IV Encontro Anual AL4AnimalS 2025

15|16 maio
Grande Auditório

Faculdade de Medicina Veterinária
Universidade de Lisboa

PROGRAMA

ABSTRACTS

GABINETE DE GESTÃO DE CIÊNCIA

Fundação para a Ciência e a Tecnologia

IV Encontro Anual AL4AnimalS | 2025

15 e 16 maio | Grande Auditório da Faculdade de Medicina Veterinária da Universidade de Lisboa

Caros Colegas Investigadores e Estudantes,

Sejam bem-vindos ao IV Encontro Anual do Laboratório Associado para a Ciência Animal e Veterinária (AL4AnimalS)!

Na edição deste ano, trouxemos à discussão e reflexão temáticas da atividade de investigação e conteúdos centrais da ciência animal e veterinária. Na sessão da manhã de quinta-feira será debatida a temática da avaliação da ciência, no seu binómio Instituição – Cientista. Trata-se de um tema de grande atualidade e relevância, com impacto direto no estatuto e financiamento das instituições, e no reconhecimento e carreira do investigador. Trouxemos para esta discussão o External Advisory Board, o Conselho Nacional de Ciência, Tecnologia e Inovação e, as três Universidades anfitriãs do AL4AnimalS (ULisboa, UPorto e UTAD).

Na sessão da tarde de quinta-feira será debatida a cooperação entre instituições ao redor do conceito de Uma Só Saúde, discutindo as oportunidades de investigação e de consolidação de parcerias e consórcios. Para este efeito trouxemos à discussão as entidades reguladoras da saúde animal e humana, para apreciação dos desafios atuais e das necessidades de investigação para dar suporte às políticas públicas.

Na sessão da manhã de sexta-feira o AL4AnimalS voltou-se sobre si próprio, apreciando o trabalho do gabinete de gestão de projetos e, durante a assembleia geral, o relatório de atividades e financeiro, traçando o caminho futuro até à próxima avaliação do Laboratório Associado.

Estarão presentes 95 trabalhos em painel dos quais 55 concorrentes aos prémios de melhor trabalho de estudante de mestrado e de doutoramento.

Espero que este encontro seja palco de ampla convivência, partilha de ideias e consolidação de colaborações entre equipas e membros.

Luís Lopes da Costa

Diretor AL4AnimalS

IV Encontro Anual AL4AnimalS | 2025

15 e 16 maio | Grande Auditório da Faculdade de Medicina Veterinária da Universidade de Lisboa

PROGRAMA

15 MAIO

08:00 | Receção dos participantes e colocação de painéis científicos

09:30 | ABERTURA

Luís Costa | Diretor do Laboratório Associado AL4AnimalS

Luís Ferreira | Reitor da Universidade de Lisboa

SESSÃO 1 | AVALIAÇÃO DA CIÊNCIA E DO CIENTISTA

09:45 | *Avaliação da ciência: exemplo dos modelos dos EUA e Brasil*

Hércules de Moura | Senior scientist at the Atlanta Centre for Disease Control (EUA)

10:15 | *Avaliar para Valorizar a Investigação: Compromissos e Caminhos*

Cecília Rodrigues | Vice-Reitora para a Investigação e Inovação, Universidade de Lisboa

10:45 | *Networking, sessão de Painéis Científicos e café*

11:15 | Mesa Redonda: Avaliação do Cientista

Moderadora: Anabela Raymundo | Conselho Nacional de Ciência, Tecnologia e Inovação

Cecília Rodrigues | Vice-Reitora para a Investigação e Inovação, Universidade de Lisboa

Pedro Rodrigues | Vice-Reitor para a Investigação e Inovação, Universidade do Porto

Eduardo Rosa | Vice-Reitor para a Investigação, Universidade de Trás-os-Montes e Alto Douro

SESSÃO 2 | *ONE HEALTH*

14:30 | Desafios emergentes em saúde animal e oportunidades de investigação

Susana Pombo | Presidente do Conselho da Organização Mundial de Saúde Animal (OMSA); Diretora, Direção Geral de Alimentação e Veterinária

15:10 | Uma Só Saúde: Projeto Campus Torres Vedras

Joaquim Ferreira | Sub-Diretor, Faculdade Medicina ULisboa; Coordenador Projeto Torres Vedras

15:50 | Desafios emergentes em saúde humana e oportunidades de investigação

André Peralta Santos | Sub-Diretor, Direção Geral de Saúde

16:30 | *Networking, sessão de Painéis Científicos e café*

17:00 | Mesa Redonda: One Health

Moderador: Telmo Nunes | Professor da Faculdade de Medicina Veterinária - ULisboa

Susana Pombo

Joaquim Ferreira

18:00 | Encerramento da sessão

19:30 | Jantar: Restaurante São Bernardo

Fundação para a Ciência e a Tecnologia

IV Encontro Anual AL4AnimalS | 2025

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PROGRAMA

16 MAIO

09:30 | Gabinete de Gestão de Ciência e Oportunidades de Financiamento

Ana Ramalho | AL4AnimalS

09:30 | ABERTURA

Luís Costa | Diretor do Laboratório Associado AL4AnimalS

Luís Ferreira | Reitor da Universidade de Lisboa

10:00 | *Networking, sessão de Painéis Científicos e café*

11:00 | Assembleia Geral AL4AnimalS

12:30 | Entrega de Prémios e Encerramento

13:00 | Almoço buffet

Fundação para a Ciência e a Tecnologia

AL4ANIMALS / ASSOCIATE LABORATORY FOR ANIMAL AND VETERINARY SCIENCE

CIISA FMV - ULisboa

ceca

CECAV ANIMAL AND VETERINARY RESEARCH CENTRE

ABSTRACTS

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MESTRADOS

DOUTORAMENTOS

PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

fct Fundação para a Ciência e a Tecnologia

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

LT 2
Emergent Infectious Diseases and Zoonosis

MSc LT2.01	First Detection of Rickettsia Helvetica and Rickettsia Conorii Subsp. Raoultii in Ixodes Ricinus Ticks Collected from Domestic Dogs in Luxembourg
MSc LT2.02	Detection of Cryptosporidium Spp. in Bile of Small Ruminants
MSc LT2.03	Antimicrobial Activity of Electrolyzed Water Against Foodborne Path ogens
MSc LT2.04	Screening for the Ciliate Buxtonella Sulcata in Free-Ranging Dairy Cattle on Terceira Island, Azores Archipelago
MSc LT2.06	Molecular Prevalence of Equine Piroplasms in Horses in Mainland Portugal – Preliminary Results
MSc LT2.07	Targeted-Amplicon NGS for Blastocystis Sp. in Shepherd Dogs of Portugal Discriminates Co-Colonization with Multiple Zoonotic Subtypes

LT 3
Comparative and Translational Medicine and Biotechnology

MSc LT3.01	Can Urothelial Carcinomas in Dogs' Benefit from the use of Toceranib Phosphate (Palladia®) as the First Therapeutic Option?
MSc LT3.02	Changes in the Endometrial Proteome Induced by Eosinophils in the Jenny
MSc LT3.03	Ctrl+Alt+Degenerate: Creating Cell Models for Sanfilippo Syndrome
MSc LT3.04	Retrospective Evaluation of Toceranib Phosphate Use in Cats With Solid Tumours
MSc LT3.05	Development of novel immunotherapies for the treatment of canine lymphoma based on immunotoxins derived from Pseudomonas aeruginosa and Corynebacterium diphtheriae
MSc LT3.06	Development of Novel Immunotoxins Using Trastuzumab-Derived Single-Chain Variable Fragments for Breast Cancer Treatment
MSc LT3.07	Case Series of Diopathic Chronic Kidney Disease in Cats: A Retrospective Study
MSc LT3.08	Precision Medicine for Canine Lymphoma: Identification of Novel and Potent Cytotoxic Compounds for Conjugation into an Antibody-Drug Conjugate
MSc LT3.09	Evaluation of PD-L2 as a serological biomarker of feline mammary carcinoma

FIRST DETECTION OF RICKETTSIA HELVETICA AND RICKETTSIA CONORII SUBSP. RAOULTII IN IXODES RICINUS TICKS COLLECTED FROM DOMESTIC DOGS IN LUXEMBOURG

Guilherme Moreira[1], Rafaela S. S. Moreira[1], Floriane André das Neves[1], Vanessa Swiontek[1], Patrícia F. Barradas[2345], Sara Gomes-Gonçalves[1], João R. Mesquita[167]

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[5] 1H-TOXRUN, One Health Toxicology Research Unit, CESPU, Portugal
[6] Centro de Estudos de Ciência Animal (CECA), Universidade do Porto, Portugal
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Introduction: Tick-borne diseases caused by Rickettsia species are an emerging public health concern in Europe. While neighbouring countries have documented Rickettsia in ticks, Luxembourg lacks data on these pathogens in ticks from domestic animals, creating a critical knowledge gap.

Objectives: This study aimed to investigate the presence and molecular characteristics of Rickettsia spp. in *Ixodes ricinus* ticks collected from domestic dogs across Luxembourg.

Methodology: Between April 2023 and April 2024, 61 *I. ricinus* ticks were collected from dogs in southern Luxembourg. DNA was extracted and screened via PCR targeting the ompB gene, with positive samples further analysed using ompA and gltA markers. Phylogenetic analysis compared sequences with global strains.

Results: Overall *Rickettsia* prevalence: 4.9% (3/61 ticks)
Two ticks harboured *R. helvetica* (100% genetic match to strains from Siberia and the USA)
One tick carried *R. conorii* subsp. *raoultii* (100% identical to Turkish strains)
All positive ticks were morphologically confirmed as *I. ricinus*

Conclusions: This first report of pathogenic Rickettsia species in Luxembourg's dog-attached ticks highlights their potential role in zoonotic transmission. The findings align with expanding distributions of tick-borne diseases in Europe, warranting enhanced surveillance and public health measures.

Funding: This work was supported by the project PRR-C05-i03-I-000190—RumiRes: Epidemiological Surveillance and Awareness of Antimicrobial Resistance and Drug Residues in Small Ruminants in the Central Region.

FIRST DETECTION OF RICKETTSIA HELVETICA AND RICKETTSIA CONORII SUBSP. RAOULTII IN IXODES RICINUS TICKS COLLECTED FROM DOMESTIC DOGS IN LUXEMBOURG

Guilherme Moreira[1], Rafaela S. S. Moreira[1], Floriane André das Neves[1], Vanessa Swiontek[1], Patrícia F. Barradas[2,3,4,5] Sara Gomes-Gonçalves[1], João R. Mesquita[1,6,7]

Introduction

Tick-borne rickettsioses are emerging vector-borne diseases caused by *Rickettsia* species of the spotted fever group (SFG). These bacteria are transmitted primarily through tick bites and can infect both humans and animals. While countries across Europe have reported various *Rickettsia* spp., Luxembourg had remained unexamined for their presence in ticks parasitizing domestic dogs.

Materials and methods

Sample Collection
Timeframe: April 2023 – April 2024
Ticks (n=61) collected from domestic dogs in various parts of Luxembourg.
Morphologically identified as ***Ixodes ricinus.***

Molecular Detection
DNA extraction from tick specimens.
PCR targeting three genes:
ompA (outer membrane protein A)
ompB (outer membrane protein B)
gltA (citrate synthase gene)

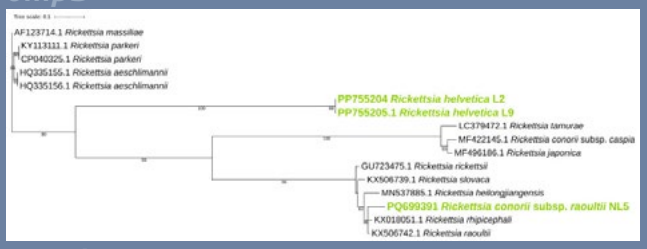
Results

Sequencing of PCR amplicons confirmed species identity through **BLASTn** comparisons:

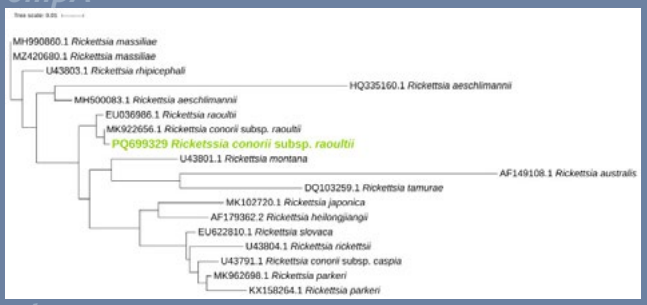
- ***R. helvetica*** sequences showed 100% identity with European strains (GenBank Accession: KY618935)
- ***R. conorii subsp. raoultii*** sequence had 99.8% identity with a strain reported in China (GenBank Accession: CP003340)

Phylogenetic analysis:
Performed using MEGA 11 with the Maximum Likelihood method (GTR + G + I model).
Sequences clustered within their respective clades with high bootstrap support (>90%):
- ***R. helvetica*** formed a tight cluster with central European isolates.
- ***R. conorii* subsp. *raoultii*** grouped within the *R. conorii* complex, closely related to Asian strains.

ompB



ompA



gltA

Discussion

- First evidence of ***R. conorii*** and ***R. helvetica*** in ticks from domestic dogs in Luxembourg.

- Dogs may act as sentinels for tick-borne pathogens relevant to public health.

- The findings suggest an underrecognized risk for rickettsioses in the region.

- **Climate change** and **increased host movement** may be facilitating the spread of these bacteria..

This work was supported by the project PRR-C05-i03-I-000190—RumiRes: Epidemiological Surveillance and Awareness of Antimicrobial Resistance and Drug Residues in Small Ruminants in the Central Region.

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PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 2 Emergent Infectious Diseases and Zoonosis

LT2.02

DETECTION OF *CRYPTOSPORIDIUM* SPP. IN BILE OF SMALL RUMINANTS

Jaqueline T. Bento [1], Sara Gomes-Gonçalves [1], Sérgio Santos-Silva [1], Andreia V. S. Cruz [1] and João R. Mesquita [1,2,3]

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INTRODUCTION: Cryptosporidiosis is a parasitic disease caused by protozoa of the genus *Cryptosporidium* spp., which can infect a wide variety of vertebrate hosts, including humans and livestock. The parasite completes its entire life cycle within a single host, primarily affecting the gastrointestinal tract and causing diarrhoeal disease, especially in young or immunocompromised individuals. Although intestinal cryptosporidiosis is well-documented, extraintestinal manifestations, such as involvement of the biliary system, have also been described, particularly in immunocompromised humans. In ruminants, *Cryptosporidium* is a known cause of neonatal diarrhoea but reports of its presence in the biliary tract are scarce, and its role in this context remains unclear.

AIMS: This study aimed to investigate, the presence of *Cryptosporidium* spp. in bile samples from small ruminants from a slaughterhouse in the central region of Portugal, contributing to a better understanding of the possible hepatobiliary involvement of this parasite in livestock.

METHODOLOGY: Bile samples were collected from small ruminants at a slaughterhouse located in central Portugal. A total of 75 pooled samples were analysed, each consisting of bile from five individual animals (375 individual samples in total). The DNA extraction followed a custom protocol. Molecular screening was conducted using PCR, and positive amplicons were purified and sequenced using the Sanger method. The obtained sequences were compared with reference sequences available in the NCBI database to confirm the identity of the parasite.

RESULTS: Out of the 75 pooled bile samples analysed, two tested positive for *Cryptosporidium* spp. DNA. The presence of the parasite was confirmed through Sanger sequencing, showing high similarity to *Cryptosporidium* spp. sequences deposited in GenBank. These findings indicate the occurrence of *Cryptosporidium* in the biliary system of small ruminants in Portugal.

CONCLUSIONS: This appears to be the first study documenting the presence of *Cryptosporidium* spp. in bile samples of small ruminants in Portugal. Further studies involving histopathological analyses and broader molecular screening are needed to assess the clinical significance and epidemiological implications of these findings.

ACKNOWLEDGMENTS/FUNDING: Sérgio Santos-Silva thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of his Ph.D work under the scholarship 2021.09461.BD contract through the Maria de Sousa-2021 program. Andreia V. S. Cruz thanks FCT for the financial support of his PhD work under the scholarship 2022.15408.BD contract through the Maria de Sousa-2022 program. This work was supported by the project PRR-C05-i03-I-000190 – RumiRes: Epidemiological Surveillance and Awareness of Antimicrobial Resistance and Drug Residues in Small Ruminants in the Central Region.

Detection of *Cryptosporidium* spp. in Bile of Small Ruminants

Jaqueline T. Bento [1], Sara Gomes-Gonçalves [1], Sérgio Santos-Silva [1], Andreia V. S. Cruz [1], João R. Mesquita [1, 2, 3]

[1] School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal.
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[3] Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente (ICETA).

Introduction

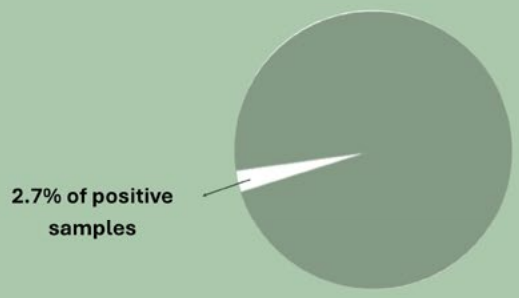
Cryptosporidiosis is a parasitic disease caused by protozoa of the genus *Cryptosporidium*, which can infect a wide variety of vertebrate hosts, including humans and livestock. The parasite completes its life cycle within a single host, primarily affecting the gastrointestinal tract and causing diarrhoeal disease.

While intestinal cryptosporidiosis is well-documented, extraintestinal forms—such as biliary involvement—have been reported, particularly in immunocompromised individuals. In ruminants, *Cryptosporidium* spp. is a known cause of neonatal diarrhoea, but its presence in the biliary tract is rarely reported, and its significance remains uncertain.

This study aimed to investigate, the presence of *Cryptosporidium* spp. in bile samples from small ruminants at a slaughterhouse in central Portugal, contributing to a better understanding of the possible hepatobiliary involvement of this parasite in livestock.

Results

Out of the 75 pooled bile samples analysed, **two pools tested positive for *Cryptosporidium* spp. DNA.** The presence of the parasite was confirmed through Sanger sequencing. These findings indicate the **presence of *Cryptosporidium* spp. in the biliary system** of small ruminants in Portugal.



Methodology

1. Sampling

Bile samples were collected from **small ruminants** at a slaughterhouse located in central Portugal. A total of **75 pooled samples** were analysed.

2. Molecular Analysis

The DNA extraction followed a custom protocol. Molecular screening was conducted using PCR, and positive amplicons were purified and sequenced using the Sanger method.

3. Sequencing Analysis

The obtained sequences were compared with reference sequences available in the NCBI database to confirm the identity of the parasite.

75 pools, each one consisting of bile from 5 individual animals - **375 individual samples** in total

Conclusion

This appears to be the first study documenting the presence of *Cryptosporidium* spp. in bile samples of small ruminants in Portugal. Further studies involving histopathological analyses and broader molecular screening are needed to assess the clinical significance and epidemiological implications of these findings.

Acknowledgments

Sérgio Santos-Silva thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of his Ph.D work under the scholarship 2021.09461.BD contract through the Maria de Sousa-2021 program. Andreia V. S. Cruz thanks FCT for the financial support of his PhD work under the scholarship 2022.15408.BD contract through the Maria de Sousa-2022 program.

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Supported by LA/P/0059/2020

ANTIMICROBIAL ACTIVITY OF ELECTROLYZED WATER AGAINST FOODBORNE PATHOGENS

Margarida Almeida[1], Márcio Moura Alves[2,3], José António Silva[2,3,4], Cristina Saraiva[2,3,4]

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INTRODUCTION: In recent years, there has been an increased interest in the application of alternative and sustainable disinfection technologies in the food industry in order to ensure food safety. Among several technologies, electrolyzed water (EW) has emerged as a promising candidate due to its broad-spectrum of antimicrobial activity and minimal environmental impact.

AIMS: Evaluate the antimicrobial efficacy of EW at two different pHs (6 and 4.5) and two inoculation times (0 and 10 min.) for pure cultures of *Listeria monocytogenes, Salmonella* Typhimurium, *Staphylococcus aureus* and *Escherichia coli.*

METHODOLOGY: EW was produced by the electrolysis of 5g NaCl/L using an AquaVolta® Water Tractor to obtain solutions of pH 6 and of 4.5. Subsequently, different concentrations of active chlorine (5, 25, 50 and 100 ppm) were prepared. Tubes with 9 mL of EW were inoculated with $5x10^7$ CFU/mL of each microorganism standardized by OD600. After the exposure time (0 and 10 min), the reaction was stopped with a tube containing 9 mL of sterile neutralisation solution (0.85% NaCl containing 0.5% $Na_2S_2O_3$). Then, decimal dilutions were prepared and spread-plated onto selective agar plates for enumeration. Distilled water and tap water were used as controls. The results were expressed as log CFU/mL.

RESULTS: In general, EW reduced microbial counts resulting in significant reductions or complete elimination at both pH levels. For *L. monocytogenes*, EW was most effective at concentrations of 50 to 100 ppm at both pH levels and exposure times, with reductions greater than 6 log. EW effectively reduced *S.* Typhimurium counts by >6 log at higher concentrations (50 and 100 ppm), with significant reductions at both pH levels and exposure times. For *E. coli*, significant reductions (>6 log) were observed at both pH levels, especially at 100 ppm and 10 min of exposure. For *S. aureus*, significant reductions were observed from 50 ppm at both pHs, with complete elimination after 10 min of exposure. At 100 ppm, total elimination occurred at time 0. Control treatments with distilled water and tap water showed no antimicrobial activity.

CONCLUSION: EW has been shown to be particularly effective from 25 ppm, with total microbial reduction observed at 50 and 100 ppm after 10 min of exposure at pH 4.5 and 6 for all tested microorganisms. These findings highlight its potential as a safe and sustainable sanitising alternative for microbiological control.

ACKNOWLEDGMENTS/FUNDING: This work was supported by the projects UIDB/00772/2020 (doi:10.54499/UIDB/00772/2020) and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Márcio Moura-Alves would also like to thank the financial support by a Ph.D. fellowship UI/BD/150835/2021 supported by FCT (doi:10.54499/UI/BD/150835/2021). The authors would like to thank Ana Leite, Kamila Soares and Helena Santos for their valuable contributions.

ANTIMICROBIAL ACTIVITY OF ELECTROLYZED WATER AGAINST FOODBORNE PATHOGENS

Margarida Almeida[1], **Márcio Moura Alves**[2,3], José António Silva[2,3,4], Cristina Saraiva[2,3,4]

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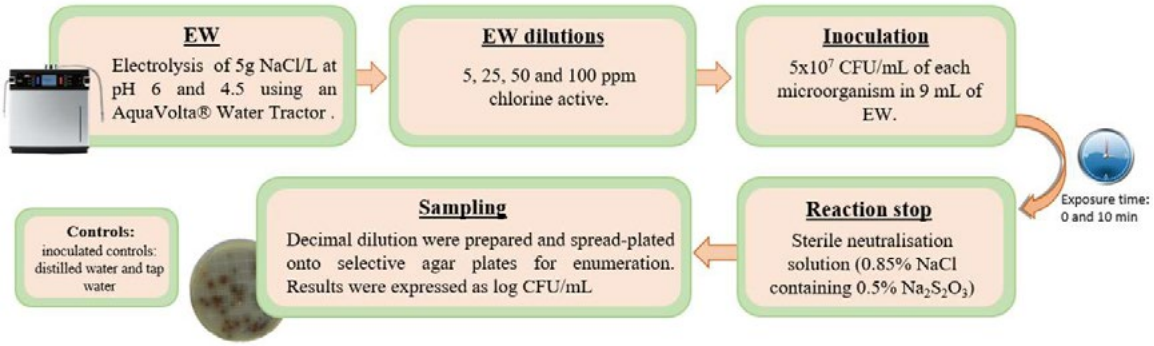
Introduction

Food industry is increasingly adopting news sustainable disinfection methods. Electrolyzed water (EW) is a promising solution, offering broad antimicrobial effectiveness with minimal environmental impact.

Aims

This study aimed to evaluate the antimicrobial activity of EW at two different pHs (6 and 4.5) and two exposure times (0 and 10 min.) for pure cultures of *Listeria monocytogenes, Salmonella* Typhimurium, *Staphylococcus aureus* and *Escherichia coli.*

Materials and Methods



Results

Table A– Counts (mean ± standard deviation, log CFU/mL) of *Listeria monocytogenes*

	L. monocytogenes					
	pH 4.5			pH 6		
Treatment	0	10	*P*	0	10	*P*
5	7.37±0.19^{a}	6.68±0.31^{b}	ns.	6.17±0.25^{a}	6.31±0.18^{a}	ns.
25	2.88±0.48^{b}	0.00±0.00^{c}	*	1.21±1.71^{b}	0.89±1.26^{b}	ns.
50	0.00±0.00^{c}	0.00±0.00^{c}	ns.	0.00±0.00^{b}	0.00±0.00^{b}	ns.
100	0.00±0.00^{c}	0.00±0.00^{c}	ns.	0.00±0.00^{b}	0.00±0.00^{b}	ns.
DW	7.11±0.30^{a}	7.34±0.11^{a}	ns.	6.74±0.13^{a}	7.02±0.14^{a}	ns.
TW	7.10±0.45^{a}	7.67±0.21^{a}	ns.	7.21±0.29^{a}	7.84±0.53^{a}	ns.
P	***	***		***	***	

Table B– Counts (mean ± standard deviation, log CFU/mL) of *Salmonella* Typhimurium

	S. Typhimurium					
	pH 4.5			pH 6		
Treatment	0	10	*P*	0	10	*P*
5	7.40±1.13^{a}	6.48±0.32^{b}	ns.	7.45±0.08^{a}	6.12±0.05^{b}	**
25	1.65±2.33^{b}	3.54±0.72^{c}	ns.	0.00±0.00^{b}	0.00±0.00^{c}	ns.
50	1.33±1.88^{b}	0.00±0.00^{d}	ns.	0.00±0.00^{b}	0.00±0.00^{c}	ns.
100	0.65±0.92^{b}	0.00±0.00^{d}	ns.	0.50±0.71^{b}	0.00±0.00^{c}	ns.
DW	7.89±0.05^{a}	8.12±0.18^{a}	ns.	7.50±0.14^{a}	7.35±0.04^{a}	ns.
TW	8.14±0.15^{a}	7.74±0.34^{ab}	ns.	7.51±0.40^{a}	7.41±0.52^{a}	ns.
P	**	***		***	***	

Table C– Counts (mean ± standard deviation, log CFU/mL) of *Staphylococcus aureus*

	S. aureus					
	pH 4.5			pH 6		
Treatment	0	10	*P*	0	10	*P*
5	7.71±0.26^{a}	7.66±0.58^{a}	ns.	6.03±0.42^{b}	6.92±0.10^{b}	ns.
25	4.43±0.70^{ab}	5.05±0.55^{b}	ns.	3.21±0.23^{c}	2.69±0.00^{c}	ns.
50	3.56±1.14^{b}	0.00±0.00^{c}	*	3.45±0.21^{c}	0.00±0.00^{c}	**
100	1.31±1.85^{b}	0.00±0.00^{c}	ns.	0.00±0.00^{d}	0.00±0.00^{d}	ns.
DW	8.17±0.28^{a}	7.77±0.03^{a}	ns.	7.87±0.11^{a}	7.94±0.05^{a}	ns.
TW	8.09±0.08^{a}	7.78±0.21^{a}	ns.	7.90±0.40^{a}	7.91±0.38^{a}	ns.
P	**	***		***	***	

Table D– Counts (mean ± standard deviation, log CFU/mL) of *Escherichia coli*

	E. coli					
	pH 4.5			pH 6		
Treatment	0	10	*P*	0	10	*P*
5	6.77±0.30^{a}	6.09±0.30^{a}	ns.	5.30±0.08^{ab}	5.56±0.33^{b}	ns.
25	2.78±0.90^{b}	0.42±0.60^{b}	ns.	1.45±2.06^{bc}	1.65±0.75^{c}	ns.
50	2.51±0.08^{b}	0.00±0.00^{b}	***	1.25±1.77^{bc}	0.00±0.00^{d}	ns.
100	0.00±0.00^{c}	0.00±0.00^{b}	ns.	0.00±0.00^{c}	0.00±0.00^{d}	ns.
DW	7.08±0.00^{a}	7.03±0.03^{a}	ns.	7.55±0.05^{a}	7.66±0.05^{a}	ns.
TW	6.90±0.06^{a}	6.99±0.08^{a}	ns.	7.54±0.04^{a}	7.32±0.13^{a}	ns.
P	***	***		**	***	

DW – distilled water; TW – tap water; ns., non-significant. For exposure times, means with different letters (lowercase) differ significantly, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

EW showed high antimicrobial efficacy against all microorganisms. Reductions greater than 6 log CFU/mL were observed at 50–100 ppm at both tested pHs (4.5 and 6). The complete elimination of microorganisms occurred especially after 10 minutes of exposure. Control treatments with distilled water and tap water showed no antimicrobial activity.

