cultural exchange. He further notes that globalization drives the expansion of sports brands and the integration of different cultures but warns of increased competition and the risk of homogenization of local cultures, which poses challenges to the preservation of regional identities. In summary, the author argues that, despite the opportunities created, it is essential to strike a balance between global development and the preservation of local cultures to ensure the sustainability of the sports sector.

Drawing on Appadurai, the growing presence of European players in the NBA reflects the intensification and interconnection of various global flows: technoscapes (the movement of athletes and coaches), finances capes (wage differentials and transnational investments), mediascapes (the global dissemination of the league's images and narratives), and ideoscapes (models of success, meritocracy, and professionalism associated with the NBA) (Appadurai, 1990, 1996). Thus, the migration of European players to the North American league does not appear as an isolated phenomenon, but as an expression of a global system of highly skilled labor mobility, in which structural asymmetries between sports markets are simultaneously reinforced and partially reconfigured through the selective circulation of talent. Europe emerges as a space for training and exporting talent, with players seeking better contracts and greater visibility. This migration is not merely economic; it also involves the circulation of tactical ideas and styles of play (Maguire, 2011; Bale & Maguire, 1994).

The comparison between the NBA and European basketball illustrates two distinct organizational and economic models in a globalized context. The NBA is a closed league, structured around high salaries, with internal economic regulation mechanisms aimed at preserving competitive balance and maximizing the league's aggregate commercial value, in line with the logic of sports entertainment described by authors in the field of sports economics (Neale, 1964; Sloane, 1971; Gratton & Taylor, 2000). European basketball, on the other hand, is based on an open, pyramid-shaped model, featuring promotion and relegation in national championships, a multitude of supranational competitions, and fragmented governance among federations and leagues, which limits the development of a single global brand and economic coordination on a continental scale. In light of the broader framework of commercialization and globalization presented by Gratton et al. (2012), the NBA emerges as an integrated global media product, while European basketball remains structurally more fragile and vulnerable to the departure of its best players.

In this sense, the migration of European players to the NBA should therefore be understood not merely as an individual upward career trajectory, but as a phenomenon with economic, sporting, and symbolic impacts on multiple levels. In economic and media terms, the presence of European stars reinforces the NBA's influence in European markets, contributing to increased television viewership, higher values for international broadcast contracts, and the expansion of global sponsorships, in line with the model of "transnational league" described in the literature on the globalization of sport (Smart, 2005, 2007; Gratton et al., 2012). From a sporting perspective, several authors associate the entry of Europeans with a hybridization of playing styles, combining the North American athletic tradition with tactical and technical influences characteristic of FIBA basketball (Maguire et al., 2002; Stokvis, 2000). Finally, in their countries of origin, the success of these players generates symbolic capital and prestige effects, boosting young people's interest in basketball and strengthening commercial ties with the NBA, albeit at the cost of a sports "brain drain" that illustrates the asymmetries inherent in the international division of labor in sports (Bale & Maguire, 1994; Thibault, 2009).

The European presence is no longer marginal. Eschker et al. (2004) show that, since the 1990s, the number of foreign players in the NBA has grown exponentially. Mills & Fort (2014) point out that this has altered the competitive balance of the league. We refer to Luka Dončić, a EuroLeague product, who quickly proved to be one of the most influential players in the NBA, demonstrating the adaptability and collective awareness typical of European basketball (Goldsberry, 2019). Tony Parker, trained in France, brought to the NBA a style of play based on tactical intelligence and efficient penetration, characteristics valued in European contexts (Elliott & Weedon, 2011).

When comparing different competitive models, it is essential to understand the structural and tactical characteristics that define each league. The specialized literature highlights significant differences between the NBA and the major European competitions. These distinctions encompass competitive rules and formats, such as game duration, court dimensions, possession time, defensive allowances, number of games per season, and playoff structure—factors that directly influence the pace of play, effort management, and the tactical solutions adopted.

In the context of performance analysis, Sampaio et al. (2008) identified key indicators associated with success in elite men's basketball, namely field goal and free throw efficiency, control of the defensive rebound, and a reduction in turnovers. Similarly, Gómez et al. (2015), in analyzing the EuroLeague, found that winning teams exhibit greater three-point shooting efficiency, better control of the defensive rebound, and fewer turnovers, suggesting that European basketball prioritizes pace control, judicious selection of outside shots, and possession management within highly collective offensive and

defensive structures. In contrast, comparative studies indicate that the NBA is characterized by a higher number of possessions per game, a high volume of shots, and a predominance of offensive transitions and fast-break situations (Sampaio et al., 2018).

Goldsberry (2019) also documents the recent tactical transformation of the North American league, marked by an increase in the use of the three-point shot, the widespread adoption of “small ball” lineups, and a greater emphasis on offensive spacing and positional versatility. This context favors interior players and wings with outside shooting ability, defensive mobility, and a broader understanding of the game—attributes often developed in European systems. These differences raise relevant questions for the next section, which discusses the implications of these trends for training, development, and player recruitment in international contexts.

As shown in Figure 1, we can see an increase in the number of European players in the NBA.

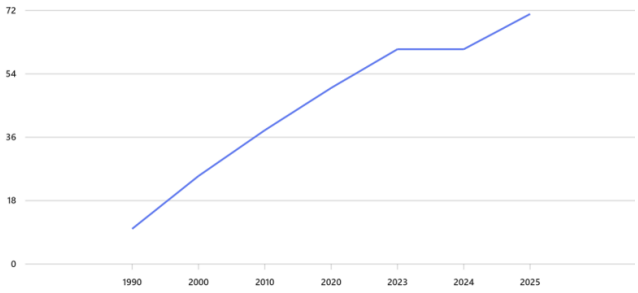

Figure 1 - Trend in the number of European players in the NBA, 1990–2025.

The differences between the NBA and the EuroLeague are evident in Figure 2, which we created based on the studies by Sampaio et al. (2018); Gómez et al. (2015); and Goldsberry (2019).

	NBA	Europe
Game duration	48 minutes	40 minutes
Field dimensions	28,7m x 15,2m	28m x 15m
Possession time	24 seconds	24 seconds
Game number per season	82 games	30-34 games

Figure 2 - Comparison between the NBA and European championships (Gómez et al. 2015; Sampaio et al. 2018; Goldsberry, 2019).

The study by Paulauskas et al. (2018) aimed to identify the game statistics that best distinguish the performance of European players in the NBA from that of EuroLeague players when competing in the same competitive setting (EuroBasket 2015). The sample included 26 European players who competed in the 2014–2015 NBA season and 82 players who played in the EuroLeague during the same season, analyzing 78 games. Individual statistics (field goals, free throws, rebounds, assists, steals, turnovers, fouls committed and drawn, and blocked shots) were normalized by minutes played and compared using discriminant analysis.

The results showed, first, that European NBA players were, on average, taller, heavier, and played more minutes than their EuroLeague counterparts. The discriminant function was statistically significant ($p = 0.008$; canonical correlation = 0.51; $\Lambda = 0.74$) and revealed that the variables with the greatest discriminant power were: made and missed two-point shots, made free throws, defensive rebounds, shot blocks, and fouls drawn. In all these categories, NBA players had higher values per minute of play, suggesting greater influence on the game in areas near the basket and in situations involving physical contact. No significant differences were observed in indicators more closely associated with perception and decision-making (e.g., assists, steals, turnovers).

The authors conclude that the main differences between European players in the NBA and the EuroLeague stem from physical characteristics (height and body mass) and game actions that are heavily dependent on these characteristics, while technical and tactical aspects related to perception and decision-making appear to be similar across groups. Additionally, the greater height of NBA players is associated with greater performance variability within the group itself, which has implications for scouting and training planning, particularly regarding the emphasis placed on actions such as defensive rebounding, controlling the space near the basket, and the ability to draw fouls and capitalize on free throws.

Zuccolotto, Manisera, and Sandri (2018) demonstrate how the shooting angle can influence the probability of scoring in basketball. Through big data analyses, the authors studied players from the NBA and European leagues, concluding that this factor is relevant in any competitive context. Although the effect is consistent, there are nuances related to playing style and athlete characteristics, which reinforces the importance of considering these technical and contextual aspects when evaluating performance. These results have important practical implications: coaches and analysts can use this information to adjust offensive strategies, optimize shooting mechanics, and customize training, thereby increasing players’ effectiveness in real game situations.

Although this study does not demonstrate the impact of FIBA players in the NBA, the analytical model could serve as a starting point for analyzing their impact in terms of game decisions and strategies.

Discussion

Analyzing these results considering the ideas of Maguire, Bourdieu, and Appadurai helps to better understand their sociological significance. According to Maguire (1999, 2011), the NBA occupies a central and hegemonic position in the global field of basketball, concentrating economic, media, and

symbolic capital. The major European leagues, in turn, occupy dominated or partially dominated positions within a transnational sports field, as Bourdieu would put it. The migration of European players to the NBA reflects a typical movement from the periphery or semi-periphery to the center: athletes seek to maximize economic and symbolic capital (better contracts, greater visibility), while European leagues function as training grounds whose most valuable talent is often captured by the dominant league. This pattern confirms what Frick (2009), Kahn (2000), and Poli (2010) describe: that sports labor markets are highly hierarchical, where major leagues have clear advantages in attracting talent.

Appadurai (1990, 1996) helps us understand this migration as part of various global "flows": people (ethnoscapes, players and coaches moving between continents), images and narratives (mediascapes, with the massive spread of the NBA in Europe and the visibility of Europeans on a global stage), and capital (financescapes, through contracts, television rights, and transnational sponsorships). These flows are not merely economic: they also involve the transfer of tactical knowledge and the reshaping of playing styles. The literature shows that European basketball prioritizes team play, control of the tempo, and efficiency in outside shooting (Sampaio et al., 2008; Gómez et al., 2015), while the NBA, historically more focused on individual heroics and transition play, has evolved toward a model that values spacing, three-point shooting, and positional versatility (Goldsberry, 2019). The integration of European players contributes to this hybridization: the "NBA style" incorporates elements of European basketball and, at the same time, exports organizational, media, and sports consumption models to Europe.

In terms of identity, the presence of European players in the NBA reinforces the perception of European basketball's quality, as fans see their athletes recognized on the world's premier competitive stage. It also creates national and regional role models who serve as symbols of success and modernity in sports. At the same time, tensions regarding loyalty arise within the NBA, European clubs, and national teams—a phenomenon already noted in the literature on athlete migration (Bale & Maguire, 1994; Maguire, 2011): NBA schedules and interests constrain players' participation in national team competitions, placing them in a constant balancing act between contractual obligations and national expectations. Finally, studies such as those by Eschker et al. (2004) and Mills & Fort (2014) show that the influx of international players has diversified the talent pool and affected the league's competitive balance, expanding the range of elite players available. European players are central figures in this redefinition of the NBA's image.

According to Schroeder & Janssen (2013), the migration of basketball players from FIBA leagues to the NBA is presented as an emblematic phenomenon of sports globalization, with an impact on both the development of athletes and the competitiveness of the leagues of origin and destination. The NBA benefits from diversity and international talent, while FIBA leagues face the challenge of retaining their best players.

Even if AI-assisted search engines helped find pertinent material, the authors' critical assessment was still the only way to understand, choose, and synthesize the evidence. As a result, rather than being automated results produced by digital techniques, the conclusions offered represent an analytical evaluation of the studied material.

Conclusions

The literature review conducted leads to the conclusion that the impact of European players in the NBA is part of a broader process of basketball globalization, marked by significant structural disparities among sports markets. The NBA asserts itself as the hegemonic center of a transnational sports field, concentrating economic, media, and symbolic capital, while European leagues primarily serve to develop and export talent (Bale & Maguire, 1994; Maguire, 1999, 2011). The migration of European athletes to the North American league thus reflects a typical movement from the periphery/semi-periphery to the center, sustained by global flows of people, capital, and media attention (Appadurai, 1990, 1996), and accompanied by a sports "brain drain" that reinforces European dependence on the North American market. From a tactical and performance perspective, the studies analyzed suggest that European players bring to the NBA skills associated with FIBA basketball—greater emphasis on team play, pace control, and efficiency in specific areas of the court—which align with the league's recent evolution toward models based on spacing, three-point shooting, and positional versatility (Sampaio et al., 2008, 2018; Gómez et al., 2015; Goldsberry, 2019; Paulauskas et al., 2018). Convergence is fostering a blending of playing styles, with the "NBA style" absorbing European influences and exporting organizational models and consumption patterns to Europe.

On a cultural and symbolic level, the growing visibility of European star players in the NBA helps to bolster the international prestige of European basketball and to create role models that fuel national and regional identification, yet intensifies conflicts of loyalty between clubs and national teams, in a context where NBA schedules and commercial interests increasingly constrain the decision-making space of European stakeholders (Bale & Maguire, 1994; Elliott & Weedon, 2011). From a scientific standpoint, this study is limited by its sources—it was an exploratory literature review—and by the absence of systematic longitudinal data on the individual trajectories of European players in the NBA. In future research, combining quantitative methods (game analysis, advanced statistics, transfer networks) with qualitative approaches (interviews, case studies) will be necessary to more clearly understand how European training translates into specific competitive advantages in the NBA, what the return effects of this migration are on national sports systems, and how selection, training, and regulatory policies in European

leagues can be rethought in a scenario where the global circulation of talent has become structural and irreversible.

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The Burden of Injuries in Padel: A Systematic Synthesis of Risk Factors and Biomechanical Implications

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Summary

Background: To synthesize the recent literature on injuries in padel players, identifying patterns of occurrence, risk factors, and preventive strategies, with a focus on amateurs, youth, and recreational players.

Methods: Thirty studies published between 2014 and 2025 were included, comprising cross-sectional studies, systematic reviews, and biomechanical analyses. Article selection was based on searches in PubMed, ScienceDirect and Google Scholar, using keywords such as “padel”, “injuries” and “musculoskeletal injuries”. Inclusion criteria encompassed studies analyzing musculoskeletal injuries in padel players, while studies involving other sports or lacking injury assessment were excluded.

Results: The prevalence of injuries among amateur players ranged from 40% to 55%, with an average incidence of 3–8 injuries per 1,000 hours of training or matches. Tendinous and muscular injuries were most common, predominantly affecting the shoulder, elbow, knee, and ankle. Risk factors included limited experience, high training volume, inadequate warm-up, psychological load, lifestyle, and specific biomechanical patterns. Sex and age differences were observed, with women more prone to upper limb tendinous injuries and men to muscular and ligament injuries. Imaging techniques and biomechanical analyses were effective in early injury identification and prevention.

Conclusions: Padel carries a significant risk of injury, particularly among amateurs and youth. Preventive strategies should include structured warm-up and strengthening programs, training load monitoring, biomechanical assessment, and attention to psychophysiological factors. Future studies should investigate preventive interventions and longitudinal cohorts to reduce injury incidence in this sport.

Keywords: padel; sports injuries; injury prevention; musculoskeletal injuries; amateur athletes.

Resumo

Introdução: Sintetizar a literatura recente sobre as lesões em praticantes de padel, identificando os padrões de ocorrência, os fatores de risco e as estratégias de prevenção, com especial enfoque em praticantes amadores, jovens e recreativos.

Métodos: Foram incluídos 30 estudos publicados entre 2014 e 2025, compreendendo estudos transversais, revisões sistemáticas e análises biomecânicas. A seleção dos artigos baseou-se em pesquisas nas bases de dados PubMed, ScienceDirect e Google Scholar, utilizando palavras-chave como padel, injuries e musculoskeletal injuries. Os critérios de inclusão abrangeram estudos que analisavam lesões musculoesqueléticas em praticantes de padel, tendo sido excluídos estudos relativos a outras modalidades desportivas ou que não incluíssem a avaliação de lesões.

Resultados: A prevalência de lesões entre os praticantes amadores variou entre 40% e 55%, com uma incidência média de 3 a 8 lesões por cada 1.000 horas de treino ou competição. As lesões tendinosas e musculares foram as mais frequentes, afetando predominantemente o ombro, o cotovelo, o joelho e o tornozelo. Os principais fatores de risco identificados incluíram a reduzida experiência na modalidade, elevados volumes de treino, aquecimento inadequado, carga psicológica, estilo de vida e padrões biomecânicos específicos. Observaram-se diferenças relacionadas com o sexo e a idade, sendo as mulheres mais suscetíveis a lesões tendinosas dos membros superiores e os homens a lesões musculares e ligamentares. As técnicas de imagiologia e as análises biomecânicas revelaram-se eficazes na identificação precoce e na prevenção de lesões.

Conclusões: O padel apresenta um risco significativo de lesão, particularmente entre praticantes amadores e jovens. As estratégias preventivas devem incluir programas estruturados de aquecimento e fortalecimento muscular, monitorização da carga de treino, avaliação biomecânica e atenção aos fatores psicofisiológicos. Estudos futuros deverão avaliar a eficácia de intervenções preventivas e desenvolver estudos longitudinais, de forma a reduzir a incidência de lesões nesta modalidade desportiva.

Palavras-chave: padel; lesões desportivas; prevenção de lesões; lesões musculoesqueléticas; atletas amadore

Introduction

Padel is a racket sport of Spanish origin that combines elements of tennis and squash. It is played on a smaller court surrounded by walls, which significantly affects the dynamics of the game. The sport is characterized by intermittent high-intensity actions, involving rapid movements, sudden changes of direction, trunk rotations, frequent accelerations and decelerations, as well as the repetitive execution of technical overhead movements, such as smashes and volleys (Alhammad et al., 2025; Muñoz et al., 2022). These demands place a high mechanical load on the musculoskeletal and neuromuscular systems, particularly in the upper and lower limbs. The sport has experienced exponential growth worldwide, becoming one of the fastest-growing racket sports, especially in Europe and Latin America (Alhammad et al., 2025; Dahmen et al., 2023). This growth is particularly evident among amateur players, young people, and recreational players, many of whom take up the sport without specific physical preparation, adequate technical supervision, or previous sporting history, which can increase their vulnerability to injury (Declève et al., 2025; Rocamora-López & Mateo-Orcajada, 2025).

Despite the recognized benefits of padel for cardiovascular health, overall physical fitness, and psychosocial well-being, recent literature has warned of a considerable incidence and prevalence of injuries associated with its practice (Ferreira et al., 2025; Muñoz et al., 2022). Epidemiological studies indicate that musculoskeletal injuries predominantly affect the shoulder, elbow, knee, and ankle, with emphasis on tendon, muscle, and ligament injuries (de Sire et al., 2024; Tagliafico et al., 2023). The so-called “padel elbow,” like lateral epicondylitis observed in other racket sports, has been frequently reported, as well as shoulder pathologies related to overload and muscle imbalances.

Several risk factors have been associated with injuries in padel players, including limited experience, high training volume and intensity, inadequate warm-up, deficits in strength and neuromuscular control, as well as specific biomechanical patterns of the technical movement (Marotta et al., 2025; Muñoz et al., 2022; Pérez et al., 2023; Ryman Augustsson & Durdel, 2025).

In addition to physical factors, psychophysiological aspects such as stress, perceived well-being, fatigue, and lifestyle have emerged as relevant elements in injury predisposition, particularly in young and recreational populations (Alhammad et al., 2025; Rocamora-López & Mateo-Orcajada, 2025).

Despite the increase in the number of studies on padel injuries, the literature still shows significant methodological heterogeneity, with a predominance of cross-sectional studies, frequent use of self-reported questionnaires, and a scarcity of longitudinal studies and structured preventive interventions. In addition, most studies focus on professional players, with a clear gap in knowledge regarding amateur, young, and recreational players, who constitute most of the population practicing the sport (Dahmen et al., 2023; Declève et al., 2025).

In this context, it is pertinent to conduct a literature review that integrates epidemiological, physiological, and biomechanical data, allowing the identification of injury patterns, risk factors, and specific preventive strategies for padel (Belmar-Arriagada et al., 2025; Rocamora-López &

Mateo-Orcajada, 2025). A structured synthesis of the available scientific evidence can help guide health professionals, coaches, and practitioners in implementing effective prevention measures, promoting safer and more sustainable practice of the sport (Declève et al., 2025; Thomas et al., 2025).

The objective of this review is to synthesize recent scientific literature on injuries associated with playing padel, analyzing their prevalence, the most frequent types and locations, as well as the main risk factors involved. Additionally, it aims to integrate epidemiological, physiological, and biomechanical evidence, identifying differences related to age, gender, and level of experience, and to discuss strategies for prevention and early identification of injuries. This review focuses particularly on amateur, young, and recreational players, with the aim of providing scientific support for the implementation of preventive practices and for guiding future research in this sport.

Methodology

The bibliographic search was conducted in the PubMed, ScienceDirect, and Google Scholar databases, The search approach integrated free-text and controlled vocabulary terms associated with sports injuries and padel. In order to increase the identification of pertinent research and refine the searches, boolean operators (AND/OR) were employed. In order to find additional publications that were not found by the first database search, reference lists of eligible articles were additionally manually searched, selected for their comprehensiveness and relevance to the literature on sports and musculoskeletal injuries. The keywords used included: “padel,” “injuries,” and “musculoskeletal injuries.

The period considered covered studies published between 2019 and 2025, ensuring the inclusion of recent articles on padel injuries. Original studies, systematic reviews, and biomechanical analyses published in English or Spanish and focusing on amateur, young, and recreational players were included. Studies that did not specifically address padel injuries, articles on other sports, reports without injury assessment, or without full access to the text were excluded.

The selection of sources was carried out in two stages. Initially, titles and abstracts were screened to identify thematic relevance. Subsequently, potentially eligible articles were evaluated in full, recording information on the study population, type and location of injuries, risk factors, and main results. This analysis allowed us to synthesize epidemiological, physiological, and biomechanical data, ensuring the integrity and comparability of the information included in the review.

No AI-assisted tools were used during the literature search or study selection process.

In total, 30 studies met the inclusion criteria and were incorporated into the review, providing a solid basis for the analysis of injury patterns, risk factors, and preventive strategies in padel. Seventeen of these studies were not cited throughout this article (Alito et al., 2024; Arteaga et al., 2025; Cocco et al., 2024; de Almeida Fagundez et al., 2024; de Sire, 2022; Demeco et al., 2022; Foesch et al., 2025; García-Fernández et al., 2019; García–Fernández et al., 2019; Latino et al., 2025; Palacio, 2024; Priego Quesada et al., 2018; Quesada et al., 2014; Sánchez-Alcaraz et al., 2021; Teixeira et al., 2025; Thomas et al., 2025; Tooth et al., 2023; Viera et al., 2025).

There were two phases to the selecting procedure. First, titles and abstracts were examined to make sure they were pertinent to the review's goals. Second, the pre-established inclusion and exclusion criteria were used to evaluate the entire texts of possibly eligible research. The final synthesis did not include any research that did not specifically address injury occurrence, risk factors, prevention, or biomechanical elements of padel.

Results

Analysis of the studies included identified consistent patterns of injury occurrence, risk factors, and physiological and biomechanical characteristics in padel players.

Table 1: Characteristics and main findings of selected studies on padel-related injuries

Author	Country	Type of study	Sample	Player Level	Main results
(Dahmen et al., 2023)	Various	Systematic review	—	Amateur and professional	Prevalence between 40–95%; shoulder and elbow most affected
(Muñoz et al., 2022)	Spain	Transversal	n=120	Amateur	Incidence 3–8 lesions/1,000 hours; predominance MS
(Ferreira et al., 2025)	Portugal	Transversal	n=164	Recreational	Differences by gender; frequent shoulder and knee injuries
(Declève et al., 2025)	Belgium	Transversal	n=278	Recreational	High prevalence among recreational users
(Alhammad et al., 2025)	South Arabia	Transversal	n=310	Amateur	Relationship between psychological stress and injuries
(de Sire et al., 2024)	Italy	Biomechanics	n=30	Competitive	Kinematic changes in the shoulder are associated with risk
(de Sire et al., 2024)	Italy	Biomechanics	n=20	Amateur	Predictive sEMG of shoulder injury
(Ryman Augustsson & Durdel, 2025)	Sweden	Transversal	n=89	Youth	Association between training load and injuries

Prevalence of Injuries

The prevalence of injuries in amateur players ranged from 40% to 55%, while systematic reviews indicate values between 40% and 95%, depending on the population studied and the assessment method used (Dahmen et al., 2023; Muñoz et al., 2022; Rocamora-López & Mateo-Orcajada, 2025). The average incidence reported was 3 to 8 injuries per 1,000 hours of training or games (Ferreira et al., 2025; Muñoz et al., 2022). Studies show that young players are more vulnerable to overuse injuries, while more experienced players tend to have more localized and predictable injury patterns (Alhammad et al., 2025; Ryman Augustsson & Durdel, 2025).

Table 2: Prevalence and incidence of injuries reported in padel players according to population and assessment method

Author	Sample	Prevalence (%)	Incidence	Evaluation method
(Dahmen et al., 2023)	Amateurs/professionals	40–95	—	Systematic review
(Muñoz et al., 2022)	Amateurs	45–55	3–8 / 1.000 h	Questionnaire
(Ferreira et al., 2025)	Recreational	42	4,2 / 1.000 h	Questionnaire
(Declève et al., 2025)	Recreational	50	—	Online Questionnaire
(Belmar-Arriagada et al., 2025)	Amateurs	48	—	Cross-sectional evaluation

Type and Locations of Injuries

Upper limbs: the shoulder and elbow were the most affected, with a predominance of tendon and muscle injuries. “Padel elbow” was reported as frequent (de Sire et al., 2024; Muñoz et al., 2022; Tagliafico et al., 2023).

Lower limbs: knee, ankle, and foot, usually with ligament and muscle injuries, especially in rapid change of direction movements (Ferreira et al., 2025; Rocamora-López & Mateo-Orcajada, 2025).

Gender differences: women had a higher incidence of tendon injuries in the upper limb, while men had more muscle and ligament injuries (Ferreira et al., 2025; Ryman Augustsson & Durdel, 2025).

Table 3: Most frequently affected anatomical regions and types of injuries reported in padel players

Body region	Type of injury	Studies reporting
Shoulder	Tendinitis, overload	(de Sire et al., 2024; Marotta et al., 2025; Muñoz et al., 2022)
Elbow	Lateral epicondylitis	(Muñoz et al., 2022; Tagliafico et al., 2023)
Knee	Ligament/muscle injuries	(Ferreira et al., 2025; Rocamora-López & Mateo-Orcajada, 2025)
Ankle	Sprains	(Declève et al., 2025; Ferreira et al., 2025)
Foot	Plantar overload	(Quesada et al., 2014; Rocamora-López & Mateo-Orcajada, 2025)

Table 4: Differences in injury patterns according to sex, age, and playing experience in padel players

Variable	Key findings	Studies
Female	More tendon injuries MS	(Ferreira et al., 2025; Ryman Augustsson & Durdel, 2025)
Male	More muscle and ligament injuries	(Ferreira et al., 2025)
Young people	Increased risk due to excessive use	(Alhammad et al., 2025; Ryman Augustsson & Durdel, 2025)
Experienced adults	More localized injuries	(Muñoz et al., 2022)

Risk Factors

Multiple factors associated with an increased risk of injury have been identified:

Training and experience: less experience (<35 years) and high weekly training volume increase the incidence of injuries (Muñoz et al., 2022; Ryman Augustsson & Durdel, 2025). Physical preparation: inadequate warm-up and lack of specific strengthening (Pérez et al., 2023; Rocamora-López & Mateo-Orcajada, 2025).Psychophysiological and lifestyle: high stress, low well-being, diet, and lifestyle habits influence risk, especially in young athletes (Alhammad et al., 2025; Ryman Augustsson & Durdel, 2025).Biomechanics and technique: shoulder movement patterns and poor posture contribute to increased risk, according to studies using sEMG (surface electromyography) and kinematic analysis (de Sire et al., 2024; Marotta et al., 2025).

Table 5: Main modifiable and non-modifiable risk factors associated with injuries in padel players.

Category	Risk factor	Studies
Training	High volume	(Muñoz et al., 2022; Ryman Augustsson & Durdel, 2025)
Physical preparation	Inadequate heating	(Pérez et al., 2023; Rocamora-López & Mateo-Orcajada, 2025)
Biomechanics	Kinematic changes in the shoulder	(de Sire et al., 2024; Marotta et al., 2025)
Psychophysiological	Stress and low well-being	(Alhammad et al., 2025; Rocamora-López & Mateo-Orcajada, 2025)
Lifestyle	Diet and recovery	(Rocamora-López & Mateo-Orcajada, 2025)

Diagnosis and Prevention

Imaging techniques (ultrasound) and biomechanical analyses have proven effective for early identification of injuries (Pérez et al., 2023; Thomas et al., 2025). The literature recommends integrated preventive programs, including warm-up, strengthening, training load monitoring, and individualized technical assessment (Declève et al., 2025; Rocamora-López & Mateo-Orcajada, 2025).

Discussion & Conclusions

This review consistently shows that injuries in padel are frequent and result from a complex interaction between biomechanical, physiological, psychosocial, and contextual factors. The studies analyzed converge in identifying the shoulder, elbow, knee, and ankle as the most affected anatomical segments, reflecting the specific demands of the sport, characterized by high-speed repetitive movements, rapid changes of direction, and predominantly asymmetrical actions (Ferreira et al., 2025; Muñoz et al., 2022; Rocamora-López & Mateo-Orcajada, 2025). The high prevalence of tendon and muscle injuries, especially in the upper limbs, seems to be directly associated with the repetitive nature of the technical movements in padel, namely the smash, volley, and overhead shots. These movements place high loads on the structures of the shoulder and elbow complex, especially when performed with inadequate biomechanical patterns or in conditions of fatigue. At the same time, lower limb injuries reflect the demands of frequent acceleration, deceleration, and rotation in confined spaces, which increases mechanical stress on the knee and ankle (Dahmen et al., 2023; Pérez et al., 2023; Ryman Augustsson & Durdel, 2025).

The literature also shows consensus on the role of modifiable factors in injury risk, including limited experience, high training volumes, and inadequate warm-up strategies. Recreational and beginner players seem particularly vulnerable, possibly due to lower technical proficiency, deficits in strength and neuromuscular control, as well as lower literacy in injury prevention. These findings reinforce the importance of an early preventive approach, even in non-competitive contexts (Ryman Augustsson & Durdel, 2025). Additionally, recent studies have highlighted the relevance of psychophysiological factors, such as high stress levels, lower perceived well-being, and accumulated fatigue, in predisposing individuals to injuries. These factors can negatively influence motor control, reaction time, and decision-making during play, increasing the risk of uncoordinated or delayed movements. The integration of psychophysiological assessments with biomechanical data, namely through sEMG and kinematic analyses, has allowed the identification of inadequate muscle activation patterns and functional asymmetries associated with a higher risk of injury, especially in young players and in the early stages of sports specialization (Alhammad et al., 2025; de Sire et al., 2024; Marotta et al., 2025).

Despite these convergences, there is considerable heterogeneity in the reported prevalence and incidence of injuries, ranging from 40% to 95% of injured players and from 3 to 8 injuries per 1,000 hours of practice. These discrepancies can be attributed to substantial methodological differences between studies, including the use of self-reported questionnaires versus clinical assessments, variations in the recall period, differences in the competitive level of participants, and distinct geographical contexts. This heterogeneity limits the direct comparability of results and highlights the need for standardized protocols for injury surveillance in padel (Dahmen et al., 2023; Ferreira et al., 2025; Muñoz et al., 2022; Rocamora-López & Mateo-Orcajada, 2025).

Among the main limitations of the available literature is the predominance of cross-sectional and observational studies, often with small samples, which hinder causal inference and understanding of the temporal evolution of injuries. The scarcity of longitudinal studies and limited research on structured preventive programs represent significant gaps, particularly considering the exponential growth of padel at the recreational level. In addition, the application of advanced biomechanical analyses has been largely restricted to high-performance contexts, reducing the transfer of knowledge to the general population of practitioners. Furthermore, pertinent material published in other languages might have been overlooked due to the restriction to research published in English and Spanish.

Given these limitations, future research should focus on longitudinal designs, with objective monitoring of external and internal training load, integration of detailed biomechanical assessments, and consideration of psychosocial factors. The implementation and evaluation of multimodal preventive programs, adapted to the specific characteristics of padel and different levels of practice, could contribute significantly to reducing the incidence of injuries and promoting safer and more sustainable practice of the sport (Declève et al., 2025; Rocamora-López & Mateo-Orcajada, 2025).

This review demonstrates that injuries associated with playing padel are common and result from a multifactorial etiology, predominantly affecting the shoulder, elbow, knee, and ankle, with a higher incidence of tendon and muscle injuries (Ferreira et al., 2025; Muñoz et al., 2022; Rocamora-López & Mateo-Orcajada, 2025). Evidence suggests that amateur, young, and less experienced players are more vulnerable to injury, particularly when exposed to high training volumes, inadequate warm-up strategies, unfavorable biomechanical patterns, and high levels of psychophysiological load (Alhammad et al., 2025; Dahmen et al., 2023; de Sire et al., 2024).

Additionally, the literature shows that variability in assessment methods and study designs limits the comparability of available epidemiological data. Nevertheless, the results converge on the need for an integrated preventive approach that simultaneously considers physical, biomechanical, and psychosocial factors. In summary, injury prevention in padel should be understood as a multidimensional and continuous process, essential to ensure safe, effective, and sustainable practice of the sport (Declève et al., 2025; Rocamora-López & Mateo-Orcajada, 2025).

Practical Applications

From a practical application standpoint, the results suggest that injury prevention programs for padel should include integrated strategies such as: Implementation of sport-specific warm-ups and muscle strengthening programs (Pérez et al., 2023; Ryman Augustsson & Durdel, 2025); Systematic monitoring of training load to prevent overload and fatigue (Belmar-Arriagada et al., 2025; Dahmen et al., 2023); Biomechanical assessment of movement patterns, especially at the shoulder and lower limbs (de Sire et al., 2024; Marotta et al., 2025); Consideration of psychophysiological and lifestyle factors is essential for promoting the overall well-being of practitioners and reducing the risk of injury (Alhammad et al., 2025).

Regarding future research, it is recommended that the following be carried out:

Longitudinal studies to monitor the evolution of injuries over time (Belmar-Arriagada et al., 2025); Development and evaluation of the effectiveness of structured preventive interventions, including specific training programs and appropriate recovery strategies (Declève et al., 2025); Exploring the use of biomechanical analysis and surface electromyography (sEMG) technologies in recreational settings to identify risk patterns that have not yet been properly detected (Marotta et al., 2025; Thomas et al., 2025).

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Injuries in Youth Futsal Players: A Systematic Review

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Summary

Background: Futsal is a high-intensity intermittent sport associated with considerable injury risk, particularly in youth populations. Despite its growing global participation, the epidemiology of injuries in young players remains insufficiently synthesized.

Objective: To synthesize available evidence on injury incidence, characteristics, and contextual risk factors in youth futsal players.

Methods: A systematic search was conducted in Scopus, Web of Science, and Google Scholar. Observational studies reporting epidemiological injury data in youth futsal were included. Methodological quality was assessed using the Downs and Black checklist. Given heterogeneity in injury definitions and exposure metrics, a narrative synthesis was performed.

Results: Five studies were included. Injury incidence was consistently higher during matches than training, ranging from 208.6 to 292.42 injuries per 1,000 match hours. Lower-limb injuries predominated (70–88%), with ankle sprains as the most frequent diagnosis, while knee injuries imposed the greatest burden (>28 days of time-loss). Female players consistently presented higher injury incidence than males. Playing position and match timing were associated with elevated injury risk.

Conclusions: Youth futsal carries a high injury risk, particularly during competition. Prevention strategies should address contextual demands and prioritize standardized injury surveillance to improve comparability and support long-term athlete development.

Keywords: futsal; youth athletes; sports injuries; injury incidence; injury epidemiology; maturation

Resumo

Introdução: O futsal é uma modalidade desportiva intermitente de elevada intensidade, associada a um risco considerável de lesão, particularmente nas populações jovens. Apesar do crescente número de praticantes a nível mundial, a epidemiologia das lesões em jovens jogadores continua insuficientemente sintetizada.

Objetivo: Sintetizar a evidência disponível sobre a incidência, as características e os fatores de risco contextuais das lesões em jovens praticantes de futsal.

Métodos: Foi realizada uma pesquisa sistemática nas bases de dados Scopus, Web of Science e Google Scholar. Foram incluídos estudos observacionais que reportavam dados epidemiológicos sobre lesões em jovens praticantes de futsal. A qualidade metodológica foi avaliada através da lista de verificação de Downs e Black. Atendendo à heterogeneidade das definições de lesão e das métricas de exposição utilizadas, foi realizada uma síntese narrativa dos resultados.

Resultados: Foram incluídos cinco estudos. A incidência de lesões foi consistentemente superior durante a competição do que durante o treino, variando entre 208,6 e 292,42 lesões por 1.000 horas de jogo. As lesões dos membros inferiores foram predominantes (70–88%), sendo a entorse do tornozelo o diagnóstico mais frequente, enquanto as lesões do joelho representaram a maior carga lesional, com períodos de afastamento da prática desportiva superiores a 28 dias. As jogadoras apresentaram, de forma consistente, uma incidência de lesões superior à dos jogadores. A posição em campo e o momento da partida estiveram associados a um risco acrescido de lesão.

Conclusões: O futsal praticado por jovens apresenta um elevado risco de lesão, particularmente durante a competição. As estratégias de prevenção deverão considerar as exigências específicas do contexto competitivo e privilegiar a implementação de sistemas padronizados de vigilância das lesões, de modo a melhorar a comparabilidade entre estudos e a apoiar o desenvolvimento desportivo a longo prazo dos atletas.

Palavras-chave: futsal; atletas jovens; lesões desportivas; incidência de lesões; epidemiologia das lesões; maturação.

Introduction

Futsal is a high-intensity intermittent sport characterized by frequent accelerations and decelerations, rapid changes of direction, repeated one-on-one situations, and continuous technical involvement in reduced playing spaces. These demands, combined with the characteristics of indoor playing surfaces, impose substantial mechanical loads on the musculoskeletal system, particularly at the ankle and knee joints, contributing to an increased risk of injury (Ahmad-Shushami & Abdul-Karim, 2020; Ribeiro & Costa, 2006).

Epidemiological evidence suggests that injury incidence in futsal may exceed that observed in football under comparable competitive contexts, particularly during match play. This difference is likely related to the specific characteristics of futsal, including reduced playing space, higher player density, frequent high-intensity actions, and the use of high-friction indoor surfaces, which may increase mechanical stress and contact frequency (Ahmad-Shushami & Abdul-Karim, 2020).

Injuries in futsal predominantly affect the lower extremities, with the ankle and knee consistently reported as the most frequently involved anatomical regions (Ribeiro & Costa, 2006; Varkiani et al., 2013). While ankle sprains are the most common injury type, knee injuries are often associated with greater clinical severity and longer time-loss, representing a higher burden for athlete development (Ribeiro & Costa, 2006).

Injury occurrence is further influenced by contextual and developmental factors. Match play is typically associated with higher injury risk due to increased intensity and contact exposure, while fatigue may impair neuromuscular control and increase susceptibility to injury. In youth populations, periods of rapid growth, particularly around peak height velocity (PHV), are associated with transient reductions in coordination and neuromuscular control, potentially increasing injury risk (Malina et al., 2015).

Even though futsal is becoming more popular all over the world, the literature on injuries sustained by young players is still somewhat small and inconsistent in methodology, which emphasizes the need for a more comprehensive synthesis of the available data. Variability in study design, injury definitions, and exposure metrics complicates direct comparisons across studies and may contribute to an incomplete understanding of injury burden.

Therefore, the aim of this systematic review is to synthesize the available evidence on injury incidence, characteristics, and contextual patterns in youth futsal players, with particular attention to methodological differences and their implications for interpreting injury risk.

Methods

Study Design and Reporting Guidelines

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines, ensuring a structured and transparent approach to study identification, selection, and synthesis.

The aim was to identify, analyze, and synthesize the available evidence regarding injury incidence and characteristics in youth futsal players.

There was no potential registration for the review protocol. All review techniques were predetermined and applied methodically throughout the review process, even if this can lessen transparency regarding methodological decisions.

Given the characteristics of the available literature, only observational studies were considered eligible. Due to expected heterogeneity in study design, injury definitions, and exposure metrics, a structured narrative synthesis approach was adopted.

Search Strategy

A systematic literature search was conducted in the Scopus, Web of Science, and Google Scholar electronic databases. The search strategy combined keywords related to futsal, injuries, and youth populations using Boolean operators: “futsal AND injury AND (youth OR adolescent OR junior OR child)”.

In order to balance comprehensiveness and feasibility, screening was restricted to the first 200 records sorted by relevance due to Google Scholar's wide scope and lesser specificity. This methodological decision aimed to balance sensitivity with feasibility, while reducing the inclusion of non-relevant records.

No restrictions were applied regarding publication year. Similarly, no language restrictions were applied during the search process; however, studies published in English, Portuguese, or Spanish were included based on accessibility and relevance.

PubMed was not included in the search strategy due to time and access constraints, which may represent a limitation and may have resulted in the omission of relevant studies.

Eligibility Criteria

Study eligibility was defined a priori using a PECO framework (Population, Exposure, Comparison, Outcome), as presented in Table 1.

The population of interest included youth futsal players aged under 18 years. However, studies involving slightly older categories (e.g., Sub-20 or <21 years) were considered when representative of late adolescent development and biological maturation, which is particularly relevant given the focus on growth-related injury risk.

Eligible studies were required to report epidemiological injury data in futsal contexts (training and/or competition), including at least one of the following: injury incidence, type, anatomical location, mechanism (contact or non-contact), or severity (e.g., time-loss or medical attention).

Given the heterogeneity of the literature, studies adopting different injury definitions (e.g., “any physical complaint”, “medical attention”, or “time-loss”) were considered eligible. Likewise, studies reporting incidence using different denominators (e.g., per 1,000 exposure hours or per number of athletes) were included, and these methodological differences were considered during data synthesis.

Studies were excluded if they: (i) focused exclusively on adult populations; (ii) included multiple sports without isolating futsal-specific data; (iii) reported only performance or intervention outcomes without epidemiological injury data; or (iv) were non-original research (e.g., reviews, editorials, conference abstracts, theses, or case reports).

Table 1. PECO Framework used to define eligibility criteria

PECO Element	Inclusion Criteria	Exclusion Criteria
Population	Youth futsal players (<18 years), including late adolescent categories (e.g., Sub-20 or <21 years) when representative of final maturation stages	Adult-only populations; recreational players without competitive context
Exposure	Participation in futsal training and/or competition, including specific contexts such as preseason periods characterized by increased training load	Other sports or mixed samples without disaggregated futsal-specific data
Comparison	Not required; may include comparisons across sex, competitive level, or context (training vs. match) when available	Not applicable
Outcome	Epidemiological injury data, including incidence rates (per 1,000 hours or per athletes), injury type, anatomical location, mechanism (contact/non-contact), and severity (e.g., time-loss or medical attention)	Performance-only outcomes; prevention-only studies without injury incidence data
Study Design	Observational studies, including prospective cohorts, retrospective analyses, and injury surveillance systems	Reviews, editorials, conference abstracts, theses, and case reports

Study Selection

The study selection process was conducted in accordance with PRISMA guidelines. After removal of duplicate records, titles and abstracts were screened to identify potentially eligible studies, followed by full-text assessment based on predefined inclusion and exclusion criteria.

The selection process was conducted by a single reviewer, which may increase the risk of selection bias; however, procedures were performed systematically, and eligibility decisions were verified through repeated full-text assessment.

A flow diagram summarizing the study selection process is presented in Figure 1.

Data Extraction

Data was extracted using a standardized form designed for this review. The extraction process included key study characteristics and epidemiological variables to ensure consistent and structured data collection across studies.

The following information was extracted from each included study: author and year of publication, country, sample size and age, sex, competitive level, study design, and context of exposure (training and/or match). In addition, methodological and epidemiological variables were collected, including the operational definition of injury, the unit of exposure (e.g., hours, athlete-exposures), the denominator used to report injury incidence (e.g., injuries per 1,000 hours or per 1,000 athletes), and the main findings related to injury incidence and characteristics.

The data extraction process was conducted by a single reviewer, and all extracted data were subsequently checked for accuracy and consistency.

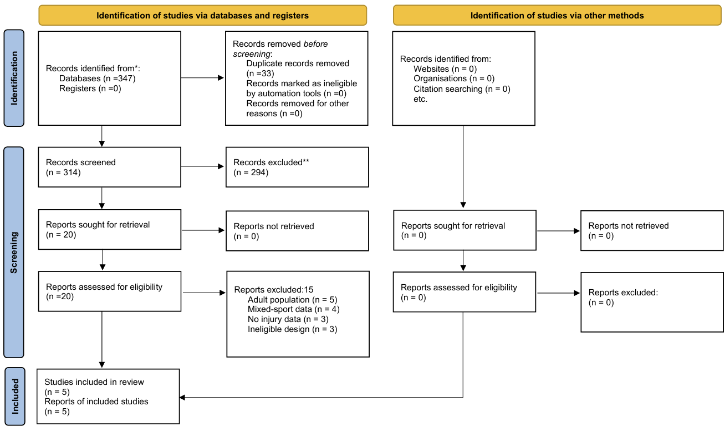

Figure 3. PRISMA 2020 flow diagram ilustrating the study selection process.

Methodological Quality Assessment

The methodological quality of the included studies was assessed using the Downs and Black checklist, which is appropriate for evaluating both randomized and non-randomized studies, including observational designs.

This checklist evaluates multiple domains, including reporting quality, external validity, internal validity (bias and confounding), and statistical power. Each study was assessed and assigned an overall methodological quality score based on the checklist criteria.

Given the observational nature of the included studies, particular attention was paid to aspects related to study design, clarity of reporting, and control of potential sources of bias.

Based on total scores, studies were classified as follows: 20–25 (good), 15–19 (fair), and <15 (poor).

The results of the methodological quality assessment are presented in Table 3.

Risk of Bias

The risk of bias was evaluated using the domains included in the Downs and Black checklist, particularly those related to reporting, external validity, internal validity (bias and confounding), and statistical power.

A qualitative synthesis of the risk of bias across studies was conducted to identify common methodological limitations and potential sources of bias.

The results are presented using descriptive summaries to facilitate interpretation.

Data Synthesis

Due to the heterogeneity observed across the included studies in terms of study design, injury definitions, and exposure metrics, a meta-analysis was not performed. Instead, a structured narrative synthesis was conducted.

The synthesis of results was organized according to key variables, including sex, age group, context of exposure (training vs. match), and type of injury metrics reported.

Particular attention was given to differences in exposure units and incidence denominators, including injuries per 1,000 hours of exposure and injuries per number of athletes.

Due to these methodological differences, direct comparisons of injury incidence rates between studies were not performed, and findings were interpreted with caution.

Results

Study Selection

The initial database search identified a total of 347 records, including Scopus (n = 30), Web of Science (n = 117), and Google Scholar (n = 200), with the latter limited to the first 200 results sorted by relevance. After the removal of duplicates (n = 33), 314 records remained for title and abstract screening.

Following this stage, 294 records were excluded, and 20 reports were sought for retrieval and assessed in full text. After full-text evaluation, 5 studies met the inclusion criteria and were included in this systematic review, while 15 reports were excluded for not meeting the predefined eligibility criteria. The study selection process is presented in Figure 1.

Study Characteristics of Included Studies

The five included studies were conducted across different geographical contexts, including Malaysia, Brazil, Portugal, and Iran, and presented heterogeneous samples in terms of age, competitive level, and methodological design. Most studies adopted prospective injury surveillance approaches in competitive match settings, while one study used a retrospective design based on administrative data.

Methodological variability was evident across studies, particularly regarding injury definitions and exposure metrics. Injury definitions ranged from inclusive criteria, such as “any physical complaint”, to more restrictive approaches based on medical attention or time-loss. Similarly, incidence was reported using different denominators, including injuries

per 1,000 exposure hours and per number of registered athletes.

Despite these methodological differences, consistent epidemiological patterns were observed. Lower-limb injuries, particularly affecting the ankle and knee, were the most frequently reported across all studies. In addition, female players consistently presented higher injury incidence compared to males. Contextual factors such as playing position and match timing also emerged as relevant contributors to injury occurrence.

Table 2. Characteristics of the included studies

Study	Country	Sample	Sex	Level	Design	Context	Injury Definition	Exposure Metric	Key Findings
Ahmad-Shushami & Abdul-Karim (2020)	Malaysia	352 (<21y)	Both	Amateur	Prospective	Match	Any complaint	per 1,000 match hours	292.4/1000h; higher in females; knee & thigh most affected
Ribeiro & Costa (2006)	Brazil	180 (17–20y)	Male	Elite youth (U20)	Prospective	Match	Any complaint	per 1,000 match hours	208.6/1000h; contusions & sprains predominant; ankle & knee
Serrano et al. (2020)	Portugal	711 (U16–U18)	Both	National youth	Prospective	Match	Any complaint	per 1,000 match hours	Higher in females; 67.5% in wingers/goalkeepers; late-game peak
Varkiani et al. (2013)	Iran	1045 athletes	Both	Registered	Retrospective	Training & Match	Medical attention	per 1,000 athletes	8.1/1000 athletes; highest in 15–24y; females > males
Marques et al. (2024)	Portugal	68 (24.3±4.6y)*	Male	Mixed levels	Prospective	Training	Time-loss	per 1,000 exposure hours	Higher in amateurs; preseason peak (38.5%)

Note: Differences in exposure metrics (hours vs. number of athletes) are present across studies. Marques et al. (2024) was included for contextual purposes due to its relevance in training-related injury patterns, particularly preseason load dynamics.

Methodological Quality and Risk of Bias

The methodological quality and risk of bias of the five included studies were assessed using the Downs and Black Quality Assessment Checklist (Table 3), which evaluates reporting, external validity, internal validity (bias and confounding), and statistical power (Downs & Black, 1998).

Overall, four studies were classified as having good methodological quality (scores between 20 and 25), while one study was rated as fair quality. Higher scores were generally associated with prospective designs, clearer and more standardized injury definitions, and more accurate exposure measurement.

In terms of reporting, most studies provided adequate descriptions of sample characteristics and data collection procedures, although some limitations were observed, particularly regarding injury characterization and exposure quantification in retrospective designs.

External validity varies across studies, with broader and more representative samples contributing to greater generalizability, whereas smaller or more specific samples limited external validity.

Regarding internal validity, prospective studies using structured and consistent data collection procedures demonstrated lower risk of bias. In contrast, retrospective approaches based on administrative or registry data were more susceptible to information bias, particularly due to potential underreporting of minor or overuse injuries.

Additionally, heterogeneity in injury definitions and exposure metrics across studies represents a relevant source of measurement bias, limiting direct comparability of incidence rates. Potential confounding factors, such as differences in competitive level, sex distribution, and exposure context, were not consistently controlled.

Overall, the included studies demonstrated acceptable to good methodological quality; however, methodological heterogeneity and potential sources of bias should be considered when interpreting the findings.

Table 3. Methodological Quality Assessment (Downs and Black)

Study	Reporting	External Validity	Internal Validity (Bias)	Internal Validity (Confounding)	Power	Total Score	Quality Level
Serrano et al. (2020)	9	3	6	5	1	24	Good
Ahmad-Shushami & Abdul-Karim (2020)	9	2	6	4	1	22	Good
Marques et al. (2024)	10	1	6	4	0	21	Good
Ribeiro & Costa (2006)	8	2	5	4	1	20	Good
Varkiani et al. (2013)	7	2	4	2	1	16	Fair

Note. Studies were classified as low, moderate, or high methodological quality based on total scores: 20–25 (good), 15–19 (fair), and <15 (poor).

Injury Incidence

Injury incidence across the included studies showed substantial variability, largely driven by differences in injury definitions and exposure metrics. Most studies reported incidence rates per 1,000 exposure hours, particularly during match play, while one study expressed incidence relative to the number of registered athletes, limiting direct comparability.

Match injury incidence was consistently higher than training incidence across studies using exposure-based metrics. Ahmad-Shushami and Abdul-Karim (2020) reported 292.42 injuries per 1,000 match hours, whereas Ribeiro and Costa (2006) reported 208.6 per 1,000 match hours. These values considerably exceed those typically observed in football, reflecting futsal's high-intensity and contact demands.

Sex-based differences were also evident, with female players consistently presenting higher injury incidence rates than male counterparts. This pattern held across different

geographical contexts, suggesting a robust epidemiological trend rather than a context-specific finding.

Despite higher incidence during match play, a greater proportion of injuries may occur in training due to greater cumulative exposure. Marques et al. (2024) identified the preseason as a particularly critical period, with a disproportionate share of total injuries concentrated in this phase despite lower incidence rates relative to competition.

Notably, Varkiani et al. (2013) expressed incidence as 8.1 injuries per 1,000 registered athletes rather than per exposure hours, a methodological difference that precludes direct comparison of absolute values, though it still contributes to identifying broader injury patterns.

Overall, variability in incidence rates reflects methodological heterogeneity in injury definitions, exposure measurement, and study design, supporting the use of a narrative synthesis rather than a quantitative meta-analysis.

Injury Characteristics

Across the included studies, injuries predominantly affected the lower limbs, with the ankle and knee consistently identified as the most frequently affected anatomical regions (Ribeiro & Costa, 2006; Serrano et al., 2020). Ankle sprains were among the most frequently reported injury types, followed by knee and thigh injuries (Ahmad-Shushami & Abdul-Karim, 2020).