Conclusion

EW has been shown to be particularly effective from 25 ppm, with total microbial reduction observed at 50 and 100 ppm after 10 min of exposure at pH 4.5 and 6 for all tested microorganisms. These findings highlight its potential as a safe and sustainable sanitising alternative for microbiological control.

ACKNOWLEDGMENTS/FUNDING: This work was supported by the projects UIDB/00772/2020 (doi:10.54499/UIDB/00772/2020) and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Márcio Moura-Alves would also like to thank the financial support by a Ph.D. fellowship UI/BD/150835/2021 supported by FCT (doi:10.54499/UI/BD/150835/2021). The authors would like to thank Ana Leite, Kamila Soares and Helena Santos for their valuable contributions.

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 2 Emergent Infectious Diseases and Zoonosis

LT2.04

SCREENING FOR THE CILIATE BUXTONELLA SULCATA IN FREE-RANGING DAIRY CATTLE ON TERCEIRA ISLAND, AZORES ARCHIPELAGO

Mário Ribeiro[1], Sara Gomes-Gonçalves[1], Alexandra Silva[1], Guilherme Moreira[1], Eric Viscogliosi[2], Magali Chabé[2], and João Rodrigo Mesquita[1,3,4]

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INTRODUCTION: Buxtonella sulcata, a ciliate protist historically regarded as a commensal in cattle gastrointestinal (GI) tracts, has recently been implicated as a potential pathogen due to molecular advances. Despite its cysts' morphological similarity to Balantioides coli, its pathogenic role remains understudied. This study aimed to assess the prevalence and genetic diversity of Bu. sulcata in asymptomatic dairy cattle on Terceira Island, Azores, where cattle farming is a key economic activity.

OBJECTIVES: The primary objective was to determine the presence and genetic variability of Bu. sulcata in free-ranging dairy cows using molecular tools, while evaluating its potential implications for animal health and productivity.

METHODOLOGY: A total of 116 faecal samples were collected from healthy adult Holstein-Friesian cows across Terceira Island. DNA was extracted and subjected to PCR targeting the ITS1–5.8S–ITS2 rRNA region. Positive amplicons were sequenced and phylogenetically analysed alongside global reference strains.

RESULTS: PCR detected Bu. sulcata in 49.1% (57/116) of samples, with 12 distinct genetic sequences identified. Phylogenetic analysis revealed close matches to isolates from cattle in Belgium (98.8–99.76% identity) and India (99.76–100%). Geographic distribution analysis showed sequence-specific clustering, with some variants restricted to southern parishes (e.g., V60) while others (e.g., V3) were widespread.

CONCLUSIONS: This study confirms a high prevalence of Bu. sulcata in Azorean dairy cattle, with significant genetic diversity. The absence of clinical signs suggests commensalism or subclinical infection, warranting further investigation into its pathogenicity, transmission dynamics, and impact on herd health. Future research should focus on calves, co-infections, and zoonotic potential, given the ciliate's detection in primates.

fct Fundação para a Ciência e a Tecnologia

SCREENING FOR THE CILIATE *BUXTUNELLA SULCATA* IN FREE-RANGING DAIRY CATTLE ON TERCEIRA ISLAND , AZORES ARCHIPELAGO

Mário Ribeiro[1], Sara Gomes-Gonçalves[1], Alexandra Silva[1], Guilherme Moreira[1], Eric Viscogliosi[2], Magali Chabé[2], João Rodrigo Mesquita[1]

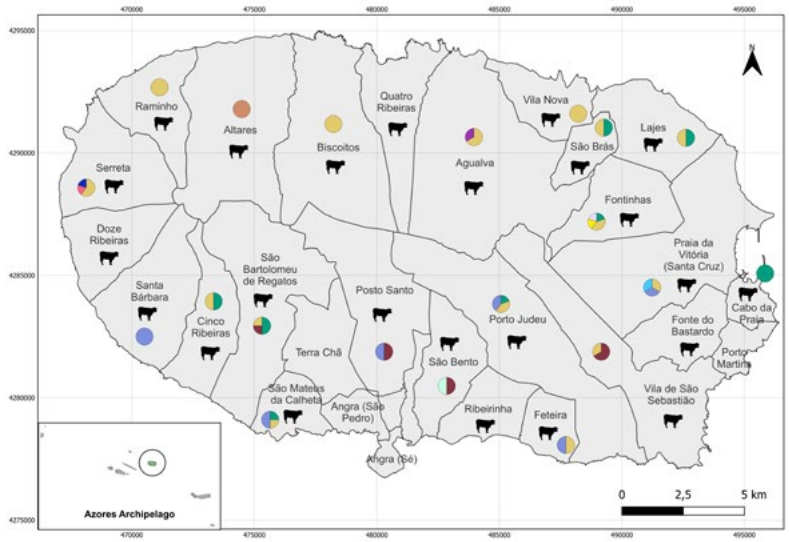
INTRODUCTION

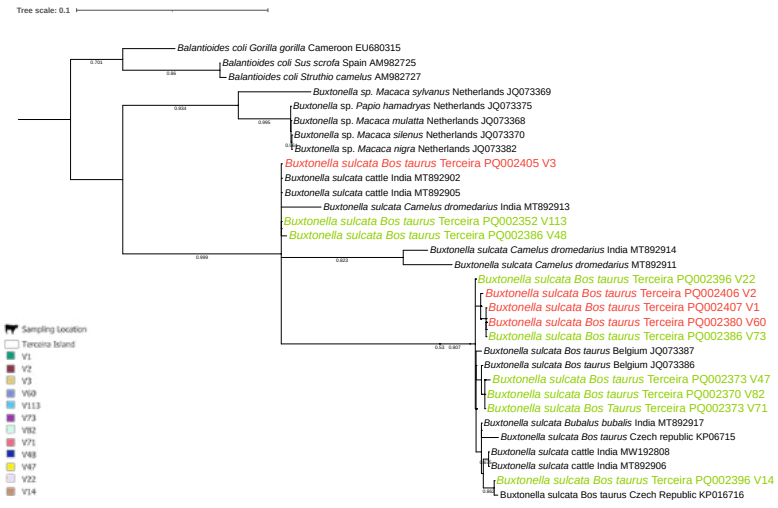
Buxtonella sulcata is a ciliate protist traditionally considered a commensal organism in the bovine gastrointestinal tract. However, morphological similarities with the pathogenic ***Balantioides coli*** have led to reevaluation of its role, especially with advancements in molecular identification techniques.

METHODS

- Fecal samples **(n=116)** were collected from clinically healthy, free-ranging Holstein-Friesian dairy cows on Terceira Island. DNA was extracted from ~200 mg of each sample using the NZY Tissue gDNA Isolation Kit.

- A semi-nested PCR targeting the **ITS1–5.8S–ITS2** rRNA region was performed for ***Bu. sulcata*** detection. Positive amplicons were sequenced, and sequences were analyzed using **BLASTn** and aligned for phylogenetic comparison.





RESULTS

- 49.1% (57/116) of samples tested positive for ***B. sulcata.***

- Identified 12 distinct genetic sequences among the positive

CONCLUSIONS

This study revealed a high prevalence (49.1%) of *Buxtonella sulcata* in asymptomatic, free-ranging dairy cattle on Terceira Island, suggesting that this ciliate is commonly present in the bovine gut microbiota. The detection of 12 distinct genotypes based on ITS1–5.8S–ITS2 rRNA sequences indicates considerable genetic diversity within the local population. These findings raise questions about the biological significance of ***Bu. sulcata*** in cattle, particularly its potential role as an **opportunistic pathogen or as an indicator of gut health.** Molecular screening proved essential for accurate detection, highlighting the need to integrate such approaches in future epidemiological and veterinary parasitology studies. Further investigations are warranted to assess the pathogenicity, transmission dynamics, and possible zoonotic implications of ***Bu. sulcata.***

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PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 2 Emergent Infectious Diseases and Zoonosis

LT2.06

MOLECULAR PREVALENCE OF EQUINE PIROPLASMS IN HORSES IN MAINLAND PORTUGAL – PRELIMINARY RESULTS

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INTRODUCTION: Equine piroplasmosis, caused by the tick-borne hemoprotozoans Babesia caballi and Theileria equi, impacts animal health and the economy by impairing athletic performance, restricting movement, and causing mortality. Although both pathogens may coexist in endemic areas, T. equi is generally more pathogenic and persistent. Disease epidemiology is influenced by tick distribution, management practices, and climate.

AIMS: To investigate the molecular prevalence of Babesia caballi and Theileria equi in horses from mainland Portugal.

METHODOLOGY: Blood samples from 339 equids were collected using a stratified sampling strategy, based on the registered horse population across NUTS II regions. Genomic DNA was extracted and screened for B. caballi and T. equi by PCR targeting the 18s rRNA gene. Positive amplicons were randomly selected for sequencing, and phylogenetic analyses conducted for species confirmation.

RESULTS: Phylogenetic analysis confirmed Theileria equi infection in 44.1% of horses, with the highest prevalence observed in the Setúbal Peninsula (50.9%). DNA of Babesia caballi was not detected.

CONCLUSIONS: Theileria equi appears to be the predominant species infecting equids in mainland Portugal. Giving the challenges that piroplasmosis presents to international horse movement and trade, controlling the disease in endemic countries like Portugal is essential to maintain market access.

ACKNOWLEDGMENTS/FUNDING: FMV-ULusófona Exploratory Project 2023-24 (Acronym: EquiVBD).

Molecular prevalence of equine piroplasms in mainland Portugal– preliminary results

PATRÍCIA PEREIRA[1], MARIA MARGARIDA ALVES[1,2,3], JOANA SIMÕES[1,2,3], RICARDO PARREIRA[4], CARLA MAIA[4], MÓNICA NUNES[5], CÁTIA MARQUES[1,2,3,6], JOSÉ MEIRELES[6], MÁRIO COTOVIO[1,3,7], ANDRÉ PEREIRA[1,2,3,4,8], DAVID W. RAMILO[1,2,3]

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Introduction

Equine piroplasmosis (EP) is a tick-borne disease caused by the haemoprotozoan parasites *Theileria equi* and *Babesia caballi*, affecting equids worldwide and posing significant challenges to equine health and international movement [1,2]. Clinical signs include fever, anaemia, lethargy, icterus, and in severe cases, haemoglobinuria and in severe cases, infection may result in the death of the animal [2]. *T. equi* is generally more pathogenic and persistent than *B. caballi*, often resulting in chronic infections that complicate disease management [2,3]. The epidemiology of EP is influenced by the distribution of tick vectors, management practices, and climatic factors, which together shape the risk of infection and parasite persistence [4]. In Portugal, EP is considered endemic, with variable prevalence reported across regions, but comprehensive molecular data on current prevalence and parasite distribution are limited [1,3]. This study aims to assess the molecular prevalence of *T. equi* and *B. caballi* in horses across mainland Portugal, providing updated insights into their epidemiology.

Objectives

To investigate the molecular prevalence of *Babesia caballi* and *Theileria equi* in horses from mainland Portugal.

Methodology

Stratified sampling strategy using NUTS II regions

Results

Phylogenetic analysis confirmed *Theileria equi* infection in 44.1% of horses (Figure 1), with the highest prevalence observed in the Setúbal Peninsula (50.9%) (Figure 2).

DNA of *Babesia caballi* was not detected.

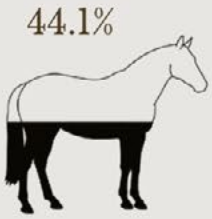

Fig. 1- Molecular prevalence of Theileria equi infection in horses across mainland Portugal

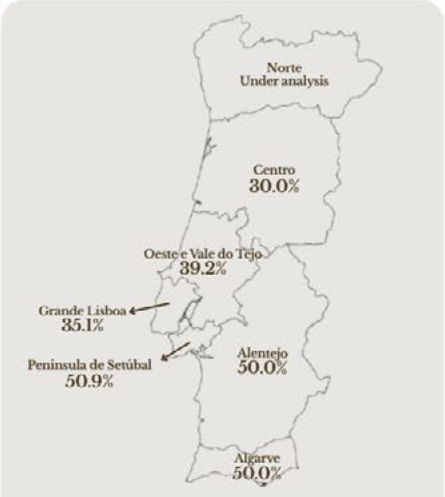

Fig. 2- Molecular prevalence of Theileria equi across the NUTS II regions

Conclusions

Theileria equi appears to be the predominant species infecting equids in mainland Portugal.

Giving the challenges that piroplasmosis presents to international horse movement and trade, controlling the disease in endemic countries like Portugal is essential to maintain market access.

Acknowledgments/Funding

FMV-ULusófona Exploratory Project 2023-24 (Acronym: EquiVBD).

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Bibliografia:

[1] Fuehrer H-P, Alho, A. M., Kayikoi, F.N, Barogh, B.S., Rosa, H, Tomás, J., Rocha, H, Harl, J. & Madeira de Carvalho, L. (2020). Survey of zoonotic and non-zoonotic vector-borne pathogens in military horses in Lisbon, Portugal. Frontiers in Veterinary Science. doi: 10.3389/fvets.2020.591943.

[2] Onyiche, T. E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X. & Thekisoe, O. (2019). A review on equine piroplasmosis: Epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. International Journal of Environmental Research and Public Health, 16(10):1736. doi: 10.3390/ijerph16101736.

[3] Ribeiro, A. J., Cardoso, L., Maia, J. M., Coutinho T. & Cotovio, M. (2013). Prevalence of Theileria equi, Babesia caballi, and Anaplasma phagocytophilum in horses from the north of Portugal. Parasitology Research, 112(7):2611-7. doi: 10.1007/s00436-013-3423-9.

[4] Scoles, G. A. & Ueti, M. W. (2015). Vector ecology of equine piroplasmosis. Annual Review of Entomology, 60:561-80. doi: 10.1146/annurev-ento-010814-021110.

TARGETED-AMPLICON NGS FOR *BLASTOCYSTIS* SP. IN SHEPHERD DOGS OF PORTUGAL DISCRIMINATES CO-COLONIZATION WITH MULTIPLE ZOONOTIC SUBTYPES

Sara Gomes-Goncalves [1], Maria João Feiteiro [2], Guilherme Moreira [1] , Rita Cruz [2,3] , Fernando Esteves [2,4], Helena Vala [2,4,5] and João R. Mesquita [1,6,7,]

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INTRODUCTION: *Blastocystis* sp. is a globally distributed intestinal protist, infecting 1–2 billion people. The parasite shows genetic diversity, with 44 subtypes (STs) identified. Transmission is primarily fecal-oral through contaminated food or water. Notably, certain subtypes are now known to be of zoonotic origin.

AIMS: This study aimed to assess the prevalence and genetic diversity of *Blastocystis* sp. in shepherd dogs from central Portugal, focusing on circulating subtypes, mixed infections, and zoonotic potential.

METHODOLOGY: Fecal samples were collected from 50 shepherd dogs across seven municipalities of central Portugal, 2024. DNA extraction was followed by SYBR Green-based qPCR targeting the SSU rRNA gene. Positive samples were sequenced using Sanger and mixed infections by Oxford Nanopore technologies. Bioinformatics analysis included subtyping, clustering, and phylogenetic analysis with MAFFT and IQ-TREE.

RESULTS: *Blastocystis* sp. was detected in 60% (30/50) samples. Zoonotic ST1–ST4 predominated, with ST4 (n = 13) most frequent. One ST14 case was also detected. Nanopore sequencing revealed mixed infections in four of five suspected samples. Phylogenetic analysis confirmed subtype assignments.

CONCLUSIONS: This study is the first to characterize *Blastocystis* sp. in Portuguese shepherd dogs, showing high prevalence of zoonotic subtypes. These dogs may serve as reservoirs for zoonotic transmission due to their close contact with livestock and humans, highlighting the need for further surveillance.

ACKNOWLEDGMENTS/FUNDING: This work is supported by Project No. PRR-C05-i03-I-000190 “RumiRes”, National Funds by FCT – Portuguese Foundation for Science and Technology, under the projects UIDB/00681/2020 (CERNAS; https://doi.org/10.54499/UIDB/00681/2020), UI/04033, LA/P/0126/2020 (CITAB; https://doi.org/10.54499/LA/P/0126/2020) and GHTM UID/04413/2020

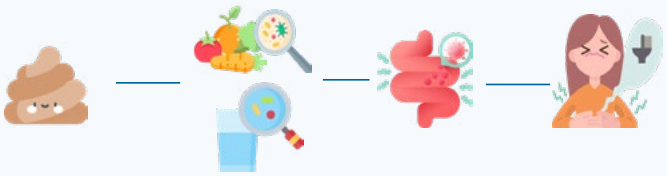
Targeted-Amplicon NGS for *Blastocystis* sp. in Shepherd Dogs of Portugal Discriminates Co-colonization with Multiple Zoonotic Subtypes

Sara Gomes-Gonçalves[1], Maria João Feiteiro[2], Guilherme Moreira [1], Rita Cruz [2,3], Fernando Esteves [2,4], Helena Vala [2,4,5] and João R. Mesquita [1,6,7,]

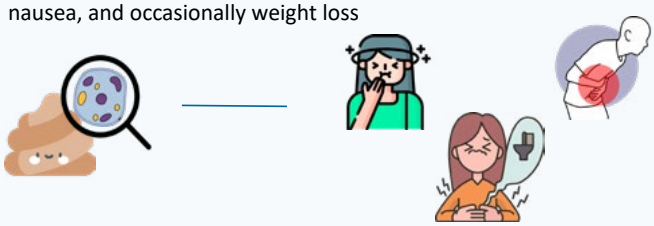
1 U.PORTO Instituto de Ciências Biomédicas Abel Salazar Universidade do Porto
2 P. Viseu ESAV
3 EPIUnit Epidemiology Research Unit
4 CERNAS Centro de Estudos de Recursos Naturais, Ambiente e Sociedade
5 CITAB
6 AL4ANIMALS Associate Laboratory for Animal and Veterinary Science
7 CECA

Introduction

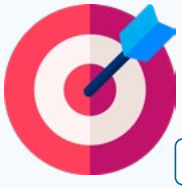
The World Health Organization (WHO) recognized diarrhoeal disease as the **third leading cause** of death in children 1–59 months of age, killing around 443 832 children every year. Disease is spread by feces-contaminated food/water or direct contact with infected individuals (humans or animals)



Blastocystis sp. is a globally distributed intestinal protist, infecting 1–2 billion people. Althought its uncertain pathogenicy, *Blastocystis* has been associated with diarrhea, abdominal pain, bloating, gas, nausea, and occasionally weight loss



Study Aim



- Calculate the occurrence of *Blastocystis* sp. in shepherd dogs
- Study the genetic diversity of *Blastocystis* sp.
- Circulating subtypes, mixed infections and zoonotic potential

Experiment Design

Collection and DNA extraction of 50 fecal samples from shepherd dogs of central Portugal

Screening for mixed infections by SYBR Green-based qPCR targeting the SSU rRNA gene

Positive samples showing mixed infections after Sanger Sequencing will be subjected to Targeted-Amplicon NGS by Oxford Nanopore Technologies

Occurence of *Blastocystis* sp.

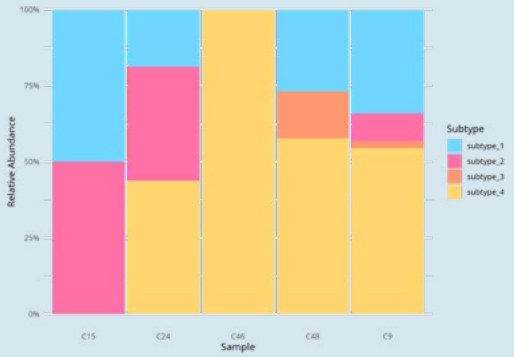
The screening for *Blatocystis* sp. scored an occurrence of 60% Blastocystis-positive samples for at least one subtype (ST).

A total 25 samples showed single-peaked chromatograms by Sanger sequencing while the remaing five presumptively showed mixed infections by visual inspection of chromatograms.

Genetic diversity and Zoonotic Potential

Based on BLASTn analysis, the STs identified in this study were ST1–ST4 and ST14, all of which are considered zoonotic. Subtypes ST1–ST4 are of particular concern, as they are responsible for approximately 90% of human infections.

Among the five presumed mixed-infection samples, four were confirmed to be true mixed infections, with up to four subtypes (ST1–ST4) detected within a single sample.



Conclusion

This study reports the first detection of *Blastocystis* in shepherd dogs in Portugal, with a 60% prevalence of zoonotic subtypes. Their close contact with livestock, especially sheep, suggests a possible role in cross-species transmission. Further studies comparing subtypes in dogs, sheep, and humans are needed to clarify transmission routes and assess public health risks.

ACKNOWLEDGMENTS/FUNDING

This work is supported by Project No. PRR-C05-i03-I-000190 "RumiRes", National Funds by FCT – Portuguese Foundation for Science and Technology, under the projects UIDB/00681/2020 (CERNAS; https://doi.org/10.54499/UIDB/00681/2020), UI/04033, LA/P/0126/2020 (CITAB; https://doi.org/10.54499/LA/P/0126/2020) and GHTM UID/04413/2020

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Supported by LA/P/0059/2020

CAN UROTHELIAL CARCINOMAS IN DOGS' BENEFIT FROM THE USE OF TOCERANIB PHOSPHATE (PALLADIA®) AS THE FIRST THERAPEUTIC OPTION?

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INTRODUCTION: The use of tyrosine kinase inhibitors, such as toceranib phosphate, has been transposed from human to veterinary medicine, authorized only for the treatment of certain canine mastocitomas. However, it has been shown that toceranib phosphate seems to be beneficial in other neoplasm types, such as urothelial carcinomas, a disease that accounts for around 1% of all canine neoplasms.

AIM: This study has as objective the evaluation of the efficacy of toceranib phosphate as a first therapeutic option in urothelial carcinomas in dogs.

METHODOLOGY: For the study 13 canines, predominantly females, neutered/sterilised, of the Beagle breed and with an average age of 11 years, followed by the Oncology department of the Veterinary School Hospital of the Faculty of Veterinary Medicine of the University of Lisbon, with urothelium carcinoma and treated with toceranib phosphate were included. Toceranib phosphate was administered every other day, at doses between 1.9 to 2.75 mg/kg, and associated with a non-steroidal anti-inflammatory drug.

RESULTS: More than half of the animals showed a positive response to the treatment, keeping the disease stable for an average of 242.4 days. The average survival time was 203.1 days, with most of the animals dying due to the progression of the disease.

CONCLUSIONS: The results obtained suggest that toceranib phosphate seems to be a promising therapeutic option for urothelial carcinomas, highlighting the importance of further research. This approach could improve the quality of life of affected dogs and expand the therapeutic options available in the field of veterinary oncology.

ACKNOWLEDGMENTS/FUNDING: A special thanks to my supervisors, Professor Berta São Braz and Dr Gonçalo Vicente, as well as to Dr Ana Coelho. I would also like to thank the Veterinary School Hospital of the Faculty of Veterinary Medicine of the University of Lisbon, especially Professor António Ferreira.

CAN UROTHELIAL CARCINOMAS IN DOGS' BENEFIT FROM THE USE OF TOCERANIB PHOSPHATE (PALLADIA®) AS THE FIRST THERAPEUTIC OPTION?

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[3]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

INTRODUCTION

Toceranib phosphate, a tyrosine kinase inhibitors, has been transposed from human to veterinary medicine, authorized only for the treatment of certain canine mastocitomas.

However, it has been shown that toceranib phosphate seems to be beneficial in other neoplasm types, such as urothelial carcinomas, a disease that accounts for around 1% of all canine neoplasms.

OBJECTIVE

Evaluate the efficacy of toceranib phosphate as a first therapeutic option in urothelial carcinomas in dogs.

METHODOLOGY

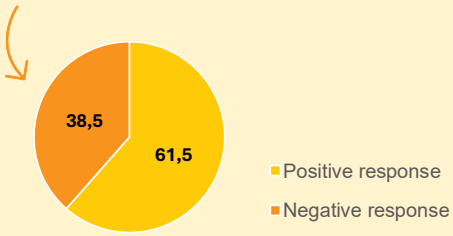
This study included 13 dogs, predominantly females, neutered/sterilised, of the Beagle breed and with an average age of 11 years, with urothelium carcinoma and treated with toceranib phosphate.

Toceranib phosphate was administered **every other day** (EOD), at doses between **1.9 to 2.75 mg/kg**, and associated with a **non-steroidal anti-inflammatory drug.**

RESULTS

In this sample uroythelium carcinoma mostly affects females (53,8%; 7/13), neutered/sterilised animals.

More than half of the animals showed a **positive response** to the treatment, keeping the **disease stable** for an average of **242.4 days**



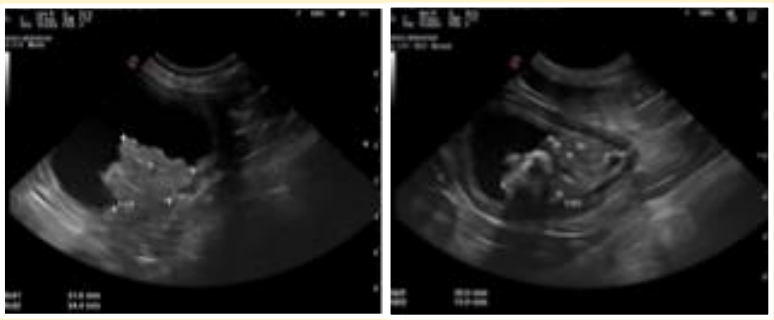


Figure 1: Echographic images of a canine with urothelial carcinoma, before and after treatment with toceranib phosphate, respectively

Average **survival time** was **203.1 days** → **76,9%** animals dying due to the progression of the disease

The use of **lower dose (1.9mg/kg)** of Toceranib phosphate demonstrated too be **equally effective** as the recommend dose (2,75mg/kg)

CONCLUSIONS

The results obtained suggest that toceranib phosphate seems to be a promising therapeutic option for urothelial carcinomas.

This approach could improve the quality of life of affected dogs and expand the therapeutic options available in the field of veterinary oncology.

ACKNOWLEDGMENTS/FUNDING

The authors thank the Fundação para a Ciência e Tecnologia (FCT), Portugal for the financial support: projects UIDB/00276/2020 (CIISA/FMV), LA/P/0059/2020- AL4AnimalS

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PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 3 Comparative and Translational Medicine and Biotechnology

LT3.02

CHANGES IN THE ENDOMETRIAL PROTEOME INDUCED BY EOSINOPHILS IN THE JENNY

Ariana Radar-Chafirovitch[1,2,3], Elisabete Silva[2,3], Miguel Quaresma[2,3], Jordi Miró[5], Inês Leites[3,4], Graça-Ferreira Dias[3,4], Maria dos Anjos Pires[3,4]

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[5]Equine Reproduction Service, Department of Animal Medicine and Surgery, Autonomous University of Barcelona, Barcelona.