Regarding injury type, contusions and ligament injuries were the most commonly observed. Contusions were particularly prevalent in tournament-based studies, likely reflecting the high physical contact demands of futsal. Ligament injuries, especially ankle sprains, were associated with greater clinical relevance due to their recurrence and associated time-loss (Ribeiro & Costa, 2006; Serrano et al., 2020).

Muscle injuries, predominantly affecting the thigh, were also reported, although less frequently than joint-related injuries, and were commonly associated with high-intensity actions such as sprinting and rapid changes of direction (Ahmad-Shushami & Abdul-Karim, 2020).

Regarding injury severity, most cases were classified as mild to moderate. More severe injuries, particularly those involving the knee, were associated with prolonged absence from sport participation (Ribeiro & Costa, 2006).

Overall, these findings highlight the predominance of lower-limb injuries and the clinical importance of ankle and knee involvement in youth futsal.

Contextual Factors

Injury occurrence in youth futsal is shaped by several contextual factors, including sex, playing position, match dynamics, and training period.

Sex-based differences were consistently observed across studies, with female players presenting higher injury incidence rates than males (Ahmad-Shushami & Abdul-Karim, 2020; Serrano et al., 2020; Varkiani et al., 2013). This pattern held across different geographical contexts and competitive levels, indicating a stable epidemiological trend rather than a context-specific finding.

Playing position was also identified as a relevant determinant. Serrano et al. (2020) reported that wingers and goalkeepers accounted for 67.5% of injuries, suggesting that positional demands influence injury risk. The distinct physical and tactical requirements of each position likely contribute to differentiated injury profiles.

Temporal patterns within matches further implicate fatigue as a contributing mechanism. A substantial proportion of injuries (63.2%) occurred during the final stages of each half, suggesting that accumulated fatigue and declining neuromuscular control heighten injury susceptibility as matches progress (Serrano et al., 2020).

Training periodization also plays a critical role. Marques et al. (2024) identified the preseason as a particularly vulnerable period, with an elevated proportion of injuries likely attributable to abrupt increases in training load and insufficient physiological adaptation.

Collectively, these findings underscore the importance of integrating contextual and situational factors into the interpretation of injury risk in youth futsal, particularly regarding sex differences, positional demands, fatigue, and training periodization.

Discussion

The findings of this systematic review indicate that youth futsal is associated with a high risk of injury, with epidemiological patterns influenced by both biological and contextual factors. Injury incidence was consistently high across studies, reaching values such as 292.42 injuries per 1,000 match hours in Malaysian tournaments and 208.6 injuries per 1,000 match hours in Brazilian youth competitions (Ahmad-Shushami & Abdul-Karim, 2020; Ribeiro & Costa, 2006).

A clear predominance of lower-limb injuries was observed (approximately 70–88%), with the ankle being the most frequently affected region, while knee injuries contributed the greatest injury burden due to longer time-loss (>28 days) (Ribeiro & Costa, 2006; Serrano et al., 2020). Ligament injuries, particularly ankle sprains, were the most frequently reported, while contusions were especially prevalent in tournament contexts.

Additionally, contextual factors such as female sex, playing position, match timing, and preseason periods were consistently associated with increased injury risk (Marques et al., 2024; Serrano et al., 2020).

Injury Patterns and Contributing Factors in Youth Futsal

The findings of this systematic review indicate that injury occurrence in youth futsal is not random but influenced by a multifactorial interaction of contextual, physiological, and developmental factors. Across the included studies, a clear predominance of lower limb injuries was observed (70%–88%), with the ankle and knee being the most frequently affected joints (Ahmad-Shushami & Abdul-Karim, 2020; Marques et al., 2024; Serrano et al., 2020; Varkiani et al., 2013). This pattern is consistent with the biomechanical demands of futsal, characterized by frequent accelerations, decelerations, changes of direction, and repeated high-intensity actions (Naser et al., 2017).

From a contextual perspective, injury distribution appears to be influenced by playing position and match dynamics. A higher proportion of injuries was reported among wingers and goalkeepers (67.5%) (Serrano et al., 2020). Additionally, a substantial proportion of injuries occurred during the final stages of each half (63.2%), suggesting a potential association with fatigue-related mechanisms (Serrano et al., 2020). This is supported by evidence indicating that futsal is a high-intensity intermittent sport in which players experience progressive fatigue due to repeated sprint efforts and limited recovery time (Naser et al., 2017).

Developmental factors also play a relevant role. Periods of rapid growth, particularly around peak height velocity (PHV), are associated with temporary reductions in coordination and neuromuscular control, which may increase injury susceptibility (Caine et al., 2006). Although not directly assessed in all studies, the reported age ranges suggest that many players were within this critical developmental window (Serrano et al., 2020; Varkiani et al., 2013).

Seasonal variations were also identified, with higher injury frequency during the preseason, likely reflecting abrupt increases in training load and insufficient adaptation (Ahmad-Shushami & Abdul-Karim, 2020).

Overall, these findings reinforce that injury risk in youth futsal is multidimensional, shaped by the interaction between game demands, fatigue, development, and training context.

Mechanisms and Interpretation of Injury Risk

The injury patterns identified in this review reflect the interaction of mechanical, physiological, and developmental factors in youth futsal. The high incidence of lower-limb injuries aligns with the biomechanical demands of the sport, involving frequent accelerations, decelerations, and cutting movements (Naser et al., 2017).

Fatigue represents a key contributing mechanism. The higher proportion of injuries during the final stages of each half suggests that accumulated fatigue may impair neuromuscular control and joint stability (Serrano et al., 2020). Fatigue has been shown to reduce proprioceptive capacity and alter movement mechanics, increasing susceptibility to non-contact injuries (Bahr & Krosshaug, 2005).

Sex-related differences may be partially explained by neuromuscular and biomechanical factors, including differences in strength, movement patterns, and joint stability (Malina et al., 2015).

Biological maturation is also a critical determinant. Periods around peak height velocity (PHV) are associated with transient reductions in coordination and increased mechanical stress on musculoskeletal structures (Caine et al., 2006).

Training-related factors further modulate injury risk. Periods of rapid workload increase, particularly during preseason, may elevate injury risk due to insufficient adaptation, consistent with load–injury frameworks (Gabbett, 2016).

Overall, injury risk in youth futsal is multifactorial, resulting from the interaction between sport demands, fatigue, maturation, and training load.

Practical Implications

The findings of this review provide guidance for the development of injury prevention strategies in youth futsal. Given the high incidence of lower-limb injuries, training programs should prioritize neuromuscular training, balance, and strength development, with emphasis on movement control during cutting and landing tasks (Bahr & Krosshaug, 2005).

Considering the role of fatigue, practitioners should implement appropriate load management strategies, including monitoring exposure, optimizing player rotation, and ensuring adequate recovery. Avoiding sudden increases in workload is essential to minimize injury risk (Gabbett, 2016).

The influence of biological maturation highlights the need to individualize training based on maturation status. During peak height velocity, particular attention should be given to coordination, movement quality, and progressive strength development (Caine et al., 2006).

Gradual progression of training load during preseason is essential to ensure adequate physical preparedness (Marques et al., 2024).

Finally, prevention strategies should consider sex-specific characteristics, with targeted interventions addressing neuromuscular control and strength in female athletes (Malina et al., 2015).

Limitations

This systematic review presents several limitations that should be considered when interpreting the findings. First, the small number of included studies (n = 5) limits the generalizability of the results to the broader youth futsal population.

Second, substantial methodological heterogeneity was observed across studies, particularly regarding injury definitions and exposure metrics. While some studies adopted time-loss criteria (e.g., Marques et al., 2024; Ribeiro & Costa, 2006), others used broader definitions such as any physical complaint or medical attention, potentially contributing to variability in reported incidence rates (Ahmad-Shushami & Abdul-Karim, 2020; Serrano et al., 2020).

Additionally, differences in incidence denominators, including injuries per 1,000 exposure hours versus injuries per number of registered athletes, prevented direct comparison across studies and precluded the use of meta-analytic techniques (Varkiani et al., 2013). The lack of standardized injury surveillance methodologies further limits cross-study comparability.

Some studies relied on retrospective designs or administrative data sources, which are inherently susceptible to recall bias and potential underreporting of less severe or overuse injuries, possibly leading to an underestimation of the true injury burden.

Finally, although the search strategy was systematic, it may not have captured all relevant studies. Moreover, the absence of detailed data on biological maturation, including peak height velocity, restricts a more in-depth analysis of developmental risk factors.

Future Research

Improving the methodological consistency of epidemiological research in youth futsal should be a priority. Standardized injury definitions and exposure metrics are essential to enhance cross-study comparability. Consensus-based approaches combining time-loss or medical attention criteria with exposure expressed per 1,000 hours would improve the consistency and interpretability of findings.

Well-designed prospective studies with larger, more representative samples are also needed, encompassing different competitive levels, age groups, and both sexes. This would enable more robust estimates of injury incidence and a deeper understanding of injury patterns across developmental stages.

Incorporating detailed assessments of biological maturation, such as peak height velocity, is crucial to clarify the role of growth-related factors in injury risk. Integrating maturation status with workload monitoring may provide valuable insights into the interaction between developmental and training-related determinants.

Finally, greater emphasis should be placed on evaluating targeted injury prevention strategies, particularly those addressing neuromuscular training, load management, and sex-specific adaptations. Assessing the implementation and effectiveness of these interventions in real-world training environments is essential to translate epidemiological findings into practical applications.

Conclusion

According to this systematic review, there is a significant risk of injury when playing youth futsal, characterized by elevated incidence rates reaching up to 292.42 injuries per 1,000 match hours and a well-defined epidemiological profile. Injuries predominantly affect the lower limbs (70–88%), particularly the ankle and knee, reflecting the sport's biomechanical demands: frequent high-intensity actions, rapid directional changes, and repeated mechanical loading.

Beyond descriptive patterns, injury occurrence is shaped by a multifactorial interaction between sport-specific demands, biological development, and contextual factors. Female sex, playing position, match-related fatigue, and preseason periods were consistently identified as relevant contributors, highlighting the importance of considering both intrinsic and extrinsic determinants when interpreting injury mechanisms in this population.

Methodological heterogeneity across studies, particularly regarding injury definitions and exposure metrics, limits direct comparisons and reinforces the need for standardized reporting frameworks. This remains a critical barrier to advancing the epidemiological understanding of youth futsal injuries.

From a practical standpoint, these findings support targeted prevention strategies encompassing neuromuscular training, progressive load management, and context-specific interventions adapted to sex, maturation status, and positional demands. Particular attention should be directed toward preseason and late-match phases, where injury risk appears consistently elevated.

In conclusion, youth futsal must be regarded as a high injury-risk sport. Reducing the burden of injuries and encouraging safe and sustainable involvement may be facilitated by ongoing advancements in methodological quality and the

application of evidence-based preventative techniques. Future research should prioritize methodological standardization to enable robust epidemiological comparisons and support the development of effective injury prevention frameworks.

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Can computer vision “see” the rider’s and surface’s effect on the horse’s symmetry?

Using SLEIP AI™ to monitor horses’ locomotor asymmetry

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Summary

Background: Objective assessment of equine locomotor asymmetry is increasingly important for improving lameness detection and monitoring horse welfare. Markerless computer vision systems may provide a practical alternative to traditional gait assessment, although the effects of rider experience and surface type remain unclear.

Objective: To investigate whether a markerless computer vision system (SLEIP AI™) can detect the effects of rider and surface type on locomotor asymmetry and to quantify between-horse variability.

Methods: Four horses were evaluated under six conditions combining rider presence (no rider, novice and experienced rider) and footing type (hard and soft surfaces). Locomotor asymmetry was measured using a smartphone-based system. Linear mixed-effects models assessed the effects of rider and footing, with horse included as a random effect. Intraclass correlation coefficients (ICC) and Fleiss' kappa were used to evaluate variability and asymmetry consistency.

Results: Footing had no significant effect on asymmetry. Rider significantly affected forelimb impact asymmetry ($p = 0.030$), with greater asymmetry under the novice rider. Between-horse variability explained 36–37% of forelimb asymmetry but only 3–8% of hindlimb asymmetry. Agreement in asymmetry sidedness was moderate overall.

Conclusions: Rider experience influenced locomotor asymmetry more than surface type. Markerless computer vision appears to be a practical tool for objective gait assessment, lameness evaluation and training monitoring.

Keywords: horse locomotion; asymmetry; rider; surface; forelimbs; hindlimbs; push-off; impact; computer vision.

Resumo

Introdução: A avaliação objetiva da assimetria locomotora em equinos tem assumido uma importância crescente na melhoria do diagnóstico de claudicações e na monitorização do bem-estar animal. Os sistemas de visão por computador sem marcadores constituem uma alternativa promissora aos métodos tradicionais de avaliação da marcha, embora a influência da experiência do cavaleiro e do tipo de piso nas medições permaneça insuficientemente esclarecida.

Objetivo: Investigar se um sistema de visão por computador sem marcadores (SLEIP AI™) é capaz de detetar os efeitos do cavaleiro e do tipo de piso na assimetria locomotora de cavalos, bem como quantificar a variabilidade entre indivíduos.

Métodos: Foram avaliados quatro cavalos em seis condições resultantes da combinação da presença do cavaleiro (sem cavaleiro, cavaleiro inexperiente e cavaleiro experiente) e do tipo de piso (superfície dura e superfície macia). A assimetria locomotora foi quantificada através de um sistema baseado em smartphone. Foram ajustados modelos lineares mistos para avaliar os efeitos do cavaleiro e do tipo de piso, considerando o cavalo como efeito aleatório. A variabilidade entre indivíduos foi avaliada através do coeficiente de correlação intraclasse (ICC) e a consistência da lateralidade da assimetria pelo coeficiente kappa de Fleiss.

Resultados: O tipo de piso não teve efeito significativo em nenhuma das medidas de assimetria. O cavaleiro influenciou significativamente a assimetria de impacto dos membros anteriores ($p = 0,030$), verificando-se maior assimetria com o cavaleiro

inexperiente. A variabilidade entre cavalos explicou 36–37% da assimetria dos membros anteriores e apenas 3–8% da dos membros posteriores. A concordância da lateralidade da assimetria foi moderada.

Conclusões: A experiência do cavaleiro influenciou mais a assimetria locomotora do que o tipo de piso. A visão por computador sem marcadores revelou-se uma ferramenta prática para a avaliação objetiva da marcha, podendo constituir um apoio útil no diagnóstico de claudicações e na monitorização do treino.

Palavras-chave: locomoção equina; assimetria; cavaleiro; tipo de piso; membros anteriores; membros posteriores; propulsão; impacto; visão por computador.

Introduction

Lameness in horses has posed challenges to riders, trainers and veterinarians for a very long time. In recent years, the generalized use of social media and growing awareness about animal welfare have raised scrutiny on equestrianism and controversial practices that can potentially inflict suffering to horses are decreasingly tolerated. To keep a social licence to operate, the equestrian industry is obliged to adopt the best practices and assure the horses' welfare.

Lameness examination includes visual observation of the horse trotted in hand, among a comprehensive set of procedures that can include imaging exams and nerve blocks (Baxter e Stashak, 2020), to reach a diagnostic. Lameness diagnostics is complex and time-consuming, and this has led to the development of several technological tools, designed to help recognising lameness and identifying the lame limb or limbs. Lately, applications using computer vision associated with machine learning became commercially available, and increasing sophistication of such systems will probably revolutionize not only lameness diagnostics but also training and rehabilitation monitoring.

Many factors can affect the manifestation of lameness, or locomotor irregularity, including the type of surface where horses are being observed. Lameness and the phase of lameness manifestation can vary between track surfaces, and some horses show impact lameness on soft ground and elevation lameness on hard ground (Azevedo et al., 2015). Pfau et al. (2014) reported a significant effect of surface type (hard/soft) on forelimb-lame horses trotted on the lunge. When comparing ridden horses on three different surfaces, greatest movement asymmetry was recorded during trotting on the least firm (softer) surface (Barstow et al., 2019).

The rider and their individual features also play a part in the locomotor irregularities of horses. The rider's weight has implications in the biomechanics of the thoracolumbar spine (Clayton et al., 2023), causing its extension (hence, the hollowing of the back). Even though an increase of 15% to 25% in the rider's weight had no effect on horses behavioural and physiological parameters (Christensen et al., 2020), there is evidence that if the rider's weight exceeds 15% to 25% of the horse's weight, the rider can induce temporary lameness and behavioural signs of musculoskeletal pain in the horse (Dyson et al., 2019).

Another factor that can interfere with the horse's locomotor regularity is the rider's experience. An experienced rider manages their weight distribution and body movements during predictable gait patterns and reacts quickly to unexpected responses from the horse. To achieve this, the rider's pelvis moves in coordination with the horse's back and the saddle, while the trunk stabilizes the upper body over the pelvis. Both innate and learned asymmetries are common in riders and horses and tend to persist across different gaits (Clayton et al., 2023).

The aim of this study was to investigate whether a smartphone-based computer vision system can detect the effects of rider and surface on locomotor asymmetry at trot and quantify the contribution of interindividual variability. In the future, it is expected that knowledge of these factors will increase exponentially, contributing to a better understanding of horse-rider interaction, ultimately benefiting equine welfare.

Materials and Methods

Four horses, used daily for riding lessons with elementary level riders, were selected for this study. A general characterization of each horse is presented in table 1. Horses were deemed sound or mildly lame before the study. All horses were shod with conventional steel shoes, except for horse 2, which was unshod in the hindlimbs.

Table 1. Age, height and estimated weight of horses

Horse	Age (yrs)	Height (cm)	Estimated weight (kg)
1	14	172	565
2	14	155	522
3	14	167	553
4	9	150	420

The horses' locomotor asymmetries were assessed using a markerless, smartphone-based, computer vision technology that tracks over 100 anatomical key points to quantify equine gait asymmetry and detect lameness, SLEIP AI™ (Lawin et al., 2023; SLEIP AI AB., 2024). Video recordings were acquired

using an iPad™ 13 Pro. Each horse was trotted back and forth on a 30 m track on a hard (crushed sand) and a soft (silica + textile fibres) surface, led in-hand (no rider), ridden by an elementary level male rider (rider 1) and an experienced male rider (rider 2). Overall, each horse was trotted 6 times. The riders' age, height and weight are presented in table 2.

Table 2. Age, height and weight of riders

Rider	Age (yrs)	Height (cm)	Weight (kg)
1	20	185	71.5
2	56	182	90

In-hand trot-ups were performed at a comfortable speed, consistent with the conditions used in lameness examinations. In ridden trot-ups, riders were asked to exert minimal interference. Both riders spontaneously adopted posting trot, although no instructions on trotting rider position were given. To assess the presence of baseline lameness, a threshold of >0.5 on the absolute measures of asymmetry when horses were conducted in-hand at trot on a hard surface was considered as clinically relevant (Meistro et al., 2025).

Data resulting from the SLEIP AI™ analysis were retrieved. The numerical asymmetry values represent the mean of all valid strides recorded by the application, with an average of 30.58 strides for the forelimbs (range: 15-52) and 23.46 strides for the hindlimbs (range: 15-31). For forelimbs, a unitary value (1) represents the inter-diagonal difference in vertical head displacement, expressed as a proportion of the horse's withers height, corresponding to 2.5% of that height. For hindlimbs, a unitary value (1) represents the inter-diagonal difference in vertical pelvic displacement, also normalized to withers height, corresponding to 1.4% of that height (SLEIP AI AB., 2024).

To evaluate the effects of rider and footing on asymmetry magnitude (absolute value of asymmetry measurements), four linear mixed models were fitted, with Forelimb Push-Off, Forelimb Impact, Hindlimb Push-Off, and Hindlimb Impact as dependent variables. In each model, rider and footing (and their interaction) were included as fixed effects, and horse was included as a random intercept:

$$Y_{ij} = \beta_0 + \beta_1 \text{Rider}_{ij} + \beta_2 \text{Footing}_{ij} + \beta_3 (\text{Rider} \times \text{Footing})_{ij} + u_j + \varepsilon_{ij}$$

The contribution of the random effect of horse was assessed using the intraclass correlation coefficient (ICC), calculated from the estimated variance components.

Individual horse's asymmetry plots were built using Ms Excel graphs from Microsoft Office 325™. The asymmetry values were considered as negative when associated with the left limbs, and positive when associated with the right limbs. Consistency in sidedness of the affected limb was evaluated using Fleiss' kappa coefficient (κ), Kappa values were calculated for each condition (forelimb and hindlimb; push-off and impact phases) as well as across all conditions combined. The strength of agreement was interpreted according to standard benchmarks (values ≤0 indicating no agreement, 0.01-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial, and >0.80 almost perfect agreement). Approximate 95% confidence intervals (95% CI) for κ were computed using asymptotic standard errors. Statistical significance of agreement was assessed by testing the null hypothesis of κ = 0 using a z-test. All statistical analyses were conducted with significance set at $p < 0.05$. Statistical analysis used IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., 2020).

Results

Baseline of registered lameness

The absolute measures of asymmetry when horses were conducted in-hand at trot on a hard surface (i.e., the conditions which are usually considered for lameness inspection) are presented in table 3.

Table 3. Asymmetry values at in-hand trot in a hard surface. Negative values point to left limb; positive values point to right limb.

Horse	Forelimb Push-Off	Forelimb Impact	Hindlimb Push-Off	Hindlimb Impact
1	-0.5	-0.1	0.1	-0.3
2	0.3	0	0.8	0.5
3	0.1	0.1	0.9	-0.1
4	0.3	0.4	0.7	0.2

Thresholds for asymmetry severity defined for the SLEIP AI™ software (Meistro et al., 2025) are as follows: very mild (0.2-0.49), mild (0.5-0.9), moderate (1.0-1.49), and severe 1.5-2.0). According to this classification, horse 1 exhibited mild forelimb asymmetry, and the remaining horses exhibited mild hindlimb asymmetries.

Effect of the rider and the type of surface

Table 4 summarises the fixed effects tests in the four linear mixed models. No significant effects of rider, footing or their interaction were detected for forelimb push-off, hindlimb push-off or hindlimb impact asymmetry measures ($p>0.05$). For forelimb impact, a significant effect of rider was observed ($p=0.03$). Bonferroni-adjusted pairwise comparisons revealed a significant difference between the 'No Rider' and 'Rider 1' conditions, with lower values observed in the 'No Rider' condition (*M*diff = -0.200, SE = 0.067, $p=0.029$). No significant differences were found between 'No Rider' and 'Rider 2' ($p=0.251$) or between 'Rider 1' and 'Rider 2' ($p=0.852$).

Table 4. Type III tests of fixed effects (F.E.) in the four models

F.E - Forelimb Push-Off	df1	df2	F	p-value
• Rider	2	15	1.439	0.268
• Footing	1	15	0.154	0.700
• Rider x Footing	2	15	1.320	0.297
F.E. – Forelimb Impact	df1	df2	F	p-value
• Rider	2	15	4.482	0.030
• Footing	1	15	0.366	0.554
• Rider x Footing	2	15	0.640	0.541
F.E. – Hindlimb Push-Off	df1	df2	F	p-value
• Rider	2	15	0.159	0.854
• Footing	1	15	0.110	0.745
• Rider x Footing	2	15	0.478	0.629
F.E. – Hindlimb Impact	df1	df2	F	p-value
• Rider	2	15	0.016	0.984
• Footing	1	15	0.147	0.707
• Rider x Footing	2	15	2.106	0.156

The proportion of variance attributable to differences between horses varied across models (table 6). For forelimb variables, the horse accounted for a substantial proportion of the variance (Forelimb Push-Off: 36.1%; Forelimb Impact: 36.9%). In contrast, for hindlimb variables, the contribution of the horse was considerably smaller (Hindlimb Push-Off: 3.0%; Hindlimb Impact: 7.6%), indicating that most of the variability in these outcomes was explained at the observation level rather than between horses.

Table 5. Variance components and intraclass correlation coefficients (ICC) for each model

Stride phase	Horse (τ^2)	Residual (σ^2)	ICC (%)
Forelimb Push-Off	0.055	0.098	36.1
Forelimb Impact	0.011	0.018	36.9
Hindlimb Push-Off	0.004	0.136	3.0
Hindlimb Impact	0.002	0.026	7.6

The asymmetries registered in each individual horse were plotted to allow for visual interpretation of each individual horse.

Figure 1 presents the values obtained for horse 1. Both in-hand trot-ups point to a push-off asymmetry in the left forelimb. This asymmetry accentuates with both riders, as well as a right hindlimb push-off asymmetry, more pronounced over soft footing.

Horse 2 (figure 2) presents a consistent right hindlimb push-off asymmetry, more apparent in the hard track surface and combined with a very mild right forelimb push-off asymmetry. However, and depending on the rider and track, this horse also presents inconsistent forelimb and hindlimb impact asymmetries in both left and right limbs.

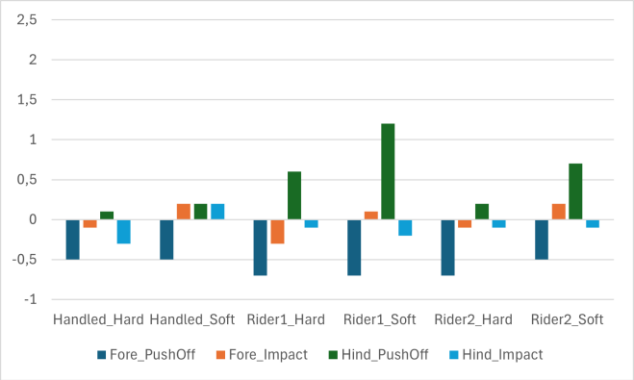

Figure 1. Registered asymmetries for horse 1

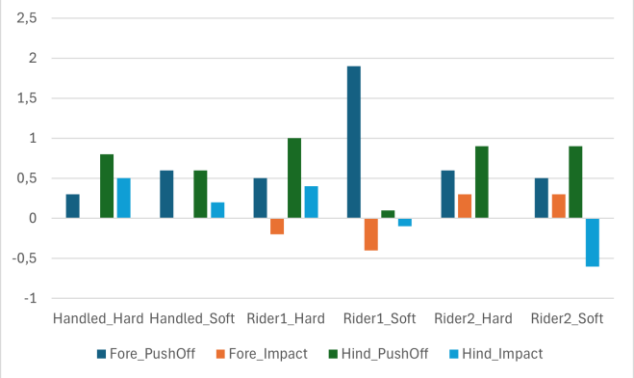

Figure 2. Registered asymmetries for horse 2

Horse 3 (figure 3) presents a right hindlimb push-off asymmetry at in-hand trot, coupled with a right forelimb impact asymmetry on hard footing. The magnitude of the hindlimb asymmetry decreases under both riders. Inconsistent forelimb and hindlimb impact asymmetries appear under rider in the right limbs.