Introduction: Infiltration of large numbers of eosinophils is a common feature of jenny's endometrium. Eosinophils have several biological functions, including tissue homeostasis, host defense against infectious agents, and immune regulation. Nevertheless, the role of eosinophils remains unclear and may influence endometrial function and pregnancy outcome.

Aimes: To gain insights in the role of eosinophils in the endometrium, by assessing the proteome of jenny endometrium, with low and high count of eosinophils.

Methodology: Endometrial biopsies were collected from 8 jenny's endometrium (7-16 years old), histologically evaluated (H&E staining). Eosinophil count was performed in 5 microscopic fields (x400), and samples grouped based on eosinophil count: endometria with low count of eosinophils (≤10, n=4) and endometria with high eosinophil count (≥130, n=4). The proteome was assessed by liquid chromatography-mass spectrometry. The DIA-NN algorithm validated the peptide/protein identification (FDR=1%), using the *Equus asinus* reference proteome. Enrichment analysis was performed using DAVID, with significance assessed by the Benjamini-Hochberg method. Proteomes were compared using the t-test and the differentially expressed proteins (DEPs) were statistically tested considering a P-value< 0.05 and -1≥Log2FC≥1.

Results: A total of 3,599 proteins were quantified in jenny's endometrial, 3,339 of which were found in both low and high eosinophil endometria. The analysis of the DEPs identified 551 DEPs in the endometria with high count of eosinophils, 98 of which were upregulated and 453 downregulated. The upregulated proteins were enriched in 26 biological processes (BP), including those related to negative regulation of endopeptidase activity, complement activation, blood coagulation, neutrophil aggregation, leukocyte migration, and response to oxidative stress. This suggests that eosinophils enhance immune activation, increase inflammation, and raise reactive oxygen species, contributing to tissue damage and potential fibrosis. The downregulated proteins were enriched in 29 BP, including processes related to Golgi organization, protein glycosylation, cytoskeleton structure, intracellular protein transport and fatty acid biosynthetic process. This suggests that eosinophils activate pathways that may disrupt extracellular matrix synthesis and the antioxidant defenses, favoring chronic inflammation and impaired tissue homeostasis.

Conclusion: Eosinophils might play a physiological regulatory role on endometrial inflammatory and immune response, when present in low count, while they might have a deleterious effect on tissue homeostasia, when their count increases.

Acknowledgments/Funding: APEGA Association. This work was funded by FCT (project No. 2022.09161; UIDB/00276/2020-CIISA; LA/P/0059/2020- AL4AnimalS- project No. 2023.LT3.3). Elisabet Silva by FCT (https://doi.org/10.54499/CEECINST/00140/2021/CP2807/CT0001);

Ariana Radar UI/BD/150839/202.

CHANGES IN THE ENDOMETRIAL PROTEOME INDUCED BY EOSINOPHILS IN THE JENNY

Ariana Radar-Chafirovitch [1,2,3]; Elisabete Silva [3,4]; Migue Quaresma [2,3]; Jordi Miró[5]; Inês Leites [3,4]; Graça-Ferreira Dias [3,4] ; Maria dos Anjos Pires[2,3]

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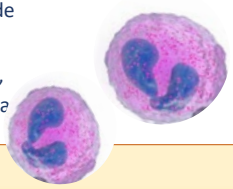


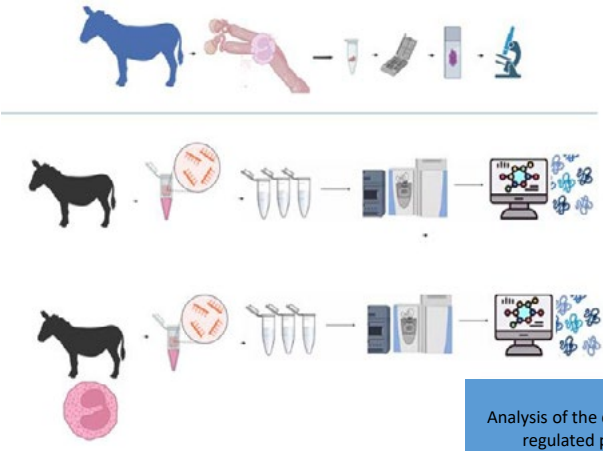
INTRODUCTION

Infiltration of large numbers of eosinophils is a common feature of jenny's endometrium. Eosinophils have several biological functions, including tissue homeostasis, host defense against infectious agents, and immune regulation. Nevertheless, the role of eosinophils remains unclear and may influence endometrial function and pregnancy outcome.

AIMS

To gain insights in the role of eosinophils in the endometrium, by assessing the proteome of jenny endometrium, with low and high count of eosinophils.

METHODOLOGY



Endometrial biopsies were collected from 8 jenny's endometrium:
* 7-16 years old
* Diestrus phase
* Endometria with low count of eosinophils (≤10, n=4) and endometria with high eosinophil count (≥130, n=4).

Endometrial proteome was assessed by LC-MS/MS.
The DIA-NN algorithm validated the peptide/protein identification (FDR=1%), using the *Equus asinus* reference proteome.

Analysis of the differentially regulated proteins

Enrichment analysis was performed using DAVID, with significance assessed by the Benjamini-Hochberg method. Proteomes were compared using the t-test and the differentially expressed proteins (DEPs) were statistically tested considering a P-value< 0.05 and -1≥Log2FC≥1

RESULTS AND DISCUSSION

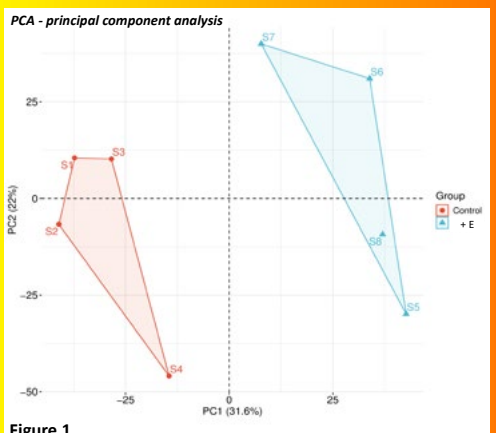


Figure 1

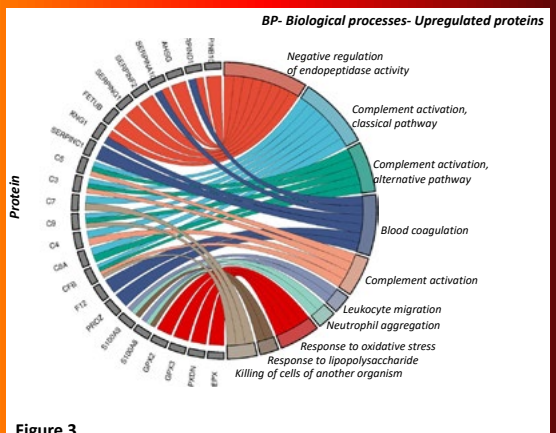


Figure 3

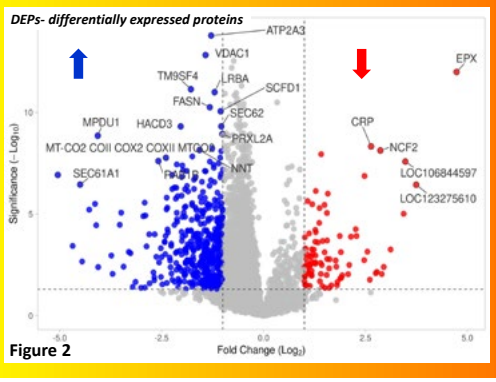


Figure 2

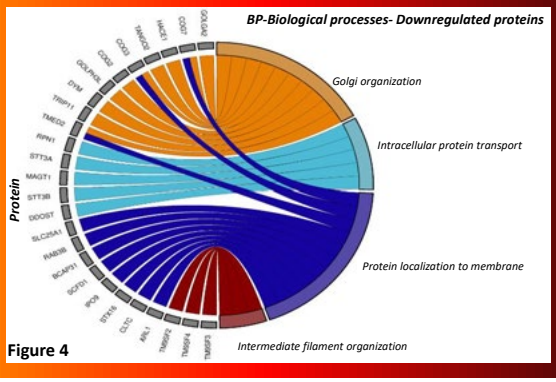


Figure 4

PCA analysis showed a clear differentiation in the endometrial proteome of jenny's with low and high eosinophil counts (Figure 1).

The analysis of the DEPs identified 551 DEPs in the endometria with high count of eosinophils, 98 of which were upregulated and 453 downregulated (Figure 2).

Upregulated proteins were enriched in 26 BP, including those related to negative regulation of endopeptidase activity, complement activation, blood coagulation, neutrophil aggregation, leukocyte migration, and response to oxidative stress (Figure 3). This suggests that eosinophils enhance immune activation, increase inflammation, and raise reactive oxygen species, contributing to tissue damage and potential fibrosis.

Downregulated proteins were enriched in 29 BP, including processes related to Golgi organization, protein glycosylation, cytoskeleton structure, intracellular protein transport and fatty acid biosynthetic process (Figure 4).
This suggests that eosinophils activate pathways that may disrupt extracellular matrix synthesis and the antioxidant defenses, favoring chronic inflammation and impaired tissue homeostasis.

CONCLUSIONS

Eosinophils might play a physiological regulatory role on endometrial inflammatory and immune response, when present in low count, while they might have a deleterious effect on tissue homeostasia, when their count increases.

Acknowledgments/Funding: APEGA Association, Zelia Cruz, that helped sample collection**.** This work was funded by FCT (project No.2022.09161; UIDB/00276/2020-CIISA; LA/P/0059/2020-AL4AnimalS- project No. 2023.LT3.3). Elisabet Silva by FCT (https://doi.org/10.54499/CEECINST/00140/2021/CP2807/CT0001); **Ariana Radar** UI/BD/150839/202.

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 3 Comparative and Translational Medicine and Biotechnology

LT3.03

CTRL+ALT+DEGENERATE: CREATING CELL MODELS FOR SANFILIPPO SYNDROME

Francisca Gonçalves[1,2,3,4]; Matilde Almeida[2,3,4,5]; Luciana Moreira[2,3,6]; Paulo Gaspar[2,3,4,7]; Hugo Rocha[7]; Maria Francisca Coutinho[2,3,4]; Sandra Alves[2,3,4]

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INTRODUCTION: Mucopolysaccharidosis type III or Sanfilippo Syndrome is a rare, autosomal recessive inherited disorder characterised by the accumulation of heparan sulphate (HS) due to mutations in enzymes involved in its degradation pathway. It is characterised by progressive and multisystemic effects, with pronounced neurological symptoms, such as early-onset childhood dementia, for which curative therapies remain unavailable.

AIMS: Aiming at generating a disease-relevant in vitro model for MPS III, which may be used not only for pathophysiological assessments but also for drug development studies, we are now producing expandable neural progenitor cells and terminally differentiated neurons from two previously generated MPS III iPSC lines.

METHODOLOGY: First, previously generated iPSCs obtained from two fibroblast cell lines from MPS III (MPS IIIC and MPS IIID) were evaluated regarding/for three key parameters associated with its primary manifestation. Briefly, we quantified the enzymatic activity of the hydrolases involved in HS degradation using fluorometric assays, assessed the intracellular accumulation of HS via LC-MS/MS and investigated lysosomal distribution through immunofluorescence staining of LAMP1, a lysosomal membrane marker. Finally, we performed the neurodifferentiation of two MPS III-derived iPSC cell lines using a commercial kit.

RESULTS: We demonstrated that MPS IIIC and MPS IIID-derived iPSCs exhibit a significant reduction in the activity of HS-degrading enzymes compared to the control. In parallel, we observed an increased accumulation of this substrate in MPS III-derived iPSCs, as well as alterations in lysosomal size and positioning, evidenced by higher LAMP1 fluorescence intensity compared to control cells. Lastly, we successfully induced the neurodifferentiation of one MPS III-derived iPSC line, and its phenotypic and biochemical characterisation is ongoing.

CONCLUSIONS: Up to this point, all our preliminary data on both cell models support the assumption that they will serve as a valuable platform for pathophysiological studies as well as for preclinical drug development.

ACKNOWLEDGMENTS/FUNDING: This work was financed by national funds through FCT—Fundação para a Ciência e a Tecnologia, I.P., within the scope of two independent projects: EXPL/BTM SAL/0659/2021 (http://doi.org/10.54499/EXPL/BTM-SAL/0659/2021) and ASOS2cureMPSIII-2022.04667.PTDC (https://doi.org/10.54499/2022.04667.PTDC).

CTRL+ALT+DEGENERATE: CREATING CELL MODELS FOR SANFILIPPO SYNDROME

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What is Sanfilippo Syndrome also known as Mucopolyssacharidoses Type III (MPS III)?

- A subgroup of **rare**, **autosomal recessive** lysosomal storage disorders caused by the **accumulation of heparan sulphate (HS)** due to mutations in enzymes responsible for its degradation.
  - 4 clinical subtypes (MPS III A-D), each resulting from deficiency in a distinct enzyme in the HS catabolic pathway.

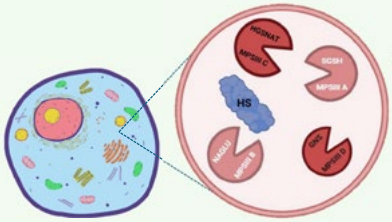

Figure 1. Simplified representation of the MPS III related lysosomal enzymes involved in HS breakdown (SGSH: N-Sulfoglucosamine sulfohydrolase, NAGLU: N-Acetyl-α glucosaminidase, HGSNAT: Heparan-α-glucosaminide-N-acetyltransferase, GNS: N-acetylglucosamine-6-sulfatase). Image created with BioRender, 2025.

Symptoms

- **Progressive, multisystemic disorder** with **early-onset** and **severe neurodegeneration,** marked by **pronounced neurological symptoms.**

Curative therapies remain unavailable

Aim

Generation of expandable neural progenitor cells and terminally differentiated neurons from MPS IIIC and MPS IIID iPSC lines

Why?

To establish a disease-relevant *in vitro* model for pathophysiological studies and drug screening

Methods

Primary MPS III Phenotype

Enzymatic Activity
Fluorimetric Assay

HS Accumulation
LC-MS/MS

Lysosomal Size and Abundance
LAMP1 Immunofluorescence

Figure 2. Schematic overview of the assays used to evaluate the primary phenotype of MPS III. Images created with BioRender, 2025.

Human iPSCs Neurogenesis

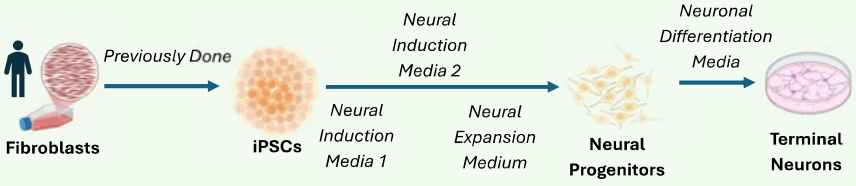

Figure 3. Schematic representation of the neurodifferentiation protocol applied to iPSCs derived from MPS IIIC and MPS IIID patient fibroblasts. First, patient-derived fibroblasts were reprogrammed into iPSCs and then subjected to neuronal differentiation using a commercial kit: EMD Millipore's Human ES/iPS Neurogenesis Kit (Cat. No. SCR603). As a control group, human dermal fibroblasts from adult (HDFa) were similarly reprogrammed into iPSCs and neurodifferentiated. Images created with BioRender, 2025.

Results

Enzyme Activity (nmol/h/mg protein)

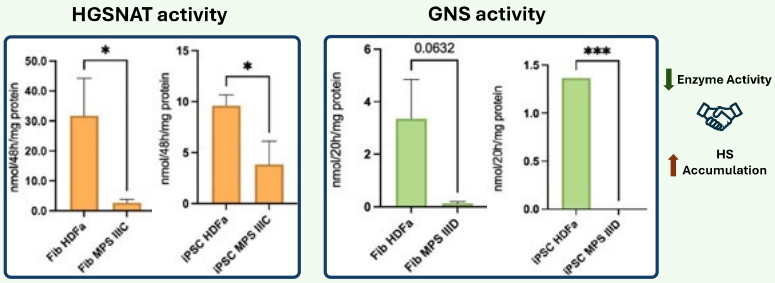

Figure 4. HGSNAT (heparan-α-glucosaminide-N-acetyltransferase) activity in MPS IIIC fibroblasts and their respective iPSC lines, and GNS (N-acetylglucosamine-6-sulfatase) activity in MPS IIID fibroblasts and derived iPSC lines, compared to corresponding control cell lines (HDFa). Graphs were generated using GraphPad Prism version 9.5.0 (525) for macOS. *p ≤ 0.05, *** p-value ≤ 0.001.

HS Content (mg/protein)

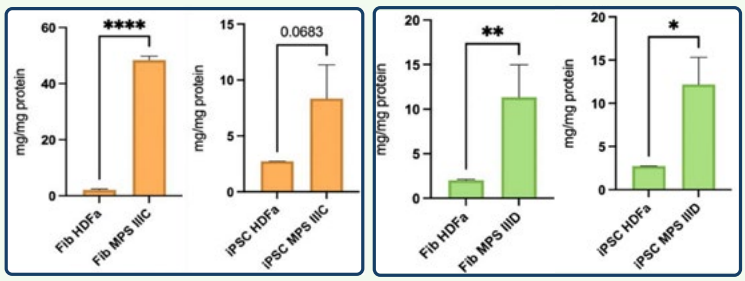

Figure 5. Heparan sulphate (HS) storage in parental fibroblasts and iPSC-derived cells from MPS IIIC and IIID patients. Adult human dermal fibroblasts (HDFa) and their respective iPSCs line were used as controls. Graphs were generated using GraphPad Prism version 9.5.0 (525) for macOS. . *p ≤ 0,05, **p ≤ 0,01, ****p ≤ 0,0001.

Lysosomal Size and Abundance (LAMP1 Expression)

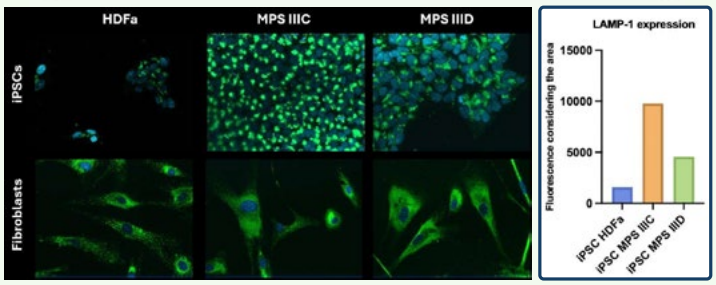

Figure 6. LAMP-1 expression in control (HDFa), MPS IIIC and MPS IIID fibroblasts and their respective iPSCs lines. Images were captured, all with the same ampliation and parameters and processed with Leica Application Suite v.3.7.0 software. Quantification of fluorescence intensity was performed in ImageJ version Java 1.8.0_345 (64-bit), and the respective graphs in GraphPad Prism version 9.5.0 (525) for macOs .

Terminal Neurons

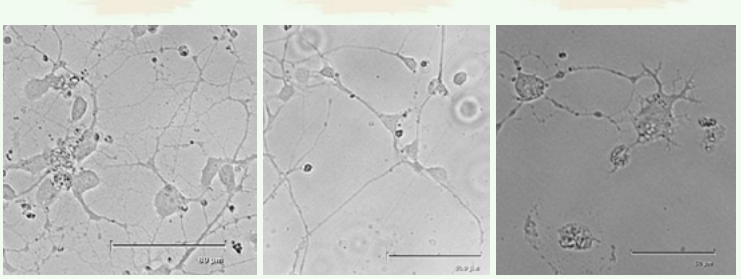

Figure 7. Terminal neurons obtained from HDFa (control group), MPS IIIC, and MPS IIID iPSCs after neurodifferentiation using EMD Millipore's Human ES/iPS Neurogenesis Kit (Cat. No. SCR603). Cells were observed using Invitrogen EVOS imaging system.

Conclusion and Future Perspectives

- Successful neurogenesis protocol confirmed morphologically.
- Phenotypic and biochemical characterisation of the MPS III expandable neural progenitor cells and terminally differentiated neurons is ongoing.
- Preliminary data **support their potential as robust *in vitro* platforms** for **pathophysiological investigation** and **preclinical drug development.**

This work was financed by national funds through FCT—Fundação para a Ciência e a Tecnologia, I.P., within the scope of two independent projects: EXPL/BTM SAL/0659/2021 (http://doi.org/10.54499/EXPL/BTM-SAL/0659/2021) and ASOS2cureMPSIII-2022.04667.PTDC (https://doi.org/10.54499/2022.04667.PTDC).

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc LT3.04

LT 3 Comparative and Translational Medicine and Biotechnology

RETROSPECTIVE EVALUATION OF TOCERANIB PHOSPHATE USE IN CATS WITH SOLID TUMOURS

Inês Mont'Alverne de Sequeira de Sousa[1], Gonçalo Eduardo Vicente[1], Ana Coelho[1], Berta Ferreira São Braz[2, 3]

[1]Veterinary school Hospital- Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[3]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

INTRODUCTION: Preliminary studies developed in dogs, in relation to the biologic activity of Toceranib Phosphate in solid tumours, demonstrate the efficacy of this target therapy with promising results comparing to the conventional chemotherapy. According to these results, the interest to understand the outcome in cats with solid neoplasia, treated with the same therapy, arises. Consequently, some studies were developed, however the biologic activity of this molecule remains, until today, not well defined in cats, namely due to the few investigations available in this species.

AIMS: The aim of this study is to analyse retrospectively the clinical response of cats with solid tumours, treated with the molecular target therapy, Toceranib Phosphate, originally developed only for the treatment of mast cell tumours in dogs. We hypothesize that Toceranib Phosphate has potential as an alternative treatment, in cat solid tumours, to conventional chemotherapy, extending the survival-time of the patients, and providing a good quality of life simultaneously.

METHODOLOGY: Evaluation of the response was performed according to the type of tumour and in the general specimen, to understand the potential of Toceranib. Response evaluation criteria for solid tumours in dogs (v1.0): A Veterinary Cooperative Oncology Group (VCOG) consensus document (cRECIST v1.0) was essential to define the tumour response. For survival analyses, a Kaplan-Meier analysis was crucial for determination of the median survival time (MST), progression-free survival (PFS) and overall survival (OS). It was also performed a Log-Rank Test to determine the influence of different variables in the efficacy of Toceranib Phosphate.

RESULTS: The specimen of 26 cats demonstrated a clinical benefit of 69.2%, with the majority of the cats achieving a stable disease. The median survival time was significantly higher than expected, with 46.2% of the specimen with a survival time above 365 days, 23.0% died 2 years after the diagnose, and one patient lived for longer than 4 years (3.8%). The progression free survival has a median of 428 days and an amplitude quite wide of 61-1612 days.

CONCLUSIONS: The obtained results suggest the existence of Toceranib Phosphate biologic activity in a variety of solid tumours in cats and, simultaneously, keeping the quality of the patient's life. Besides that, it was possible to understand that its use for an extended period of time is well tolerated, which is a factor in favour of its implementation. With this, it's expected that future studies, mainly prospective ones, with bigger dimensions, with standard methods for data collection, with control groups and with the analyse of RTK's profile, became developed to support the existence of Toceranib Phosphate clinical efficacy, in a variety of solid tumours in cats.

ACKNOWLEDGMENTS/FUNDING: Inês Mont'Alverne de Sequeira de Sousa acknowledges the Veterinary Hospital of the Faculty of Veterinary Medicine of the University of Lisbon and the financial support by CIISA/FMV project UIDB/00276/2020, through a MSc grant.

RETROSPECTIVE EVALUATION OF TOCERANIB PHOSPHATE USE IN CATS WITH SOLID TUMOURS

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Introduction

Preliminary studies developed in dogs, in relation to the biologic activity of Toceranib Phosphate in solid tumours, demonstrate the efficacy of this target therapy with promising results comparing to the conventional chemotherapy. According to these results, the interest to understand the outcome in cats with solid neoplasia, treated with the same therapy, arises. Consequently, some studies were developed, however the biologic activity of this molecule remains, until today, not well defined in cats, namely due to the few investigations available in this species.

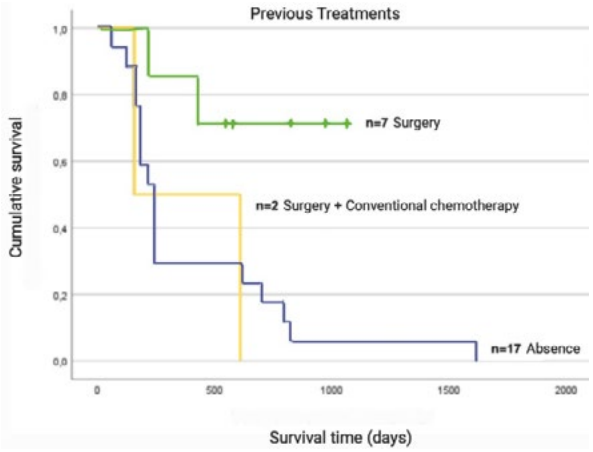
Methods

For survival analyses, a Kaplan-Meier analysis was crucial for determination of the median survival time (MST), progression-free survival (PFS) and overall survival (OS). It was also performed a Log-Rank Test to determine the influence of different variables in the efficacy of Toceranib Phosphate.

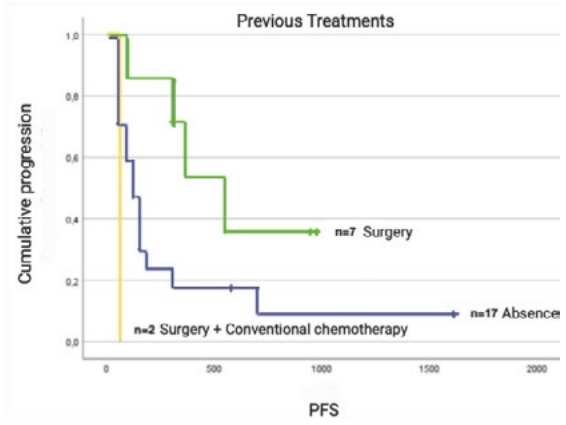
Results

The specimen of 26 cats demonstrated a clinical benefit of 69.2%, with the majority of the cats achieving a stable disease. The median survival time was significantly higher than expected, with 46.2% of the specimen with a survival time above 365 days, 23.0% died 2 years after the diagnose, and one patient lived for longer than 4 years (3.8%). The progression free survival median was 428 days, with a quite wide amplitude of 61-1612 days.

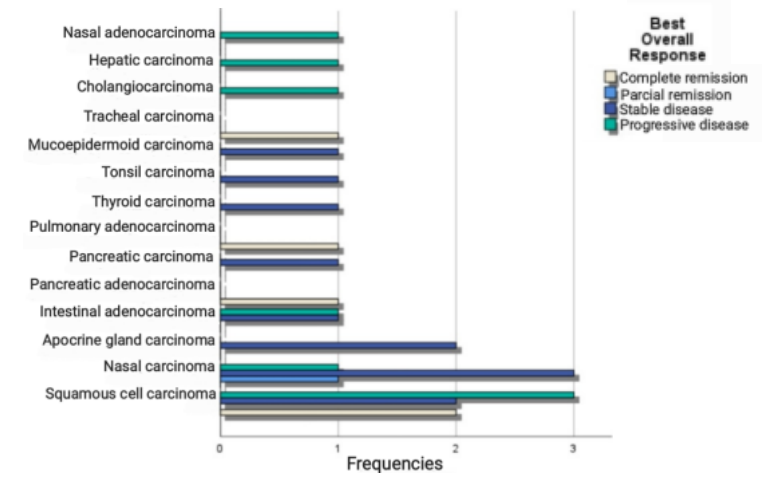
Graphic 1. Kaplan-Meier curve for MST, according to prior treatment or its absence



Graphic 2. Kaplan-Meier curve for PFS, according to prior treatment or its absence



Graphic 3. Best clinical response distribution, according to the tumour type



Conclusion

The obtained results suggest the existence of Toceranib Phosphate biologic activity in a variety of solid tumours in cats and, simultaneously, keeping the quality of the patient's life. It was possible to understand that its use for an extended period of time is well tolerated, which is a factor in favour of its implementation. With this, it's expected that future studies, mainly prospective ones, became developed to support the existence of Toceranib Phosphate clinical efficacy, in a variety of solid tumours in cats.

ACKNOWLEDGMENTS/FUNDING: The authors thank the Fundação para a Ciência e Tecnologia (FCT), Portugal for the financial support: projects UIDB/00276/2020 (CIISA/FMV), LA/P/0059/2020- AL4AnimalS.

Supported by LA/P/0059/2020

DEVELOPMENT OF NOVEL IMMUNOTHERAPIES FOR THE TREATMENT OF CANINE LYMPHOMA BASED ON IMMUNOTOXINS DERIVED FROM *PSEUDOMONAS AERUGINOSA* AND *CORYNEBACTERIUM DIPHTHERIAE*

João Mota[1,2], Rafaela Marimon[1,2], Ana Leonardo[1,2], Isa Moutinho[1,2], Afonso Basto[1,2], Luís Tavares[1,2], Frederico Aires da Silva[1,2*]

[1]Center of Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal.

INTRODUCTION: Canine multicentric lymphoma and human diffuse large B-cell lymphoma (DLBCL), the most common non-Hodgkin lymphoma in humans, share similar incidence rates, biology, genetic features and pathology characteristics. These similarities support the translational use of canine lymphoma as a relevant model for studying human DLBCL, including in the development of new therapies. Although the initial response to treatment is usually positive, recurrence of the disease is frequent and is often associated with resistance to chemotherapy which also has many associated adverse effects, making it necessary to develop new therapeutic approaches. Immunotherapy, particularly immunotoxins, has emerged as a promising strategy due to its enhanced specificity, ability to overcome drug resistance, and potent cytotoxic effects against cancer cells.

AIMS: Develop new immunotoxins by combining single-chain (scFv) and single-domain (sdAb) antibody fragments with PE38 and DT toxins, that bind specifically to B-cell lymphoma cancer cells and are able to eliminate them directly sparing healthy cells.

METHODOLOGY: Two immunotoxins were developed, scFv-HA22 conjugated with PE38, and sdAb-C5 conjugated with DT. scFv-HA22 was expressed in BL21 *E. coli*, purified by immobilized metal affinity chromatography (IMAC) and confirmed with Western blot and Coomassie stain. The scFv-HA22 binding to cancer cells was validated against a canine large B-cell lymphoma cell line (CLBL-1) through flow cytometry and confocal microscopy.

RESULTS: Western blot and Coomassie stain revealed a good expression and purification of scFv-HA22-PE38. Flow cytometry showed positive results confirming binding to the target CLBL-1 cell line. Ongoing studies are being conducted to evaluate internalisation by confocal microscopy and to assess cytotoxic activity in vitro. In parallel, expression and functional characterisation of the sdAb-C5-DT immunotoxin are currently in progress.

CONCLUSIONS: Our results show that scFv-HA22-PE38 is able to be produced and specifically binds to canine lymphoma cells which supports its potential as a candidate for internalisation and cytotoxicity activity.

ACKNOWLEDGMENTS/FUNDING: This work was supported by CIISA and AI4Animals through the project UIDB/00276/2020 and LA/P/0059/2020-AL4AnimalS, funded by FCT.

Development of novel immunotherapies for the treatment of canine lymphoma based on immunotoxins derived from Pseudomonas aeruginosa and Corynebacterium diphtheriae

João Mota[1,2,*], Rafaela Marimon[1,2], Ana Leonardo[1,2],Isa Moutinho[1,2], Afonso Basto[1,2], Luís Tavares[1,2], Frederico Aires da Silva[1,2]

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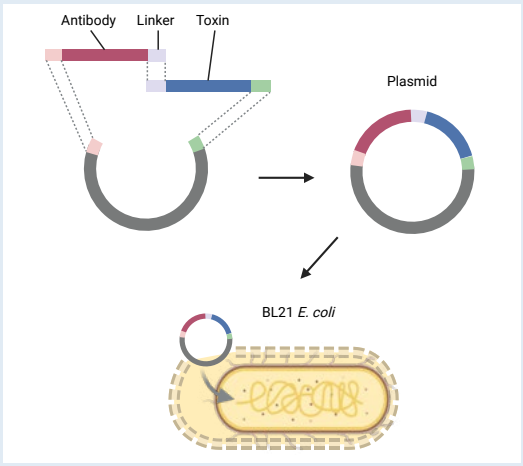
INTRODUCTION

Canine multicentric lymphoma and human diffuse large B-cell lymphoma (DLBCL), the most common non-Hodgkin lymphoma in humans, share similar incidence rates, biology, genetic features and pathology characteristics. These similarities support the translational use of canine lymphoma as a relevant model for studying human DLBCL, including in the development of new therapies. Although the initial response to treatment is usually positive, recurrence of the disease is frequent and is often associated with resistance to chemotherapy which also has many associated adverse effects, making it necessary to develop new therapeutic approaches. Immunotherapy, particularly immunotoxins, has emerged as a promising strategy due to its enhanced specificity, ability to overcome drug resistance, and potent cytotoxic effects against cancer cells.

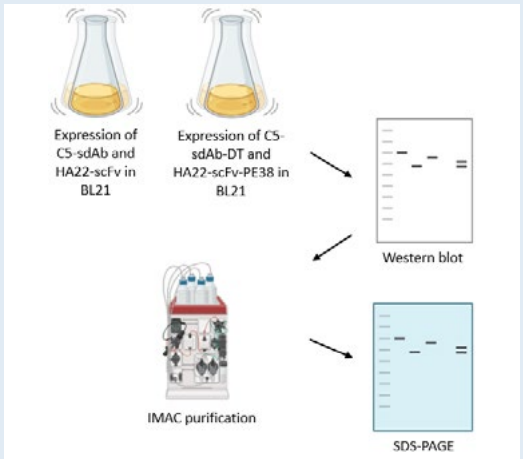
OBJECTIVE

Develop new immunotoxins by combining single-chain (scFv) and single-domain (sdAb) antibody fragments with PE38 and DT toxins, that bind specifically to B-cell lymphoma cancer cells and are able to eliminate them directly sparing healthy cells.

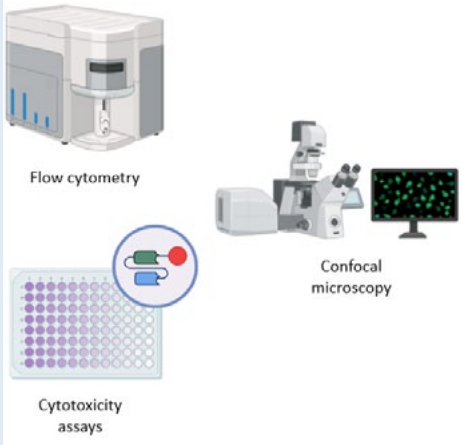
METHODS



Construction of immunotoxins in pET-21 and pHTP1 plasmid vectors and tranformation into BL21 *E. coli* strain.



Expression of the immunotoxins in 2, 4 and 20 hour timepoint and at 19 ºC and 30 ºC. Confirming best production conditions through western blot. Purification by His-tag afinity and confirmation with SDS-PAGE.



Flow cytometry, confocal microscopy and cytotoxicity assays to confirm binding, internalization and cytotoxicity of the immunotoxins to CLBL-1 canine lymphoma cells and Raji human lymphoma cells.

RESULTS

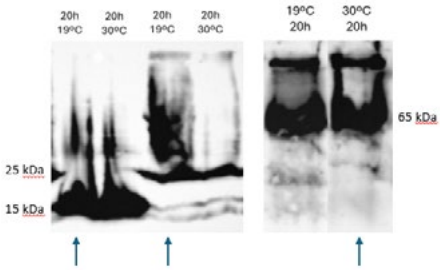
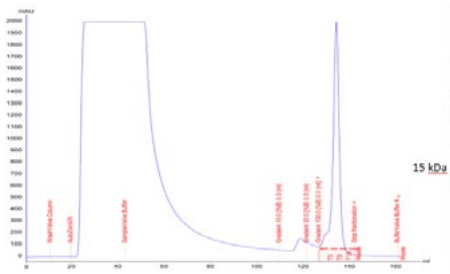


Figure 1: Western blot of C5-sdAb (15 kDa), HA22-scFv (25 kDa) and HA22-scFv-PE38 (65 kDa). Best conditions for C5 and HA22 were 19 ºC 20h and for HA22-PE38 were 30 ºC 20h.



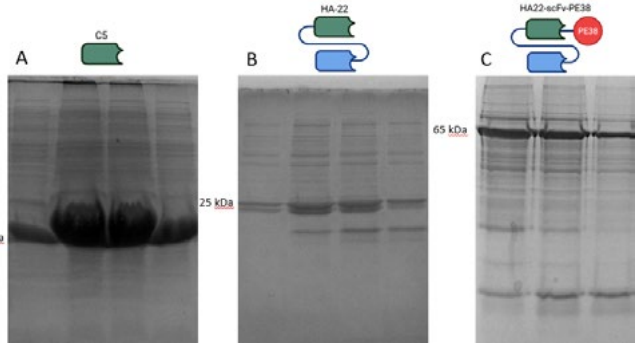


Figure 2: IMAC purification and confirmation through SDS-PAGE. A) SDS-PAGE of C5 purification; B) SDS-PAGE of HA22 purification; C) SDS-PAGE of HA22-PE38 purification.

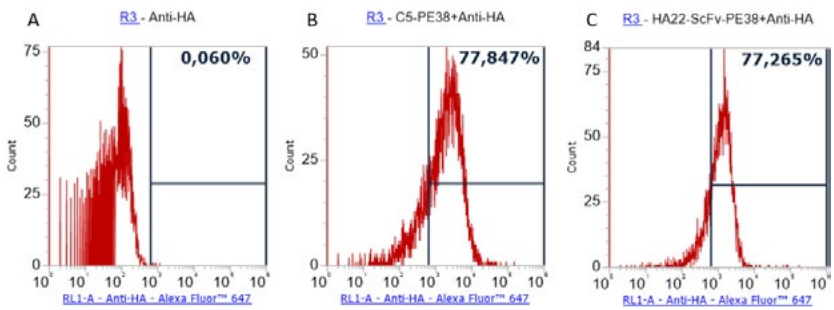


Figure 3: Flow cytometry results of HA22-PE38 binding to CLBL-1 canine lymphoma cell line. A) Negative control; B) Positive control; C) HA22-PE38.

CONCLUSION

We were able to express and purify the HA22, C5 and HA22-PE38 antibodies. Through flow cytometry we confirmed that HA22-PE38 has good binding to CLBL-1 cells of canine lymphoma. We are finalizing confocal microscopy and citotoxicity assays for HA22-scFv-PE38 and working on expression and characterization of C5-sdAb-DT.

ACKNOWLEDGEMENTS

This work was supported by CIISA and Al4Animals through the project UIDB/00276/2020 and LA/P/0059/2020-AL4AnimalS, funded by FCT.

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc LT3.06

LT 3 Comparative and Translational Medicine and Biotechnology

DEVELOPMENT OF NOVEL IMMUNOTOXINS USING TRASTUZUMAB-DERIVED SINGLE-CHAIN VARIABLE FRAGMENTS FOR BREAST CANCER TREATMENT

Juan Silva[1,2], Rafaela Marimon[1,2], Ana Leonardo[1,2], Isa Moutinho[1,2], Afonso Basto[1,2], Luís Tavares[1,2], Frederico Aires da Silva[1,2*]

[1] Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

INTRODUCTION: Breast cancer remains one of the leading causes of cancer-related deaths worldwide, largely due to its heterogeneity and aggressive subtypes like HER2-positive breast cancers. Conventional therapies, including chemotherapy, radiotherapy, and surgery, have limitations, including high recurrence rates and significant side effects. Among targeted therapies, monoclonal antibodies (mAbs) have had success, with trastuzumab being the first-line antibody used against HER2-positive tumours but faces resistance and limited penetration, indicating the need for new treatments. To overcome these limitations, single-chain variable fragments (scFv), consisting of the variable regions of heavy and light chains joined by a flexible linker, are promising alternatives due to their smaller size and enhanced tumour penetration. This project was tested in feline mammary carcinoma cells, leveraging HER2 similarity with humans to support translational One Health research.

AIMS: This project aims to develop a novel immunotoxin by fusing the trastuzumab-derived scFv to potent bacterial toxins, specifically diphtheria toxin (DT) and *Pseudomonas* exotoxin A (PE38). These immunotoxins are designed to specifically recognise and bind to the HER2 receptor, overexpressed in approximately 20% of breast cancers, thereby delivering a cytotoxic payload directly into cancer cells while sparing healthy tissue.

METHODOLOGY: To construct the recombinant immunotoxins, DNA sequences encoding the trastuzumab-derived scFv were fused via a flexible peptide linker to the catalytic domains of DT and PE38. The constructs were cloned into an appropriate expression vector and transformed into *Escherichia coli* (BL21) for expression. Protein expression was optimized and confirmed by ELISA, SDS-PAGE and Western blot. Purification was performed through affinity chromatography. Binding specificity was assessed by ELISA using recombinant HER2 protein and flow cytometry was also employed on HER2-positive breast cancer cell lines to confirm the binding of the immunotoxins to target cells.

RESULTS: Preliminary results indicate successful expression and purification of the fusion proteins, confirming target specificity. Expression assays were successfully performed with the scFv-DT toxin, yielding promising results. Additionally, binding assays using ELISA and flow cytometry confirmed the effective binding of the immunotoxins.

CONCLUSIONS: These findings support the therapeutic potential of scFv-based immunotoxins for HER2-positive breast cancer. This approach combines the specificity of antibody recognition with the potent cell-killing capability of bacterial toxins, offering a promising alternative for patients who develop resistance to conventional HER2-targeted therapies. Future work will focus on cytotoxicity assays, improving the half-life of this immunotoxin through conjugation with the ABD domain of ZAG, as well as performing the same procedure used with the DT toxin but with PE38.

ACKNOWLEDGMENTS/FUNDING: This work was supported by CIISA and Al4Animals through the project UIDB/00276/2020 and LA/P/0059/2020-AL4AnimalS, funded by FCT.

Development of novel Immunotoxins using Trastuzumab-derived single-chain variable fragments for HER2-positive breast cancer treatment

Juan Silva[1,2*], Rafaela Marimon[1,2], Ana Leonardo[1,2], Isa Moutinho[1,2], Afonso Basto[1,2], Luís Tavares[1,2], Frederico Aires da Silva[1,2*]

[1] Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal.

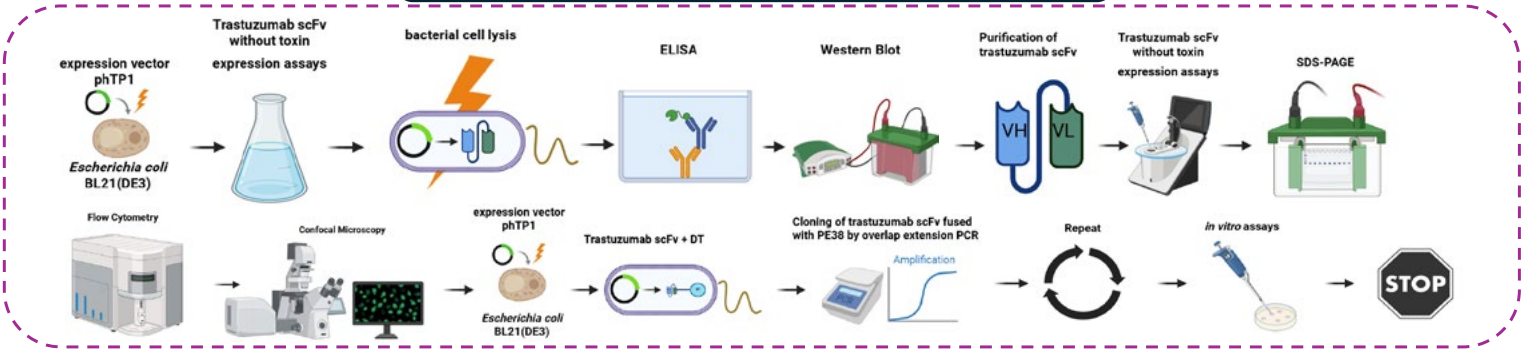
Introduction

Breast cancer remains one of the leading causes of cancer-related deaths worldwide, largely due to its heterogeneity and aggressive subtypes like HER2-positive breast cancers. Conventional therapies, including chemotherapy, radiotherapy, and surgery, have limitations, including high recurrence rates and significant side effects. Among targeted therapies, monoclonal antibodies (mAbs) have had success, with trastuzumab being the first-line antibody used against HER2-positive tumours but faces resistance and limited penetration, indicating the need for new treatments. To overcome these limitations, single-chain variable fragments (scFv), consisting of the variable regions of heavy and light chains joined by a flexible linker, are promising alternatives due to their smaller size and enhanced tumour penetration. This project was tested in feline mammary carcinoma cells, leveraging HER2 similarity with humans to support translational One Health research.

Objectives

1. Develop a HER2-targeted immunotoxin by fusing trastuzumab scFv with diphtheria toxin.
2. Expression and purification of trastuzumab scFv, scFv-DT, and scFv-PE38 constructs.
3. Evaluate binding via ELISA, flow cytometry, and confocal microscopy.

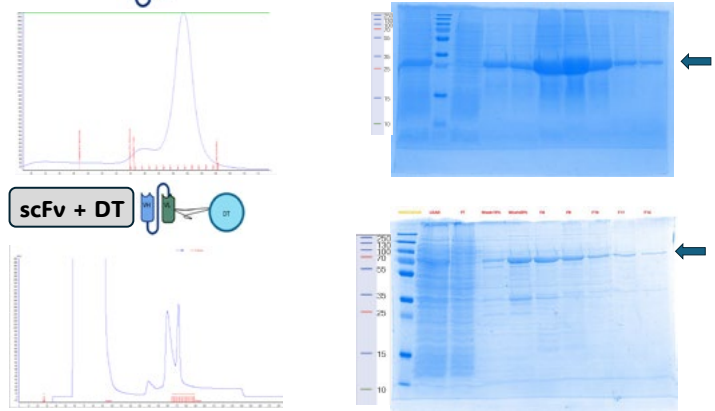
Methods



Results

1.Expression and purification of antibodies.

scFv



scFv + DT

Figure 1. Expression and purification of trastuzumab scFv and scFv + DT using a His-tag affinity column. Following elution, protein fractions were analyzed by SDS-PAGE to evaluate purity and confirm successful expression.

2.Binding via ELISA

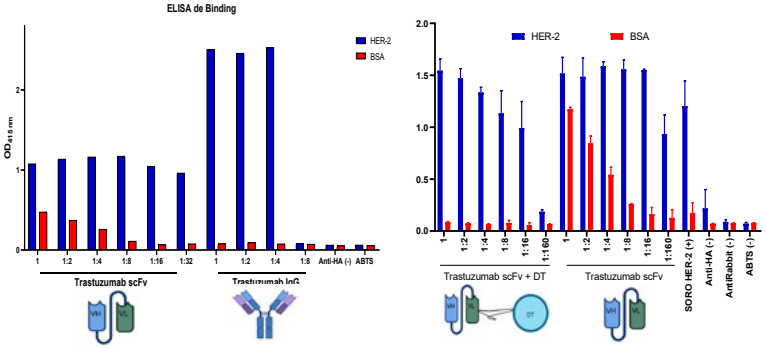


Figure 2. Binding ELISA of trastuzumab scFv and trastuzumab scFv-DT constructs against recombinant HER2.

3.Binding via Flow cytometry

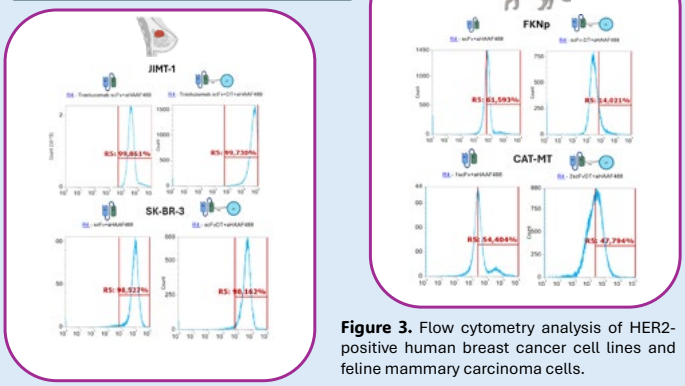


Figure 3. Flow cytometry analysis of HER2-positive human breast cancer cell lines and feline mammary carcinoma cells.

4.Binding via confocal microscopy

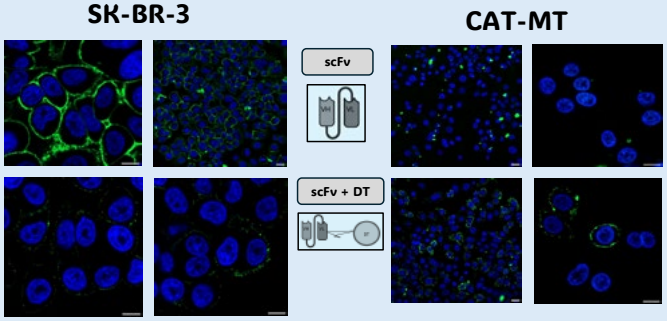


Figure 4. Confocal microscopy of HER2-positive human breast cancer cell line and feline mammary carcinoma cells.

Acknowledgment

CIISA has provided support via Project UIDB/00276/2020, funded by FCT, and LA/P/0059/2020-AL4AnimalS.

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 3 Comparative and Translational Medicine and Biotechnology

LT3.07

CASE SERIES OF DIOPATHIC CHRONIC KIDNEY DISEASE IN CATS: A RETROSPECTIVE STUDY

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[2] CIISA – Interdisciplinary Research Centre in Animal Health, Faculty of Veterinary Medicine, University of Lisbon.
[3] AL4AnimnalS-Associate Laboratory for Animal and Veterinary Sciences
[4] Veterinary Clinic Dra. Elsa Pereira

INTRODUCTION: Chronic Kidney Disease (CKD) in cats is a progressive and irreversible condition mostly of idiopathic origin that negatively impacts the quality of life of both cats and their owners.

AIMS: This study aimed to characterise idiopathic CKD in 50 cats focusing on diagnostic and clinical management and to identify prognostic indicators across CKD stages.

METHODOLOGY: Clinical records (2018–2023) from the HEV Lisbon University Hospital were retrospectively reviewed. Cats were screened based on elevated blood creatinine and SDMA levels (IRIS stages II–IV). CKD was staged according to the 2023 IRIS guidelines. A convenience group of stage I cats was also included. Cats with extra-renal diseases were excluded. The median time for CKD progression to the next stage (mDP) and the median survival time (mST) were determined. Statistical analysis was performed with significance set at $p < 0.05$.

RESULTS: 50 cats were included. In stage I (*n*=6), in the absence of clinical signs, only ultrasonographic changes (100%) and decreased urine specific gravity (USG) (50%) were present. The mDP was 943 (IQR 488-1434) and mST was 1678 (IQR 1129-2349) days.

All stage II cats (100%, *n*=28) showed azotemia, decreased USG, followed by ultrasonographic changes (96.4%), polyuria/polydipsia (PU/PD) (57.4%), dehydration (50%), proteinuria (46.4%), hyporexia (42.9%), lethargy (36%), weight loss (WL) (32.1%), vomiting (19%), anorexia (10%), hyperphosphataemia (10.7%), and neurological signs (NS) (3.6%). Cats were hospitalized 1 to 2 times, with a median duration of 3 days (IQR 0.5-4). The mDP was 848 (IQR 488-1464) and mST was 885 (IQR 488-1159) days.

All stage III cats (100%, *n*=10) presented azotemia, decreased USG, dehydration, proteinuria, ultrasonographic changes, followed by WL (80%), lethargy (70%), hyperphosphataemia (70%), vomiting (50%), PU/PD (57.14%), anorexia (50%), hyporexia (20%), NS (20%), and hypocalcaemia (10%). Cats were hospitalized 1 to 2 times, with a median duration of 4 days (IQR 3-4). The mDP was 336 (IQR 182-366) and mST was 345 (IQR 214-519) days.

All stage IV cats (100%, *n*=6) exhibited anorexia, azotemia, dehydration, WL, decreased USG, ultrasonographic changes, and hyperphosphataemia, followed by hypocalcaemia (83.3%) lethargy (83.3%) vomiting (50%), proteinuria (46.4%), NS (33.3%) and PU/PD (16.7%). Cats were hospitalized 3 to 5 times, with a median duration of 5 days (IQR 5-6). Both mDP and mST were 114 (IQR 110-150) days.

CONCLUSION: Factors significantly associated with stage III and IV included anaemia, anorexia, NS, WL hyperphosphataemia and hypocalcaemia. These findings underscore the importance of early CKD diagnosis in cats, as the disease advances through the IRIS stages, it is associated with worsening clinical signs, more frequent and prolonged hospitalizations and a decrease in median survival time. Further studies on CKD etiology are urgently needed to foster targeted therapeutics and preventive strategies.

fct Fundação para a Ciência e a Tecnologia

CASE SERIES OF IDIOPATHIC CHRONIC KIDNEY DISEASE IN CATS: A RETROSPECTIVE STUDY

Luís Vasconcelos Pessoa [1] ; Gonçalo Santos [1,2,3] ; Elsa Pereira [4] ; Maria Teresa Villa de Brito [1,2,3]

1- FMV - Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal;
2-CIISA - Interdisciplinary Research Centre in Animal Health, Faculty of Veterinary Medicine, University of Lisbon;
3-Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal
4-Veterinary Clinic Dra. Elsa Pereira, Praceta D.Luís I, 6, 2610-197 Alfragide, Portugal

INTRODUCTION

Chronic Kidney Disease (CKD) in cats is a **progressive and irreversible condition**, predominantly of idiopathic origin. Its high prevalence among senior and geriatric individuals, coupled with its significant impact on quality of life, renders CKD **one of the foremost diseases under scientific scrutiny.**

AIM

Deliver a comprehensive clinical and prognostic characterisation of idiopathic CKD in cats:

- **Unveiling stage-specific indicators of disease progression and survival.**
- **Enhancing understanding of the disease's clinical course.**
- **Reinforcing the critical importance of early diagnosis** to transform outcomes in feline nephrology.

METHODOLOGY

- **Data Source:** Clinical records (2018–2023) from the HEV Lisbon University Veterinary Hospital were retrospectively reviewed.
- **Inclusion Criteria:** Cats with elevated serum creatinine and SDMA (IRIS stages II–IV); a convenience sample of stage I cats was also included.
- **Staging:** CKD classified according to the 2023 IRIS guidelines.
- **Exclusion Criteria**: Cats with extra-renal comorbidities were excluded.
- **Outcomes**: Median time to progression (mDP) and median survival time (mST) were calculated.
- **Statistical Analysis:** Performed with significance set at $p < 0.05$.

Results

Clinical Signs

Stage II

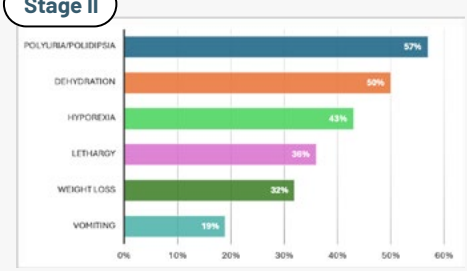

Figure 1. Prevalence of clinical signs observed in feline patients diagnosed with IRIS stage II CKD.

Stage III

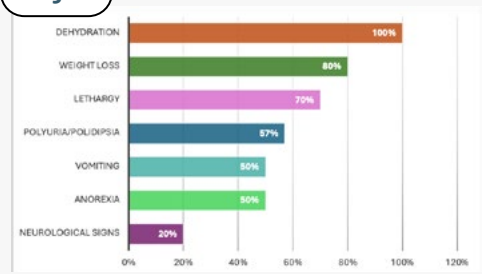

Figure 2. Prevalence of clinical signs observed in feline patients diagnosed with IRIS stage III CKD.