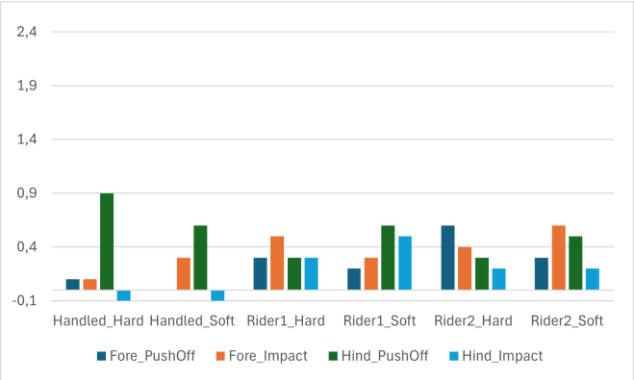

Figure 3. Registered asymmetries for horse 3

Horse 4 (figure 4) presents a push-off right hindlimb asymmetry in in-hand trot, coupled with a right forelimb impact asymmetry. With rider 1, the right hindlimb push-off asymmetry disappears, and the right forelimb impact asymmetry accentuates and with rider 2, both asymmetries

decrease when compared to handled trot. No substantial differences are observed between hard and soft footing.

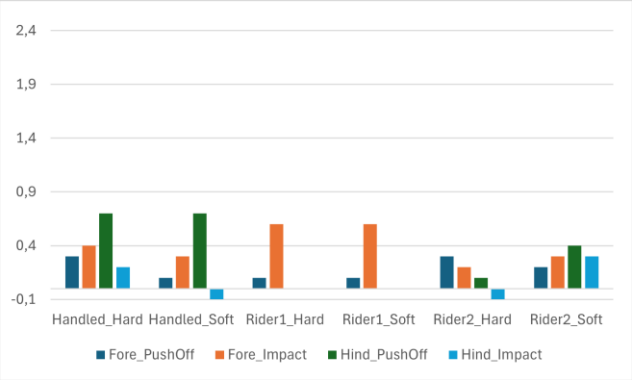

Figure 4. Registered asymmetries for horse 4

Consistency in asymmetry sidedness

For consistency analysis, asymmetry values up to 0.1 (absolute value) were considered negligible (indicating absence of asymmetry). Data are shown in table 6 and Fleiss k values, confidence intervals and p-values in table 7.

Table 6. Observations in asymmetry sidedness

Stride Phase	Horse	Left	None	Right
Forelimb Push-Off	Horse 1	6	0	0
	Horse 2	0	0	6
	Horse 3	0	2	4
	Horse 4	0	3	3
Forelimb Impact	Horse 1	1	3	2
	Horse 2	2	2	2
	Horse 3	0	1	5
	Horse 4	0	0	6
Hindlimb Push-Off	Horse 1	0	1	5
	Horse 2	0	1	5
	Horse 3	0	0	6
	Horse 4	0	3	3
Hindlimb Impact	Horse 1	2	3	1
	Horse 2	1	2	3
	Horse 3	0	2	4
	Horse 4	0	4	2

Table 7. Fleiss k values, confidence intervals and p-values

Stride phase	Kappa	CI 95%	p-value
All	0.41	0.28-0.54	<0.0001
Forelimb Push-Off	0.53	0.30-0.76	0.0002
Forelimb Impact	0.28	0.05-0.51	0.028
Hindlimb Push-Off	0.49	0.26-0.72	0.0004
Hindlimb Impact	0.22	0.00-0.44	0.058

The results show a moderate overall agreement between observations. Consistency in asymmetry sidedness can be interpreted as moderate in forelimb and hindlimb push-off phases, and as fair in forelimb and hindlimb impact phases. K value for hindlimb impact phase was borderline non-significant (p=0.058).

Discussion

Baseline lameness assessment

The horses included in this study were previously deemed as sound or mildly lame by riders (equestrian pupils and instructors). The registered measures point to mild asymmetries in all horses, with potential clinical relevance of hindlimb asymmetries in horses 2, 3 and 4. This points to the need of a more comprehensive examination, to assess the eventual presence of injury that could compromise the horses' welfare on their daily ridden routines. Even though asymmetry is not always associated with pain (Marunova et al. 2025), it has been established that subjective assessment of equine lameness by owners, and even by veterinarians, can be unreliable and contrast with objectively assessed asymmetry levels (Greve & Dyson, 2014; Müller-Quirin et al., 2020). Additionally, establishing the presence of lameness based solely on straight line, in-hand trot-ups may prove insufficient, and lungeing and ridden work observation can contribute with valuable information for a complete assessment (Dyson, 2014).

Effects of the footing and the rider

Surface properties influence hoof-ground interactions and impact loading. Hard surfaces increase impact forces and vibration, whereas more compliant surfaces reduce these effects, partly by prolonging the braking phase following impact (Parkes & Witte, 2015). In this study, the hardness of the footing's effect on registered asymmetries was non-significant. This agrees with previous studies that assessed the effect of surface type on push-off and impact asymmetries in trotting horses (Azevedo et al., 2015; Marunova et al., 2024).

One of this study's aims was to investigate the relevance of rider's weight on the horses' locomotor asymmetries. Hence, the two male riders were selected based on their biometrics, as both had approximately the same height, but rider 1 had a substantially lower body weight than rider 2. Paradoxically, the only significant effect detected in the study was that of rider 1, who aggravated forelimb impact asymmetry. Rider 2's body weight exceeded rider 1's in 20.6%, falling within the range previously determined to produce no significant effects (Christensen et al., 2020). The rider/horse bodyweight ratios in this study varied between 12.39% (rider 1/horse 1) and 21.43% (rider 2/horse 4), under the maximum acceptable value of 25% from which the rider can induce temporary lameness and behavioural signs of musculoskeletal pain in the horse (Dyson et al., 2019). Roost et al. (2020) reported differences in magnitude and distribution of pressures among riders according to their bodyweight, which may have contributed to the development of musculoskeletal pain, but acknowledged that the differences might also have been

influenced by saddle fit and riders' positions. We can speculate that the results in this study can be due to the other factor that distinguishes both riders, i.e., experience. Rider 1 is an elementary level, 20-year-old student with under 5 years of riding experience, while rider 2 is an advanced level riding instructor, with over 40 years of riding experience. Rider 2's experience may have contributed to a better balance and greater ability of the rider to adjust to the horses' motion, hence having a lower effect on gait asymmetry, as can be inferred from the observation of plots in figures 1 to 4. A previous study reported that horses showed significant differences, namely in hindlimb lameness, when ridden, even though the influence of the rider could not be predicted for an individual horse and recommended that the level of training of the rider should be taken into consideration (Licka et al., 2010).

Another factor to consider is the fact that horses were ridden in posting trot. Lameness can be altered by the diagonal on which the rider sits; usually in horses with hindlimb lameness it becomes more apparent when the rider sits on the diagonal of the lame(r) limb. Forelimb lameness may also be affected, but in a less predictable way (Dyson, 2014). During posting trot, maximum croup height becomes asymmetrical, likely because of rider-induced downward forces when rising from the saddle. This can mimic hindlimb push-off lameness and results in increased asymmetry of back movement compared with in-hand trotting (Clayton et al., 2023). Visualizing the videos recorded with SLEIP AI™ allowed noticing that rider 1 sat consistently on the right forelimb/left hind diagonal (probably due to being less experienced), while rider 2 systematically changed diagonals. This may help to explain why with horse 4, rider 1 apparently caused the "disappearance" of right hindlimb asymmetry (as he was consistently exerting downward forces in this limb's diagonal, when rising, hence masking the asymmetry). According to Persson-Sjodin et al. (2018), a push-off hindlimb lameness is expected to increase when the rider sits during the lame hindlimb stance, whereas an impact hindlimb lameness is expected to decrease.

Compensatory lameness must also be considered when interpreting the results, as Reed et al. (2020) reported that 56.6% of horses presented for lameness evaluation showed combined head and pelvis asymmetries, which in most cases were attributed to compensatory asymmetries. For instance, horse 1 presented a forelimb push-off asymmetry, and an accentuated right hindlimb push-off asymmetry when ridden. Contralateral hindlimb push-off asymmetry is the most common compensatory pattern associated with forelimb lameness – the first law of sides (Reed et al., 2020), and the riders' weight probably accentuated this pattern in horse 1. The second law of sides can probably help explaining the pattern shown by horse 3, where the right hindlimb push-off asymmetry coupled with an ipsilateral forelimb impact (compensatory) asymmetry, accentuated by the riders' weight. Horse 2 is the case where the asymmetry pattern is harder to explain, posing the hypothesis of multi-limb lameness, with a possible influence of hindlimbs being unshod in discrepancies between riders and surfaces. Panos et al. (2023) reported a positive effect on lameness scores and hock angles of shoeing the hindlimbs of previously barefoot horses.

The fact that the study lacked thorough lameness diagnostics limits the ability to explain some of the observed patterns. Nevertheless, inter-individual differences between horses accounted for a substantial proportion of the variance, impacting over 36% of the variance of forelimb asymmetries, although only between 3 and 8% of hindlimb asymmetries. The reasons for a higher inter-individual variance in forelimb asymmetry may involve the fact that head movements are more susceptible to external influences and distractions than those of the pelvis, and that head oscillation pattern is more dependent on active muscular contractions at trot (Clayton et al., 2025).

Consistency in sidedness of asymmetry

In this study, there was a moderate overall agreement between observations, regarding left and right sided asymmetries. Push-off asymmetries exhibited a higher consistency than impact asymmetries. Possible explanations may involve the impact of riders' weight in the weight-bearing phase of the stance associated with sitting or rising trotting position, as well as firmness or compliance of the footing and the effect of compensatory lameness. However, it has been reported that horses regularly switch between small left- or right-sided movement symmetries, although they less frequently switch between more severe left- and right-sided movement symmetries (Pfau et al., 2025).

Limitations of this study include the lack of significance of some of the analysed factors which likely reflects the insufficient statistical power resulting from the reduced number of horses involved and precludes a generalization of conclusions without further confirmation. Additionally, the fact that riders were not instructed to ride in sitting trot adds confusion to an already complex interpretation, and lack of definitive lameness diagnostics prevents a more consistent analyses of primary and compensatory lameness. The findings should therefore be interpreted with caution. The software that was used (SLEIP AI v. 2.8.1) has subsequently been updated, and now includes a feature that uses the motion of the head and the withers to differentiate between primary and compensatory asymmetries (for instance, pointing to primary forelimb issue if the withers moves in the same direction as the head, or compensatory if they move in opposite directions). This feature could have helped in the interpretation of the results in this study and should be used in future attempts.

Nevertheless, the study points to the negligible effect of trotting surface in asymmetry, consistent with previous studies, and the more noticeable effect of the rider, also in agreement with previous reports. Interindividual factors affect forelimb asymmetry more than hindlimb asymmetry. Markerless computer vision technology allows for non-invasive, time-

efficient data retrieval of locomotor asymmetry in horses, and machine learning algorithms will likely revert in growing accuracy of these systems, making them a valuable resource in veterinary lameness diagnostics, injury rehabilitation and training monitoring.

Ethical statement: this study involved no procedures on animals, according to the definition stated in the Decree of Law nr. 113/2013 of August 7th.

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20 Anos de Ensino de Enfermagem Veterinária em Portugal: *Evolução, Especialização e Perspetivas Futuras*

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Introdução

Nas últimas duas décadas, a Medicina Veterinária tem assistido a profundas transformações científicas, tecnológicas e organizacionais, acompanhando a crescente valorização do bem-estar animal, o desenvolvimento dos cuidados médico-veterinários e a diversificação das áreas de intervenção clínica. Neste contexto, a necessidade de profissionais qualificados capazes de assegurar cuidados técnicos especializados e de apoiar de forma diferenciada a prática clínica veterinária tornou-se progressivamente mais evidente (Royal College Veterinary Surgeon, [RCVS],s.d.). Foi neste cenário de mudança que emergiu, em Portugal, o ensino da Enfermagem Veterinária, constituindo um importante marco na profissionalização e qualificação das equipas de saúde animal.

Antes da criação dos primeiros cursos superiores especializados, muitas das funções atualmente desempenhadas pelos enfermeiros veterinários eram asseguradas de forma empírica e sem enquadramento formativo estruturado, baseando-se sobretudo na aprendizagem em contexto de trabalho (RCVS, s.d.). Contudo, a crescente complexidade dos procedimentos clínicos, o avanço das técnicas de diagnóstico e terapêutica, bem como a necessidade de assegurar elevados padrões de qualidade e segurança nos cuidados prestados aos animais, impulsionaram a criação de programas de formação específicos nesta área (European Association of Establishments for Veterinary Education [EAEVE], s.d.).

Paralelamente, a crescente sofisticação da Medicina Veterinária conduziu ao aparecimento de novas áreas de intervenção e especialização, incluindo anestesia, emergência, cuidados intensivos, reabilitação, imagiologia e medicina de animais exóticos. Esta evolução reforçou a importância da formação contínua e da especialização profissional, colocando novos desafios ao ensino superior e ao reconhecimento da profissão no panorama nacional e europeu (EAEVE, s.d.).

Atualmente, os enfermeiros veterinários desempenham um papel essencial na promoção da saúde e bem-estar animal (Figura 1). Simultaneamente, persistem desafios relacionados com a harmonização curricular, o reconhecimento profissional, a regulamentação da profissão e a adaptação às novas exigências científicas e tecnológicas (Matos, 2022).

Figura 1. Funções definidas na equipa veterinária de um Centro de atendimento veterinário.

A evolução da enfermagem veterinária tem sido particularmente evidente em países com sistemas regulatórios consolidados, onde existe reconhecimento formal da profissão, definição clara de competências e exigência de formação acreditada, como no Reino Unido (RCVS, 2020; FVE, 2023). Em contraste, em países como Portugal, o desenvolvimento tem ocorrido de forma mais gradual, refletindo a ausência de regulamentação específica e a diversidade de percursos formativos (FVE, 2023).

Assim, o presente artigo pretende analisar a evolução do ensino da Enfermagem Veterinária em Portugal ao longo dos últimos 20 anos, abordando o surgimento e desenvolvimento dos cursos, a evolução das competências profissionais, a necessidade crescente de especialização, o enquadramento europeu e internacional, bem como os desafios e perspetivas futuras para o ensino e para a profissão.

Evolução do Ensino da Enfermagem Veterinária em Portugal

O ensino de Enfermagem Veterinária em Portugal surgiu como resposta à crescente necessidade de profissionais qualificados para apoiar a prática médico-veterinária, num contexto marcado pela modernização das clínicas e hospitais veterinários, pela valorização do bem-estar animal e pela crescente sofisticação dos cuidados prestados aos animais de companhia e de produção. Até ao início dos anos 2000, muitas das funções atualmente atribuídas aos enfermeiros veterinários eram desempenhadas de forma informal, através da aprendizagem prática em contexto laboral, sem formação superior específica ou enquadramento académico estruturado.

A criação dos primeiros cursos de Enfermagem Veterinária constituiu, assim, um marco importante na profissionalização das equipas veterinárias em Portugal. Entre as instituições pioneiras destacou-se a atual Instituto Politécnico de Portalegre, através da Escola Superior de Biociências de Elvas, considerada uma das primeiras escolas portuguesas a oferecer formação superior nesta área, inicialmente sob a forma de bacharelato em 2003, posteriormente adaptado ao modelo de licenciatura no âmbito do Processo de Bolonha (IPP, s.d.; DGES, 2026).

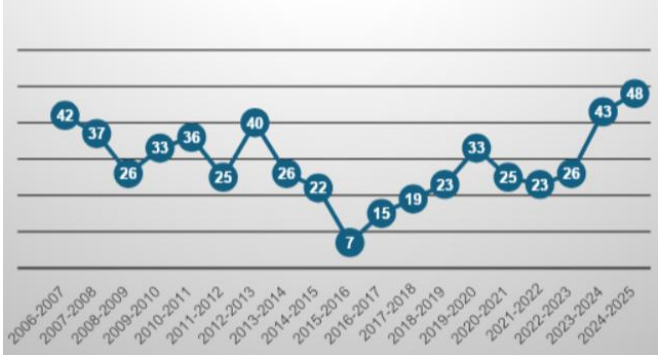

Figura 2. Evolução do número de diplomados em Enfermagem Veterinária na Escola Superior de Biociências de Elvas desde o curso Académico 2006/2007

Ao longo das últimas duas décadas, a oferta formativa expandiu-se progressivamente para outras instituições do ensino superior politécnico, refletindo o aumento da procura estudantil e das necessidades do setor veterinário. Atualmente, várias escolas superiores públicas e privadas disponibilizam licenciaturas em Enfermagem Veterinária (Tabela 1).

Tabela 1. Instituições de ensino superior em Portugal que oferecem o curso de Enfermagem Veterinária no ano letivo 2025/2026.

Tipo de instituição	Ano de inicio	Escola	Entidade
Públicas	2003	Escola Superior de Biociências de Elvas	Instituto Politécnico de Portalegre
	2004	Escola Superior Agrária de Viseu	Instituto Politécnico de Viseu
	2005	Escola Superior Agrária de Bragança	Instituto Politécnico de Bragança
	2006	Escola Superior Agrária de Ponte de Lima	Instituto Politécnico de Viana do Castelo
	2008	Escola Superior Agrária de Castelo Branco	Instituto Politécnico de Castelo Branco
	2020	Escola Superior Agrária de Coimbra	Instituto Politécnico de Coimbra
Privadas	2018	Escola Superior de Tecnologias da Saúde do Tâmega e Sousa	CESPU – Cooperativa de Ensino Superior Politécnico e Universitário
	2020	IPLUSO - Instituto Politécnico da Lusofonia	SESC – Sociedade de Estudos Superiores e Culturais, S.A.
	2021	Escola Superior de Saúde Egas Moniz	Egas Moniz School of Health & Science

A consolidação destes cursos acompanhou a evolução da própria Medicina Veterinária, marcada pelo desenvolvimento tecnológico, pela diferenciação clínica e pela necessidade crescente de equipas multidisciplinares altamente qualificadas. Os planos curriculares passaram a integrar componentes científicas, laboratoriais e clínicas mais avançadas, incluindo áreas como anestesia, imagiologia, cuidados intensivos, comportamento animal e enfermagem médico-cirúrgica. Paralelamente, reforçou-se a importância do ensino prático, dos estágios curriculares e da ligação entre as instituições de ensino e os hospitais veterinários (Bok et al,2013).

Neste contexto, a Escola Superior de Biociências de Elvas assumiu um papel particularmente relevante no desenvolvimento da Enfermagem Veterinária em Portugal, não apenas pela sua posição pioneira, mas também pelo crescimento sustentado do número de diplomados ao longo dos anos. A evolução destes números evidencia a consolidação da área e o aumento do interesse pela profissão, refletindo igualmente a crescente integração dos enfermeiros veterinários no mercado de trabalho nacional (IPP, s.d.).

A análise da tendência de diplomados demonstra uma trajetória globalmente crescente, acompanhando a expansão do setor veterinário e a valorização progressiva destes profissionais nas equipas clínicas. Este crescimento poderá igualmente estar relacionado com a diversificação das saídas profissionais, a melhoria das infraestruturas hospitalares veterinárias e o aumento da procura de cuidados especializados para animais de companhia (Dunne, 2020, Bok, 2013).

Evolução do Perfil Formativo e das Competências Profissionais

A evolução do ensino da Enfermagem Veterinária em Portugal acompanhou as profundas transformações verificadas na Medicina Veterinária contemporânea. Numa fase inicial, os cursos de Enfermagem Veterinária centravam-se sobretudo na aquisição de competências práticas de apoio à atividade clínica, incluindo contenção animal, assistência em consulta, cuidados básicos de internamento, higiene hospitalar e apoio cirúrgico. Contudo, a rápida modernização dos hospitais e

clínicas veterinárias, associada ao desenvolvimento tecnológico e ao aumento da exigência dos cuidados prestados, conduziu à necessidade de formação mais abrangente e especializada.

Segundo Bok et al. (2013), os modelos contemporâneos de educação veterinária têm vindo a privilegiar abordagens pedagógicas centradas na aprendizagem em contexto clínico e no desenvolvimento de competências profissionais integradas, combinando conhecimento científico, capacidade técnica, pensamento crítico e competências de comunicação. A evolução curricular acompanha tendências internacionais de ensino baseado em competências, procurando preparar profissionais capazes de atuar em contextos clínicos complexos e multidisciplinares (ACOVENE, 2021). Esta abordagem tem igualmente influenciado os cursos portugueses de Enfermagem Veterinária, onde o ensino prático, os estágios curriculares e a simulação clínica assumem um papel cada vez mais relevante na formação dos estudantes.

Paralelamente, o perfil do enfermeiro veterinário evoluiu significativamente ao longo das últimas duas décadas. Para além das competências técnicas tradicionais, estes profissionais desempenham atualmente funções de elevada responsabilidade no acompanhamento anestésico, monitorização de pacientes críticos, gestão de internamento, colheita e processamento de amostras laboratoriais, apoio em exames complementares de diagnóstico e educação dos tutores para os cuidados de saúde animal. Em muitos contextos clínicos, assumem ainda um importante papel na coordenação das equipas de enfermagem e na gestão operacional dos serviços veterinários (Ferrinho R et al., 2025).

Dunne et al. (2020) destacam que a perceção das competências profissionais na Enfermagem Veterinária está fortemente associada à capacidade de integração entre conhecimento teórico e experiência prática, reforçando a importância de modelos formativos orientados para a prática clínica supervisionada. Esta perspetiva tem sido progressivamente incorporada nas instituições portuguesas, que têm vindo a reforçar protocolos com hospitais e clínicas veterinárias para proporcionar experiências clínicas diversificadas aos estudantes (Cooper, 2011, Orpet, 2011).

Além das competências técnicas e científicas, a evolução curricular tem igualmente valorizado competências transversais, nomeadamente comunicação interpessoal, ética profissional, trabalho em equipa multidisciplinar e gestão emocional. Estas competências tornaram-se particularmente relevantes perante o aumento da complexidade das relações entre profissionais veterinários e tutores, bem como face aos desafios emocionais frequentemente associados à prática clínica veterinária (Orpet 2011).

No contexto europeu, organismos como a *Accreditation Committee for Veterinary Nurse Education* têm contribuído para a harmonização das competências esperadas dos enfermeiros veterinários, promovendo referenciais comuns de formação e qualidade pedagógica. Esta harmonização tem incentivado as instituições portuguesas a aproximarem os seus modelos curriculares dos padrões internacionais, facilitando igualmente a mobilidade académica e profissional no espaço europeu (Lane & Bogue,2018).

Assim, a evolução do perfil formativo da Enfermagem Veterinária em Portugal reflete não apenas o crescimento académico da área, mas também a crescente valorização destes profissionais no contexto da saúde animal, acompanhando as exigências de uma Medicina Veterinária cada vez mais tecnológica, diferenciada e especializada (Lane & Bogue,2018).

Necessidade Crescente de Especialização

O desenvolvimento tecnológico, a modernização dos hospitais veterinários e a expansão das áreas clínicas de intervenção contribuíram para a transformação do papel do enfermeiro veterinário, que passou progressivamente de uma função predominantemente assistencial para uma atividade altamente diferenciada e tecnicamente especializada (Ancheta et al., 2024; Piccione et al., 2025).

A necessidade de especialização reflete igualmente a transformação dos próprios modelos hospitalares veterinários. Muitos hospitais e centros de referência passaram a organizar-se segundo modelos semelhantes aos da medicina humana, integrando equipas multidisciplinares e serviços clínicos diferenciados. Neste contexto, os enfermeiros veterinários passaram a assumir funções específicas em determinadas áreas clínicas, contribuindo para uma maior eficiência dos serviços e para a melhoria da qualidade dos cuidados prestados (Ancheta et al., 2024; Piccione et al., 2025).

Segundo Cooper et al. (2011), a crescente especialização da Enfermagem Veterinária constitui uma consequência natural da evolução científica e tecnológica da Medicina Veterinária, sendo essencial para garantir padrões elevados de qualidade clínica e segurança dos pacientes. Paralelamente, o desenvolvimento da especialização tem impulsionado a necessidade de formação pós-graduada e de aprendizagem contínua. Em diversos países europeus, nomeadamente no Reino Unido, existem sistemas de certificação profissional e programas de especialização reconhecidos em áreas específicas da Enfermagem Veterinária. Esta realidade tem vindo a influenciar progressivamente o contexto português, onde se observa uma crescente oferta de cursos avançados, pós-graduações e programas de mestrado dirigidos à especialização profissional.

A criação de programas de formação avançada, como o Mestrado em Enfermagem Veterinária em Animais de Companhia desenvolvido por vários institutos politécnicos portugueses, representa um importante passo na consolidação científica e académica da profissão em Portugal. Estes programas procuram responder às exigências de uma prática clínica cada vez mais diferenciada, promovendo competências avançadas em áreas técnicas, científicas e de investigação (Ferrinho R, et al. 2025).

Além das competências clínicas especializadas, a evolução da profissão exige igualmente competências transversais relacionadas com comunicação, gestão emocional, trabalho em equipa e tomada de decisão ética. O contacto permanente com situações críticas, sofrimento animal e comunicação com tutores em contextos emocionalmente exigentes reforça a importância do desenvolvimento de competências interpessoais e de estratégias de bem-estar profissional.

Dunne et al. (2020) salientam que a especialização e a formação contínua são fatores determinantes para o fortalecimento da identidade profissional dos enfermeiros veterinários e para a valorização da profissão no contexto das equipas de saúde animal. Os autores sublinham ainda que a crescente diferenciação das funções destes profissionais contribui para uma maior autonomia técnica e reconhecimento profissional.

No contexto europeu, organismos como a *Accreditation Committee for Veterinary Nurse Education* têm promovido referenciais comuns de competências avançadas e incentivado a harmonização da formação especializada em Enfermagem Veterinária. Esta harmonização poderá desempenhar um papel importante no futuro da profissão em Portugal, favorecendo a mobilidade profissional, a internacionalização e o reconhecimento académico dos programas formativos (ACOVENE, 2021).

A Enfermagem Veterinária na Prática Clínica

O enfermeiro veterinário desempenha hoje um papel central na prática clínica veterinária, refletindo a evolução da profissão ao longo das últimas duas décadas e a crescente complexidade dos cuidados de saúde animal (Dunne et al., 2020). Em Portugal, a consolidação da formação superior nesta área permitiu a integração progressiva destes profissionais nas equipas clínicas, onde assumem funções técnicas, assistenciais e organizacionais fundamentais para o funcionamento eficiente de clínicas e hospitais veterinários.

A monitorização anestésica constitui uma das áreas de maior relevância da intervenção destes profissionais. O enfermeiro veterinário assegura a preparação do paciente, a avaliação pré-anestésica, a vigilância contínua durante o procedimento cirúrgico e os cuidados pós-operatórios imediatos, garantindo a estabilidade fisiológica e o bem-estar do animal. Para além do bloco operatório, os enfermeiros veterinários assumem igualmente um papel essencial nos serviços de internamento e cuidados intensivos, onde a monitorização contínua de pacientes críticos é fundamental.