Stage IV

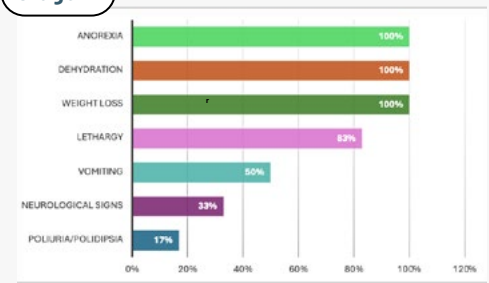

Figure 3. Prevalence of clinical signs observed in feline patients diagnosed with IRIS stage IV CKD.

Analytical Biomarkers

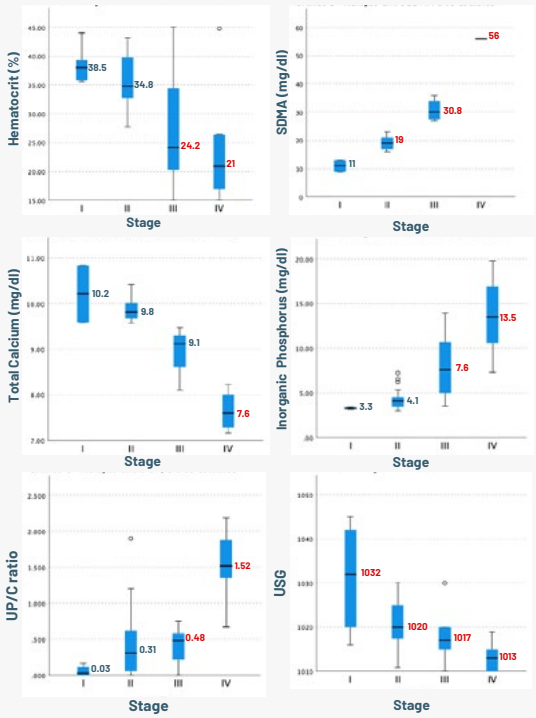

Figure 4. Association between renal biomarkers and IRIS stages of CKD. The depicted biomarkers demonstrated statistically significant differences between the early stages (I and II) and the advanced stages (III and IV) of CKD. Values highlighted in red fall outside the established reference intervals.

Ultrasound

	Stage I	Stage II	Stage III	Stage IV
Reduced Kidney Size	0%	35,7%	70%	100%
Irregular Kidney Margins	16,67%	50%	80%	100%
Corticomedullary definition loss	100%	96,4%	100%	100%
Increased cortical echogenicity	100%	92,9%	100%	100%
Renal parenchymal mineralisation	33,3%	10%	70%	100%

Table 1. Ultrasonographic findings prevalence acording to the stages of IRIS CKD guidelines.

IRIS stages duration time

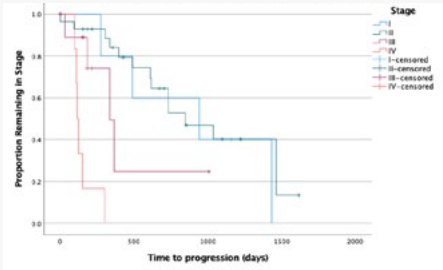

Figure 5. Time to progression between IRIS CKD stages. Median time: Stage I - 943 (IQR 488-1434) days ; Stage II-848 (IQR 488-1464) days ; Stage III - 336 (IQR 182-366) days ; Stage IV -114 (IQR 110-150) days.

Survival time

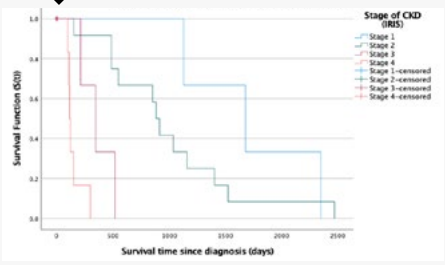

Figure 6. Median survival time: Stags I - 1678 (IQR 1129-2349) days ; Stage II- 885 (IQR 488-1159) days ; Stage III - 345 (IQR 214-519) days ; Stage IV - 114 (IQR 110-150) days.

Conclusion

Factors significantly associated with **stage III** and **IV** included **anaemia, anorexia, neurological signs, weight loss, hyperphosphataemia, hypocalcaemia** and **proteinúria.** These findings underscore the importance of **early CKD diagnosis** in cats. As the disease advances through the **IRIS stages**, it is associated with **worsening clinical signs**, **more frequent and prolonged hospitalizations** and a **decrease in median survival time.** Further studies on CKD etiology are urgently needed to foster **targeted therapeutics** and **preventive strategies.**

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PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc

LT 3 Comparative and Translational Medicine and Biotechnology

LT3.08

PRECISION MEDICINE FOR CANINE LYMPHOMA: IDENTIFICATION OF NOVEL AND POTENT CYTOTOXIC COMPOUNDS FOR CONJUGATION INTO AN ANTIBODY-DRUG CONJUGATE

Rita Pedrosa[1,2,3], Rafaela Marimon[1,2], Ana André[1,2], Gonçalo Vicente[1,2,3], Solange Gil[1,2,3], Luís Tavares[1,2], Frederico Aires-da-Silva[1,2]

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INTRODUCTION: Canine lymphoma is the most common hematopoietic malignancy in dogs, accounting for 7% to 24% of all diagnosed canine cancers. Despite its significant impact on canine health, treatment options remain limited. Due to its frequent systemic presentation, multiagent chemotherapy is the treatment of choice in most cases. However, fewer than 10% of affected dogs achieve the cure and this therapeutic approach has limitations such as the development of drug resistance and untargeted cytotoxicity. In human medicine, immunotherapy has emerged as a complementary or adjunctive therapeutic strategy to conventional chemotherapy, specifically targeting cancer cells and using alternative mechanisms of action. One example are antibody-drug conjugates (ADCs). These molecules consist of an antibody linked to a cytotoxic payload, combining the specificity of the antibody moiety with the potency of the cytotoxic payload. Bifunctional ADCs enable the simultaneous delivery of two molecules with distinct mechanisms of action, aiming to mitigate the development of drug resistance. Therefore, immunotherapy hold the potential to deliver safer and more effective clinical outcomes.

OBJECTIVES: The aim of this study is to identify potent cytotoxic compounds with novel mechanisms of action against canine lymphoma cells. These compounds are intended for conjugation into a previously studied ADC targeting canine diffuse large B-cell lymphoma, with the goal of developing a bifunctional ADC. This therapeutic approach has the potential to improve treatment outcomes and provide more options for cases that are refractory to conventional therapies.

METHODOLOGY: A panel of eight promising compounds was tested in two well-established canine lymphoma cell lines (CLBL-1 and 17-71). Microtubule inhibitors (MMAF, MMAE, and DM1) and targeted protein degraders (MS4078, DT22-16, NX-2127, ARV-825, and ARV-771) were the selected compounds. Cytotoxicity assays were conducted, and the half-maximal inhibitory concentration (IC_{50}) values were determined for each compound in both cell lines. In addition to assessing their individual potency, the synergistic effects when combining SN-38, MMAE, and ARV-825 were evaluated.

RESULTS: Most compounds exhibited a concentration-dependent cytotoxicity in both cell lines, with some demonstrating extremely high potency. The most potent compounds were MMAE, DM1, and ARV-825 with IC_{50} values ranging from 380 pM to 67,41 nM. In addition, the combination of SN-38 with ARV-825 demonstrated a potential synergistic effect.

CONCLUSIONS: This study identified MMAE, DM1, and ARV-825 as highly potent cytotoxic compounds against canine lymphoma cell lines, supporting their potential as ADC payloads for future targeted therapies. Furthermore, it was demonstrated that combining molecules with distinct mechanisms of action has the potential to enhance therapeutic efficacy, thereby validating the development of bifunctional ADCs. These findings provide a foundation for developing novel targeted therapies in veterinary oncology.

ACKNOWLEDGEMENTS/FUNDING: This work was supported by CIISA through Project UIDB/00276/2020, funded by Fundação para a Ciência e a Tecnologia (FCT) and LA/P/0059/2020-AL4AnimalS.

Precision medicine for canine lymphoma: Identification of novel and potent cytotoxic compounds for conjugation into an antibody-drug conjugate

Rita Pedrosa [1,2,3 (*)], Rafaela Marimon [1,2], Ana André [1,2], Gonçalo Vicente [1,2,3], Solange Gil [1,2,3], Luís Tavares [1,2], Frederico Aires-da-Silva [1,2]

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BACKGROUND

Canine Lymphoma (cL) is the most common hematopoietic malignancy diagnosed in dogs. Multi-agent chemotherapy is, in most cases, the treatment of choice for this type of neoplasia. However, these protocols lack specificity since they target dividing cells. The onset of drug resistance is also a growing concern, particularly given the absence of effective alternatives for cL treatment. In human medicine, immunotherapy has emerged as an alternative or adjunctive therapeutic strategy to conventional chemotherapy. Antibody-drug conjugates (ADCs) are an example of an innovative immunotherapy that combines payload cytotoxicity with antibody selectivity. It is possible to conjugate payloads with distinct mechanisms of action (MoA), which can help overcome drug resistance.

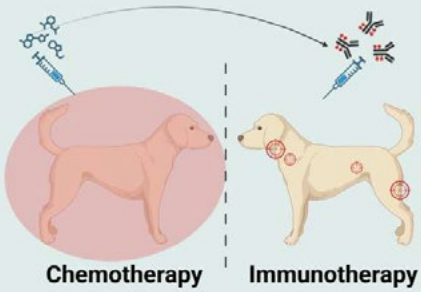


Figure 1. Schematic representation of the different areas affected by chemotherapy and immunotherapy.

The aim of this study is to **select the most promising compounds** for further conjugation with ADCs to **develop a bifunctional canine lymphoma-targeting molecule.** Thus, improving treatment outcomes and providing more options for refractory cases.

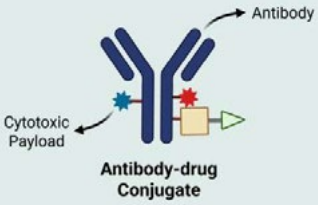


Figure 2. ADC structure.

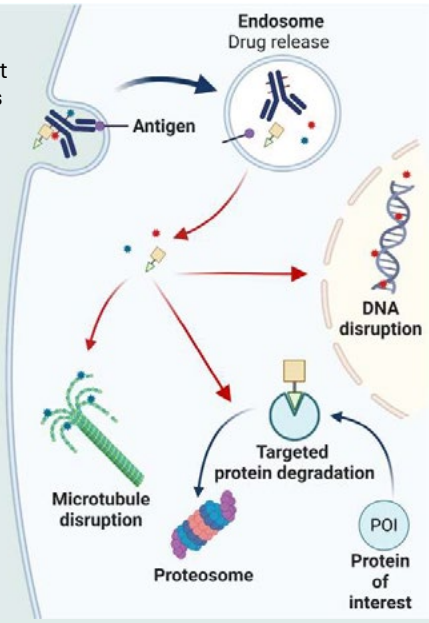


Figure 3. Illustration of the ADC targeting and its mechanism of action with different cytotoxic payloads.

METHODOLOGY

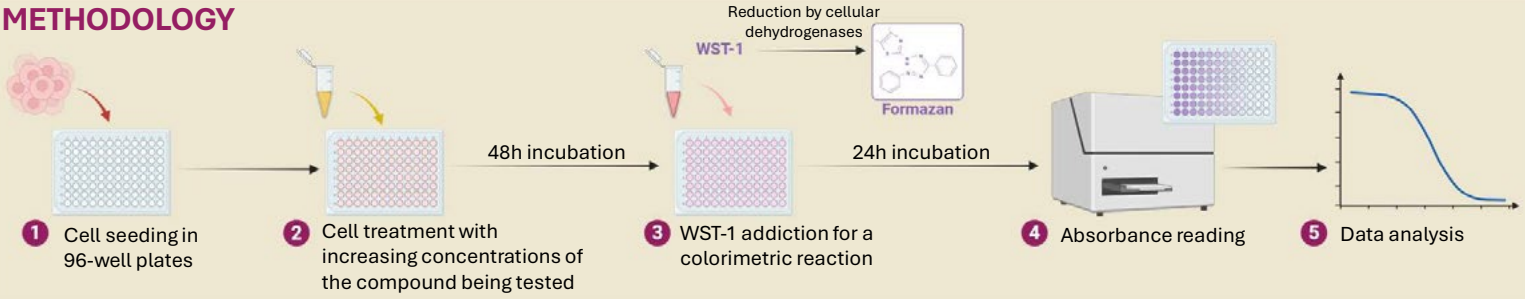
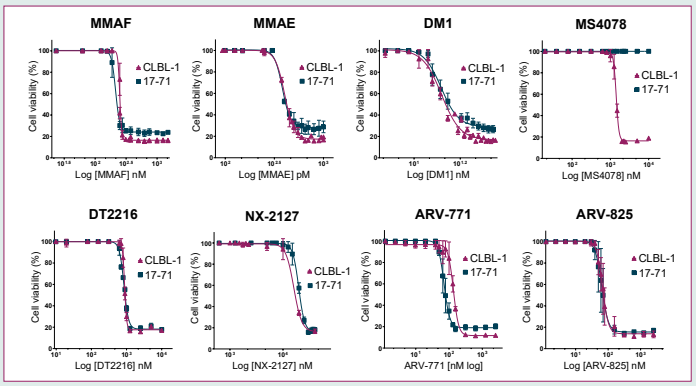


Figure 4. Schematic representation of the methodology used for the cytotoxicity assays.

RESULTS

A



B

	IC_{50} (nM)	CLBL-1	17-71
Microtubule inhibitors	MMAF	249.7	214.3
	MMAE	0.4	0.38
	DM1	13.16	13.11
PROTACs	MS4078	1421	-
	DT2216	882.1	844.9
	NX-2127	15 988	19 785
	ARV-825	67.41	63.60
	ARV-771	135.20	71.01

C

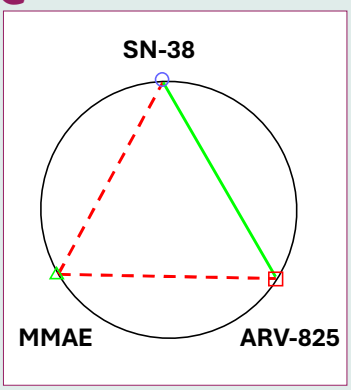


Figure 5. (**A**) Results obtained from cytotoxicity assays of the various compounds on two canine lymphoma cell lines. (**B**) Best-fit IC_{50} values of each molecule. (**C**) Polygonogram image where synergism is represented by a solid green line and antagonism by a dashed red line.

CONCLUSION

- All compounds demonstrated a concentration-dependent cytotoxicity.
- The IC_{50} values were similar between both cell lines except for MS4078.
- The most potent compound was **MMAE** followed by **DM1** and **ARV-825**.
- SN-38 and ARV-825 showed a synergistic effect when combined.

Our study identified extremely potent molecules with distinct mechanisms of action that can be further incorporated into ADCs, offering a more targeted, effective and safe way to approach cL treatment.

ACKNOWLEDGEMENTS

CIISA has provided support through Project UIDB/00276/2020, funded by FCT and LA/P/0059/2020-AL4AnimalS.

PAINÉIS CIENTÍFICOS A CONCURSO

MESTRADOS

MSc LT3.09

LT 3 Comparative and Translational Medicine and Biotechnology

EVALUATION OF PD-L2 AS A SEROLOGICAL BIOMARKER OF FELINE MAMMARY CARCINOMA

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INTRODUCTION: Feline mammary carcinoma is the third most common tumor in cats, presenting an aggressive behavior, low survival rate and limited treatment options. So, the identification of diagnostic tools for earlier detection and more effective treatment is crucial. Considering the clinicopathological similarities between the feline mammary carcinoma and the human mammary cancer, this animal model is an invaluable source for comparative oncology studies. PD-1/PD-L1/PD-L2 axis is nowadays under investigation, because of its role in human breast cancer, presenting promising results in immunotherapy. However, some breast cancer patients have an incomplete response to this treatment, while some PD-L1 negative patients have a positive response, with more studies on PD-L2 role being needed. To the best of our knowledge, no studies evaluating soluble PD-L2 levels in cats with mammary carcinoma had been conducted before.

AIMS: Validate serum PD-L2 as a diagnostic and prognostic biomarker in feline mammary carcinoma.

METHODOLOGY: Serum PD-L2 levels in 52 female cats with mammary carcinoma were compared with 28 healthy controls, through enzyme-linked immunosorbent assay (ELISA). In the study, associations between serum PD-L2 levels and clinicopathological features or between mammary carcinoma subtypes were investigated. In addition, correlations between soluble PD-L2, PD-L1 and PD-1 levels were evaluated.

RESULTS: Cats with mammary carcinoma showed significantly higher serum PD-L2 levels than healthy controls ($p<0,0001$) and ROC analysis revealed that the best cut-off value to differentiate sick from healthy animals was 1989 pg/ml (specificity: 96,5%; sensitivity: 93,7%; AUC=0,98). HER2-positive and TNBC cats exhibited significantly higher serum PD-L2 levels than other subtypes ($p<0,0001$) and ROC analysis revealed that the best cut-off value to differentiate cats with these subtypes from luminal A and luminal B subtypes was 5622 pg/ml (specificity: 95,3%; sensitivity: 82,6%; AUC=0,92). Moreover, a positive correlation was demonstrated between serum levels of PD-L2, PD-L1 and PD-1. No statistical associations were found between serum PD-L2 and the different clinicopathological features. No statistical associations were also found between serum PD-L2 and Ki67 index, ER and CK5/6 status. However, an association between serum PD-L2 levels and PR ($p<0,045$) and HER2 status ($p<0,06$) was verified. Addicionally, no statistical associations were found between serum PD-L2 and disease-free survival (DFS) ($p<0,28$) and overall survival (OS) ($p<0,82$).

CONCLUSIONS: Results suggest that serum PD-L2 levels might have value as a diagnostic and prognostic biomarker feline mammary carcinoma, especially in HER2 positive and TNBC. To confirm the predictive value of serological PD-L2 in feline mammary carcinoma further studies are needed.

ACKNOWLEDGMENTS/FUNDING: This research was funded by Fundação para a Ciência e a Tecnologia (Portugal) through the project CIISA-UIDP/CVT/00276/2020. V.S.J is receipt of a MSc fellowship from CIISA.

Evaluation of PD-L2 as a serological biomarker of feline mammary carcinoma

Vitória Silva João[1,2], Jorge Correia[1,2], João Ferreira[3], Fernando Ferreira[1,2]

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[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal.

[3] Gulbenkian Institute for Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Introduction

- Feline mammary carcinoma is the third most common tumor in cats, characterized by aggressive behavior, with low survival rate and limited treatment options.
- This disease shares several clinicopathological features with human mammary cancer, being a model for comparative oncology studies.
- PD-1/PD-L1/PD-L2 axis plays an important role in immune evasion and in breast cancer progression, presenting promising results in immunotherapy.
- However, some human patients do not respond completely to the immune checkpoint inhibitors and some PD-L1 negative patients have a positive response, being a deeper study on PD-L2 involvement needed.
- To the best of our knowledge, no studies evaluating soluble PD-L2 levels in cats with mammary carcinoma had been conducted before.

Objectives

- Validate serum PD-L2 as a diagnostic and prognostic biomarker in feline mammary carcinoma.

Methods

- **Biological Sample Collection:** Serum PD-L2 levels in 52 female cats with mammary carcinoma were compared with 28 healthy controls collected from Small Animal Hospital of FMV ULisboa and Assoc. Zoófila Portuguesa.
- **ELISA kit used**: commercially available PDCD1LG2 ELISA kit from WUHAN HUAMEI BIOTECH Co
- **ELISA procedure:** 96 multi-well plates peptide coated were incubated with standard or serum sample, followed by incubation with a Biotin-antibody, and wash 3 times. Then an incubation with HRP-avidin was performed, followed by an aspiration and a 5 times wash. Next, TMB substrate was add and the multi-well plates were incubated. After the addition of the Stop solution, the absorbance was read at 450 nm.
- **Evaluations**: Associations between serum PD-L2 levels and clinicopathological features or between mammary carcinoma subtypes were validated. Correlations between soluble PD-L2, PD-L1 and PD-1 levels were also checked.
- **Statistical Analysis**: The two spreadsheets of the Microsoft Excel program (version 16.30, Microsoft Corporation, Redmond, WA, USA) containing the data of the cats used in the present study were imported into the SAS program (SAS 9.4, SAS Institute Inc., Cary, NC, USA), in which the statistical analysis was performed.

Results

- Cats with mammary carcinoma showed significantly higher serum PD-L2 levels than healthy controls ($p<0.0001$, Fig.1).
- ROC analysis revealed that the best cut-off value to differentiate diseased from healthy animals was 1989 pg/ml (specificity: 96.5%; sensitivity: 93.7%; AUC=0,98, Fig.2)
- HER2-positive and TNBC cats exhibited significantly higher serum PD-L2 levels than other subtypes ($p<0.0001$, Fig.3).
- ROC analysis revealed that the best cut-off value to differentiate cats with these subtypes from luminal A and luminal B subtypes was 5622 pg/ml (specificity: 95.3%; sensitivity: 82.6%; AUC=0.92, Fig.4).
- A positive correlation was observed between PD-L2, PD-L1 and PD-1 serum levels.
- No statistical associations were found between serum PD-L2 and the different clinicopathological features.
- No statistical associations were also found between serum PD-L2 and Ki67 index, ER and CK5/6 status.
- An association between serum PD-L2 levels and PR ($p<0,045$) and HER2 status ($p<0.06$) was verified.
- No statistical associations were found between serum PD-L2 and disease-free survival (DFS) ($p<0.28$) and overall survival (OS) ($p<0.82$).

Conclusion

- Results suggest that serum PD-L2 levels might have value as a diagnostic and prognostic biomarker of feline mammary carcinoma, especially in HER2 positive and TNBC. To confirm the predictive value of serological PD-L2 in feline mammary carcinoma further studies are needed.

Acknowledgments/Funding

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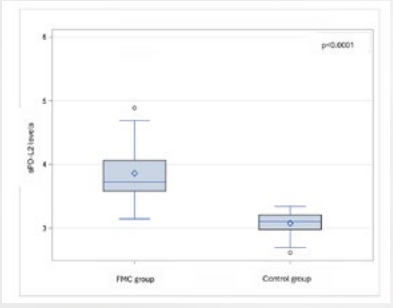

Figure 1: Box plot diagrams representing the serum PD-L2 levels of queens with mammary carcinoma (FMC group) and healthy cats (control group) after transformed by the equation log (x+1).

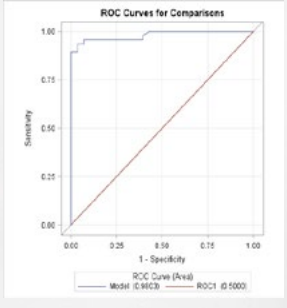

Figure 2: Receiver-operating characteristic (ROC) curve of serum PD-L2 levels for ELISA.

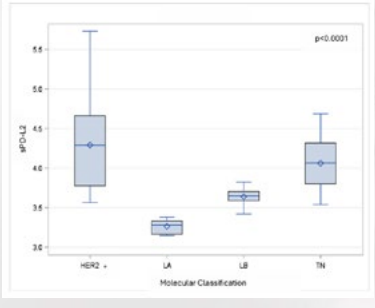

Figure 3: Cats with HER2-overexpressing mammary carcinoma and TNBC show higher serum PD-L2 levels than animals with other FMC molecular subtypes

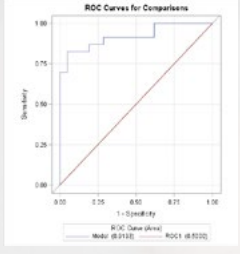

Figure 4: Receiver-operating characteristic (ROC) curve of serum PD-L2 levels of cats with the most aggressive subtypes (HER-2-overexpressing and TNBC) and cats with less aggressive subtypes (Luminal A and Luminal B).

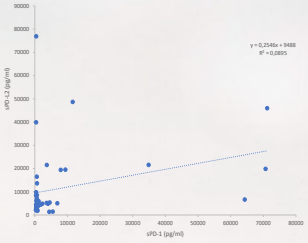

Figure 5: sPD-L2 shows a positive correlation with sPD-1 levels in cats with FMC.

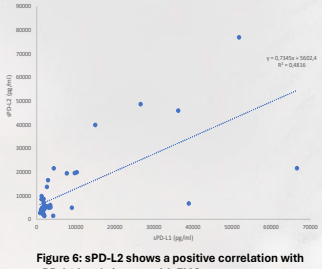

Figure 6: sPD-L2 shows a positive correlation with sPD-L1 levels in cats with FMC.

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

LT 1
Green Animal Production

PhD LT1.01	Chlorella Vulgaris in Broiler Diets: Synergistic Effects of Extrusion And Enzyme Supplementation on Growth Performance
PhD LT1.02	Fatty Acid Composition of Fat From Goat Kids Produced In Alentejo – Portugal
PhD LT1.03	Genomic Detection of Selection Signatures Linked to Reproduction and Morphology in South Angolan Sheep
PhD LT1.04	Feeding Sunflower Oil Enriched With Bromoform From Asparagopsis Taxiformis On Young Bulls' Growth, Health and Ruminal Methane Emissions
PhD LT1.05	Genetic Characterization of Native Sheep (Ovis Aries) From South of Angola
PhD LT1.06	Genomic Characterization of Autochthonous Cattle From Angola
PhD LT1.07	Effect of Pre-Weaning Feeding Management On Fatty Acid Composition of The Fat of Lambs Finished With a High-Cereal Diet
PhD LT1.08	The Inclusion of Almond Hulls in Lamb Diets Improves the Fatty Acid Composition of Intramuscular Fat
PhD LT1.09	Genetic Diversity and Demographic Structure of The Lusitano Horse Breed

LT 2
Emergent Infectious Diseases and Zoonosis

PhD LT2.08	Phylogenetic Analysis of Asfv Sf2 Helicase Proteins Reveals Distinct Evolutionary Patterns Across Isolates
PhD LT2.09	*Encephalitozoon Cuniculi* In Pet Rabbits – A One Health Issue?
PhD LT2.10	A Single-Domain Antibody Platform Targeting Sars-Cov-2 and Future Viral Threats
PhD LT2.11	Viral Diversity in Portuguese Bats: A Metagenomic Study
PhD LT2.12	Effect of Alginate Coatings With Laurel and Olive Plant Extracts Against L. Monocytogenes And Salmonella Inoculated in Chicken Meat
PhD LT2.13	Combining Tlr2 With Tlr4 or Tlr9 Agonists In Liposomal Formulations to Fine-Tune Vaccine-Induced Immune Responses
PhD LT2.14	Diagnostics and Diversity: Molecular Characterization of Cryptosporidium Spp. in Reptiles
PhD LT2.15	Bat-Associated Coronavirus Found in Pigs: Insights Into Potential Cross-Species Transmission
PhD LT2.16	Molecular Detection of Canine Gastrointestinal Viruses: A Call For One Health Surveillance
PhD LT2.33	A Deep Look at Canine Monocyte-Derived Cells in The Context of Canine Leishmaniosis: Phase Contrast and Scanning Electron Microscopy

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

LT 3

Comparative and Translational Medicine and Biotechnology

PhD LT3.10	Desialylation-Based Immunotherapy for Feline Mammary Carcinoma
PhD LT3.11	Electroretinography as a Measure of Retinal Tolerance to Conditioned Media Application in Healthy Rats
PhD LT3.12	Short-Term Safety of Topical and Oral CBD in Dogs: A Preclinical Evaluation
PhD LT3.13	Ovarian Metastases in a Queen With Synchronous Mammary and Endometrial Adenocarcinoma
PhD LT3.14	Advanced Biomaterials for Bone Regeneration: Combining Composites and Bioactive Hydrogels
PhD LT3.16	Adipokines and Macrophage Interplay in Cow Postpartum Subclinical Endometritis
PhD LT3.17	Biodegradable Polymers For the Fabrication of Feline Ureteral Stents: Mechanical and Topographical Characterization in an Feline Artificial Urine Model
PhD LT3.18	Chitosan and Hyaluronic Acid Nanoparticulate System Development for Enhanced Ocular Delivery of Dorzolamide
PhD LT3.19	Global Profiling of Testicular and Epididymal Sperm Proteomes: Protein Markers of Sperm Post-Testicular Maturation
PhD LT3.20	Cannabinoid Receptors Cb1 and Cb2 in Canine Amelanotic Oral Melanomas: A Spontaneous Model To Explore Translational Targets In Immuno-Oncology
PhD LT3.21	Is Exon Skipping the Key to Correct N-Acetylglucosamine-1-Phosphotransferase Deficiency? An Antisense Oligonucleotide Therapeutic Approach
PhD LT3.22	Immune Landscapes of The Canine Oral Mucosa: Optimising Targeted Oro-Mucosal Immunotherapy
PhD LT3.23	Canine Melanoma and Focal Adhesion Kinase-Targeted Strategies In Veterinary Oncology
PhD LT3.24	3d Bioprinting of A Secretome-Infused Contact Lens for Corneal Wound Healing
PhD LT3.25	Comparison of NGF and Receptors Gene Transcription in Equine Endometrium
PhD LT3.26	Immunohistochemical Characterization and Localization 11 β – Hydroxysteroid Dehydrogenase Type 2 in the Feline Kidney and Adrenal Gland – Is There a Link to Chronic Kidney Disease?
PhD LT3.27	Lymphocyte Profile of Healthy Cats: Preliminary Results of a Flow Cytometry Study
PhD LT3.28	Grape Stem Polyphenol Extracts on Prostate Cancer Cell Modulation
PhD LT3.29	Silanized Hyaluronic Acid Hydrogels for Advanced Skin Therapy

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT1.01

LT 1 Green Animal Production

CHLORELLA VULGARIS IN BROILER DIETS: SYNERGISTIC EFFECTS OF EXTRUSION AND ENZYME SUPPLEMENTATION ON GROWTH PERFORMANCE

Ana Rita Mendes[1,2,3]; Daniela Carvalho[1]; Obete Madacussengua[1]; Cátia Martins[1,4]; Maria Spínola[2,3]; Joana Ferreira[2,3]; Ana Fontes[2,3]; José Pestana[2,3]; André Almeida[1,4]; Madalena Lordelo[1,4], José Prates[2,3]

[1] LEAF – Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal
[2] CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal
[3] AL4AnimalS – Laboratório Associado para Ciência Animal e Veterinária, Universidade de Lisboa, Lisboa, Portugal
[4] TERRA – Associate Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal

INTRODUCTION: *Chlorella vulgaris* (MCV) is a sustainable and protein-rich microalga with potential as a feedstuff for poultry. However, its rigid cell wall limits nutrient digestibility and bioavailability. Pretreatment methods such as extrusion and enzymatic hydrolysis have shown promise in enhancing these properties.

AIM: Building on recent studies investigating MCV incorporation, this research evaluated the effects of dietary inclusion of MCV at 15%, with extrusion alone or combined with pancreatin treatments, on broiler performance.

METHODS: From day 7 to 35, 120 male Ross 308 broilers were randomly assigned to four diets: CTR (maize–soybean meal based), CV (15% MCV), CVEX (15% extruded MCV), and CVEXEZ (CVEX + 0.30% pancreatin). Animals were housed in 10 cages per treatment (3 birds per cage), with *ad libitum* feeding. Feed intake (FI), body weight (BW), daily body weight gain (DBWG), feed conversion ratio (FCR), and beak adhesion scores (BS) were recorded weekly. On day 35, one animal per cage was euthanised for organ weight and intestinal viscosity assessment.

RESULTS: BW was significantly lower in the CV group in comparison to CTR on days 14 and 21 (p = 0.037 and p = 0.018), indicating a temporary adaptation phase. FCR varied across weeks - initially higher in MCV-based diets, then aligning with or improving with time, suggesting a progressive physiological adjustment to the diets. No significant differences in BW, DBWG, or FCR were observed between treatments across the full trial period (p > 0.050), while FI was higher in CVEXEZ compared to CV (p = 0.012). Animals on all MCV-based diets had lower relative weights of the proventriculus, liver, and jejunum (p < 0.001), but showed higher carcass and breast meat yields compared to CTR (p < 0.001). All MCV diets increased BS (p < 0.002), though this did not appear to negatively impact FI, and excreta viscosity remained unchanged across treatments (p > 0.050), suggesting no detrimental impact on gut health.

CONCLUSIONS: Despite minor early setbacks, broilers adapted well to 15% MCV inclusion, with no negative effects on growth performance by day 35. Enhanced carcass yields and stable overall performance support the feasibility of using MCV in broiler diets, without requiring pretreatment for digestibility enhancement.

ACKNOWLEDGMENTS/FUNDING: This research was funded by grants from the Foundation for Science and Technology (FCT, Lisbon, Portugal) through LEAF (UIDB/04129/2020), TERRA (LA/P/0092/2020), CIISA (UIDB/00276/2020), and AL4AnimalS (LA/P/0059/2020). Financial support was also provided by FCT through PhD grants awarded to A.R.M. (2022.11690.BD), M.S. (UI/BD/153071/2022), O.M. (SFRH/BD/151524/2021), and a Post-Doctoral fellowship to J.M.P. (SFRH/BPD/116816/2016). The Portugal 2020 project (P2020/17/SI/70114/2019) also contributed to funding.

Chlorella vulgaris in Broiler Diets: Synergistic Effects of Extrusion and Enzyme Supplementation on Growth Performance

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Supported by LA/P/0059/2020

INTRODUCTION

Chlorella vulgaris, a microalgae, is an alternative ingredient to soybean meal in poultry diets

Its **recalcitrant cell wall** limits its digestibility, restricting its inclusion at high levels for broilers

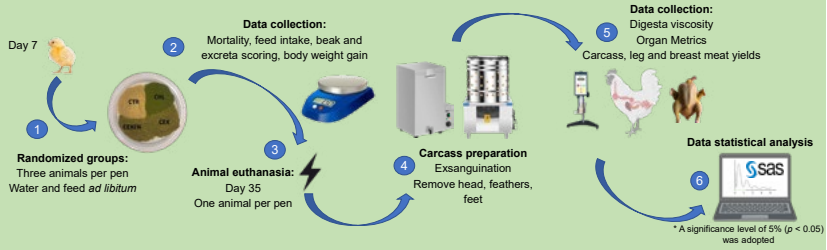
Pancreatin and Extrusion

Have been shown to improve *Chlorella vulgaris's* digestibility ***in vitro***

Aim: To evaluate the inclusion of 15% *Chlorella vulgaris* in broilers diets and asses animal performance as well as two processing strategies – extrusion and extrusion combined with super-dose pancreatin – to improve digestibility

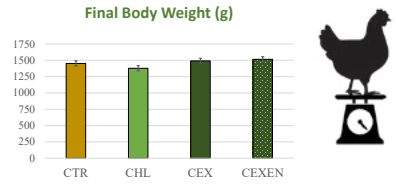
MATERIALS AND METHODS

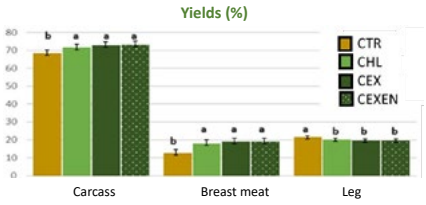
Strain: Ross 308
Microalgae: *Chlorella vulgaris* at 15% incorporation level
Experimental design: 120 male broilers:
10 replicates per treatment of 3 animals each
Four Treatments (from day 7 to day 35):
- **CTR** - Control diet (maize-soybean meal);
- **CHL** - 15% incorporation of *Chlorella vulgaris;*
- **CEX** - 15% incorporation of extruded *Chlorella vulgaris*;
- **CEXEN** - CEX + 0.3% pancreatin enzyme.

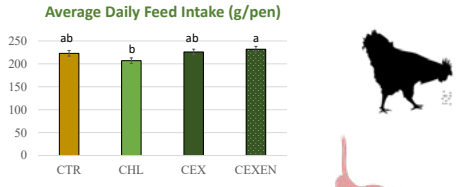


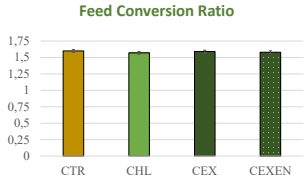
RESULTS*

PERFORMANCE PARAMETERS









ORGAN METRICS

	CTR	CHL	CEX	CEXEN	SEM	*p-value*
Relative weight, g/kg body weight						
Crop	3.87	3.86	3.83	3.38	0.327	0.668
Proventriculus	5.32^{a}	3.43^{b}	3.47^{b}	3.66^{b}	0.256	< .001
Gizzard	16.0	14.9	15.8	15.7	0.61	0.583
Liver	24.4^{a}	18.7^{b}	19.9^{b}	18.8^{b}	0.92	<.001
Duodenum	6.88^{a}	6.21^{ab}	5.78^{b}	6.19^{ab}	0.273	0.034
Jejunum	12.49^{a}	10.10^{b}	10.02^{b}	9.60^{b}	0.484	< .001
Ileum	10.62	9.72	10.08	9.14	0.417	0.100
Caecum	6.14^{a}	5.12^{b}	5.62^{ab}	5.19^{b}	0.248	0.023
Relative length, cm/kg body weight						
Duodenum	19.3	20.4	18.2	18.8	0.67	0.108
Jejunum	51.8	52.8	49.3	47.4	1.96	0.224
Ileum	56.1^{ab}	59.5^{a}	53.0^{bc}	51.3^{c}	1.27	< .001
Caecum	12.4	14.1	12.6	11.9	0.67	< .001

VISUAL BEAK SCORING

Visual Beak Scoring ranged from 1 (clean beak) to 5 (severe feed adhesion)

	CTR	CHL	CEX	CEXEN	SEM	*p-value*
Day 14	1.67^{b}	2.87^{a}	3.13^{a}	2.63^{a}	0.222	< .001
Day 21	1.28^{c}	2.08^{b}	2.88^{a}	2.33^{ab}	0.160	< .001
Day 28	1.47^{b}	2.34^{a}	2.51^{a}	2.38^{a}	0.192	0.002
Day 35	1.28^{b}	1.90^{a}	2.21^{a}	1.97^{a}	0.160	0.002

DIGESTA VISCOSITY

cP	CTR	CHL	CEX	CEXEN	SEM	*p-value*
Duodenum + Jejunum	5.19	6.16	5.15	5.52	0.469	0.411
Ileum	9.32	8.20	8.45	8.74	0.765	0.758

VISUAL EXCRETA SCORING

Visual Excreta Scoring ranged from 1 (firm, normal) to 5 (watery, diarrhea-like consistency)

	CTR	CHL	CEX	CEXEN	SEM	*p-value*
Day 14	1.00	1.00	1.00	1.00	-	-
Day 21	1.00^{b}	1.60^{a}	1.60^{a}	1.60^{a}	0.101	< .001
Day 28	1.10^{b}	1.70^{a}	1.30^{ab}	1.45^{ab}	0.127	0.016
Day 35	1.00^{b}	1.35^{a}	1.15^{ab}	1.05^{ab}	0.079	0.018

CONCLUSIONS & TAKE HOME MESSAGE

Incorporating 15% *Chlorella vulgaris* in broiler diets from day 7:
- Did not impair growth performance
- Increased carcass and breast meat yield across all *Chlorella* groups
- With combined extrusion & pancreatin increased feed intake compared to untreated *Chlorella*
- Increased beak scores ➡ potentially decreased feed intake
- Increased excreta scores ➡ pre-treatments mitigated the effect
- Induced organ-level physiological adaptations ➡ decreased length/weight
- Did not significantly affect digesta viscosity

***Chlorella vulgaris* can be incorporated at 15% from day 7 without pre-treatments, sustaining performance and increasing carcass yield**

This research was funded by FCT - Fundação para a Ciência e a Tecnologia, I.P. grants: 2022.11690.BD to A.R.M., SFRH/BD/151524/2021 to O.M., UI/BD/153071/2022 to M.P.S., project UID/04129 of LEAF, UIDB/00276/2020 to CIISA and LA/P/0059/2020 to AL4AnimalS.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT1.02

LT 1 Green Animal Production

FATTY ACID COMPOSITION OF FAT FROM GOAT KIDS PRODUCED IN ALENTEJO – PORTUGAL

Andreia Silva[1,2,3]; Olinda Guerreiro[1,4]; Susana P. Alves[2,3,5]; José Santos-Silva[2,6]; Rui J. B. Bessa[2,3,5] and Eliana Jerónimo[1,4]

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[2] CIISA – Center for Interdisciplinary Research in Animal Health Lisbon, Portugal.
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INTRODUCTION: Information on the fatty acid (FA) composition of fat from small ruminants produced in Portugal is currently limited. The FA composition of ruminant fat depends on its complex lipid metabolism, which is influenced by several factors, including diet.

AIM: This study aimed to characterize the FA composition of goat kids' fat produced in the Alentejo region, in two production seasons (autumn/winter (A/W) vs spring (Sp)).

METHODOLOGY: A total of 70 goat kids' samples of kidney knob channel fat (KKCF) were collected from 11 producers from Alentejo region in two production seasons – A/W (36 samples) and Sp (34 samples) characterized by lower and higher pasture availability, respectively. The KKCF samples were collected in slaughterhouse, after carcass cooling. Lipid extraction and combined transesterification of FA were performed. The FA methyl esters were analysed through GC-FID. SAS mixed procedure was used for statistical analysis.

RESULTS: The proportions of linear chain saturated (LC-SFA), monounsaturated (MUFA) and polyunsaturated FA (PUFA) were not affected by production seasons (P>0.05). Branched chain FA (BCFA) were higher in Sp than in A/W (1.37 vs. 0.99 g/100g total FA, respectively). Vaccenic acid (18:1*t*11) was the main *trans* FA in both seasons (1.09 g/100g total FA), followed by 18:1*t*10 (0.38 g/100g total FA), with no effect of the production season on the proportions of both *trans* FA. Rumenic acid (18:2*c*9,*t*11) was higher in Sp than in A/W (0.42 vs 0.21 g/100g total FA, respectively).

CONCLUSIONS: Despite the differences in pasture availability throughout the year, there were not many differences between seasons in the FA composition of KKCF from kids produced in the Alentejo region. However, the KKCF from kids produced in Sp had higher levels of rumenic acid, a FA with potential beneficial effects on human health.

ACKNOWLEDGMENTS/FUNDING: This work was funded under the "Val+Alentejo – Valorisation of small ruminant products from Alentejo" project, Alentejo 2020 (ALT20-03-0246-FEDER-000049), European Regional Development Fund (ERDF) and Portuguese Foundation for Science and Technology (FCT), under the projects UIDB/05183/2020 and UIDP/05183/2020 to MED, UIDB/00276/2020 to CIISA, LA/P/0121/2020 to CHANGE, LA/P/0059/2020 to AL4AnimalS and PhD studentship of A.S. (2022.12759.BD).

4th Annual Meeting
AL4AnimalS
15 / 16 MAY 2025

Supported by LA/P/0059/2020

FATTY ACID COMPOSITION OF FAT FROM GOAT KIDS PRODUCED IN ALENTEJO – PORTUGAL

Why study fatty acid composition of goat fat?

- Information on the fatty acid composition of small ruminants, such as goat kid, produced in Alentejo, is currently limited.
- The fatty acid composition of ruminant fat depends on its complex lipid metabolism, which is influenced by several factors, including diet.

Why compare different production seasons?

- In the Mediterranean area, the availability of natural resources for animal feed varies throughout the year.
- Seasonal variation in the availability of natural feed sources and the need for dietary supplementation during periods of scarcity can influence the fatty acid composition of goat kids' fat throughout the year.

CONCLUSIONS

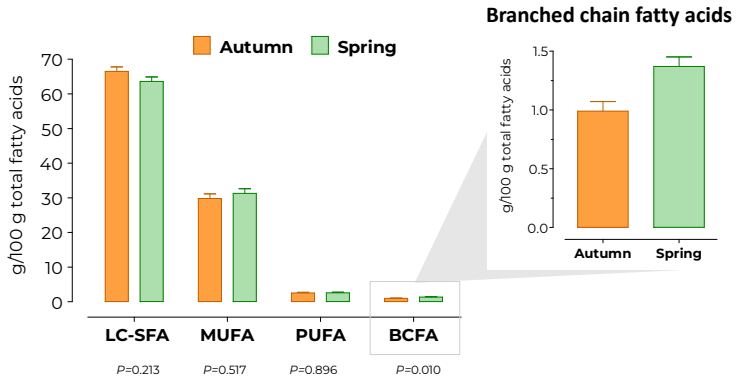
Despite the differences in pasture availability throughout the year, there were **not many differences between seasons** in the fatty acid composition of fat from got kids produced in Alentejo region.

Fat from **goat kids produced in Spring** had **higher levels of rumenic acid**, a fatty acid with potential beneficial effects on human health.

STUDY AIM to characterize the fatty acid composition of goat kids' fat produced in the Alentejo region, in two production seasons - autumn *vs* spring

RESULTS

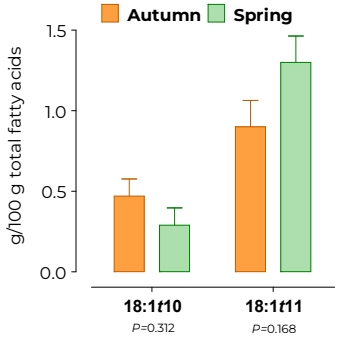
General fatty acids composition of goat kids' fat by season



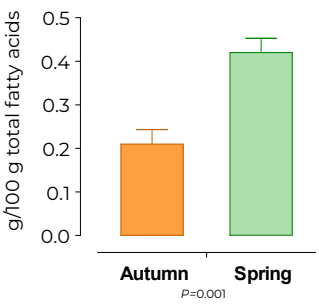
LC-SFA – linear chain saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; BCFA – Branched chain fatty acid

✓ LC-SFA, MUFA and PUFA proportions were **not affected** by production seasons.
✓ **Branched chain fatty acids** were **higher** in **Spring** than in Autumn.

Trans fatty acids by season (18:1*t*10 and 18:1*t*11)



Rumenic acid by season (18:2*c*9,*t*11)



✓ **Vaccenic acid** (18:1*t*11) was the **main *trans* fatty acid in both seasons**
✓ 18:1*t*10 and 18:1*t*11 were **not affected** by production seasons

✓ Rumenic acid (18:2*c*9*t*11) ↑ **in Spring**

METHODOLOGY

SAMPLE COLLECTION:

Autumn	Spring
December 2020	March - June 2021
Pasture SCARCITY	**Pasture ABUNDANCE**
36 samples	34 samples
Kidney knob channel fat	

*collected in slaughterhouse, after carcass cooling

PRODUCTION REGION: **Alentejo** – Portugal
(11 commercial farms)

Laboratory Analysis:

- Lipid extraction with dichloromethane:methanol (2:1, v/v)
- Fatty acids transesterification with combined basic - acid catalysis
- Gas Chromatography with Flame Ionization Detector (GC-FID) quantification

Alves SP et al. **2015**, *PLoS ONE*, *10* (12), 3-5.

Statistical Analysis:

- SAS - Mixed Procedure

AUTHORS:

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VAL+ALENTEJO - Valorisation of small ruminant products from Alentejo (ALT20-03-0246-FEDER-000049)
UIDB/05183/2020, UIDP/05183/2020 (MED); LA/P/0121/2020 (CHANGE); UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS)

PhD studentship:
A. Silva (2022.12759.BD)

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT1.03

LT 1 Green Animal Production

GENOMIC DETECTION OF SELECTION SIGNATURES LINKED TO REPRODUCTION AND MORPHOLOGY IN SOUTH ANGOLAN SHEEP

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INTRODUCTION: Natural and artificial selection in African sheep populations has left distinctive genomic signatures. In southern Angola, pastoralist communities rear fat-tailed sheep, valued for their adaptability, protein source, and cultural significance, contributing to the sustainability of traditional agropastoral systems.

AIMS: This study aims to identify candidate genes associated with productivity and adaptation in these sheep.

METHODOLOGY: We sequenced genomes from Namibe (N=3) and Cunene (N=5) and compared them with publicly available data from African and commercial breeds. After quality control, mapping (BWA), and SNP calling (samtools, bcftools) with a minimum 10x coverage and observation in at least three reads, ~20 million high-confidence SNPs were identified. Selection signatures were detected using the within-breed Integrated Haplotype Score (iHS) and Pairwise Cross Population Extended Haplotype Homozygosity (XP-EHH) methods (R package rehh), followed by gene set enrichment analysis for biological processes (ShinyGO) and QTL overlap analysis.

RESULTS: We identified 65 candidate regions (1,052 kb, 39 genes) through iHS and 88–278 regions using XP-EHH, with an average of 51 genes per population. To identify candidate genes under selection exclusively in southern Angolan sheep, we selected the genes identified by both methods for functional analysis. Thus, eight genes were revealed, enriching 65 biological processes grouped into 11 major GO terms, in which growth hormone regulation, maternal process involved in parturition, and neuromuscular junction development were most represented. QTLs for body weight and bone density frequently overlapped with selected regions.

CONCLUSIONS: In conclusion, we identified novel gene candidates of selection that are related to relevant biological processes that should be further investigated.

ACKNOWLEDGMENTS/FUNDING: This work was supported by FCT 2022.10733.PTDC. AJA was supported by CEEC 4th edition (2021.02058.CEECIND). FT was supported by PhD fellowship PRT/BD/154780/2022. HC and KS were supported by PDCT PhD fellowship N012/D-UL/PDCT-M003/2022 and N015/D-UL/PDCT-M003/2022.

GENOMIC DETECTION OF SELECTION SIGNATURES LINKED TO REPRODUCTION AND MORPHOLOGY IN SOUTH ANGOLAN SHEEP

FÁBIO TEIXEIRA [1,2] ; HERMENEGILDO CHIAIA [2,3,4] ; KIALA SEBASTINO [5,3,4] ; JOAQUIM GASPAR [2] ; PEDRO AFONSO [2] ; SEBASTIÃO NGOLA [6] ; CLÁUDIO SIMÃO [6] ; JOSÉ CASEMIRO [6] ; JERUSA PIRES [5] ; ADELINO MIGUEL [6] ; LADISLAU GOMES [7] ; EZEQUIEL DALA [6] ; MAVILDE PIRES [5] ; PACIÊNCIA NANGA [5] ; DULCE SANTOS [8] ; ALEXANDRE LEITÃO [3,4] ; JOAQUIM MORAIS [2] ; JOSÉ MORAS CORDEIRO [2] ; LUÍS TELO GAMA [3,4] ; JOHANN SÖLKNER [9] ; ANDREIA JESUS AMARAL [10,3,4]

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INTRODUCTION

Natural and artificial selection in African sheep populations has left distinctive genomic signatures. In southern Angola, pastoralist tribes breed fat-tailed sheep adapted to arid and mesic savannas for protein and cultural purposes. This study aims to identify candidate genes associated with productivity and adaptation in these sheep.

CONCLUSIONS

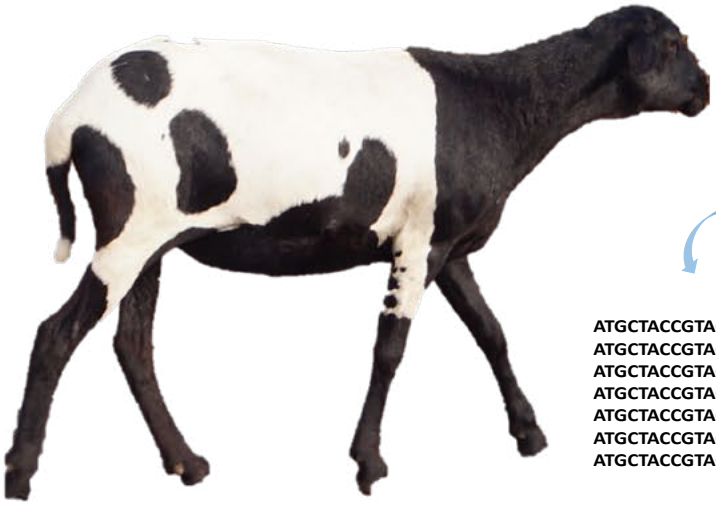
- This study represents the first exploration of the genetic background of native sheep in Angola.
- We identified novel gene candidates of selection that are related to relevant biological processes that should be further investigated.

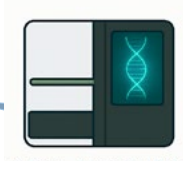
METHODOLOGY

DNA from Southern Angolan sheeps N=8

Whole genome sequencing 150 bp-10x

~20 million SNPs identified





ATGCTACCGTACGATCGA
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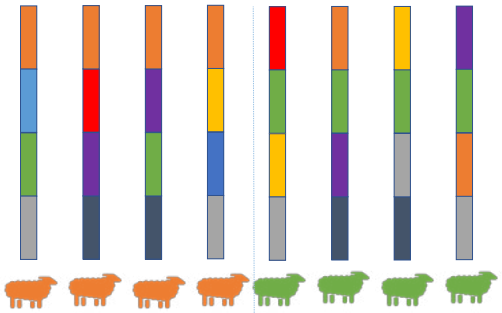
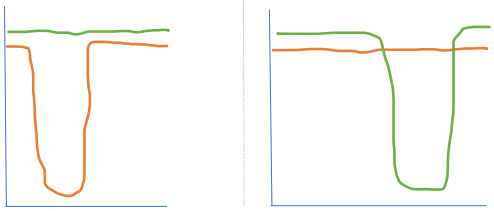
SINGLE NUCLEOTIDE POLIMORPHISM (SNP)

Identification of selective sweeps (iHS and xpEHH)

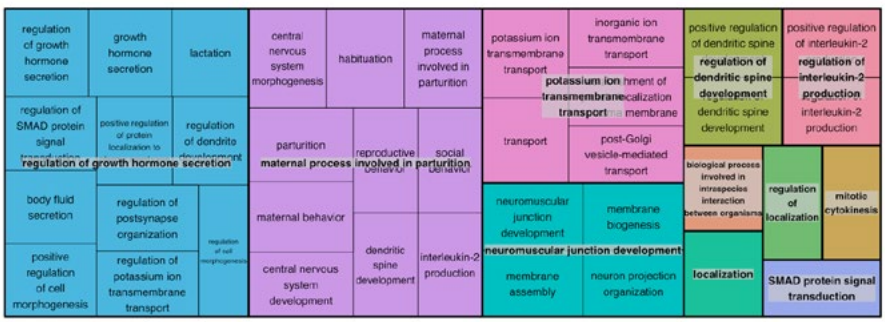




RESULTS

- ***CNBD1***
- ***OR6A2***
- ***GALNT17***
- ***KALRN***
- ***U6***
- ***SPTBN1***
- ***KCNIP4***
- ***KCNA4***

Eight common genes identified with both methods



Genes enriched 65 biological processes grouped into 11 major GO terms. Most represented: Growth hormone regulation, Maternal process involved in parturition, and Neuromuscular junction development

ACKNOWLEDGMENTS/FUNDING

This work was supported by FCT 2022.10733.PTDC. AJA was supported by CEEC 4th edition (2021.02058.CEECIND). FT was supported by PhD fellowship PRT/BD/154780/2022. HC and KS were supported by PDCT PhD fellowship N012/D-UL/PDCT-M003/2022 and N015/D-UL/PDCT-M003/2022.