Outro domínio relevante é a educação dos tutores e a promoção da saúde animal. O enfermeiro veterinário desempenham um papel ativo na sensibilização dos proprietários para a prevenção de doenças, cumprimento de planos vacinais, desparasitação, nutrição adequada e bem-estar geral dos animais. Esta função educativa reforça a ligação entre a clínica veterinária e a comunidade, contribuindo para a melhoria global da saúde animal. Segundo Cooper et al. (2011), a integração eficaz dos enfermeiros veterinários nas equipas clínicas permite otimizar os recursos humanos e melhorar a qualidade dos cuidados prestados, promovendo uma abordagem mais estruturada e eficiente da prática veterinária.

Mais recentemente, o conceito de *One Health* – Uma Só Saúde tem vindo a reforçar o papel dos enfermeiros veterinários na interface entre saúde animal, saúde humana e saúde ambiental. Neste contexto, estes profissionais participam ativamente em programas de prevenção de doenças zoonóticas, vigilância sanitária e promoção do bem-estar animal, contribuindo para a saúde pública de forma integrada (Ferrinho et al, 2025). Estes autores referem que os enfermeiros veterinários assumem um papel crescente no contexto *One Health*, sendo reconhecidos como elementos fundamentais na implementação de estratégias de prevenção e controlo de doenças, bem como na promoção de práticas sustentáveis de saúde animal.

Deste modo, o papel dos enfermeiros veterinários na prática clínica e na saúde animal tem vindo a evoluir de forma consistente, passando de uma função predominantemente auxiliar para uma atuação altamente qualificada, técnica e indispensável no funcionamento das equipas veterinárias modernas.

Desafios Atuais e Perspetivas Futuras

A Enfermagem Veterinária em Portugal encontra-se numa fase de consolidação académica e profissional, mas ainda enfrenta desafios significativos que condicionam o seu pleno reconhecimento e desenvolvimento.

Um dos principais desafios prende-se com o reconhecimento e enquadramento profissional dos enfermeiros veterinários. Apesar da crescente importância destes profissionais nas equipas clínicas, a definição clara de competências, responsabilidades e estatuto profissional ainda apresenta variações entre países e, em alguns casos, dentro do próprio contexto nacional. Esta indefinição pode limitar a autonomia técnica e a valorização plena da profissão (Kogan, L. R., et al. (2020). NO mo modelo existente no Reino Unido, a enfermagem veterinária é uma profissão regulamentada e plenamente integrada no sistema veterinário. Neste país, o *Royal College of Veterinary Surgeons* é responsável pelo registo profissional obrigatório, definição de normas de formação e supervisão do exercício profissional (RCVS, 2020). Paralelamente, a *British Veterinary Nursing Association* desempenha um papel fundamental na representação dos profissionais, promoção da formação contínua e desenvolvimento da identidade profissional.

Outro desafio relevante é a necessidade de uniformização curricular entre instituições de ensino superior. Embora exista uma base comum de formação, persistem diferenças na abordagem pedagógica, na carga prática e na profundidade de algumas áreas clínicas. Esta heterogeneidade pode influenciar a preparação dos diplomados e a sua integração no mercado de trabalho (Ancheta et al., 2024).

A retenção de profissionais no setor veterinário constitui igualmente uma preocupação crescente. O elevado nível de exigência emocional, a carga horária intensa e a responsabilidade associada ao cuidado de animais em estado crítico podem contribuir para fenómenos de desgaste profissional e burnout. Segundo Kogan et al. (2020), o bem-estar das equipas veterinárias é um fator essencial para a qualidade dos cuidados prestados, sendo necessário investir em estratégias de suporte emocional e organizacional.

Adicionalmente, a rápida evolução tecnológica exige uma constante atualização de competências. O uso crescente de equipamentos avançados de diagnóstico, sistemas digitais de registo clínico e novas abordagens terapêuticas implica uma formação contínua e adaptativa por parte dos enfermeiros veterinários (Ancheta et al., 2024).

Conclusão

Ao longo das últimas duas décadas, o ensino da Enfermagem Veterinária em Portugal evoluiu de forma significativa, passando de uma formação emergente e ainda pouco estruturada para um ensino superior consolidado, integrado no sistema académico nacional e alinhado com tendências europeias. Este percurso reflete não apenas a expansão das instituições de ensino que passaram a oferecer esta formação, mas também a crescente valorização do papel do enfermeiro veterinário no contexto da prática clínica e da saúde animal.

A análise desenvolvida ao longo deste artigo evidencia que o surgimento e desenvolvimento dos cursos de Enfermagem Veterinária estiveram intimamente ligados à modernização da Medicina Veterinária, à necessidade de equipas clínicas mais diferenciadas e à crescente complexidade dos cuidados prestados aos animais.

A crescente necessidade de especialização constitui hoje um dos principais motores de desenvolvimento da profissão, refletindo a diversificação das áreas clínicas e a sofisticação dos serviços veterinários. Áreas como anestesia, cuidados intensivos, imagiologia e medicina de espécies exóticas demonstram a importância de uma formação contínua e avançada, capaz de responder às exigências de uma prática cada vez mais diferenciada.

No plano europeu, a Enfermagem Veterinária tem vindo a consolidar-se através de processos de harmonização curricular e definição de competências comuns, promovidos por organismos internacionais, reforçando a mobilidade académica e profissional. Este enquadramento tem contribuído para aproximar a realidade portuguesa dos referenciais europeus, embora persistam desafios relacionados com o reconhecimento profissional e a uniformização das competências.

Em perspetiva, o futuro da Enfermagem Veterinária em Portugal dependerá da capacidade de integração entre ensino, investigação e prática clínica, bem como da aposta na especialização, na internacionalização e na inovação pedagógica. A consolidação desta área como profissão autónoma, qualificada e cientificamente sustentada será determinante para responder aos desafios crescentes da Medicina Veterinária contemporânea e às exigências de uma sociedade cada vez mais consciente da importância do bem-estar animal.

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Comparison of Two Commercial Extenders for Refrigerated Stallion Semen: Effects on Sperm Quality over 48 h

Refrigerated Stallion Semen Extenders

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Summary

Artificial insemination in horses relies on effective semen preservation to maintain sperm function during storage and transport, particularly for refrigerated semen used in national and international shipment within 24 to 48 h. This study compared the effect of two commercial extenders, INRA 96® and Equiplus®, on the quality of refrigerated stallion semen. Semen from ten stallions of proven fertility was collected and diluted to a final concentration of 25 × 10⁶ spermatozoa/mL in each extender. Samples were evaluated at 0, 24, and 48 h for total motility (TM) and progressive motility (PM) using computer-assisted sperm analysis (CASA), and for functional parameters using flow cytometry, including viability, mitochondrial membrane potential (MMP) and acrosome status. Sperm motility decreased over time after dilution with both extenders; however, TM and PM were significantly higher in samples diluted with INRA 96® at 48 h ($p < 0.05$), with no significant differences at 0 or 24 h ($p > 0.05$). Sperm viability declined during storage but remained significantly higher in samples diluted with INRA 96® at 48 h ($p < 0.05$). No significant differences were observed in MMP between extenders at any time point ($p > 0.05$). Acrosome damage differed between extenders, being significantly higher in samples diluted with Equiplus® at 48 h ($p < 0.05$), with no significant changes over time within each extender ($p > 0.05$). These results suggest that INRA 96® may be associated with improved preservation of motility and viability, and reduced acrosome damage during refrigerated storage for up to 48 h.

Keywords: stallion semen, semen extender, INRA 96®, Equiplus®, refrigeration, sperm quality

Resumo

A inseminação artificial em equinos depende de uma preservação eficaz do sémen para manter a funcionalidade dos espermatozoides durante o armazenamento e o transporte, particularmente no caso do sémen refrigerado utilizado em transportes nacionais e internacionais num período de 24 a 48 horas. Este estudo comparou o efeito de dois diluentes comerciais, INRA 96® e Equiplus®, na qualidade do sémen refrigerado de garanhões. O sémen de dez garanhões de fertilidade comprovada foi colhido e diluído até uma concentração final de 25 × 10⁶ espermatozoides/mL em cada um dos diluentes. As amostras foram avaliadas às 0, 24 e 48 horas quanto à motilidade total (MT) e à motilidade progressiva (MP), recorrendo à análise computorizada do sémen (CASA), bem como quanto a parâmetros funcionais determinados por citometria de fluxo, incluindo viabilidade espermática, potencial da membrana mitocondrial (PMM) e integridade do acrossoma. A motilidade espermática diminuiu ao longo do tempo após a diluição com ambos os diluentes; no entanto, a MT e a MP foram significativamente superiores nas amostras diluídas com INRA 96® às 48 horas ($p < 0,05$), não se verificando diferenças significativas às 0 ou 24 horas ($p > 0,05$). A viabilidade espermática diminuiu durante o período de armazenamento, mas manteve-se significativamente superior nas amostras diluídas com INRA 96® às 48 horas ($p < 0,05$). Não foram observadas diferenças significativas no potencial da membrana mitocondrial entre os dois diluentes em nenhum dos momentos de avaliação ($p > 0,05$). Os danos no acrossoma diferiram entre os diluentes, sendo significativamente superiores nas amostras diluídas com Equiplus® às 48 horas ($p < 0,05$), sem alterações significativas ao longo do tempo dentro de cada diluente ($p > 0,05$). Estes resultados sugerem que o INRA 96® poderá proporcionar uma melhor

preservação da motilidade e da viabilidade espermática, bem como uma menor ocorrência de danos no acrossoma durante a conservação do sémen refrigerado por um período até 48 horas.

Palavras-chave: sémen de garanhão; diluente de sémen; INRA 96®; Equiplus®; refrigeração; qualidade espermática.

Introduction

Artificial insemination has become a cornerstone of modern equine reproduction, allowing genetic improvement, wider dissemination of desirable traits and improved biosecurity through reduced animal movement (Oliveira et al., 2019; Aurich, 2012). Therefore, the collection, evaluation and preservation of stallion semen have assumed greater importance in both clinical and commercial settings. However, the reproductive efficiency of a stallion is not always directly correlated with its athletic or genetic value, making semen handling and preservation strategies critical to ensure optimal fertility outcomes (Munroe & Weese, 2011).

Accurate assessment of semen quality relies on a combination of conventional and advanced techniques. Traditional parameters such as motility, concentration and morphology remain fundamental, but modern approaches including computer-assisted sperm analysis (CASA) and flow cytometry provide deeper insights into sperm function and fertilizing potential (Battut et al., 2016; Peña et al., 2016; Mortimer, 2000). These techniques enable the evaluation of key functional attributes such as mitochondrial activity, plasma membrane integrity and acrosomal status.

Following collection, semen must be appropriately processed and preserved to maintain sperm viability. Semen extenders play a central role in this process by protecting spermatozoa against thermal shock, providing energy substrates, stabilizing cell membranes and inhibiting microbial growth (Hernández-Avilés et al., 2020; Loomis & Graham, 2008). The effectiveness of semen preservation is influenced by several factors, including storage temperature, duration and, importantly, the composition of the extender used.

Refrigerated semen is widely used in equine practice due to its practicality and relatively good fertility outcomes when used within 24 to 48 hours after collection (Dascanio & McCue, 2021; Aurich, 2008). However, sperm survival during storage is highly variable and depends not only on the extender but also on individual stallion characteristics (Ferrer et al., 2020; Varner et al., 2015). Therefore, the selection of an appropriate extender is essential to maximize semen quality and fertility potential.

Among the commercially available extenders, INRA 96® and Equiplus® are commonly used in equine reproduction (Rečková et al., 2022; LeFrapper et al., 2010). Despite their widespread application, comparative data on their performance under practical refrigerated conditions remain limited.

The aim of this study was to compare the effects of two commercial extenders (INRA 96® and Equiplus®) on stallion semen quality during refrigerated storage at 5 ºC. Samples were evaluated at 0, 24 and 48 hours for total and progressive motility, as well as for functional sperm parameters assessed by flow cytometry, including viability, mitochondrial membrane potential and acrosome status.

Materials and Methods

Animals and Experimental Design

The study was conducted using ten stallions of proven fertility housed at the Animal Selection and Reproduction Center (CENSYRA, Badajoz, Spain). Animals ranged in age from 4 to 19 years and included several breeds (Purebred Spanish, Arabian, Anglo-Arabian, Spanish Sport Horse crossbred and Dutch Warmblood) during the 2022 breeding season. Each stallion provided three ejaculates collected on non-consecutive days to minimize the effect of collection frequency. The ejaculates were treated as independent samples.

Semen Collection and Processing

Ejaculates were obtained using a pre-warmed (45 ºC) and lubricated INRA model artificial vagina (IMV, L'Aigle, France) with an inline filter to eliminate the gel fraction. Semen was immediately transported to the laboratory for evaluation and processing. All procedures were performed under standard management conditions routinely applied at the facility and complied with national regulations on animal welfare.

Immediately after ejaculation, semen was transported to the laboratory under controlled temperature conditions to avoid thermal shock. Ejaculates were weighed to determine volume and maintained at 37 ºC prior to analysis. Sperm concentration was assessed using a hemocytometer (Mackler® chamber). Samples were then divided into two aliquots and diluted with two commercial extenders: INRA 96® and Equiplus®.

Dilutions were performed to achieve a final concentration of 25 × 10^6 spermatozoa/mL. Aliquots corresponding to each extender were prepared for each ejaculate and treated as paired samples. After initial evaluation (0 h), samples were maintained at room temperature for 10–15 minutes to allow gradual cooling and subsequently stored at 5 ºC in darkness to minimize photodamage and oxidative stress. Evaluations were conducted at three time points: immediately after dilution (0 h), after 24 hours and after 48 hours of storage. Before each evaluation, samples were rewarmed to 37 ºC for 5 minutes.

Motility Assessment

Sperm motility was evaluated using a computer-assisted sperm analysis system (ISAS®). For each sample, five microscopic fields were recorded, and the highest and lowest values were excluded to reduce variability. The final result corresponded to the mean of the remaining three fields. Both total motility (TM) and progressive motility (PM) were recorded.

Flow Cytometry Analysis

Flow cytometry was performed on samples from five stallions at 0 and 48 hours to assess sperm functional parameters. Mitochondrial membrane potential was evaluated using the JC-1 probe (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide), which distinguishes between high and low mitochondrial activity. Acrosome status was assessed using peanut agglutinin conjugated with fluorescein isothiocyanate (PNA-FITC) in combination with propidium iodide (PI), with PNA-positive spermatozoa considered to have damaged acrosomes.

Protocols, including probe concentrations and incubation conditions, were conducted according to previously described methodologies (Robles & Martínez-Pastor, 2016; Peña et al., 2016). Analyses were limited to 0 and 48 hours due to logistical and methodological constraints, including the complexity of sample processing and limited availability of equipment and reagents.

Statistical Analysis

Data were tested for normality using the Shapiro–Wilk test and are presented as mean ± standard error of the mean (SEM). Comparisons between extenders and time points were performed using analysis of variance (one way ANOVA) followed by Tukey's post-hoc test. Statistical significance was set at $p < 0.05$. All analyses were conducted using SigmaPlot software (version 12.3, Systat Software, Chicago, IL, USA).

Ethical Statement

All procedures were carried out in accordance with standard animal care practices and institutional guidelines.

Results

At 0 h, no significant differences were observed between extenders for either total motility (TM) or progressive motility (PM) ($p > 0.05$) (Table 1).

At 24 h, both TM and PM decreased in samples diluted with INRA 96® and Equiplus®. However, no significant differences were detected between extenders at this time point ($p > 0.05$) (Table 1).

At 48 h, TM and PM were significantly higher in samples diluted with INRA 96® compared to Equiplus® ($p < 0.05$). A significant time-dependent decrease in motility (0–48 h) was observed within each extender for both TM and PM ($p < 0.05$), as indicated by different superscript letters in Table 1.

Sperm viability decreased over time in both extenders, with a significant reduction observed between 0 and 48 h ($p < 0.05$). At 48 h, viability remained significantly higher in samples diluted with INRA 96® compared to Equiplus® ($p < 0.05$). No significant differences were detected between extenders in mitochondrial membrane potential (MMP) at either time point ($p > 0.05$). In contrast, acrosome damage was significantly higher in samples diluted with Equiplus® at both 0 and 48 h ($p < 0.05$), with no significant changes over time within each extender ($p > 0.05$) (Table 2).

Table 1. Mean (± SEM) total motility (TM) and progressive motility (PM) of stallion semen diluted with INRA 96® and Equiplus® at 0, 24, and 48 hours. Different superscript letters indicate significant differences ($p < 0.05$) between time points and extenders (two-way ANOVA followed by Tukey's post-hoc test).

Storage time (h)	Extender	n	TM (%) Mean ± SEM	PM (%) Mean ± SEM
0 h	INRA 96®	30	78.03 ± 2.21^{a}	61.23 ± 3.75^{a}
	Equiplus®	30	75.10 ± 2.11^{a}	56.03 ± 1.52^{a}
24 h	INRA 96®	30	69.07 ± 2.17^{b}	56.07 ± 2.05^{ab}
	Equiplus®	30	62.23 ± 2.59^{c}	52.33 ± 2.20^{b}
48 h	INRA 96®	30	55.60 ± 3.44^{d}	43.43 ± 3.21^{c}
	Equiplus®	30	46.87 ± 3.21^{e}	37.40 ± 2.63^{d}

Discussion

The present study shows that the choice of extender has a significant impact on the preservation of stallion semen quality during refrigerated storage. Overall, INRA 96® demonstrated superior performance compared to Equiplus®, particularly in maintaining sperm motility and viability after prolonged storage.

Table 2. Flow cytometry parameters (mean ± SEM) including sperm viability, sperm showing High mitochondrial membrane potential (hMMP) and Acrosome damage (% PNA-positive spermatozoa) in samples diluted with INRA 96® and Equiplus® at 0 and 48 hours. Significant differences were indicated with different letters $p < 0.05$.

Storage time Hours (h)	Extender	n	Sperm hMMP (%) Mean ± SEM	Sperm viability (%) Mean ± SEM	Acrosome damage (% PNA-positive spermatozoa) Mean ± SEM
0 h	INRA 96®	5	60.05 ± 6.61	69.26 ± 3.08 [a]	9.62 ± 1.66[a]
	Equiplus®	5	67.33 ± 3.21	65.57 ± 2.24 [a]	20.23 ± 1.75[b]
48 h	INRA 96®	5	49.90 ± 4.83	67.52 ± 2.49[a]	3.06 ± 1.08[a]
	Equiplus®	5	47.87 ± 4.96	52.14 ± 3.29[b]	15.92 ± 3.56[b]

A progressive decline in both total and progressive motility was observed in all samples, which is consistent with previous findings on refrigerated equine semen (Dascanio & McCue, 2021; Hernández-Avilés et al., 2020). The significantly higher motility observed in INRA 96® at 48 h ($p < 0.05$) suggests a greater protective capacity of this extender against storage-induced damage under refrigeration conditions. This improved preservation of motility may be related to the composition of INRA 96®, which includes components that contribute to membrane stabilization and provide adequate energy substrates to sustain sperm metabolism during storage (Hernández-Avilés et al., 2020). Differences among extenders in their ability to preserve sperm function during cooling have been previously reported and are known to influence fertility outcomes (Ferrer et al., 2020). These findings are particularly relevant under field conditions, where cooled semen is routinely transported over long distances and storage time directly impacts fertility outcomes.

Flow cytometry has proven to be a robust and reliable tool for the multiparametric assessment of sperm function, allowing simultaneous evaluation of key indicators such as membrane integrity, mitochondrial activity and acrosomal status (Umirbaeva et al., 2024). The absence of significant differences in mitochondrial membrane potential ($p > 0.05$) suggests that both extenders similarly preserved mitochondrial function, which is closely associated with sperm motility and energy production (Peña et al., 2016).

In contrast, sperm viability was significantly higher in samples diluted with INRA 96® at 48 h ($p < 0.05$), indicating better preservation of plasma membrane integrity. Membrane integrity is a key determinant of sperm survival and fertilizing capacity (Battut et al., 2016).

Acrosome damage was significantly higher in Equiplus® samples at both time points ($p < 0.05$), indicating greater structural compromise of the acrosome in this extender. The acrosome plays a crucial role in oocyte penetration, and its integrity is essential for successful fertilization; therefore, increased acrosomal damage may negatively affect fertilizing potential (Peña et al., 2016; Battut et al., 2016).

Individual variability among stallions is an important factor influencing semen preservation, as previously described in equine reproduction studies (Ferrer et al., 2020). Although multiple ejaculates per stallion were analyzed, this variability should be considered when interpreting the results.

Some limitations of this study include the relatively small number of stallions evaluated by flow cytometry and the absence of intermediate (24 h) data for these parameters, which limits the assessment of temporal changes in sperm functional characteristics. In addition, the lack of in vivo fertility data prevents direct extrapolation of laboratory findings to reproductive outcomes. Future studies should aim to correlate in vitro parameters with fertility results and explore strategies to further improve extender performance.

Overall, the results of this study support that extender selection is a key factor in equine reproductive management and that INRA 96® may provide improved preservation of sperm quality during refrigerated storage.

Conclusions

Extender selection significantly influences the preservation of stallion semen quality during refrigerated storage. Under the conditions evaluated, INRA 96® showed superior performance compared with Equiplus®, particularly in maintaining motility

and viability and reducing acrosome damage after 48 h at 5 °C. These results suggest that INRA 96® may represent a more suitable option for short-term refrigerated semen storage.

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Maneio da dor em animais de companhia

Estudo de casos clínicos em contexto hospitalar

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Summary

Appropriate pain management in companion animals is a fundamental pillar of veterinary medicine, with a direct impact on animal welfare and clinical recovery. This study aimed to evaluate pain management in a hospital setting through a retrospective analysis of clinical cases. A descriptive observational study was conducted based on the clinical records of companion animals treated at a veterinary hospital during April 2024. Three clinical cases (two involving acute pain and one involving chronic pain) were analysed, considering variables such as pain type, assessment methods, analgesic protocols, and clinical outcome. The results suggest the consistent use of multimodal analgesia, combining different pharmacological classes, including opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and other adjuvant agents. Continuous monitoring and the individualised adjustment of analgesic protocols were also found to play an important role. The analysis of the clinical cases demonstrated differences between acute and chronic pain, both in their clinical presentation and therapeutic response. It can be concluded that effective pain management requires an integrated approach based on systematic patient assessment and the implementation of individualised therapeutic strategies. Veterinary nursing plays a key role in the early recognition, monitoring, and optimisation of pain management in the hospital environment.

Keywords: animal welfare, companion animals; multimodal analgesia; pain management; veterinary nursing;

Resumo

O maneio adequado da dor em animais de companhia constitui um pilar fundamental da medicina veterinária, com impacto direto no bem-estar animal e na recuperação clínica. O presente estudo teve como objetivo avaliar o maneio da dor em contexto hospitalar, através de uma análise retrospetiva de casos clínicos. Foi realizado um estudo observacional descritivo com base nos registos clínicos de animais de companhia acompanhados num hospital veterinário durante o mês de abril de 2024. Foram analisados três casos clínicos (dois de dor aguda e um de dor crónica), considerando variáveis como tipo de dor, métodos de avaliação, protocolos analgésicos e evolução clínica. Os resultados sugerem a utilização consistente de analgesia multimodal, com recurso a diferentes classes farmacológicas, nomeadamente opióides, anti-inflamatórios não esteroides (AINE´s) e outros agentes adjuvantes. Verificou-se ainda a importância da monitorização contínua e da adaptação individualizada dos protocolos analgésicos. A análise dos casos clínicos demonstrou diferenças entre dor aguda e crónica, tanto na apresentação clínica como na resposta terapêutica. Conclui-se que o maneio eficaz da dor requer uma abordagem integrada, baseada na avaliação sistemática do paciente e na aplicação de estratégias terapêuticas individualizadas. O papel da enfermagem veterinária revela-se essencial na identificação precoce, monitorização e otimização do controlo da dor em ambiente hospitalar.

Palavras-chave: maneio da dor; analgesia multimodal; animais de companhia; enfermagem veterinária; bem-estar animal

Introdução

O reconhecimento e o maneio da dor em animais de companhia têm assumido um papel central na prática clínica veterinária, sendo atualmente considerados componentes essenciais do bem-estar animal e da qualidade dos cuidados prestados. A dor não tratada ou inadequadamente controlada pode desencadear alterações fisiológicas e comportamentais significativas, comprometendo não só a recuperação clínica, mas também a qualidade de vida dos pacientes (Alamrew, E. & Fesseha, H., 2020; Downing, R. &

Della Rocca, G., 2023; Cital, S., 2022; WSAVA, 2022).

A dor pode ser classificada em aguda ou crónica, consoante a sua duração e mecanismos fisiopatológicos subjacentes. A dor aguda está geralmente associada a lesões teciduais recentes, como trauma ou procedimentos cirúrgicos, desempenhando uma função protetora. Por outro lado, a dor crónica persiste para além do período esperado de cicatrização, podendo envolver fenómenos de sensibilização periférica e central, tornando-se mais complexa de diagnosticar e tratar (Alamrew, E. & Fesseha, H., 2020; WSAVA 2022; Cital, S. 2022). A avaliação da dor em medicina veterinária apresenta desafios particulares, uma vez que os animais não conseguem comunicar verbalmente a sua experiência dolorosa. Assim, a identificação da dor baseia-se na observação de alterações comportamentais, fisiológicas e posturais, bem como na utilização de escalas de avaliação validadas. Entre as mais utilizadas destacam-se a Glasgow Composite Measure Pain Scale, a Colorado State University Pain Scale e a escala visual analógica (VAS), que permitem padronizar a avaliação e monitorização da dor, contribuindo para uma abordagem mais objetiva e consistente (WSAVA,2022).

Neste contexto, o papel da enfermagem veterinária é fundamental, não só na administração terapêutica, mas também na deteção precoce de sinais de dor, monitorização contínua dos pacientes e adaptação dos cuidados em ambiente hospitalar. A proximidade com o animal permite uma avaliação mais frequente e detalhada, contribuindo significativamente para a otimização do controlo da dor. Apesar dos avanços na área, diversos estudos demonstram que a dor continua a ser subdiagnosticada e subtratada em medicina veterinária, especialmente em contextos hospitalares com elevada carga de trabalho. Assim, torna-se pertinente analisar práticas clínicas reais, de forma a identificar padrões, limitações e oportunidades de melhoria.

Deste modo, o presente estudo teve como objetivo avaliar o maneio da dor em animais de companhia em contexto hospitalar, através de uma análise retrospetiva de casos clínicos, complementada pela descrição de uma série de casos representativos, permitindo uma abordagem integrada entre dados quantitativos e prática clínica.

Materiais e Métodos

Foi realizado um estudo observacional de casos clínicos, com o objetivo de avaliar o maneio da dor em animais de companhia em contexto hospitalar. O estudo foi conduzido no AniCura Alma Veterinária, durante o mês de abril de 2024.