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT1.04

LT 1 Green Animal Production

FEEDING SUNFLOWER OIL ENRICHED WITH BROMOFORM FROM *ASPARAGOPSIS TAXIFORMIS* ON YOUNG BULLS' GROWTH, HEALTH AND RUMINAL METHANE EMISSIONS

Francisco Sena[1,2], Diogo Henriques[3], Maria Teresa Dentinho[2,3,4], Katia Paulos[3,5], Alexandra Francisco[2,3,4], Ana Paula Portugal[3], Artur Oliveira[6], Henrique Ramos[6], Ricardo Bexiga[1,2,4], Saeedeh Moradi[1,2], Jorge Jesus Correia[1,2,4], Diana Soares[1,2,4,7,8], Nuno Rodrigues[7], Ricardo Teixeira[8], Susana Alves[1,2,4], Tiago Domingos[7,8], Rui Bessa[1,2,4], José Santos-Silva[2,3,4]

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INTRODUCTION: Concerns regarding greenhouse gas emissions from the ruminant livestock production sector have led to research in different antimethanogenic strategies. One of these strategies consists in the supplementation with the red seaweed *Asparagopsis taxiformis*, a potent antimethanogenic species, which was also effective when immersed in vegetable oils.

AIMS: A trial was performed focusing on the impact of supplementing *A. taxiformis* macerate immersed in sunflower oil (Bromoil) on ruminal methane emissions, animal performance and health.

METHODOLOGY: Sixteen Angus-cross young bulls fed a total mixed ration diet, comprising haylage and concentrate (50:50 in DM) and 20 g of oil/kg of DM, were randomly assigned to one of three treatments varying in dietary bromoform ($CHBr_3$) concentration: B0 (0 mg/kg DM), B20 (20 mg/kg DM) and B30 (30 mg/kg DM). After a 17-day adaptation period to the diets and experimental condition, the animals were fed *ad libitum* for 77 days. Rumen methane emissions were measured using a GreenFeed unit. The animals were weighed every 14 days prior to feed distribution. Treatments were suspended 72 h before slaughter. The stability of $CHBr_3$ was also evaluated using a diet similar to that used in the experiment, prepared in triplicate, analysed using gas chromatography mass spectrometry. Physical and chemical traits were evaluated in meat.

RESULTS: The half-life of $CHBr_3$ in air-exposed diets was 3.18 h, and at 24 h about 87% of the $CHBr_3$ had disappeared from the samples. Dry matter intake and feed conversion ratio were not affected by the treatments; however, average daily gain was reduced by 10% in B30 when compared to B0. Ruminal methane production per kg of live weight gain was reduced by 33% in B20 and by 52% in B30, when compared to B0. Carcass parameters, meat physical traits and fatty acid profile were not affected by the treatments, and no $CHBr_3$ residues were detected in meat and liver. Examination of the rumen wall showed that animals of the three treatments displayed lesions, the cause of which was not conclusive.

CONCLUSIONS: Supplementation with Bromoil effectively reduced methane emissions from young bulls at doses between 20 and 30 mg $CHBr_3$/kg DM, without leaving traces of $CHBr_3$ residues in meat samples. However, the highest dose resulted in a 10% reduction in average daily gain.

ACKNOWLEDGMENTS/FUNDING: This study was supported by two PhD research grants awarded by FCT to Francisco Sena (UI/BD/152817/2022) and Diana Soares (2022.13385.BDANA). The funding from FCT CEECIND/00365/2018/CP1572/CT0012 (R. Teixeira), UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), UIDB/50009/2020 (LARSyS), UIDP/50009/2020 (MARATEC), and LA/P/0083/2020 (LARSyS) and CYTED (SISPEC-Sistemas Ganaderos Inteligentes y sostenibles, 125RT0167) is also acknowledged. Funding from projects GEEBovMit (PRR-C05-i03-I-000027 GEEBovMit) and GreenBeef (POCI-01-0247-FEDER-047050/LISBOA-01-FEDER-047050) is also acknowledged.

Feeding sunflower oil enriched with bromoform from *Asparagopsis taxiformis* on young bulls' growth, health and ruminal methane emissions

Francisco Sena[1,2*], Diogo Henriques[3], Maria Teresa Dentinho[2,3,4], Katia Paulos[3,5], Alexandra Francisco[2,3,4], Ana Paula Portugal[3], Artur Oliveira[6], Henrique Ramos[6], Ricardo Bexiga[1,2,4], Saeedeh Moradi[1,2], Jorge Jesus Correia[1,2,4], Diana Soares[1,2,4,7,8], Nuno Rodrigues[7], Ricardo Teixeira[8], Susana Alves[1,2,4], Tiago Domingos[7,8], Rui Bessa[1,2,4], José Santos-Silva[2,3,4]

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Introduction

Concerns regarding greenhouse gas emissions from the ruminant livestock production sector, specifically methane (CH_4), led to the research in different antimethanogenic strategies. One of these strategies consists in the supplementation with the red seaweed *Asparagopsis taxiformis*, a potent antimethanogenic species. When immersed in vegetable oils, a similar effect on ruminal methanogenesis has also been reported both *in vitro* and *in vivo*.

Objectives

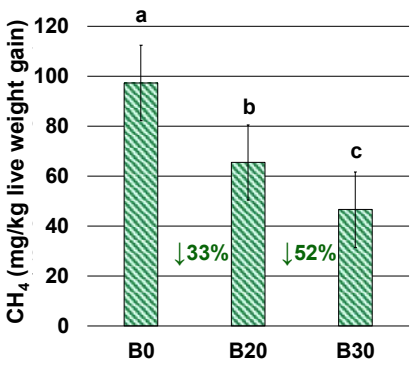
An *in vivo* trial assessed the impact of supplementing *A. taxiformis* macerate immersed in sunflower oil (Bromoil) on ruminal CH_4 emissions, animal performance and health.

Methods

Sixteen Angus-cross young bulls fed a total mixed ration diet, comprising haylage and concentrate (50:50 in DM) and 20 g of oil/kg of DM, were randomly assigned to one of three treatments varying in dietary bromoform ($CHBr_3$) concentration: B0 (0 mg/kg DM), B20 (20 mg/kg DM) and B30 (30 mg/kg DM). After a 17-day adaptation period to the diets and experimental condition, the animals were fed ad libitum for 77 days. Ruminal CH_4 emissions were measured using a GreenFeed unit. The animals were weighed every 14 days prior to feed distribution. Treatments were suspended 72 h before slaughter. The stability of $CHBr_3$ was also evaluated using a diet like that used in the experiment, prepared in triplicate, analysed using gas chromatography mass spectrometry. Physical and chemical traits were evaluated in meat.

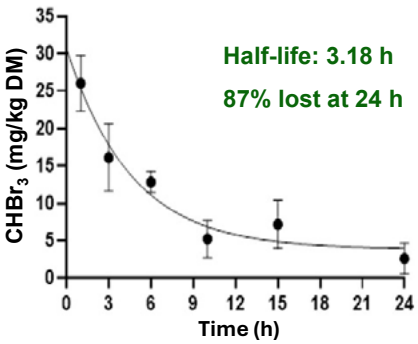
Results

CH_4 production



Variable	Effect of Bromoil
DM intake	**No**
Average daily gain	**Reduced by 10% in B30**
Feed conversion efficiency	**No**
Carcass parameters	**No**
Meat physical traits	**No**
Fatty acid profile	**No**
$CHBr_3$ residues in meat & liver	**Not detected**
Lesions in the rumen wall	**All animals (including control)**

$CHBr_3$ in air-exposed TMR



$Y = (Y_0 - Plateau)*exp(-k*hours) + Plateau$, fitted ($R^2$ = 0.868, RSD = 3.29). Equation parameters estimates: Y_0 = 30.7; Plateau = 3.84; k = 0.218.

Conclusion

Supplementation with Bromoil effectively reduced CH_4 emissions from young bulls at doses between 20 and 30 mg $CHBr_3$/kg DM, without leaving traces of $CHBr_3$ residues in meat samples. However, the highest dose resulted in a 10% decrease in average daily gain, which needs further optimisation.

Acknowledgements

PhD research grants awarded by FCT to Francisco Sena (UI/BD/152817/2022) and Diana Soares (2022.13385.BDANA). The funding from FCT CEECIND/00365/2018/CP1572/CT0012 (Ricardo Teixeira), UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), UIDB/50009/2020 (LARSyS), UIDP/50009/2020 (MARATEC), and LA/P/0083/2020 (LARSyS) and CYTED (SISPEC-Sistemas Ganaderos Inteligentes y sostenibles, 125RT0167) is also acknowledged. Funding from projects GEEBovMit (PRR-C05-i03-I-000027 GEEBovMit) and GreenBeef (POCI-01-0247-FEDER-047050/LISBOA-01-FEDER-047050) is also acknowledged.

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT1.05

LT 1 Green Animal Production

GENETIC CHARACTERIZATION OF NATIVE SHEEP (*Ovis aries*) FROM SOUTH OF ANGOLA

Hermenegildo Chiaia[1,2,3], Fábio Teixeira[2,3,4], Kiala Sebastino[1,3,5], Pedro Afonso[2], Joaquim Gaspar[2], Sebastião Ngola[6], Cláudio Simão[6], Paciência Nanga[5], Jerusa Pires[5], Adelino Suami Miguel[6], Ezequiel Dala[6], Ladislau Gomes[7], Dulce Santos[8], Joaquim Morais[2], Alexandre Leitão[1,3], José Manuel Cordeiro[2], Luís Telo da Gama[1,3], Johann Sölkner[9], Andreia Jesus Amaral[1,3,4]

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INTRODUCTION: African sheep are thought to descend from the Asiatic mouflon (*Ovis orientalis*). Domestication of sheep is thought to have begun in the central Fertile Crescent. Migration of sheep from the centre of domestication coincides with the spread of the Neolithic culture from the countries of the Fertile Crescent and has three main directions: Europe, Asia and Africa. Although several local breeds are registered, Mondombes, Angola Long-Legged and Angola Maned, their conservation status is unknown as well as their characterisation at genetic level.

AIMS: This study aims to investigate the genetic diversity of Southern Angolan sheep and their relatedness with other worldwide breeds.

METHODOLOGY: DNA was obtained from blood samples collected using FTA cards. We sequenced the genomes from Namibe (N=3) and Cunene (N=5) sheep that can be cathegorized as Long-Legged fat tailed using whole-genome sequencing (WGS). Their genomes were sequenced and compared with publicly available WGS data from African (Dorper, Djallonke, Dman and Sardi), European (Rambouillet, Churra and Merino Preta) Australian (Merino) and Chinese (Tibetan) breeds. After quality control, mapping (BWA), and SNP calling (samtools, bcftools) with a minimum 10x coverage and observation in at least three reads, ~20 million high-confidence SNPs were identified. Principal component analysis was performed to assess the population structure, using PLINK; admixture analysis from k=2 to k=10, was performed using Fastmixture to investigate ancestry; and Linkage disequilibrium (LD) decay was estimated using PopLDdecay to investigate diversity.

RESULTS: PC1 (13.3%) and PC2 (7.09%) suggest low divergence between Cunene and Namibe sheep. PC1 separates Angolan, Nigerian, and Ghanaian sheep from others; PC2 separates Angolan from Ghanaian and Nigerian sheep. Angolan sheep show higher LD than Portuguese and Moroccan breeds, but lower than Chinese, Ghanaian, Nigerian, French, and Australian ones. Admixture results show distinct profiles for Angolan sheep across all K values, indicating either long isolation or retention of ancestral variation. Elevated LD implies reduced population size. Moreover, these Angolan sheep seem to have diverged, or to belong to a different ancestral lineage than other sheep. The higher level of LD found, suggests a reduction of the population size.

CONCLUSIONS: In conclusion, these Angolan sheep present unique genetic features that should be further explored enlarging sample size.

ACKNOWLEDGMENTS/FUNDING: This work was supported by the Portuguese Foundation for Science and Technology (FCT), under projects UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); 2021.02058.CEECIND; 2022.10733.PTDC and PDCT-MESCTI (Government of Angola).

GENETIC CHARACTERIZATION OF NATIVE SHEEP (*Ovis aries*) FROM SOUTH OF ANGOLA

Hermenegildo Chiaia[1,2,3], Fábio Teixeira[2,3,4], Kiala Sebastino[1,3,5], Pedro Afonso[2], Joaquim Gaspar[2], Sebastião Ngola[6], Cláudio Simão[6], Paciência Nanga[5], Jerusa Pires[5], Adelino Suami Miguel[6], Ezequiel Dala[6], Ladislau Gomes[7], Dulce Santos[8], Joaquim Morais[2], Alexandre Leitão[1,3], José Manuel Cordeiro[2], Luís Telo da Gama[1,3], Johann Sölkner[9], Andreia Jesus Amaral[1,3,4]

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Introduction

African sheep are thought to descend from the Asiatic mouflon (*Ovis orientalis*). Domestication of sheep is thought to have begun in the central Fertile Crescent. Migration of sheep from the centre of domestication coincides with the spread of the Neolithic culture from the countries of the Fertile Crescent and has three main directions: Europe, Asia and Africa.

Although several local breeds of Angola (Mondombes, Angola Long-Legged and Angola Maned) are registered by FAO DaD-IS, their conservation status is unknown as well as their characterisation at genetic level.

Aims

This study aims to investigate the genetic diversity of Southern Angolan sheep and their relatedness with other worldwide breeds.

Methodology

DNA was obtained from blood samples collected using FTA cards. We sequenced the genomes from Namibe (N=3) and Cunene (N=5) sheep that can be cathegorized as Long-Legged fat tailed using whole-genome sequencing (WGS) (Figure 1). Their genomes were sequenced and compared with publicly available WGS data from African (Dorper, Djallonke, Dman and Sardi), European (Rambouillet, Churra and Merino Preta) Australian (Merino) and Chinese (Tibetan) breeds. After quality control, mapping (BWA), and SNP calling (samtools, bcftools) with a minimum 10x coverage and observation in at least three reads, ~20 million high-confidence SNPs were identified.

Principal component analysis was performed to assess the population structure, using PLINK; admixture analysis from k=2 to k=10, was performed using Fastmixture to investigate ancestry; and Linkage disequilibrium (LD) decay was estimated using PopLDdecay to investigate diversity.

Figure 1 – Sampling of native Angolan sheep.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology (FCT), under projects UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); 2021.02058.CEECIND; 2022.10733.PTDC and PDCT-MESCTI (Government of Angola).

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Conclusion

These findings suggest that we do not observe great genetic divergence between sheep from Namibe and Cunene. Angolan sheep present unique genetic features, suggesting harbouring genetic information from an old ancestor. The higher level of LD identified might be due to lower population or small sampling size. These results should be further explored enlarging sample size.

Results

PC1 separates Angolan, Nigerian, and Ghanaian sheep from others. PC2 separates Angolan from Ghanaian and Nigerian sheep.

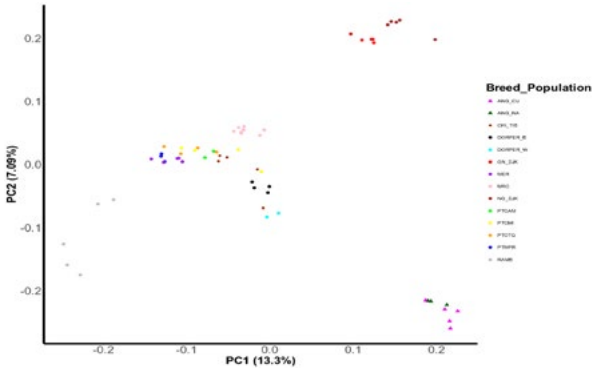

Figure 2 - Principal Component Analysis. Eigenvectors for Principal Component Analysis.

Admixture results show the existence of two genetic lines at K=2. Even at K=10, some Angolan sheep keep a high percentage of one of these strains that is absent in other breeds.

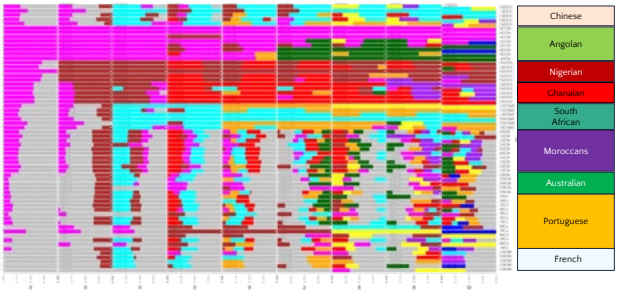

Figure 3 - Admixture-based clustering considering k between 2 and 10. For each individual on the y-axis, the amount of shared genetic material is shown on the x-axis.

In comparison with other African and French breeds, Angolan sheep show lower level of extent of linkage disequilibrium.

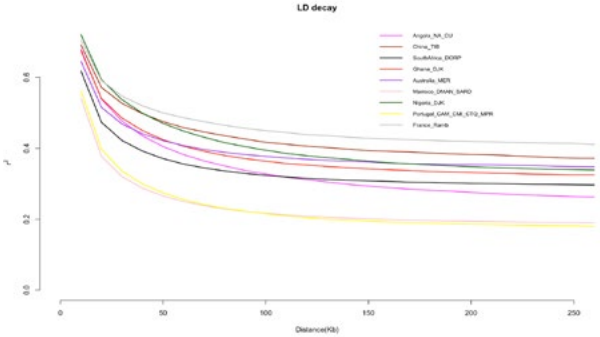

Figure 4 – Linkage disequilibrium pattern of Angolan, Ghanaian, Nigerian, South African, Moroccan, Australian, Portuguese, French and Chinese sheep breeds.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT1.06

LT 1 Green Animal Production

GENOMIC CHARACTERIZATION OF AUTOCHTHONOUS CATTLE FROM ANGOLA

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Needs led man to resort to the domestication process of several wild species, from which thousands of breeds of domestic animals emerged, with different genetic and environmental characteristics. The domestication process began 10,000 years ago, with the "Fertile Crescent" region as its center, covering the regions of the current Middle East, including southwestern Turkey, Iran, Syria and Iraq. The wild subspecies of Auroque cattle (*Bos primigenius namadicus*) is now extinct, among the first to be domesticated by man, 8,000 years ago, and the first records of cattle in Africa date back to around 2,000 and 3,000 years ago, in the North African region. From Auroque we had, Bos primigenius Taurus and Bos primigenius Indicus, from these African cows were domesticated, as well as the hybrid species (natural and artificial) *Bos Taurus* vs *Bos Indicus*, also known as Sanga cattle.

With problems of drought and lack of pastures (desertification of the Sahara), in North Africa, farmers and pastoralists were forced to immigrate to Southern Africa, taking their cattle with them, with the Sanga cattle, the main type taken by farmers and pastoralists, also known as the "Bantu" people, thus the Sanga cattle, becoming the main base of native cattle in Southern Africa.

Angola has Sanga cattle as the main cattle breed, in which the name of the cattle varies according to the location and the native language of the region with great influence on their physiognomy, among them we have, Kwanhama, Humbe, Mucubal, Mumuíla, Damara, Barotse, Cateta, Daomé, Mocho de Quilengues, Mocho de Malange, Ndama, representing cattle from the South to the North of Angola, with a population trend of stable conservation.

Objectives of the study: evaluate the level of diversity of local cattle in Angola, evaluate their genetic distance? How they relate to and differ from other races. A total of six samples collected in Namibe and Cunene were sequenced, generating a total of 339Gb of data, the depth reached was 10X for 4 samples and 30X depth for two samples.

This work was supported by FCT- Fundação para a Ciência e Tecnologia IP (UIDB/00276/2020; LA/P/0059/2020 - AL4AnimalS; 2021.02058.CEECIND; 2022.10733.PTDC) and by Projecto de Desenvolvimento de Ciência e Tecnologia (PDCT-M003/2022)

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT1.07

LT 1 Green Animal Production

EFFECT OF PRE-WEANING FEEDING MANAGEMENT ON FATTY ACID COMPOSITION OF THE FAT OF LAMBS FINISHED WITH A HIGH-CEREAL DIET

Letícia Fialho[1,2,3], Olinda Guerreiro[1,4], Susana P. Alves[2,3,5], Rui J.B. Bessa[2,3,5], Eliana Jerónimo[1,4]

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[5] Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

INTRODUCTION: The ruminant diet affects the ruminal biohydrogenation, as high-forage diets favour the ruminal production of 18:1*t*11, whereas cereals-rich diets enhance the production of 18:1*t*10. This change in ruminal biohydrogenation with higher levels of 18:1*t*10 instead of 18:1*t*11, known as "*t*10 shift", has consequences in the nutritional value of ruminant's products. The 18:1*t*11 is the precursor of rumenic acid (18:2*c*9*t*11), both fatty acids (FA) with potential beneficial effects on human's health in opposite of 18:1*t*10.

AIMS: The main goal of this work was to evaluate the effect of the pre-weaning feeding management on the FA composition of kidney knob channel fat (KKCF) of lambs finished with a high-cereal diet, with particular focus on precursors and products of the ruminal biohydrogenation.

METHODOLOGY: After birth, 32 lambs and their mothers were divided into four different groups, in the first group the lambs were separated from their mothers and fed with a milk replacer (M); in the other three groups the lambs remain with their mothers on pasture (P); or stabled being provided to ewes pellets of dehydrated lucerne (L); or concentrated for lactating ewes (C). At weaning, the lambs were housed in pairs according to the pre-weaning management, and after 7 days of adaptation, were fed with a cereal-rich diet and straw (ca 10%) for 33 days. The KKCF was collected after lamb´s slaughter for analysis of the FA composition. The data were analysed using the PROC Mixed of SAS, considering the effect of pre-weaning feeding management.

RESULTS: The proportion of 18:2n-6 was higher in groups C and M (3.94 g/100g total FA), while L group showed intermediate values and P group the lowest levels (2.87 g/100g total FA; P=0.011). Conversely, the L group (0.48 g/100g total FA) presented the highest proportion of 18:3n-3, with lower levels found in M group (0.15 g/100g total FA; P<0.001). The 18:2*c*9*t*11 proportion was lower in M group when compared to the other groups (0.13 vs 0.37 g/100g total FA; P<0.001). The levels of 18:0, 18:1*t*10 and 18:1*t*11 in KKCF were not affected by pre-weaning feeding management. The 18:1*t*10 levels were higher than 18:1*t*11 in all treatments, except for two lambs in P group.

CONCLUSIONS: The results showed that utilization of feed rich in cereals during the fattening phase of lambs promote the occurrence of "*t*10 shift" independently of the pre-weaning feeding management.

ACKNOWLEDGMENTS/FUNDING: This work has been funded for Portuguese Foundation for Science and Technology (FCT) under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), UIDB/05183/2020 (MED), LA/P/0121/2020 (CHANGE), and PhD grant awarded to LF (2020.04456.BD).

4th Annual Meeting of the Associate Laboratory for Animal and Veterinary Science

Effect of pre-weaning feeding management on fatty acid composition of the fat of lambs finished with a high-cereal diet

Letícia Fialho[1,2,3], Olinda Guerreiro[1,4], Susana P. Alves[2,3,5], Rui J.B. Bessa[2,3,5], Eliana Jerónimo[1,4]

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Introduction

- The ruminant diet affects the ruminal biohydrogenation, as high-forage diets favour the ruminal production of 18:1*t*11, whereas cereals-rich diets enhance the production of 18:1*t*10.
- The change in ruminal biohydrogenation with higher levels of 18:1*t*10 instead of 18:1*t*11, known as "*t*10 shift", has consequences in the nutritional value of ruminant's products.
- The 18:1*t*11 is the precursor of rumenic acid (18:2*c*9*t*11), both fatty acids with potential beneficial effects on human's health in opposite of 18:1*t*10.

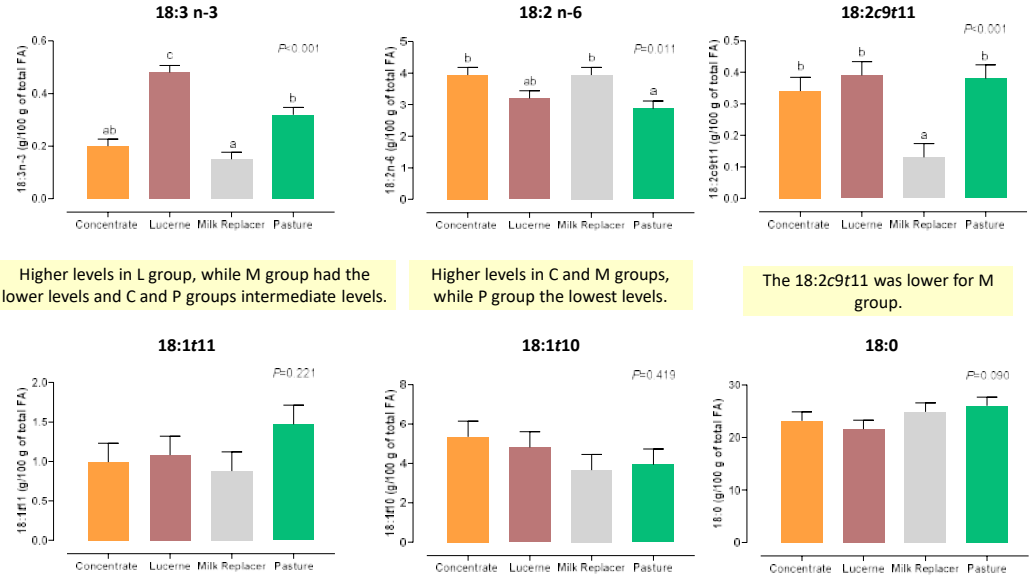
Conclusion

The utilization of feed rich in cereals during the fattening phase of lambs promote the occurrence of "*t*10 shift" independently of the pre-weaning feeding management.

Does the pre-weaning feeding management affect the fatty acid composition of kidney knob channel fat of lambs finished with a high-cereal diet?

Results

Main precursors and products of the ruminal biohydrogenation in kidney knob channel fat



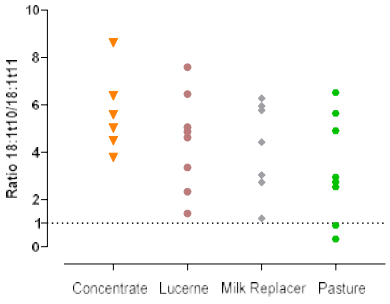
Higher levels in L group, while M group had the lower levels and C and P groups intermediate levels.

Higher levels in C and M groups, while P group the lowest levels.

The 18:2*c*9*t*11 was lower for M group.

The levels of 18:1*t*11, 18:1*t*10 and 18:0 were not affected by pre-weaning feeding management.

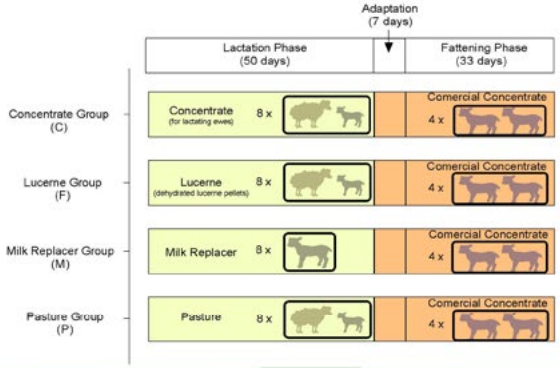
"*t*10 shift" – 18:1*t*10/18:1*t*11 > 1



Only two lambs from P group had ratio 18:1*t*10/18:1*t*11 < 1, while in all other lambs the 18:1*t*10/18:1*t*11 ratio suggest the occurrence of "*t*10 shift".

Materials and Methods

- ✓ After birth, 32 lambs and their mothers were divided into four different groups:



- ✓ At weaning, the lambs were housed in pairs according to the pre-weaning management, and after 7 days of adaptation, were fed with a cereal-rich diet and straw (ca 10%) for 33 days.
- ✓ Sample Collection: Kidney knob channel fat (KKCF).

Total lipids of KKCF were extracted with dichloromethane:methanol (2:1, v/v)
(Folch et al. 1957, J Biol Chem, 226: 497-509)

↓

Transesterification into fatty methyl esters (FAME) using combined basic and acid catalysis
(Alves SP et al. 2015, PLoS ONE, 10 (12), 3-5)

↓

FAME were analysed by gas chromatography with flame ionization detection (GC-FID)
(Oliveira et al. 2016, Anim.Feed Sci. Techn, 213, 64-73)

- ✓ Data analysed using the PROC Mixed of SAS, considering the effect of pre-weaning feeding management.