Foram selecionados três casos clínicos em canídeos, com base na sua relevância clínica e diversidade de apresentação:

- Dois casos de dor aguda
- Um caso de dor crónica

Os critérios de inclusão foram:

- Presença de sinais clínicos compatíveis com dor
- Avaliação clínica documentada
- Instituição de terapêutica analgésica
- Informação clínica completa

A recolha de dados foi realizada através da análise detalhada dos registos clínicos dos pacientes selecionados. Foram consideradas as seguintes variáveis:

- Espécie, raça, idade, peso e sexo
- História clínica pregressa
- Motivo de consulta
- Achados do exame físico
- Tipo de dor (aguda ou crónica)
- Exames complementares realizados
- Protocolos terapêuticos instituídos
- Evolução clínica

A avaliação da dor foi baseada na observação de sinais clínicos, alterações comportamentais e achados do exame físico, incluindo parâmetros como postura, resposta à manipulação, vocalização e alterações neurológicas.

Sempre que possível, a avaliação foi complementada com recurso a escalas de dor validadas, nomeadamente a *Glasgow Composite Measure Pain Scale*, permitindo uma classificação mais objetiva da intensidade da dor e facilitando a monitorização da evolução clínica dos pacientes. Os casos foram classificados em:

- Dor aguda, associada a condições de início súbito, como trauma ou situações cirúrgicas
- Dor crónica, associada a patologias degenerativas e persistentes

Os dados foram analisados de forma descritiva, permitindo a caracterização dos casos clínicos e das abordagens terapêuticas utilizadas. A comparação entre dor aguda e crónica foi realizada com base na apresentação clínica, estratégias terapêuticas e evolução dos pacientes.

Resultados

Caracterização dos casos clínicos

Foram analisados três casos clínicos de canídeos, todos do sexo feminino, com idades compreendidas entre os 4 e os 17 anos e pesos entre 2,5 kg e 57 kg (Tabela 1).

Tabela 1. Casos clínicos avaliados

Caso	Idade (anos)	Peso (Kg)	Tipo de dor
1.Suki	8	2,5	Aguda
2.Singha	4	57	Aguda
3.Nicky	17	15,5	Crónica

Apresentação clínica

Os casos de dor aguda apresentaram início súbito e sinais clínicos marcados, incluindo dor intensa, alterações neurológicas e sinais sistémicos graves. No caso do paciente 1, observaram-se alterações neurológicas associadas a dor significativa, enquanto no caso do paciente 2 se verificaram sinais compatíveis com uma emergência cirúrgica. De acordo com a aplicação das escalas de avaliação da dor, ambos os pacientes apresentaram pontuações compatíveis com dor de intensidade elevada. Por outro lado, o caso de dor crónica (paciente 3) apresentou uma evolução progressiva e persistente, caracterizada por claudicação, fraqueza muscular e alterações estruturais associadas a patologias degenerativas, tendo sido classificado, com base nas escalas de dor, como apresentando dor de intensidade moderada e carácter crónico. Com base na avaliação clínica e na aplicação de escalas de dor, os pacientes 1 e 2 apresentaram dor de intensidade elevada, enquanto o paciente 3 apresentava dor de carácter moderado e crónico.

Abordagem diagnóstica

Nos casos de dor aguda, foram realizados exames complementares de diagnóstico de forma imediata, incluindo radiografias, análises laboratoriais e avaliação neurológica. No caso do paciente 2, o diagnóstico de torção gástrica foi confirmado por radiografia abdominal, o que motivou intervenção cirúrgica urgente. No caso de dor crónica, o diagnóstico baseou-se em exames previamente realizados, nomeadamente radiografia da coluna vertebral, evidenciando alterações degenerativas severas.

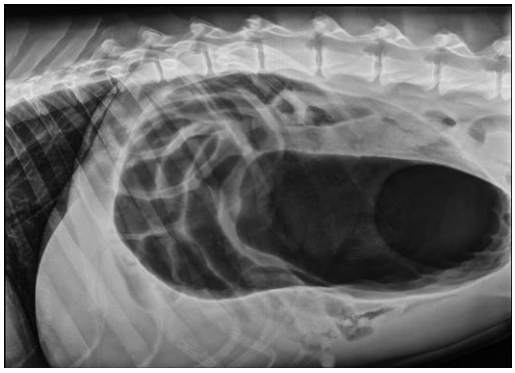

Figura 1. Radiografia latero-lateral abdominal direita evidenciando torção gástrica; Paciente: Singha.

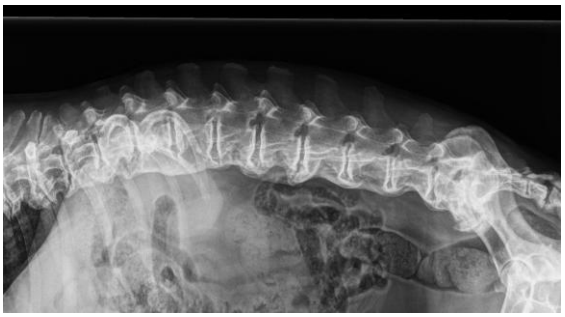

Figura 2. Radiografia latero-lateral da coluna evidenciando fusão das vértebras; Paciente: Nicky.

Estratégias terapêuticas

Observou-se a utilização de analgesia multimodal em 100% dos casos analisados (Tabela 2). Nos casos de dor aguda, os protocolos terapêuticos caracterizaram-se por:

- Utilização de opióides (2/2 casos)
- Administração de AINEs (2/2 casos)
- Recurso a fármacos adjuvantes, como lidocaína em perfusão contínua

Estes protocolos apresentaram carácter mais intensivo, com o objetivo de promover um controlo rápido e eficaz da dor.

No caso de dor crónica, verificou-se uma abordagem terapêutica mais complexa e prolongada, incluindo:

- Gabapentina
- AINE's seletivos (grapiprant)
- Anticorpo monoclonal (bedinvetmab)
- Suplementação com canabidiol

Adicionalmente, foram incluídas estratégias não farmacológicas, nomeadamente fisioterapia.

Evolução clínica

Todos os pacientes apresentaram evolução clínica favorável após a instituição do tratamento, embora com diferenças relevantes entre dor aguda e crónica.

Nos casos de dor aguda, observou-se melhoria rápida e resolução significativa dos sinais clínicos, com alta hospitalar em curto período de tempo. No caso do paciente 2, a estabilização clínica ocorreu nas primeiras 48 horas após a intervenção cirúrgica.

No caso de dor crónica, a evolução foi mais lenta e dependente de ajustes terapêuticos, verificando-se a necessidade de monitorização contínua. O controlo da dor foi alcançado, embora sem resolução completa do quadro clínico.

Discussão

Os resultados obtidos sugerem diferenças consistentes entre a dor aguda e a dor crónica em animais de companhia, tanto ao nível da apresentação clínica como das abordagens

diagnósticas, terapêuticas e da evolução clínica, em concordância com o descrito na literatura (Alamrew & Fesseha, 2020; Cital, 2022). A distinção entre estes dois tipos de dor revela-se fundamental para a definição de estratégias de maneio adequadas, uma vez que cada uma apresenta mecanismos fisiopatológicos, implicações clínicas e necessidades terapêuticas distintas.

A dor aguda caracterizou-se por um início súbito, frequentemente associada a quadros clínicos de elevada gravidade, exigindo intervenção imediata e direcionada. Este padrão encontra-se em concordância com as diretrizes da WSAVA (2022), que definem a dor aguda como uma resposta fisiológica a lesão tecidular recente, frequentemente acompanhada por alterações sistémicas e sinais clínicos evidentes. Nos casos clínicos analisados, verificou-se que este tipo de dor estava frequentemente associado a situações críticas, como no caso de torção gástrica, em que a dor se encontra diretamente relacionada com risco iminente de vida. Nestes contextos, a rapidez na intervenção revelou-se um fator crítico associado à estabilização clínica e à melhoria do prognóstico.

A dor pós-operatória, particularmente após cirurgias invasivas como a correção da torção gástrica, apresentou-se como significativa, resultante do trauma tecidular e da manipulação cirúrgica. O seu controlo eficaz exigiu a implementação de protocolos analgésicos intensivos, baseados numa abordagem multimodal (Mathews et al., 2014). Esta estratégia, amplamente recomendada na literatura, permite melhorar o controlo da dor, reduzir a necessidade de opióides em doses elevadas e promover uma recuperação mais segura e eficaz. A associação de fármacos com diferentes mecanismos de ação, nomeadamente opióides e anti-inflamatórios não esteroides (AINEs), demonstrou ser particularmente eficaz no controlo da nocicepção, prevenindo fenómenos de sensibilização periférica e central (Mathews et al., 2014; WSAVA, 2022) e reduzindo o risco de complicações pós-operatórias.

Em contraste, a dor crónica apresentou uma evolução progressiva e persistente, frequentemente com impacto funcional significativo, embora frequentemente com menor intensidade aparente nos momentos de avaliação clínica. Este tipo de dor está frequentemente associado a patologias degenerativas, como a osteoartrite, e envolve mecanismos fisiopatológicos complexos, incluindo sensibilização periférica e central (Wiese, 2015; Monteiro-Steagall et al., 2017; Cital, 2022). O caso clínico analisado reflete esta realidade, evidenciando a necessidade de abordagens terapêuticas prolongadas, ajustáveis e centradas no controlo sustentado da dor e na melhoria da qualidade de vida do paciente.

O maneio da dor crónica revelou-se, assim, mais complexo, exigindo uma abordagem integrada que combina terapêuticas farmacológicas e não farmacológicas. No presente estudo, foram utilizados fármacos moduladores da dor neuropática, como a gabapentina, AINEs seletivos e terapias inovadoras, nomeadamente anticorpos monoclonais como o bedinvetmab. Este último constitui uma abordagem terapêutica recente com evidência crescente no controlo da dor associada à osteoartrite. Adicionalmente, a integração de terapias adjuvantes, como a fisioterapia e a reabilitação funcional, reforça a importância de uma abordagem multidimensional no tratamento da dor crónica (Millis & Levine, 2014).

Métodos não farmacológicos, como termoterapia, crioterapia, laserterapia, eletroterapia, terapias manuais, cinesioterapia e a utilização de extratos medicinais, são amplamente descritos na literatura como estratégias adjuvantes no maneio da dor, contribuindo para a melhoria da mobilidade e da qualidade de vida dos pacientes quando aplicados de forma adequada (Alamrew & Fesseha, 2020; Corti, 2014). A sua integração nos protocolos terapêuticos evidencia a crescente valorização de abordagens multimodais e individualizadas.

Relativamente à abordagem diagnóstica, verificou-se que os casos de dor aguda exigiram a realização de exames complementares de diagnóstico de forma imediata, com o objetivo de identificar rapidamente a causa subjacente e orientar a intervenção terapêutica. Por outro lado, nos casos de dor crónica, o diagnóstico baseou-se frequentemente em avaliações prévias e na evolução clínica do paciente. Este achado reforça a importância da rapidez na identificação etiológica em situações agudas, conforme descrito nas diretrizes da WSAVA (2022), sendo determinante para a eficácia do tratamento.

A utilização de analgesia multimodal em todos os casos analisados encontra-se em concordância com as recomendações atuais, que defendem a combinação de fármacos com diferentes mecanismos de ação como estratégia de eleição no maneio da dor (Cital, 2022; Mathews et al., 2014; WSAVA, 2022). Esta abordagem permite potenciar o efeito analgésico, reduzir as doses individuais administradas e minimizar os efeitos adversos, contribuindo para uma terapêutica mais segura e eficaz.

No que diz respeito à evolução clínica, observou-se uma recuperação mais rápida nos casos de dor aguda, em contraste com a necessidade de monitorização contínua e ajustes terapêuticos nos casos de dor crónica. Estes resultados estão em concordância com a literatura, que indica que a dor aguda tende a resolver-se com o tratamento da causa subjacente, enquanto a dor crónica requer estratégias de controlo a longo prazo, centradas na manutenção da função e na melhoria da qualidade de vida (Cital, 2022).

A utilização de grelhas de avaliação de dor em medicina veterinária tem vindo a assumir uma importância crescente, uma vez que permite reduzir a subjetividade associada à interpretação dos sinais clínicos. Escalas como Glasgow *Composite Measure Pain Scale* e a *Colorado State University Pain Scale* são amplamente utilizadas e validadas, permitindo uma avaliação mais consistente entre diferentes observadores e ao longo do tempo.

No entanto, apesar das suas vantagens, estas ferramentas apresentam algumas limitações, nomeadamente a dependência da experiência do observador e a variabilidade

comportamental entre animais. Ainda assim, a sua aplicação em contexto clínico revela-se fundamental para orientar a decisão terapêutica, monitorizar a eficácia dos protocolos analgésicos e melhorar o bem-estar animal.

Importa ainda destacar o papel fundamental da enfermagem veterinária no maneio da dor. Os enfermeiros veterinários desempenham uma função central na monitorização contínua dos pacientes, na deteção precoce de sinais clínicos de dor e na adaptação dos cuidados prestados. A sua proximidade ao paciente permite uma avaliação mais frequente e detalhada, contribuindo significativamente para a eficácia do tratamento e para a promoção do bem-estar animal (Scarlett, 2019; Shaffran, 2008; Elder et al., 2017). Além disso, os enfermeiros veterinários constituem um elo de ligação essencial entre o médico veterinário, o tutor e o paciente, sendo responsáveis por grande parte dos cuidados prestados e pela sua qualidade. A capacidade de avaliar a dor, reconhecer alterações no estado clínico e comunicar eficazmente com a equipa clínica é determinante para a otimização dos resultados terapêuticos (Nugent-Deal, 2017).

A adoção de uma abordagem estruturada e multidimensional na avaliação da dor, conforme proposto por Clapham (2014), revela-se igualmente essencial para reduzir a variabilidade entre observadores e garantir uma avaliação mais precisa. A utilização de escalas de dor, em complemento ao exame clínico completo, permite monitorizar a resposta do paciente à terapêutica instituída, nomeadamente através de avaliações realizadas antes e após a administração de analgésicos.

Apesar da relevância dos resultados obtidos, o presente estudo apresenta algumas limitações, nomeadamente o reduzido número de casos analisados e o seu carácter observacional, o que pode limitar a generalização dos dados. No entanto, a análise detalhada dos casos clínicos permite uma compreensão aprofundada das práticas clínicas adotadas e reforça a importância da implementação de estratégias baseadas na evidência científica no maneio da dor em contexto hospitalar veterinário.

De forma global, este estudo reforça a importância do maneio da dor em animais de companhia como componente essencial na promoção do bem-estar e na recuperação clínica. A análise comparativa entre dor aguda e dor crónica destaca a necessidade de abordagens diferenciadas, adaptadas às características específicas de cada tipo de dor. A intervenção precoce em situações agudas e a gestão contínua e integrada da dor crónica revelam-se determinantes para a otimização dos resultados clínicos, reforçando a importância de uma prática clínica baseada na avaliação sistemática, terapêutica multimodal e colaboração interdisciplinar. Estes resultados reforçam a necessidade de uma abordagem baseada em evidência científica, promovendo a utilização sistemática de ferramentas de avaliação da dor e estratégias terapêuticas individualizadas em medicina veterinária.

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Bridging the Gap Between Scientific Knowledge and Owner Perception of Canine Cognitive Dysfunction Syndrome

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Summary

Canine Cognitive Dysfunction Syndrome (CCDS) is a progressive neurodegenerative disorder associated with aging, sharing clinical and pathological similarities with Alzheimer's disease in humans. Despite its increasing prevalence, CCDS remains significantly underdiagnosed, largely due to limited awareness among dog owners and challenges in early detection. This study aimed to assess the prevalence of CCDS and evaluate owner awareness in the Alentejo region of Portugal, as well as to identify associated risk factors. A cross-sectional observational study was conducted using structured questionnaires administered to owners of dogs aged eight years or older (n = 86). Results revealed that only 9.3% of dogs had a prior diagnosis, whereas 58% exhibited clinical signs consistent with cognitive impairment, indicating substantial underdiagnosis, in agreement with previous findings (Salvin et al., 2010). Additionally, a significant proportion of owners lacked awareness of CCDS. These findings highlight a critical gap between scientific knowledge and owner perception, emphasizing the need for educational strategies and early screening protocols.

Keywords: canine cognitive dysfunction syndrome; geriatric veterinary medicine; owner perception; cognitive impairment; early screening.

Resumo

Síndrome de Disfunção Cognitiva Canina (SDCC) é uma doença neurodegenerativa progressiva associada ao envelhecimento, que partilha semelhanças clínicas e patológicas com a doença de Alzheimer em humanos. Apesar da sua prevalência crescente, a SDCC continua a ser significativamente subdiagnosticada, em grande parte devido ao reduzido conhecimento dos tutores de cães sobre a doença e às dificuldades na sua deteção precoce. O presente estudo teve como objetivo avaliar a prevalência da SDCC e o nível de conhecimento dos tutores na região do Alentejo, Portugal, bem como identificar fatores de risco associados. Foi realizado um estudo observacional transversal, recorrendo à aplicação de questionários estruturados a tutores de cães com idade igual ou superior a oito anos (n = 86). Os resultados revelaram que apenas 9,3% dos cães tinham um diagnóstico prévio, enquanto 58% apresentavam sinais clínicos compatíveis com défice cognitivo, indicando um subdiagnóstico substancial, em concordância com estudos anteriores (Salvin et al., 2010). Verificou-se ainda que uma proporção significativa dos tutores desconhecia a existência da SDCC. Estes resultados evidenciam uma importante discrepância entre o conhecimento científico disponível e a perceção dos tutores, reforçando a necessidade de implementar estratégias de sensibilização e protocolos de rastreio precoce.

Palavras-chave: síndrome de disfunção cognitiva canina; cães geriátricos; medicina veterinária geriátrica; rastreio precoce; bem-estar animal.

Introduction

Advances in veterinary medicine, nutrition, and preventive care have significantly increased the lifespan of companion animals, leading to a growing population of geriatric dogs and, consequently, a higher prevalence of age-related diseases (Landsberg et al., 2012; Mazzola & Fatjó, 2018). Among these, Canine Cognitive Dysfunction Syndrome (CCDS) has emerged as a major concern in veterinary geriatrics due to its progressive nature and impact on animal welfare.

CCDS is a neurodegenerative disorder characterized by cognitive decline and behavioral alterations, with strong similarities to Alzheimer's disease in humans, both clinically and pathophysiologically (Dewey et al., 2019; Prpar Mihevc & Majdič, 2019). Neuropathological features include β-amyloid plaque deposition, oxidative stress, mitochondrial dysfunction, and neuronal loss (Head, 2008; Borràs et al., 2021). These alterations are associated with deficits in learning, memory,

and executive function, which progressively impair the animal's daily functioning.

Behaviorally, CCDS manifests through a cluster of signs commonly summarized by the acronym DISHA (Disorientation, Interaction changes, Sleep-wake disturbances, House-soiling, and Activity alterations) (Landsberg et al., 2012). These signs often develop gradually, making early detection challenging. Furthermore, studies have shown that subtle cognitive decline may begin years before clinical recognition, suggesting that CCDS is frequently identified at advanced stages (Madari et al., 2015; Szabó et al., 2016).

Epidemiological data indicate that the prevalence of CCDS increases markedly with age, affecting a significant proportion of dogs over eight years old (Salvin et al., 2010; Chapagain et al., 2018). However, prevalence estimates vary widely due to differences in diagnostic criteria and assessment tools. Importantly, several studies highlight that a large percentage of affected dogs remain undiagnosed, primarily due to under-recognition by owners and insufficient screening in clinical practice (Salvin et al., 2010).

The role of owners in the detection of CCDS is critical. Since behavioral changes are often first noticed in the home environment, owner perception directly influences the likelihood of seeking veterinary care. However, research indicates that many owners interpret cognitive changes as normal aging, leading to delays in diagnosis and intervention (Fast et al., 2013; Mazzola & Fatjó, 2018). This misinterpretation represents a major barrier to early detection and management.

In addition to age, several risk and protective factors have been identified. Environmental enrichment, regular physical activity, and social interaction have been associated with better cognitive outcomes (Mongillo et al., 2013; Chapagain et al., 2018). Nutritional interventions, particularly diets enriched with antioxidants and medium-chain triglycerides, have demonstrated neuroprotective effects and may slow cognitive decline (Pan et al., 2011; Studzinski et al., 2005).

At the neurobiological level, experimental studies in aging dogs have provided valuable insights into cognitive decline mechanisms. Longitudinal research has shown progressive impairments in learning and memory tasks, reinforcing the validity of dogs as a model for human neurodegenerative diseases (Milgram et al., 2005; Siwak et al., 2001; Tapp et al., 2004).

More recently, advances in diagnostic approaches, including neuroimaging and electrophysiological biomarkers, have opened new possibilities for early detection. Techniques such as electroencephalography (EEG) have shown potential in identifying functional brain changes associated with CCDS (Barnes Heller et al., 2022). However, these methods are not yet widely accessible in routine veterinary practice.

Despite growing scientific knowledge, a significant gap remains between research findings and their application in clinical settings. In particular, the discrepancy between owner perception and clinical reality continues to contribute to underdiagnosis and suboptimal management of CCDS.

Therefore, this study aims to evaluate both the prevalence of CCDS and the level of awareness among dog owners, contributing to a better understanding of this gap and supporting the development of more effective diagnostic and educational strategies.

Material and Methods

A cross-sectional observational study was conducted. The study included 86 dogs aged ≥8 years, recruited from a veterinary clinical setting in Évora, Portugal. Data were collected through structured questionnaires administered to dog owners. Data were collected using a structured questionnaire divided into five sections and designed to obtain detailed information on factors potentially associated with Canine Cognitive Dysfunction Syndrome (CCDS). Section I recorded general animal information, including demographic and signalment data. Subjects were divided into five age groups: 8–10 years, 11–12 years, 13–14 years, 15–16 years, and >16 years. Section II collected information on concurrent medical conditions and the animal's clinical history, including previous diagnosis or suspicion of CCDS. Section III assessed diet type and feeding habits. Section IV evaluated the dog's level of physical activity and social interaction. Section V investigated behavioural changes associated with CCDS, using six ordinal frequency scales to quantify the occurrence of clinical signs commonly associated with cognitive dysfunction. Behavioral changes were evaluated using criteria aligned with validated tools such as the Canine Cognitive Dysfunction Rating Scale (Madari et al., 2015). Behavioural frequency was assessed by owners using a 4-point scale (0 = never, 1 = once per month, 2 = once per week, 3 = daily). Total scores were obtained by summing individual items and multiplying the result by two to generate the final CCDS score. Dogs were then classified as normal ageing (0–7), mild cognitive impairment (8–40), or severe cognitive impairment (41–69).

The scoring system was based on previously established associations between behavioural alterations and neuropathological changes observed in geriatric dogs, supporting its clinical relevance.

Statistical analyses were performed using Jamovi software (version 2.7). Descriptive statistics were used to analyze the data and normality was assessed using the Shapiro–Wilk test. As the assumption of normality was violated, a one-way Welch ANOVA was applied to compare differences between groups, followed by post-hoc Tukey tests for pairwise comparisons.

Results

A total of 86 questionnaires were collected from owners of dogs aged eight years or older. The sample was predominantly composed of dogs between eight and ten years of age (43%), followed by those aged 11 to 12 years (27%), with progressively fewer individuals represented in older age groups. Males accounted for 55% of the sample, and 57% of the dogs were sterilized.

A clear discrepancy emerged between clinical diagnosis and questionnaire-based assessment of CCDS. Only 9.3% of dogs (8 individuals) had received a formal clinical diagnosis, whereas behavioral scoring indicated that 46.5% of the animals presented mild CCDS and 11.6% severe CCDS (Figure 1.). Overall, approximately 58.1% of the dogs exhibited some degree of cognitive impairment. In addition,

Evaluated CCDS	Count	% Total	% Accumulated
Light	40.0	46.5%	46.5%
Severe	10.0	11.6%	58.1%
Natural ageing	36.0	41.9%	100.0%

Figure 1. Distribution of individuals with CCDS diagnose

Analysis of potential risk factors revealed a higher presence of CCDS in small and medium-sized dogs, while no severe cases were identified among large or giant breeds (Figure 2). All clinically diagnosed cases occurred in sterilized animals, although cognitive impairment surged similarly in both reproductive status.

Evaluated CCDS	Weight	Count	% Total
Light	small	17.00	19.8%
	medium	12.00	14.0%
	large	9.00	10.5%
	giant	2.00	2.3%
Severe	small	4.00	4.7%
	medium	6.00	7.0%
	large	0.00	0.0%
	giant	0.00	0.0%
Natural ageing	small	10.00	11.6%
	medium	12.00	14.0%
	large	12.00	14.0%
	giant	2.00	2.3%

Figure 2. CCDS diagnose across different body size categories

The distribution of CCDS categories was similar between sexes. Mild cognitive impairment was the most frequent category in females (25.9% of the total sample), whereas physiological ageing was more commonly observed in males (28.2%). Severe CCDS was uncommon and occurred at the same frequency in both sexes (5.9% each) (figure 3).

Dietary patterns showed that dogs receiving senior-specific diets had higher proportions of mild and severe CCDS, a finding that likely reflects dietary adjustments following the onset of clinical signs rather than a causal relationship. Although physical activity and environmental enrichment were considered potentially relevant, no conclusive associations were established. Furthermore, no consistent relationship between breed and CCDS was identified.

Evaluated CCDS	Sex	Count	% Total
Mild	F	22.00	25.9%
	M	17.00	20.0%
Severe	F	5.00	5.9%
	M	5.00	5.9%
Natural ageing	F	12.00	14.1%
	M	24.00	28.2%

Figure 3. Distribution of dogs across CCDS categories by sex, including frequencies and percentages.

Behavioral alterations were commonly reported by owners, with reduced activity being the most prevalent sign, followed by disorientation, anxiety, learning and memory deficits, changes in social interaction, and sleep disturbances. These findings suggest that decreased activity and spatial disorientation are the most noticeable manifestations of cognitive decline in this population.

The assumption of normality was assessed using the Shapiro–Wilk test, which indicated that the data were not normally distributed. Therefore, a one-way Welch ANOVA was conducted to compare differences between groups (different ages and body size categories).

The analysis of differences between age groups revealed a statistically significant effect of group on the CCDS score ($F(4, 23.7) = 9.75$, $p < 0.001$). Post-hoc comparisons using Tukey's test showed a significant difference between group 1 (aged from 8 to 10 years old) and group 3 (aged from 13 to 15 years old) ($p < 0.05$), following the same tendency when comparing group 1 with group 4 (aged from 15 to 16 years old), while no other pairwise comparisons reached statistical significance. The analysis of differences in SDCC scores among body size categories (small, medium, large, and giant breeds) showed no statistically significant effect of body size on CCDS scores, as determined by a one-way Welch ANOVA ($F(3, 13.5) = 0.809$, $p = 0.511$). Post-hoc Tukey tests revealed no significant

pairwise differences between any of the body size groups (all $p > 0.05$).

Discussion

The present study underscores the increasing importance of CCDS in veterinary medicine, particularly in the context of extended canine lifespan resulting from advances in veterinary care and improved owner awareness (Neilson et al., 2001; Rofina et al., 2006; Salvin et al., 2010). The most notable finding is the marked discrepancy between the low rate of clinical diagnosis and the substantially higher prevalence suggested by behavioral assessment. The fact that only 9.3% of dogs had a former clinical diagnosis, despite 58.1% showed signs of cognitive impairment, strongly supports previous reports that CCDS is widely underdiagnosed (Salvin et al., 2010). This underdiagnosis is likely multifactorial. One key factor is the tendency for owners to interpret behavioral changes as a normal consequence of aging rather than as indicators of pathology (Chapagain et al., 2018). The considerable proportion of respondents unfamiliar with CCDS further reinforces the role of limited awareness in delaying or preventing diagnosis. Additionally, the reliance on subjective behavioral assessments contributes to diagnostic inconsistency. These findings highlight the need to incorporate routine cognitive screening into geriatric veterinary evaluations, as previously recommended (Landsberg et al., 2012).

The study also illustrates the inherent challenges associated with CCDS diagnosis. Currently, diagnosis is largely presumptive, based on clinical signs and the exclusion of other medical conditions (Chapagain et al., 2018; Landsberg et al., 2012). Although advanced diagnostic tools such as neuroimaging and biomarker analysis show promise, their use in clinical practice remains limited due to cost, accessibility, and procedural constraints, including the need for anesthesia (González-Martínez et al., 2011; MacQuiddy et al., 2022).

Regarding risk factors, the higher prevalence observed in smaller dogs may be related to their longer lifespan, which increases the likelihood of developing age-related neurodegenerative conditions (Azkona et al., 2009; Neilson et al., 2001). However, conflicting evidence in the literature suggests that this relationship is not yet fully understood (Katina et al., 2016). Indeed, in the present study, no significant differences in SDCC scores were found among the different body size categories, indicating that body size was not significantly associated with cognitive dysfunction in the studied population. The relatively small sample size within some body size categories may have limited the ability to detect subtle differences, highlighting the need for studies involving larger populations.

The observed lack of significant differences in SDCC scores between sterilized and non-sterilized animals in the present study does not support a strong association between reproductive status and cognitive dysfunction, although a hormonal influence has been previously suggested (Hart, 2001). This finding should be interpreted with caution and further investigated in larger studies.

Dietary patterns indicated higher proportions of mild and severe CCDS in dogs receiving senior-specific diets; however, no statistically significant association was found between diet type and CCDS severity, suggesting that this distribution may reflect dietary adaptation associated with ageing and the potential onset of clinical signs rather than a causal relationship. Existing evidence supports the neuroprotective effects of diets enriched with antioxidants and other functional components (Katina et al., 2016), which may help delay or mitigate the progression of CCDS, suggesting that owners may be acting accordingly when selecting these diets. .

The behavioral patterns identified in this study differ somewhat from previous reports. While reduced activity and disorientation were the most prominent signs in this population, other studies have emphasized changes in social interaction and memory deficits (Azkona et al., 2009). This discrepancy may reflect differences in caregiver perception, as well as the inherent difficulty in recognizing subtle cognitive impairments such as memory decline (Landsberg et al., 2012).

Overall, the findings emphasize the importance of early detection and intervention in CCDS. Improving caregiver education, implementing standardized screening protocols, and strengthening the role of veterinary professionals in geriatric care are essential steps to enhance diagnosis and management. Early identification of cognitive decline may allow for interventions that slow disease progression and improve the quality of life of affected animals (Landsberg et al., 2012).

Conclusion

CCDS is a significant and increasingly recognized condition in aging dog populations, with important implications for animal welfare and veterinary practice. The present study identified a discrepancy between behavioral signs consistent with cognitive impairment and formal clinical recognition, suggesting potential underdiagnosis, likely influenced by limited owner awareness and delayed identification of early signs.

Although nutritional, lifestyle, and biological factors are commonly proposed as modulators of cognitive health, no significant associations were found between CCDS severity and diet type, body size, or reproductive status. Nevertheless, dogs receiving senior-specific diets showed higher proportions of mild and severe CCDS, likely reflecting dietary adaptation following ageing and clinical progression rather than a causal relationship.

From a clinical perspective, early detection remains essential, and routine cognitive screening in geriatric consultations, together with improved owner education, may support earlier intervention. The limited sample size in some subgroups may

have reduced statistical power, highlighting the need for larger and more balanced studies to improve the robustness and generalizability of future findings.

Overall, improving CCDS recognition in clinical practice requires continued efforts to bridge the gap between scientific evidence and owner perception, alongside the development of standardized diagnostic approaches and preventive geriatric care strategies.

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Serogroup Distribution and High Seroprevalence of Bovine Leptospirosis in Southeastern Portalegre, Portugal

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Summary

Bovine leptospirosis is a globally distributed zoonotic disease with significant implications for animal health, public health, and livestock productivity. This study aimed to characterize the epidemiological status of leptospirosis in cattle from the Alto Alentejo region (Portugal), with particular emphasis on serogroup distribution and infection dynamics. Between February and June 2025, a total of 83 cattle from 11 farms were sampled across four municipalities. Serological analysis was performed using the microscopic agglutination test (MAT), while molecular detection of pathogenic Leptospira DNA was conducted using polymerase chain reaction (PCR) in selected calf samples. Overall, 81% of animals tested positive, suggesting widespread exposure and endemic circulation. The most prevalent serogroups were *L. Pomona* (27%), *L. Mozdok* (23%), and *L. Pyrogenes* (13%), accounting for over 78% of positive cases. Multiple serogroup reactivity was frequently observed, reflecting either repeated exposure or cross-reactivity. Infections were geographically widespread, with higher frequencies in extensive production systems. PCR confirmed active infection in calves, supporting the role of leptospirosis in acute disease and mortality events. Climatic conditions, particularly increased precipitation, likely contributed to environmental persistence and transmission. Overall, the findings suggest a complex epidemiological scenario involving multiple serogroups, environmental reservoirs, and host interactions in cattle from Alto Alentejo. However, the conclusions should be interpreted cautiously due to the relatively small sample size, limited geographic coverage, cross-sectional study design, and the restricted use of PCR analysis in selected samples. . These findings emphasize the need for improved surveillance, adapted vaccination strategies, and integrated control measures to mitigate the impact of bovine leptospirosis in southern Portugal.

Keywords: bovine leptospirosis; seroprevalence; MAT; PCR; epidemiology; Portugal; zoonosis

Resumo

A leptospirose bovina é uma doença zoonótica de distribuição mundial, com implicações significativas para a saúde animal, a saúde pública e a produtividade pecuária. O presente estudo teve como objetivo caracterizar a situação epidemiológica da leptospirose em bovinos da região do Alto Alentejo (Portugal), com especial enfoque na distribuição dos serogrupos e na dinâmica da infeção. Entre fevereiro e junho de 2025, foram amostrados 83 bovinos provenientes de 11 explorações pecuárias distribuídas por quatro concelhos. A análise serológica foi realizada através do teste de microaglutinação (MAT), enquanto a deteção molecular de ADN de *Leptospira* patogénica foi efetuada por reação em cadeia da polimerase (PCR) em amostras selecionadas de vitelos. No total, 81% dos animais apresentaram resultados seropositivos, sugerindo uma exposição generalizada e uma circulação endémica do agente. Os serogrupos mais prevalentes foram *L. Pomona* (27%), *L. Mozdok* (23%) e *L. Pyrogenes* (13%), representando, em conjunto, mais de 78% dos casos positivos. Foi frequentemente observada reatividade a múltiplos serogrupos, refletindo uma exposição repetida ou reações cruzadas. As infeções apresentaram uma distribuição geográfica alargada, com maior frequência em sistemas de produção extensivos. A PCR confirmou infeção ativa em vitelos, reforçando o papel da leptospirose em casos de doença aguda e episódios de mortalidade. As condições climáticas, em particular o aumento da precipitação, terão provavelmente contribuído para a persistência ambiental e a transmissão do agente. Globalmente, os resultados sugerem um cenário epidemiológico complexo, envolvendo múltiplos serogrupos, reservatórios ambientais e interações entre hospedeiros nos bovinos do Alto Alentejo. Contudo, as conclusões devem ser interpretadas com prudência, devido à dimensão relativamente reduzida da amostra, à cobertura geográfica limitada, ao desenho transversal do estudo e à utilização restrita da PCR apenas em amostras

selecionadas. Estes resultados reforçam a necessidade de melhorar os programas de vigilância, adaptar as estratégias de vacinação e implementar medidas integradas de controlo para mitigar o impacto da leptospirose bovina no sul de Portugal.

Palavras-chave: leptospirose bovina; seroprevalência; teste de microaglutinação; reação em cadeia da polimerase; epidemiologia; Portugal; zoonose.

Introduction

Leptospirosis is a neglected zoonotic disease caused by pathogenic spirochetes of the genus Leptospira, affecting a wide range of domestic and wild animals, as well as humans. In cattle, the disease has significant economic impact, being associated with reproductive disorders such as abortion, infertility, stillbirths, and reduced milk production (Bolin, 2003; Ellis, 2015). Chronic infection may also result in persistent renal colonisation, contributing to environmental contamination and sustained transmission cycles (Ko et al., 2009).

Despite its importance, leptospirosis remains underdiagnosed due to its frequently subclinical presentation and the limitations of laboratory diagnostics, particularly cross-reactivity in the microscopic agglutination test (MAT) (Levett, 2001; Picardeau, 2017). In Portugal, epidemiological data remain limited, especially in extensive production systems such as those predominant in the Alentejo region (Lilenbaum & Martins, 2014). Nevertheless, recent outbreaks and serological surveys in Portugal highlight the circulation of multiple pathogenic serogroups in cattle populations, particularly under extensive production systems and favorable climatic conditions (Paixão et al., 2025).Therefore, bovine leptospirosis is considered an endemic zoonotic disease in Portugal, with studies demonstrating the circulation of multiple pathogenic Leptospira serogroups among domestic and wild animal hosts, highlighting the epidemiological complexity and persistence of infection in Portuguese cattle production systems (Ferreira et al., 2020), which the present study aims to address.

Recent observations of increased calf mortality in the study area raised concerns regarding the possible involvement of leptospirosis, particularly given the increased susceptibility of young animals to acute infection (Haake & Levett, 2015). Understanding circulating serogroups is essential for effective disease control, particularly in regions where multiple transmission pathways coexist.

Therefore, this study aimed to characterise the epidemiology of bovine leptospirosis in the Alto Alentejo region, with a focus on seroprevalence, serogroup distribution, and infection dynamic.

Material and Methods

The study was conducted in the southeastern region of the Portalegre district, Portugal, encompassing the municipalities of Elvas, Campo Maior, Monforte, and Arronches. A total of 83 cattle from 11 farms were included (Figure 1). Written informed consent for sample collection, diagnostic procedures, and publication of anonymized data was obtained from all animal owners prior to the study. The farms included in the study were predominantly extensive production systems, with 10 farms dedicated to beef production involving different purebred and crossbred beef cattle breeds, and one semi-intensive dairy farm composed of Holstein–Friesian cattle. The studied population included animals from different age groups and was divided into two categories:

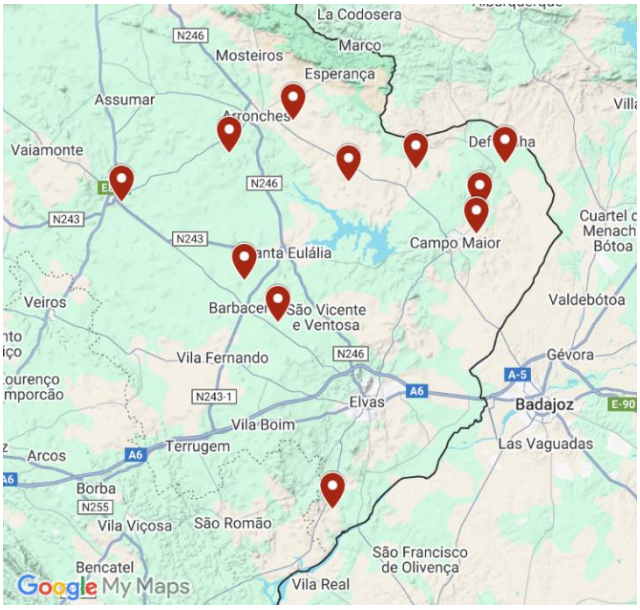

Figure 4. Representative map of the locations of the 11 farms in the municipalities of Elvas, Campo Maior, Monforte, and Arronches. Source: Google My Maps.

Group A: adult females, used for serological testing, aleatory selected from each herd; and Group B: seven calves, whose tissues were used for post-mortem analysis. Calves submitted to necropsy were between 1 and 6 months of age and had a history of sudden death or clinical signs compatible with acute disease.

Blood samples were collected from adult females by coccygeal venepuncture into plain tubes for serum separation. Samples were refrigerated immediately after collection and transported under controlled conditions to the laboratory.

In necropsied calves, urine samples were obtained by cystocentesis, and tissue samples (liver and kidney) were collected aseptically. All samples were stored at approximately 4°C until laboratory analysis.

Serological detection of Leptospira spp. was performed using the microscopic agglutination test (MAT), the reference method for leptospirosis diagnosis. A titre ≥1:100 was considered positive. Analyses were carried out at the National Institute for Agrarian and Veterinary Research (INIAV), Évora.

Statistical analyses were performed using Jamovi software (version 2.7). Data are presented as mean ± standard deviation (SD).

Results

A total of 83 cattle from 11 farms located in the Alto Alentejo region were tested for Leptospira spp. infection (Supplementary material, Figure 5). Overall, 67 animals (81%) were positive, indicating a high level of exposure within the studied population.

Table 1. Descriptive statistics of frequency of serovars/serogroups detected by the reference diagnostic method MAT in samples obtained from cattle in the Alto Alentejo region, titers reflected as 100, 200, 400 and 800.

	L. H	L. Po	L. Ca	L. M	L. G	L. Py	L. Co	L. B
N	17	41	8	36	6	20	3	22
Mean	106	161	100	156	100	100	100	123
SD	24.3	188	0.00	168	0.00	0.00	0.00	68.5
Min.	100	100	100	100	100	100	100	100
Max.	200	800	100	800	100	100	100	400

L. H = Hardjo; L. Po = Pomona; L. Ca = Canicola; L. M = Mozdok; L. G = Grippotyphosa; L. Py = Pyrogenes; L. Co = Copenhageni; L. B = Bratislava.

Spatially, infections were observed across all sampled municipalities (Elvas, Campo Maior, Monforte, and Arronches) (Supplementary material, figure 4), with no clear restriction to a geographic cluster. In Elvas, Monforte and Campo Maior, the most prevalent serovars were L. Pomona and L. Mozdok, while in Arronches, serovars L. Hardjo and L. Bratislava presented similar prevalencies as the previous ones (Figure 3).

Table 2. Descriptive statistics of frequency of Serovars/serogroups detected by the reference diagnostic method MAT in samples obtained from cattle in the Alto Alentejo region, in the four counties.

	County	L. H	L. P	L. Ca	L. M	L. G	L. Py	L. Co	L. B
N	Elvas	2	11	2	10	2	7	1	4
	Monforte	0	5	1	4	0	3	2	1
	Arronches	12	15	5	12	2	7	0	12
	Campo Maior	3	10	0	10	2	3	0	5
Mean	Elvas	100	255	100	100	100	100	100	225
	Monforte		100	100	100		100	100	100
	Arronches	100	100	100	100	100	100		100
	Campo Maior	133	180		300	100	100		100

Multiple serogroup reactivity was frequently observed within individual animals, with several cases showing simultaneous detection of two to five serogroups. This pattern may reflect co-exposure in the field, environmental contamination, or serological cross-reactivity inherent to the MAT technique.

Meteorological data from the study period showed marked fluctuations in precipitation, with periods of intense rainfall in January, March, and April 2025, exceeding the climatological average. These wet conditions, followed by warm temperatures and short dry periods, likely contributed to environmental persistence and dissemination of Leptospira spp., particularly in grazing areas and water-accumulating soils typical of extensive farming systems in the region.

From the seven cases of neonatal death in calves presenting lesions consistent with leptospirosis at necropsy, five presented lesions compatible with leptospirosis (Figure 2) supporting the involvement of pathogenic Leptospira in the acute infection and sudden mortality events observed.

Figure 25. Organs of calves after necropsy. Kidney (A) and liver (B) showing changes in color and consistency.

Discussion

The high seroprevalence observed in this study (81%) suggests that bovine leptospirosis is endemic in the Alto Alentejo region. This value exceeds those reported in several European studies, suggesting that local environmental and management conditions are particularly favourable for pathogen persistence and transmission (Costa et al., 2015). Extensive production systems, which dominate the region, likely contribute to this scenario by increasing exposure to contaminated water and soil.

The predominance of L. Pomona, L. Mozdok, and L. Hardjo and L. Bratislava represents a notable epidemiological pattern. While Pomona has been historically associated with livestock infections, the prominence of other serogroups may indicate evolving transmission dynamics involving wildlife reservoirs and multi-host interactions. This shift has also been reported in other endemic regions and reflects increasing ecological complexity (Vincent et al., 2019).

The detection of multiple serogroups within individual animals further supports the hypothesis of repeated exposure or co-circulation of strains. However, this pattern may also be influenced by cross-reactivity inherent to MAT, definitive association between Leptospira infection and the observed mortality events could not be established.

This study has several limitations that should be considered when interpreting the results. First, the relatively small sample size and the inclusion of farms from a restricted geographical area limit the generalisability of the findings to the wider cattle population in Portugal. Second, the cross-sectional design provides only a snapshot of infection status and does not allow assessment of temporal trends, transmission dynamics, or causal relationships. Third, serological diagnosis based on MAT may be influenced by cross-reactivity between serogroups and cannot distinguish between past exposure and active infection. Despite these limitations, the study provides valuable insights into the epidemiology of bovine leptospirosis in the Alto Alentejo region and supports the need for further longitudinal and large-scale investigations.

From a control perspective, the diversity of circulating serogroups and the apparent mismatch with commonly used vaccines represent a significant challenge. Current vaccination strategies may not provide adequate protection against locally prevalent strains, reinforcing the need for region-specific epidemiological data to inform control measures (Loureiro & Lilenbaum, 2020).

Conclusions

This study demonstrates that leptospirosis is apparently prevalent in cattle farms in southeastern Alentejo, with data compatible with endemic circulation and serogroup diversity. Overall, the findings suggest a complex epidemiological scenario involving environmental persistence, multiple reservoirs, and continuous exposure. Effective control of bovine leptospirosis in this context will require integrated approaches combining surveillance, vaccination, and improved management practices.

highlighting the need for complementary diagnostic approaches to improve epidemiological resolution (Levett, 2001).

Higher numbers of positive cases were recorded in extensive systems, where animals have greater environmental exposure and contact with potentially contaminated water sources.

Environmental conditions appear to play a central role in disease dynamics. The increased precipitation observed during the study period likely enhanced bacterial survival in soil and water, facilitating indirect transmission. This relationship between climatic factors and leptospirosis has been consistently demonstrated in both animal and human studies (Trueba et al., 2004).

Necropsy findings in calves were compatible with lesions that may occur in cases of acute leptospirosis, a disease to which young animals are particularly susceptible due to its potential for rapid systemic dissemination and fatal outcomes (Haake & Levett, 2015). Nevertheless, because no molecular testing was performed on calf tissues, a

The predominance of non-vaccine serogroups and the confirmation of active infections emphasise the need for improved surveillance and adapted control strategies. Future efforts should focus on integrating serological and molecular diagnostics, as well as developing vaccination programmes tailored to regional epidemiological profiles.

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Criptosporidiose Neonatal em Vitelos: Estudo Epidemiológico e Molecular no distrito de Portalegre

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Summary

Bovine cryptosporidiosis is a parasitic disease caused by protozoa of the genus *Cryptosporidium*, with *Cryptosporidium parvum* being an important agent of neonatal diarrhoea in calves. This study aimed to determine the occurrence of *Cryptosporidium* spp. in calves under one month of age from cattle farms in the district of Portalegre, Portugal, and to molecularly identify the species involved. Between February and May 2024, 55 faecal samples were collected from calves originating from 14 beef and dairy farms. Samples were analysed using parasitological methods and Ziehl–Neelsen staining, direct immunofluorescence and PCR. The overall prevalence of infection was 47% (26/55). Molecular analysis confirmed the predominance of *C. parvum*, while one sample was identified as *C. bovis*. These findings demonstrate the widespread occurrence of this parasite in cattle farms from the Alto Alentejo region and reinforce the importance of prophylactic measures and good management practices to reduce transmission.

Keywords: *Cryptosporidium parvum*; calves; prevalence; epidemiology; molecular diagnosis; Portugal.

Resumo

A criptosporidiose bovina é uma parasitose causada por protozoários do género *Cryptosporidium*, sendo *Cryptosporidium parvum* um importante agente de diarreia neonatal em vitelos. O presente estudo teve como objetivo determinar a ocorrência de *Cryptosporidium* spp. em vitelos com menos de um mês de idade provenientes de explorações bovinas do distrito de Portalegre, bem como identificar as espécies presentes através de métodos moleculares. Entre fevereiro e maio de 2024 foram recolhidas 55 amostras fecais de vitelos de 14 explorações de carne e leite. As amostras foram analisadas por técnicas parasitológicas e coloração de Ziehl-Neelsen, imunofluorescência direta e PCR. A prevalência global de infeção foi de 47% (26/55). A análise molecular confirmou a predominância de C. parvum, sendo igualmente identificada uma amostra positiva para C. bovis. Os resultados demonstram a elevada disseminação deste parasita em explorações bovinas do Alto Alentejo e reforçam a importância de medidas profiláticas e boas práticas de maneio para reduzir a transmissão da infeção.

Palavras-chave: *Cryptosporidium parvum*; vitelos; prevalência; epidemiologia; diagnóstico molecular; Portugal.

Introdução

A criptosporidiose é uma zoonose causada por protozoários do género *Cryptosporidium* spp., capazes de infetar uma vasta gama de hospedeiros animais. Trata-se de uma doença de distribuição mundial, uma vez que os oocistos esporulados, forma infetante do parasita, apresentam elevada resistência ambiental, distribuição ubiquitária e grande capacidade de disseminação (Louro et al., 2024). A transmissão ocorre principalmente por via fecal-oral, através do contacto direto com animais infetados ou indiretamente pela ingestão de água, alimentos ou fomites contaminados. Em bovinos, a infeção ocorre maioritariamente durante o primeiro mês de vida, período em que os vitelos apresentam maior suscetibilidade devido à imaturidade do sistema imunitário (Chen et al., 2023; Rodrigues, 2024).

Os vitelos neonatos constituem importantes reservatórios do parasita, estando frequentemente associados a quadros de diarreia neonatal e perdas produtivas significativas. Estudos realizados entre 2016 e 2018 em 32 explorações do distrito de Évora demonstraram que 55,7% dos vitelos com menos de um mês de idade eram positivos para *Cryptosporidium* spp., sendo 62,5% das explorações positivas para *C. parvum* (Martins, 2018). Em Espanha, foram reportadas prevalências de *C. parvum* em vitelos na ordem dos 17%, bem como a presença de *C. andersoni* em novilhos, embora com menor frequência (Diaz et al., 2021). Por sua vez, entre 2014 e 2017, um estudo realizado em vitelos de carne sintomáticos de

diferentes regiões do Norte Alentejano (Elvas, Terrugem, Monforte, Campo Maior e Arronches) revelou uma prevalência de 39% para *C. parvum* em 75 animais analisados (Santos & Minas, 2018).

O distrito de Portalegre apresenta uma importante atividade pecuária bovina, com explorações de carne e leite de elevada importância económica. Apesar da reconhecida relevância da criptosporidiose bovina nas explorações alentejanas, continuam a existir poucos estudos epidemiológicos focados em vitelos neonatos nesta região, bem como reduzida sensibilização dos produtores relativamente ao impacto da doença. O conhecimento atualizado da epidemiologia do parasita e a implementação de práticas adequadas de maneio são fundamentais para o estabelecimento de medidas preventivas eficazes e para a redução da disseminação da infeção, minimizando os impactos na saúde e bem-estar animal assim como na saúde pública.

O presente estudo teve como objetivos avaliar a ocorrência de *Cryptosporidium* spp. em vitelos com menos de um mês de idade, com ou sem sinais clínicos, provenientes de explorações bovinas do distrito de Portalegre, bem como identificar molecularmente as espécies presentes nas amostras positivas com maior carga parasitária.

Materiais e Métodos

Área de estudo e recolha de amostras

Entre fevereiro e maio de 2024, foram recolhidas 55 amostras fecais de vitelos com menos de um mês de idade, provenientes de 14 explorações bovinas localizadas em diferentes concelhos do distrito de Portalegre. As explorações incluídas no estudo foram selecionadas de forma oportunística, procurando assegurar uma distribuição geográfica representativa do distrito, no âmbito de intervenções sanitárias acompanhadas pelo médico veterinário assistente dessas explorações. A maioria das amostras foi obtida em onze explorações de bovinos de carne em regime extensivo, tendo sido igualmente incluídas três explorações leiteiras em regime intensivo.

As amostras fecais foram recolhidas por estimulação retal, de modo a minimizar contaminações ambientais, em vitelos com ou sem sinais clínicos de diarreia, recolhendo-se entre uma a sete amostras por exploração. Todas as amostras foram devidamente identificadas, acondicionadas sob refrigeração e transportadas para laboratório para posterior processamento. As colheitas foram realizadas com consentimento informado dos produtores e no âmbito de exames físicos e procedimentos clínicos de rotina, não tendo sido efetuados procedimentos invasivos adicionais aos animais.

Técnicas laboratoriais para deteção de oocistos

Para a deteção de oocistos de *Cryptosporidium* spp., as amostras fecais foram processadas e filtradas de acordo com o descrito em Louro et al. (2025), de forma a obter preparações destinadas à coloração por Ziehl-Neelsen modificada e à imunofluorescência direta. Posteriormente, procedeu-se à observação microscópica através da técnica de Ziehl-Neelsen (ZN) e da técnica de Imunofluorescência Direta (IFD), de acordo com metodologias previamente descritas na literatura (Louro et al., 2025).

Extração de DNA e PCR

Das amostras positivas para *Cryptosporidium* spp., foram selecionadas aquelas com maior carga parasitária para caracterização molecular. O DNA genómico foi extraído a partir das amostras fecais utilizando o kit NZY Tissue gDNA Isolation Kit (NZYTech, Portugal), seguindo as instruções do fabricante para amostras fecais. As amostras de DNA extraído foram armazenadas a -20 ºC até posterior análise molecular. A identificação das espécies foi realizada através de PCR seguida de análise de RFLP (*Restriction Fragment Length Polymorphism*), utilizando as enzimas de restrição *MboII* e *SspI*, de acordo com a metodologia descrita por Louro et al. (2024). Em todas as reações de PCR foi incluída uma amostra positiva de *C. parvum* como controlo positivo e água ultrapura como controlo negativo.

Análise estatística

A prevalência de infeção por *Cryptosporidium* spp. foi calculada com os respetivos intervalos de confiança a 95% (IC95%). A associação entre a presença de diarreia e a positividade das amostras foi avaliada através do teste exato de Fisher, considerando-se estatisticamente significativo um valor de $p < 0,05$.

Resultados

Foram analisadas 55 amostras fecais de vitelos com menos de um mês de idade, provenientes de 14 explorações do distrito de Portalegre. A presença de oocistos de *Cryptosporidium* spp. foi inicialmente avaliada através da técnica de ZN, tendo sido identificadas 20 amostras positivas (20/55). Posteriormente, todas as amostras foram analisadas por IFD, técnica que permitiu identificar 26 amostras positivas (26/55), incluindo as 20 amostras identificadas por ZN. A prevalência global de infeção por *Cryptosporidium* spp. foi de 47,3% (26/55; IC95%: 34,7–60,2%). Nos vitelos sem diarreia, a prevalência observada foi de 43,6% (17/39; IC95%: 29,3–59,0%), enquanto nos animais com diarreia foi de 56,3% (9/16; IC95%: 33,2–76,9%). A análise estatística não demonstrou associação significativa entre a presença de diarreia e a positividade para *Cryptosporidium* spp. (teste exato de Fisher; $p = 0,553$).

Das 14 explorações incluídas no estudo, 12 apresentaram pelo menos um animal positivo para *Cryptosporidium* spp., correspondendo a uma prevalência ao nível das explorações de 85,7%. A prevalência dentro das explorações variou entre 0% e 100%, observando-se diferenças entre explorações e concelhos do distrito de Portalegre. A distribuição dos resultados por exploração encontra-se apresentada na Tabela 1. A comparação entre os métodos laboratoriais utilizados demonstrou um maior número de deteções positivas pela técnica de IFD relativamente à coloração ZN. As amostras positivas por IFD foram classificadas de acordo com a carga parasitária observada por microscopia, considerando-se três níveis distintos em ampliação de 400x: N1 (1–5 oocistos por campo observado), N2 (<20 oocistos por campo) e N3 (>20 oocistos por campo). As amostras com maior carga parasitária foram selecionadas para posterior caracterização molecular. Para análise molecular, foram selecionadas nove amostras com elevada carga parasitária observada por IFD. A identificação molecular por PCR-RFLP confirmou a presença de *Cryptosporidium parvum* em oito amostras, enquanto uma amostra foi identificada como *C. bovis*. Relativamente à consistência fecal, observaram-se amostras positivas tanto em vitelos com diarreia como em animais sem sinais clínicos evidentes.

Tabela 1. Localização das explorações de bovinos de leite (L1 a L3) e de bovinos de carne (C4 a C14), total de amostras por exploração e resultados (positivos/negativos na cominação de ZN + IFD).

Exploração	Concelho	*n*	Resultado ZN/IFD	
			Negativo	Positivo
L1	Campo Maior	5	3	2
L2	Avis	2	0	2
L3	Nisa	2	0	2
C4	Avis	3	1	2
C5	Elvas	7	4	3
C6	Nisa	3	3	0
C7	Ponte de Sôr	1	0	1
C8	Sousel	1	0	1
C9	Monforte	5	3	2
C10	Sousel	7	5	2
C11	Arronches	5	5	0
C12	Alter Real	4	2	2
C13	Elvas	4	1	3
C14	Campo Maior	6	2	4
		55	29	26

A distribuição geográfica das explorações amostradas encontra-se representada na Figura 1, sendo igualmente apresentadas imagens representativas das observações microscópicas obtidas pelas técnicas de ZN e IFD (Figuras 2).

Figura 1. Distribuição geográfica das explorações e número de amostras recolhidos nos concelhos do distrito de Portalegre.

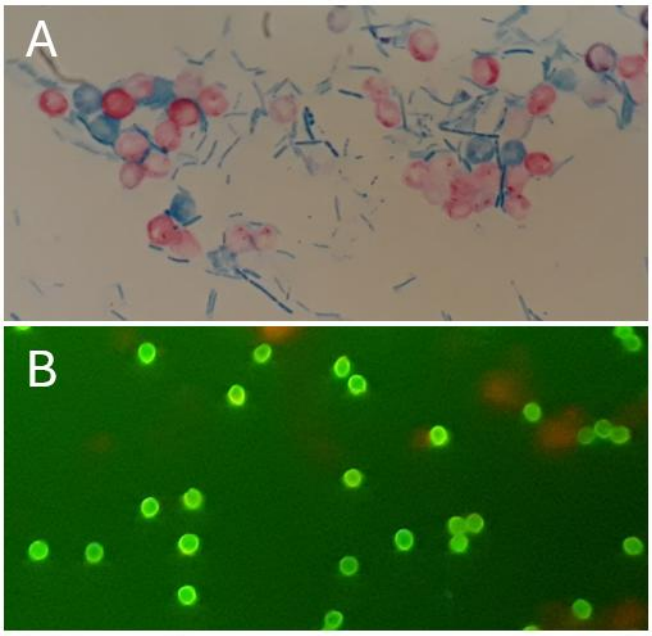

Figura 2. Oocistos de *Cryptosporidium* spp. em esfregaço fecal corado com Ziehl-Neelsen (A), ampliação 1000x e por Imunofluorescência Direta (B), ampliação 400x.

Discussão

Os resultados obtidos neste estudo demonstram uma elevada ocorrência de *Cryptosporidium* spp. em vitelos com menos de

um mês de idade no distrito de Portalegre, tendo sido observada uma prevalência global de 47% e positividade em 12 das 14 explorações estudadas. Estes dados reforçam a importância da criptosporidiose como uma das principais parasitoses associadas à diarreia neonatal bovina e estão de acordo com estudos prévios realizados noutras regiões do Alentejo e de Portugal. Apesar de a prevalência de *Cryptosporidium* spp. ter sido superior nos vitelos com diarreia, observaram-se igualmente elevadas taxas de positividade em animais sem sinais clínicos evidentes. Estes resultados reforçam a importância epidemiológica dos portadores subclínicos na disseminação ambiental do parasita nas explorações de bovinos.

A prevalência observada foi inferior à descrita por Martins (2018), que reportou 55,7% de vitelos positivos em 62,5% de explorações positivas no distrito de Évora, mas superior aos valores obtidos por Saramago (2019), que identificou uma prevalência global de 25,7% em explorações do Alentejo Central e Litoral. Por outro lado, Santos & Minas (2018) reportaram uma prevalência de 39% em vitelos sintomáticos do Norte Alentejano. Estas diferenças podem estar relacionadas com fatores como a dimensão da amostra, critérios de seleção dos animais, métodos de diagnóstico utilizados, idade dos vitelos e condições de maneio das explorações.

A elevada frequência de explorações positivas sugere uma ampla distribuição de *Cryptosporidium* spp. na região estudada, embora se tenha observado variabilidade na prevalência entre explorações, possivelmente relacionada com diferenças de maneio, higiene e pressão de infeção ambiental.

A técnica de Imunofluorescência Direta revelou maior sensibilidade diagnóstica relativamente à coloração de Ziehl-Neelsen, permitindo identificar um maior número de amostras positivas. Este resultado está de acordo com a literatura, que descreve a IFD como uma técnica mais sensível para deteção de oocistos, especialmente em infeções de baixa carga parasitária.

A caracterização molecular confirmou a predominância de *Cryptosporidium parvum*, principal espécie associada a diarreia neonatal em vitelos e reconhecida pelo seu potencial zoonótico. A identificação de *C. bovis* numa das amostras analisadas demonstra igualmente a circulação de outras espécies de *Cryptosporidium* em bovinos da região, embora aparentemente com menor prevalência. A presença de animais positivos sem sinais clínicos evidentes sugere ainda a possível importância epidemiológica de portadores subclínicos na manutenção e disseminação ambiental do parasita.

Entre as principais limitações deste estudo destacam-se o reduzido número de amostras analisadas por exploração, a ausência de avaliação de fatores de risco associados ao maneio e a subamostragem realizada para análise molecular, limitada às amostras com maior carga parasitária. Adicionalmente, o número reduzido de explorações leiteiras incluídas impossibilitou comparações robustas entre sistemas de produção intensivos e extensivos. Apesar destas limitações, o estudo fornece dados epidemiológicos relevantes sobre a ocorrência de *Cryptosporidium* spp. em vitelos do Alto Alentejo, contribuindo para o conhecimento da distribuição regional deste parasita em bovinos jovens.

Conclusão

O presente estudo demonstrou uma elevada ocorrência de *Cryptosporidium* spp. em vitelos com menos de um mês de idade no distrito de Portalegre. A análise molecular confirmou a predominância de *Cryptosporidium parvum*, principal espécie associada à criptosporidiose neonatal bovina, tendo sido igualmente identificada a presença de *C. bovis*. Os resultados obtidos reforçam a importância epidemiológica deste parasita em explorações de bovinos do Alto Alentejo, incluindo em animais sem sinais clínicos evidentes, que poderão desempenhar um papel relevante na disseminação ambiental da infeção, constituindo um risco elevado para os trabalhadores agrícolas em contacto com os animais infetados. A utilização combinada de técnicas microscópicas, imunológicas e moleculares revelou-se útil para a deteção e caracterização do agente. Entre as principais limitações do estudo destacam-se a reduzida dimensão amostral e a limitada subamostragem para análise molecular. Assim, serão necessários estudos futuros com maior número de explorações e animais, bem como avaliação de fatores de risco associados ao maneio e à transmissão da infeção, de forma a aprofundar o conhecimento epidemiológico da criptosporidiose bovina em Portugal.

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Estudo biométrico de morcegos insetívoros em Portugal Continental

Contributo para a monitorização epidemiológica da raiva em fauna selvagem

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Summary

Rabies is a viral zoonotic disease caused by *Lyssavirus*, with bats representing important natural reservoirs in Europe. Despite the absence of endemic rabies in Portugal, surveillance of wild bat populations remains epidemiologically relevant. This study aimed to characterize biometrically insectivorous bats admitted to wildlife rehabilitation centres in mainland Portugal and to contribute to passive rabies surveillance. A total of 103 bat carcasses from six species were collected between 2016 and 2023 from three rehabilitation centres. Body weight, body length and wing length were recorded and compared with reference values. Many individuals presented reduced body weight, suggesting compromised body condition, while body and wing measurements were generally within expected physiological ranges. Rabies screening by RT-PCR was performed on brain tissue from 20 individuals, with all samples testing negative. These findings contribute to the biometric characterization of bats in Portugal and highlight the importance of passive surveillance programmes for zoonotic diseases such as rabies.

Keywords: Rabies; passive surveillance; insectivorous bats; biometry;

Resumo

A raiva é uma doença viral zoonótica causada por vírus do género *Lyssavirus*, sendo os morcegos importantes reservatórios naturais na Europa. Apesar da ausência de raiva endémica em Portugal, a vigilância das populações de morcegos silvestres continua a ser epidemiologicamente relevante. O presente estudo teve como objetivo caracterizar biometricamente morcegos insetívoros admitidos em centros de recuperação de fauna selvagem em Portugal Continental e contribuir para a vigilância passiva da raiva. Foram analisados 103 cadáveres pertencentes a seis espécies de morcegos, recolhidos entre 2016 e 2023 em três centros de recuperação. Foram registados parâmetros biométricos, incluindo peso corporal, comprimento do corpo e comprimento das asas, posteriormente comparados com valores de referência. Muitos indivíduos apresentaram pesos inferiores aos valores fisiológicos esperados, sugerindo condição corporal comprometida, enquanto as restantes medições biométricas se encontravam geralmente dentro dos intervalos descritos para cada espécie. A pesquisa do vírus da raiva foi realizada por RT-PCR em tecido encefálico de 20 indivíduos no INIAV, tendo todas as amostras apresentado resultados negativos. Os resultados obtidos contribuem para a caracterização biométrica de morcegos em Portugal e reforçam a importância dos programas de vigilância passiva de doenças zoonóticas, como a raiva.

Palavras-chave: Raiva; vigilância passiva; morcegos insetívoros; biometri

Introdução

A raiva é uma doença viral zoonótica causada por vírus do género *Lyssavirus*, caracterizada por uma encefalite aguda progressiva que afeta o sistema nervoso central e que, após o aparecimento dos sinais clínicos, é quase invariavelmente fatal. (Maio, 2019) Apesar de se encontrar erradicada na maioria dos países da União Europeia, incluindo Portugal, continuam a ser reportados casos esporádicos, frequentemente associados à exposição a animais silvestres, nomeadamente morcegos, considerados importantes reservatórios naturais do vírus (Banyard, 2020; DGAV, 2020).

Na Europa, várias espécies de morcegos têm sido identificadas como hospedeiros de diferentes variantes de *Lyssavirus*, desempenhando um papel relevante na epidemiologia da doença. A transmissão ocorre

principalmente por contacto direto, através de mordeduras ou arranhões, embora a exposição a aerossóis em colónias densas também tenha sido descrita. Apesar do reduzido número de casos reportados em humanos, o risco potencial associado à interação com morcegos justifica a manutenção de sistemas de vigilância ativa, particularmente em espécies com maior proximidade a ambientes antropizados (Maio, 2019).

Em Portugal, a vigilância da raiva em fauna selvagem é limitada, sendo a testagem maioritariamente realizada em animais mortos ou suspeitos. Neste contexto, os centros de recuperação de fauna selvagem assumem um papel fundamental, não só na reabilitação de animais, mas também como fonte de informação epidemiológica relevante. Para além da deteção do vírus, a análise de parâmetros biométricos e do estado geral dos indivíduos permite avaliar condições de saúde, fatores de risco e possíveis causas de mortalidade (DGAV, 2020).

Assim, o presente estudo teve como objetivo avaliar a presença do vírus da raiva em morcegos provenientes de centros de recuperação em Portugal Continental, recorrendo a técnicas de biologia molecular, bem como caracterizar biometricamente os indivíduos analisados. Este trabalho pretende contribuir para o conhecimento da situação epidemiológica da raiva em morcegos em Portugal e para a monitorização do estado de saúde destas populações.

Materiais e métodos

O estudo foi conduzido entre fevereiro e junho de 2023, tendo sido analisados 103 cadáveres de morcegos recolhidos em Portugal Continental entre 2016 e 2023, provenientes de centros de recuperação de fauna selvagem (CRASM - Centro de Recuperação de Animais Selvagens de Montejunto, CERAS - Centro de Estudos e Recuperação de Animais Selvagens e RIAS - Centro de Recuperação e Investigação de Animais Selvagens). Foram incluídos indivíduos de diferentes espécies, idades e sexos, sendo excluídos os cadáveres em avançado estado de decomposição ou com condições inadequadas para análise. As necropsias foram realizadas em ambiente controlado, numa câmara de fluxo laminar, garantindo condições de biossegurança. Foi utilizado equipamento de proteção individual (bata, luvas e máscara), sendo empregue um novo par de luvas e lâmina de bisturi para cada indivíduo, de modo a evitar contaminações cruzadas. Antes da necropsia, os cadáveres foram descongelados à temperatura ambiente durante aproximadamente 10–15 minutos. Para cada espécime, procedeu-se à recolha de dados biométricos, incluindo peso corporal (com balança de precisão), comprimento do corpo e comprimento da asa (com régua graduada) (Figura 1). Foi ainda realizada a identificação da espécie e sexagem com base em características morfológicas externas.

Paralelamente, foi avaliado o estado geral dos indivíduos, nomeadamente condição corporal, presença de lesões, fraturas ou alterações macroscópicas. Durante a necropsia, foram recolhidos órgãos como fígado, pulmões e intestinos, quando viáveis, para estudos complementares. A cabeça completa de cada indivíduo foi separada e acondicionada para análise virológica. Todas as amostras foram devidamente identificadas e armazenadas a -20 °C até processamento laboratorial. A deteção do vírus da raiva foi realizada no laboratório de virologia através de técnicas moleculares. Após secção sagital das cabeças, foi recolhido tecido encefálico, seguido de extração de ácidos nucleicos e realização de RT-PCR, de acordo com procedimentos recomendados.

Figura 1 Medição da asa de *P.pipistrellus*

Resultados

Identificação taxonómica e por sexo

Foram analisados 103 indivíduos pertencentes a seis espécies de morcegos, provenientes de três centros de recuperação de fauna selvagem em Portugal (Tabela 1). A espécie mais representada foi *Pipistrellus pipistrellus* (Morcego-anão) seguida de *Eptesicus serotinus* (Morcego-hortelão-escuro) e *P. pygmaeus* (Morcego-pigmeu). A maioria dos indivíduos foi identificada quanto ao sexo, com predominância de machos, e distribuiu-se por diferentes classes etárias, incluindo crias, juvenis, sub-adultos e adultos.

Causas de admissão nos centros de recuperação

As causas de admissão dos morcegos nos centros de recuperação, registadas pelos respetivos centros de fauna selvagem, encontram-se apresentadas na Figura 2.

Tabela 1. Número de indivíduos por espécie e sexo

	Machos	Fêmeas	Ind.[1]	Total
Morcego-anão (*Pipistrellus pipistrellus*)	60	22	0	82
Morcego-hortelão-escuro (*Eptesicus serotinus*)	5	4	0	9
Morcego-pigmeu (*Pipistrellus pygmaeus*)	2	4	1	7
Morcego-de-peluche (*Miniopterus schreibersii*)	2	1	0	3
Morcego-rabudo (*Tadarida teniotis*)	0	1	0	1
Morcego-de-ferradura-pequeno (*Rhinolophus hipposideros*)	0	1	0	1

[1] Sexo indeterminado

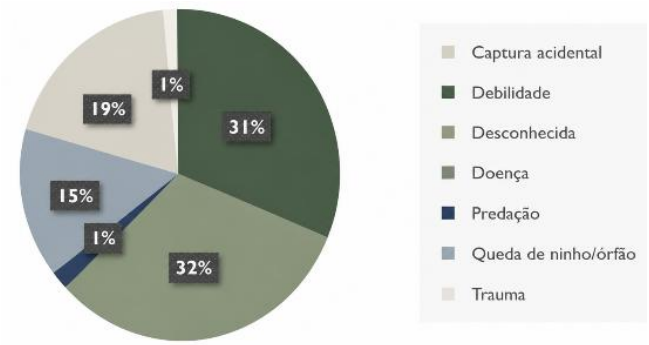

Figura 2. Causas de admissão dos morcegos

Caracterização biométrica

No que respeita aos dados biométricos, foram registados o peso corporal, comprimento do corpo e comprimento das asas (Tabela 2). De forma geral, os valores de comprimento encontraram-se dentro dos intervalos descritos para cada espécie. No entanto, observou-se que muitos indivíduos apresentavam pesos inferiores aos valores fisiológicos de referência, particularmente em *P. pipistrellus*. As médias e desvios padrão foram calculados por espécie, sexo e classe etária, evidenciando variações consistentes com o desenvolvimento dos indivíduos.

Resultados das necropsias

A avaliação macroscópica durante as necropsias revelou que vários indivíduos apresentavam condição corporal reduzida, bem como lesões externas, incluindo fraturas e danos nas membranas alares. A nível interno, foram observadas alterações como hemorragias torácicas e abdominais, presença de líquido livre e, em alguns casos, rutura do diafragma.

Deteção molecular de Lyssavirus

No âmbito da análise molecular para deteção do vírus da raiva, foram testados 20 indivíduos através de RT-PCR em amostras de tecido encefálico. Todos os exemplares analisados apresentaram resultados negativos, incluindo 19 indivíduos da espécie *P. pipistrellus* e um de *P. pygmaeus*. Estes resultados não evidenciam a presença do vírus da raiva nas amostras analisadas.

Tabela 2. Dados biométricos por espécie

Espécie		*n*	**Peso** (g) Média ± DP	**Corpo** [1] (cm) Média ± DP	**Asa** [1] (cm) Média ± DP
P. pipistrellus		**82**			
Sexo	♂	47	2,55 ± 1,17	5,6 ± 1,20	6,2 ± 2,31
	♀	35	2,58 ± 1,07	5,7 ± 1,13	6,1 ± 2,08
Idade	cria	22	1,83 ± 1,00	4,8 ± 1,03	4,2 ± 1,79
	juvenil	2	3,34 ± 0,10	6,4 ± 0,14	7,7 ± 0,99
	subadulto	1	2,18	6,5	8,2
	adulto	34	3,50 ± 0,70	6,6 ± 0,70	7,9 ± 1,30
P. pygmaeus		**7**			
Sexo	♂	2	4,80 ± 0,66	6,1	6,5
	♀	4	3,44 ± 1,13	7,2 ± 1,14	7,7 ± 0,40
Idade	subadulto	1	2,85	7,5	6,2
	adulto	5	4,10 ± 1,16	7,2 ± 1,17	7,5 ± 0,74
E. serotinus		**9**			
Sexo	♂	4	20,91 ± 4,58	13,0 ± 0,84	11,7 ± 1,86
	♀	5	20,83 ± 5,11	14,5 ± 0,46	11,8 ± 1,21
Idade	subadulto	3	20,81 ± 6,03	12,9 ± 0,71	11,3 ± 1,54
	adulto	6	20,91 ± 4,23	14,2 ± 0,57	12,0 ± 1,65
M. schreibersii		**3**			
Sexo	♂	2	10,80 ± 0,51	10,7 ± 0,21	12,5 ± 1,27
	♀	1	9,93	10,5	12,2
Idade	adulto	3	10,50 ± 0,51	10,6 ± 0,17	12,4 ± 0,92
T. teniotis		**1**			
Sexo	♀	1	22,98	12,5	13,8
R. hipposideros		**1**			
Sexo	♀	1	5,36	6,0	8,0

[1] Comprimento do corpo e da asa (membro anterior)

Discussão

Os resultados obtidos no presente estudo não evidenciaram a presença do vírus da raiva nos morcegos analisados, uma vez que todas as amostras testadas por RT-PCR apresentaram resultado negativo. Este achado está em consonância com o estatuto epidemiológico de Portugal como país indemne de raiva, onde não são registados casos autóctones desde 1961. No entanto, esta ausência de deteção não deve ser interpretada como ausência de risco, sobretudo considerando que, na Europa, os morcegos constituem reservatórios reconhecidos de diferentes variantes de *Lyssavirus*, nomeadamente o European Bat Lyssavirus (EBLV) (Banyard, 2020). Assim, a vigilância passiva, baseada na análise de indivíduos mortos ou debilitados, continua a ser fundamental para a deteção precoce de eventuais introduções ou circulação viral.

Importa salientar que apenas uma fração dos indivíduos recolhidos foi efetivamente testada para a presença do vírus, o que limita a robustez das conclusões epidemiológicas. A reduzida dimensão amostral ao nível da análise molecular, aliada ao caráter oportunista da recolha (centrada em animais admitidos em centros de recuperação), pode introduzir viés de seleção, não sendo representativa da população selvagem em geral. Adicionalmente, a degradação de algumas amostras devido ao tempo de armazenamento ou às condições de conservação pode influenciar a qualidade do material genético, afetando potencialmente a sensibilidade da técnica de deteção.

A distribuição das espécies analisadas revelou uma clara predominância de *Pipistrellus pipistrellus*, o que poderá refletir não só a sua elevada abundância e ampla distribuição geográfica em Portugal, mas também a sua maior proximidade a ambientes urbanos e periurbanos. Esta proximidade aumenta a probabilidade de deteção por parte da população e subsequente encaminhamento para centros de recuperação. Por outro lado, espécies como *Miniopterus schreibersii* ou *Rhinolophus hipposideros*, com estatutos de conservação mais sensíveis e hábitos ecológicos mais restritivos, encontram-se sub-representadas, o que pode limitar a compreensão global da dinâmica das populações de morcegos em Portugal (Rainho, 2013).

Relativamente às causas de ingresso nos centros de recuperação, a elevada proporção de casos classificados como “desconhecidos” constitui uma limitação importante, dificultando a identificação de fatores de risco específicos. Ainda assim, a debilidade e o trauma surgem como causas relevantes, sugerindo a influência de fatores ambientais e antrópicos (Razón, 2023). A debilidade, frequentemente associada a desidratação e desnutrição, poderá estar relacionada com alterações na disponibilidade de alimento, nomeadamente a diminuição de insetos (Delso, 2020), consequência do uso de pesticidas e das alterações climáticas. Por sua vez, os casos de trauma poderão resultar de colisões com infraestruturas humanas, como edifícios, veículos e parques eólicos, bem como de predação por animais domésticos, como gatos (Birdlife, 2021).

Os dados biométricos obtidos reforçam esta interpretação, evidenciando que muitos indivíduos apresentavam pesos inferiores aos valores fisiológicos de referência. Estes dados sugerem que uma proporção significativa dos morcegos admitidos nos centros de recuperação já se encontrava num estado comprometido antes da sua recolha. No entanto, é importante considerar que fatores como o tempo decorrido até à recolha, as condições de transporte e armazenamento, nomeadamente a congelação prolongada, podem também contribuir para a perda de massa corporal. Em contraste, os comprimentos corporais e das asas apresentaram-se, de forma geral, dentro dos intervalos esperados, indicando que não existem alterações estruturais significativas e que as diferenças observadas ao nível do peso são mais indicativas de alterações fisiológicas do que morfológicas.

As observações macroscópicas realizadas durante as necropsias, incluindo a presença de lesões traumáticas, hemorragias internas e alterações nos órgãos, corroboram a existência de múltiplos fatores de stress e mortalidade nestas populações (ICNF, 2017). Estes dados evidenciam o papel crucial dos centros de recuperação não apenas na reabilitação de indivíduos, mas também como plataformas de recolha de informação científica relevante para a monitorização da saúde das populações selvagens (Birdlife, 2021).

De um ponto de vista epidemiológico, este estudo contribui para o reforço do conhecimento sobre a ausência aparente de circulação do vírus da raiva em morcegos em Portugal. No entanto, considerando a mobilidade destas espécies, incluindo movimentos migratórios e a proximidade com regiões onde foram reportados casos de *Lyssavirus*, como Espanha, é fundamental manter e reforçar os sistemas de vigilância. A implementação de programas de monitorização mais abrangentes, incluindo amostragem ativa e aumento do número de indivíduos testados, poderá melhorar significativamente a capacidade de deteção precoce.

Em conclusão, apesar de não ter sido detetada a presença do vírus da raiva, os resultados obtidos evidenciam a importância de uma abordagem integrada que combine vigilância virológica com avaliação do estado de saúde dos indivíduos. Esta abordagem é essencial para compreender melhor os fatores que afetam as populações de morcegos e para desenvolver estratégias eficazes de conservação e saúde pública.

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