ACKNOWLEDGMENTS/FUNDING: This work has been funded for Portuguese Foundation for Science and Technology (FCT) under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), UIDB/05183/2020 (MED), LA/P/0121/2020 (CHANGE), and PhD grant awarded to LF (2020.04456.BD).

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT1.08

LT 1 Green Animal Production

THE INCLUSION OF ALMOND HULLS IN LAMB DIETS IMPROVES THE FATTY ACID COMPOSITION OF INTRAMUSCULAR FAT

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INTRODUCTION: Vaccenic (*t*11-18:1) and rumenic (*c*9,*t*11-18:2) acids, both fatty acids (FA) with potential beneficial effects on human health, are found in higher levels in intramuscular fat of lambs fed forage-based diets. However, in contrast to high-cereal diets, forage-based diets can reduce the animal performance and intramuscular fat deposition. Moreover, high-cereal diets favor the ruminal production of *t*10-18:1 instead of *t*11-18:1 (*t*10-*shift*), with detrimental effects on the nutritional value of lamb fat. Replacing cereals with almond hulls (AH), a sugar-rich and low-starch agricultural co-product, in a diet with a 60:40 concentrate-to-forage ratio could be a strategy to prevent the *t*10-*shift* and ensure high growth performance.

OBJECTIVES: The aim of this work was to evaluate if the partially replacing cereals with AH in a diet containing 60:40 concentrate-to-forage ratio limits the occurrence of the *t*10-*shift* and improves the intramuscular fat and FA composition, in addition to evaluate its effect on growth performance of lambs.

METHODOLOGY: The trial included 24 ram lambs assigned to one of 3 diets, in which cereals were stepwise replaced with AH, reaching 0, 9 and 18% of AH in diet. All diets included 40% dehydrated lucerne and 5% soybean oil. After 7 days of adaptation, the average daily gain (ADG) and feed intake were monitored. Total lipids were extracted from *Longissimus thoracis* (LT) muscle, with dichloromethane:methanol (2:1, v/v), transesterified into FA methyl esters and analysed by gas chromatography with flame ionization detection (GC-FID). Data was analysed using ProcMixed (SAS).
RESULTS: The partial replacement of cereals with AH up to 18% did not affect the ADG (349 g/d), but quadratically increased feed conversion ratio (P=0.020). In rumen content, the proportions of *t*10-18:1 (P=0.019) decreased linearly with increasing levels of AH in diets, while *t*11-18:1 (P=0.005) quadratically increased. In all animals fed diets containing AH, the *t*10-/*t*11-18:1 ratio as < 1. Total lipid content of LT was not affected by the diet. The proportions of *t*11-18:1 (P=0.032, + 52.5%) and *c*9,*t*11-18:2 (P=0.005, +40.5%) in intramuscular fat increased linearly with increasing levels of AH in diet, and the *t*10-18:1 decreased quadratically (P=0.022).

CONCLUSIONS: Partially replacing cereals with AH in the diet containing a 60:40 concentrate-to-forage ratio increased the health-beneficial FA in lamb meat and prevented the occurrence of the *t*10-*shift*, maintaining high growth performance.

ACKNOWLEDGMENTS/FUNDING: This work was funded under the SubProMais (PDR2020-101-030988, PDR2020-101-030993) and Portuguese Foundation for Science and Technology (FCT) under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), UIDB/05183/2020 (MED), LA/P/0121/2020 (CHANGE), and PhD grant awarded to LC (2020.05712.BD).

THE INCLUSION OF ALMOND HULLS IN LAMB DIETS IMPROVES THE FATTY ACID COMPOSITION OF INTRAMUSCULAR FAT

Liliana Cachucho[1,2,3], Susana P. Alves[2,3,4], Olinda Guerreiro[1,5], Manuel Varregoso[6,7], Cláudia Costa[6], Kátia Paulos[6], José Santos-Silva[2,3,6], M. Teresa P. Dentinho[2,3,6], Rui J. B. Bessa[2,3,4], Eliana Jerónimo[1,5]

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WHY ALMOND HULLS?

Replacing cereals with sugar-rich and low-starch co-products, such as almond hull (AH), in diets with 60:40 concentrate-to-forage ratio could:

- Improve the nutritional value of lamb intramuscular fat.
  - → Increase vaccenic (*t*11-18:1) and rumenic (*c*9,*t*11-18:2) acids – both fatty acids with beneficial health effects.
  - → Prevents the *t*10 *shift* (unfavorable increase in *t*10-18:1 linked to high-cereal diets).
- Maintain the lamb growth performance.

OBJECTIVES

To evaluate whether partially replacing cereals with almond hulls (AH) in a 60:40 concentrate-to-forage diet for lambs:

- ✓ Affects animal growth performance.
- ✓ Limits the occurrence of the *t*10 *shift*.
- ✓ Improves intramuscular fat content and fatty acid composition.

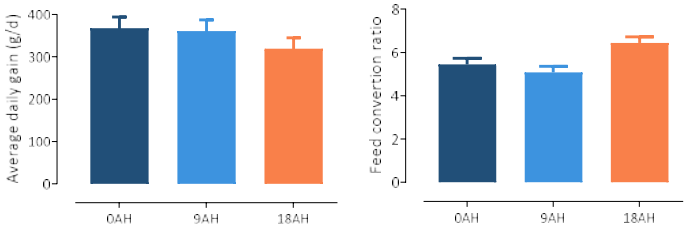
CONCLUSIONS

Partially replacing cereals with almond hulls in the diet up to 18% containing a 60:40 concentrate-to-forage ratio, increased the health-beneficial fatty acids in lamb meat and prevented the occurrence of the *t*10 *shift*, maintaining high growth performance.

- ⊕ *t*11-18:1 and *c*9,*t*11-18:2 in intramuscular fat
- ⊖ *t*10-18:1 in intramuscular fat
- ✓ Prevention of the occurrence of *t*10 *shift*

RESULTS

1 How do almond hulls impact average daily gain and feed conversion ratio?



Did not effect → 349 g/d
(*P* > 0.005)

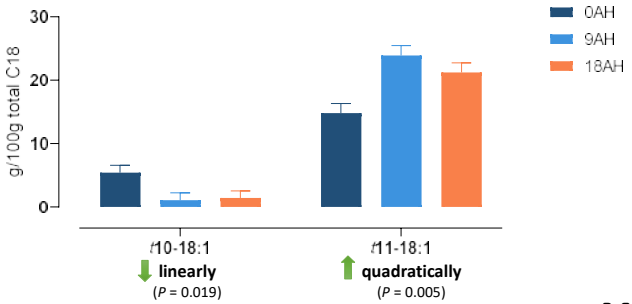
↑ quadratically
(*P* = 0.020)

2 How do almond hulls impact the rumen fatty acid composition and biohydrogenation?

Did not affect:

- Total fatty acid concentration of rumen content.
- Contents of 18:2n-6, 18:3n-3 and *c*9,*t*11-18:2.
- Contents of 18:0, total biohydrogenation intermediates, and sum of the 18:1 and 18:2 isomers.
- Estimated biohydrogenation of *c*9-18:1, 18:2n-6 and 18:3n-3 and completeness.

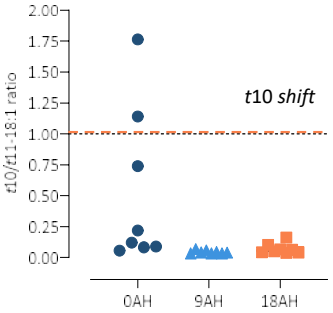
***t*10-18:1** and ***t*11-18:1**



↓ linearly (*P* = 0.019)

↑ quadratically (*P* = 0.005)

3 How do almond hulls impact the *t*10-/*t*11-18:1 ratio in the rumen?



0AH diet

Only 2 lambs had *t*10-/*t*11-18:1 > 1

9AH and 18AH diets

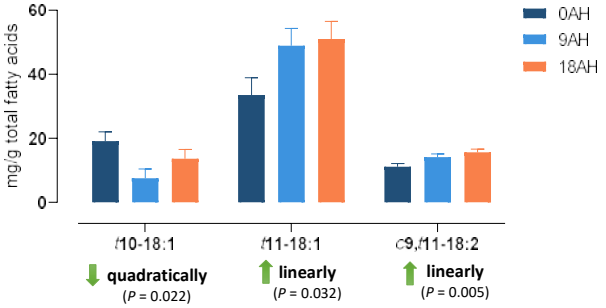
All lambs had *t*10-/*t*11-18:1 < 1

4 How do almond hulls impact the total lipids and fatty acids composition in intramuscular fat?

Did not affect:

- Intramuscular fat content (16.9 mg/g meat).
- Contents od 18:0, *c*9-18:1, 18:2n-6 and 18:3n-3.
- Contents of total n-6 and n-3 polyunsaturated fatty acids.

***t*10-18:1, *t*11-18:1 and *c*9,*t*11-18:2**



↓ quadratically (*P* = 0.022)

↑ linearly (*P* = 0.032)

↑ linearly (*P* = 0.005)

METHODOLOGY

Experimental Design

- **Animals:** 24 ram lambs
- **Dietary Groups** (n = 8 per group):
  - → Diet with 0% almond hulls: **0AH**
  - → Diet with 9% almond hulls: **9AH**
  - → Diet with 18% almond hulls: **18AH**
- **Diet composition:**
  - → 40% dehydrated lucerne
  - → 5% soybean oil

- **Trial duration:** 6 weeks
- **Adaptation Period:** 7 days
- **Feed offered *ad libitum***
- **Parameters Monitored:** Average daily gain and feed conversion ratio
- **Sample Collection:**
  - → *Longissimus thoracis* muscle
  - → Ruminal content
- **Data analysed using ProcMixed (SAS)**
  - → Orthogonal polynomial contrasts

***Longissimus thoracis* muscle**

Ruminal content

Total lipids → extracted with dichloromethane:methanol (2:1, v/v)

Folch et al. 1957, J Biol Chem, 226:497-509

Transesterification into fatty acid methyl esters

Analysed by gas chromatography with flame ionization detection (GC-FID).

Oliveira et al. 2016, Anim. Feed Sci. Techn., 213: 64-73

ACKNOWLEDGMENTS/FUNDING:
This work was funded under the SubProMais (PDR2020-101-030988, PDR2020-101-030993) and Portuguese Foundation for Science and Technology (FCT) under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), UIDB/05183/2020 (MED), LA/P/0121/2020 (CHANGE), and PhD grant awarded to LC (2020.05712.BD; https://doi.org/10.54499/2020.05712.BD).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT1.09

LT 1 Green Animal Production

GENETIC DIVERSITY AND DEMOGRAPHIC STRUCTURE OF THE LUSITANO HORSE BREED

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INTRODUCTION: The Lusitano horse is a Portuguese native horse breed with historical and cultural significance. Over the past decade, interest in the breed has grown both nationally and internationally. Monitoring the demographic evolution of the Lusitano population is crucial for supporting breeding decisions, genetic improvement programs, and conservation strategies.

AIMS: The main objective of this study was to conduct a demographic characterisation of the Lusitano horse breed over a 64-year period, using data from the breeders' association, APSL. The study aimed to describe registration trends, gender distribution, geographic dispersion, and breeding patterns, contributing to the understanding of the breed's current status and future potential.

METHODOLOGY: Data were obtained from the official Lusitano Studbook, managed by APSL. Most demographic parameters were calculated using software developed by Carolino and Gama (2002). Descriptive statistics was applied to quantify annual registration figures, sex distribution and reproductive output. Pedigree structure was analysed using SAS (SAS Institute, 2021), and genetic parameters relating to reproductive traits were estimated with MTDFREML (Boldman et al., 1995).

RESULTS: The Lusitano horse has had notable expansion both nationally and internationally, with 46% of recent births occurring outside Portugal. The number of registered mares has grown steadily, reaching a maximum of 5939, while the number of active stallions remained stable, with a peak of 2606. Breeding is concentrated among a small number of animals, and although most mares are approved for reproduction, the selection of stallions is more restrictive. A gradual decline has been observed in the proportion of stallions evaluated for the Studbook, with a peak value of 14% recorded in 2017. Genealogical depth exceeds 12 generations on average, enabling accurate genetic evaluation. Inbreeding increased after the closure of studbook in 1989, having stabilized in last decades. A yearly reduction in the inbreeding rate was observed between 2000 and 2024 (ΔF/year = 0.0038%). The effective population size has grown, and the average generation interval, for the same period, was 10.18 years, being slightly longer for males (11.10 years).

CONCLUSIONS: The demographic analysis of the Lusitano horse population reveals a healthy and expanding breed, with a strong national representation and an increasing international presence. Overall, the findings on this study provide valuable information to breeders and owners of Lusitano horses, offering them additional tools for selection and the consequent genetic improvement of this native Portuguese horse breed.

ACKNOWLEDGMENTS/FUNDING: This work has been funded by national funds through Portuguese Foundation for Science and Technology (FCT), under Projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS) and grant (2023.04918. BDANA).

GENETIC DIVERSITY AND DEMOGRAPHIC STRUCTURE OF THE LUSITANO HORSE BREED

MARGARIDA PAIS MATEUS[1,2,3]; **JOÃO RALÃO DUARTE**[3]; **ANTÓNIO VICENTE**[3,4,5]; **MARIA JOÃO FRADINHO**[1,2,6]; **MANUEL SILVEIRA**[7]; **NUNO CAROLINO**[1,2,8]

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INTRODUCTION

Lusitano Horse: Portuguese native horse breed, selected for work and performance;

Global Reach: Present in >25 countries; 46% of recent births occur abroad;

Complete Genealogical Records: Studbook data since 1967, with >12 known generations;

Why Demographic Analysis?
- Understand population structure & genetic variability;
- Support conservation and breeding strategies.

This Study uses APSL data (1970–2024) to analyse:
- Reproductive trends
- Inbreeding levels
- Generation intervals (L)
- Effective population size (Ne)

AIMS

The study aimed to conduct a demographic characterisation of the Lusitano horse breed over a 64-year period, using data from APSL, contributing to the understanding of the breed's current status and future potential.

METHODOLOGY

- **Data Source**: APSL Studbook (n = 90319 animals);
- **Software**: Software developed by Carolino and Gama (2002), MTDFREML and SAS;
- **Analyses Included**:
  - Demographic trends (births, breeders, sex ratio)
  - Pedigree structure and genealogical depth
  - Inbreeding coefficients (Fi) and relationships (aij)
  - Generation interval (L) and effective population size (Ne)
  - Inbreeding trends assessed via linear regression on birth year

RESULTS

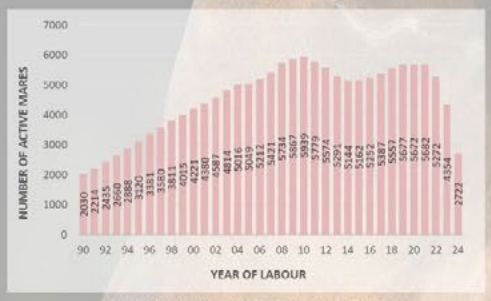

Figure 1 - Number of active breeding females present per year.
Note: Incomplete data for 2023 and 2024

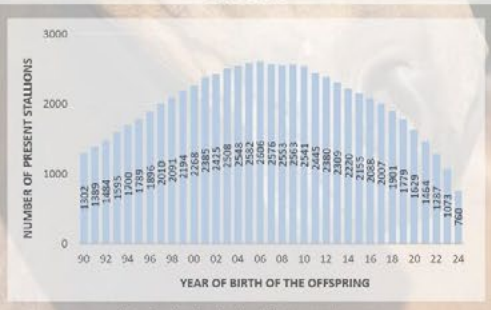

Figure 2 - Number of active stallions present per year
Note: Incomplete data for 2023 and 2024

Figure 3 - Changes in the percentage of births abroad
Note: Incomplete data for 2023 and 2024

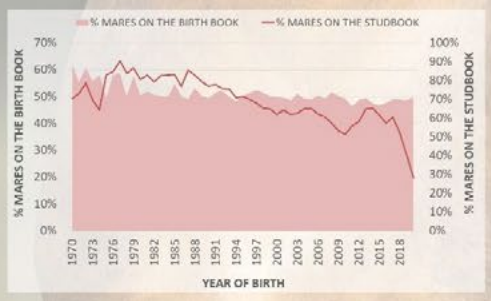

Figure 4 - Percentage of mares registered in the Studbook by year of birth compared to those registered in the Birth Book

Figure 5 - Percentage of stallions registered in the Studbook by year of birth in relation to those registered in the Birth Book

Figure 6 - Evolution of the inbreeding coefficient (in %) by year of birth and respective trend in different periods

Table 1 – Interval of Generations, Effective Population Size (Ne) and Inbreeding Rate (ΔF) in different periods

Parameter	1960-2024	1980-2024	2000-2024
Inbreeding/Year	0.0466%	0.0524%	0.0038%
Interval of Generations (years)	9.95	9.92	10.18
Inbreeding/Generation	0.464%	0.520%	0.039%
Effective Population Size (Ne)	107.81	96.15	1289.24

CONCLUSIONS

- **Lusitano population is healthy and expanding**, with steady national growth and an expanding international presence;
- **High proportion of breeding females** supports inbreeding control, but may limit selection intensity;
- **Inbreeding levels are stabilising**, supported by deep genealogical data;
- Findings offer **practical tools for breeders and owners** to support informed selection and sustainable genetic improvement;
- Contributes to the long-term preservation and valorisation of this iconic Portuguese breed.

ACKNOWLEDGMENTS/FUNDING:

This work has been funded by national funds through Portuguese Foundation for Science and Technology (FCT), under Projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS) and grant (2023.04918.BDANA).

BIBLIOGRAPHY

Carolino, N. e L. T. Gama, 2002. Manual de Utilização de Software para a Gestão de Recursos Genéticos Animais. Estação Zootécnica Nacional, Instituto Nacional de Investigação Agrária e Pescas, Portugal (policopiado).

PHYLOGENETIC ANALYSIS OF ASFV SF2 HELICASE PROTEINS REVEALS DISTINCT EVOLUTIONARY PATTERNS ACROSS ISOLATES

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INTRODUCTION: African Swine Fever Virus (ASFV) is a lethal double-stranded DNA virus causing high mortality in domestic pigs and wild boars. ASFV encodes six SF2 superfamily helicase proteins involved in nucleic acid metabolism. Although their role is believed to be critical for viral replication and immune evasion, they remain poorly characterized.

OBJECTIVES: The objective of this study was to investigate the evolutionary relationships among the ASFV SF2 helicase proteins (A859L, B962L, D1133L, F1055L, Q706L, and QP509L) across a diverse set of viral isolates from different geographic origins and hosts.

METHODOLOGY: Protein sequences were retrieved from the Identical Protein Groups (IPG) resource at NCBI. Multiple sequence alignments were performed using multiple algorithms, including MAFFT and GLProbs, and alignment quality was evaluated with Totally Conserved Columns (TC) scores in R. Phylogenetic trees were constructed using FastTree 2 with support values based on 1,000 site resamplings, and pairwise distance matrices were computed in MEGA following optimal model selection. Heatmaps, minimum spanning trees (MST), and sequence logos were generated in R to visualize genetic distances and sequence variability. Highly divergent sequences (RSA2, Zaire, and Wart80) were excluded from the sequence logo analysis to enhance resolution. Protein domain diagrams were prepared using IBS 2.0.

RESULTS: Phylogenetic analyses revealed a consistent division between older, genetically diverse African isolates and more recent, highly conserved European and Asian strains associated with genotype II. The A859L and QP509L helicase genes displayed greater sequence variability, allowing finer phylogenetic resolution between isolates, while Q706L and F1055L were more conserved. MST and heatmap analyses supported these findings, showing tight clustering of genotype II isolates and greater divergence among older African strains. Outlier sequences such as RSA2 and Zaire exhibited long branch lengths and high genetic distances, reflecting greater evolutionary divergence. Sequence logo analyses, after removal of outliers, identified conserved regions interspersed with a moderate number of polymorphic sites.

CONCLUSIONS: The ASFV SF2 helicase proteins reflect the major evolutionary split between historical African lineages and contemporary pandemic strains, with variability differing among individual genes. These findings improve the current understanding of ASFV evolution and suggest that helicase genes may serve as valuable molecular markers for epidemiological studies and as targets for antiviral strategies.

ACKNOWLEDGMENTS/FUNDING: The authors gratefully acknowledge the support of Fundação para a Ciência e a Tecnologia, I.P. (FCT, Portugal) for funding this work through the projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS). Ana Catarina Urbano further acknowledges the financial support of FCT, Portugal through the doctoral fellowship 2021.07919.BD.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.09

LT 2 Emergent Infectious Diseases and Zoonosis

ENCEPHALITOZOON CUNICULI IN PET RABBITS – A ONE HEALTH ISSUE?

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INTRODUCTION: *Encephalitozoon cuniculi* is an ubiquitarian microsporidium, which can infect a wide range of species, including man. Although considered a primary pathogen to rabbits, its importance has increased with the advent of human immunodeficiency virus infection, when it became recognized as an opportunistic agent. *E. cuniculi* can infect any tissue and inflammation seems to activate latent infection and target microsporidia towards the inflamed tissues. In humans, clinical signs include fever, headache, gastrointestinal and respiratory disease, and weakness. In rabbits, the disease is mainly ophthalmologic, renal and neurologic, with vestibular syndrome overrepresented.

The zoonotic risk of *E. cuniculi* associated with animal-assisted interventions is recognised. In addition, rabbits are the third most common pet in many countries, living in close contact with their owners, and if infected with *E. cuniculi*, their zoonotic potential should not be overlooked.

AIMS: To determine the seroprevalence of *E. cuniculi* in pet rabbits assisted at the Teaching Hospital of the Faculty of Veterinary Medicine – Lisbon University (HEV-FMV) between August 2nd 2023 and February 8th 2025, and identify risk factors for rabbit infection and human exposure.

METHODOLOGY: Upon written consent, a 2 mL blood sample was collected from rabbits, regardless of their clinical status, for *E. cuniculi* antibody detection by ELISA. Parallel to sampling, an epidemiological survey for rabbit owners was also conducted, with questions concerning the age of the family members and presence of immunocompromising disease, access to the household and free roaming time allowed to the rabbit, access to the outdoors and contact with other pets.

RESULTS: A total of 110 pet rabbits were involved in the survey. The sample included 57% (63/110) male and 43% (47/110) female rabbits, aged between 6 months and 13 years old (3.9 +/- 2.4 years). A global *E. cuniculi* seroprevalence of 51% (56/110) was determined, although only 9% (5/56) of the rabbits had clinical signs at the time of diagnosis, giving rise to a 91% rate of infections without clinical signs. 85% (93/110) of the rabbits were allowed access to the owner's house, either permanently or temporarily. Only 24.5% (27/110) rabbits contacted with children under the age of 12 years and 1.9% (2/102) with clinically immunocompromised owners were identified (one organ transplanted patient and one with chronic pancreatitis).

CONCLUSIONS: Most pet rabbits live close to their owners. Being territorial animals, sexually intact males (and some females) often mark the house and the owners with urine, increasing the possibility of zoonotic *E. cuniculi* transmission. Given the high prevalence of the infection, and a significant number of carrier rabbits, there can be a risk to their human cohabitants in a One Health perspective. Routine analysis of the infection in pet rabbits should be encouraged, and adequate prophylactic protocols should be recommended.

FUNDING: This work was financed by national funds through FCT - Foundation for Science and Technology, I.P., within the scope of the project CIISA - UIDB/00276/2020 and AI4AnimalS - LA/P/0059/2020.

Encephalitozoon cuniculi in pet rabbits – a One Health issue?

Ana Reisinho[1,2,3]; Esmeralda Delgado[1,2,3]; Gabriela Santos-Gomes[4]; Isabel Pereira da Fonseca[2,3]
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Facts

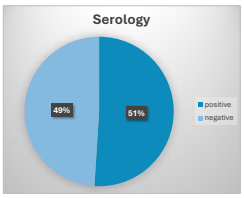
- ✓ Pet rabbit population is increasing worldwide
- ✓ *E cuniculi* is an opportunistic zoonotic agent highly prevalent in rabbit population
- ✓ The zoonotic risk of *E. cuniculi* associated with animal-assisted interventions is recognized (Santaniello et al, 2021)
- ✓ *E. cuniculi* infection has been detected in immunocompromised individuals
- ✓ Zoonotic risk for pet rabbit owners should not be overlooked

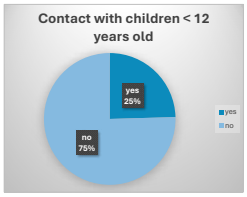
Aims

To determine the seroprevalence of *E. cuniculi* in pet rabbits assisted at the Teaching Hospital of the Faculty of Veterinary Medicine – Lisbon University (HEV-FMV) and identify risk factors for rabbit infection and human exposure

Methodology

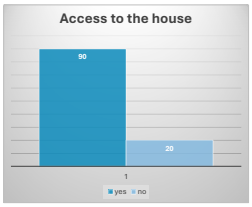
- ✓ Serologic testing (ELISA) of rabbits assisted at the HEV-FMV between August 2nd 2023 and February 8th 2025
- ✓ Epidemiological questionnaire regarding management, household environment and family in contact

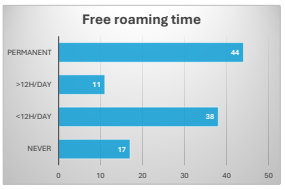




Results

- 110 pet rabbits
  - ✓57% (63/110) male and 43% (47/110) female rabbits,
  - ✓Age between 6 months and 13 years old (3.9 +/- 2.4 years).
- Global *E. cuniculi* seroprevalence: 51% (56/110)
  - ✓9% (5/56) with clinical signs at the time of diagnosis
  - ✓91% without clinical signs
- Questionnaire
  - ✓ 85% (93/110) were allowed access to the owner's house, either permanently or temporarily
  - ✓ 24.5% (27/110) contacted with children under the age of 12 years
  - ✓ 1.9% (2/102) rabbits in contact with clinically immunocompromised owners (one organ transplanted patient and one with chronic pancreatitis).





Conclusions

→ **Most pet rabbits live close to their owners.**

→ **Sexually intact males (and some females) often mark the house and the owners with urine, increasing the possibility of zoonotic *E. cuniculi* transmission.**

→ **Given the high prevalence of the infection, and a significant number of carrier rabbits, there can be a risk to their human cohabitants in a One Health perspective.**

→ **Routine analysis of the infection in pet rabbits should be encouraged, and adequate prophylactic protocols should be recommended.**

FUNDING: This work was financed by national funds through FCT - Foundation for Science and Technology, I.P., within the scope of the project CIISA - UIDB/00276/2020 and AI4AnimalS - LA/P/0059/2020.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT2.10

LT 2 Emergent Infectious Diseases and Zoonosis

A SINGLE-DOMAIN ANTIBODY PLATFORM TARGETING SARS-COV-2 AND FUTURE VIRAL THREATS

Isa Moutinho[1,2], Rafaela Marimon[1,2], Mafalda Henriques[1,2], Ana Leonardo[1,2], João Gonçalves[3], Luís Tavares[1,2], Frederico Aires-Da-Silva[1,2]

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[3] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

INTRODUCTION: The COVID-19 pandemic, caused by SARS-CoV-2, exposed critical gaps in our capacity to respond rapidly to emerging viral threats. Although mass vaccination programmes were essential to control viral spread and disease severity, the emergence of immune-evasive variants highlighted the need for complementary strategies. In addition, new pathogens with pandemic potential continue to emerge, reinforcing the urgent need for better preparedness. SARS-CoV-2 enters host cells via its spike protein (S), particularly through its receptor-binding domain (RBD), which interacts with the ACE2 cell receptor. Targeting this interaction provides an opportunity to block infection at its earliest stage. Single-domain antibodies (sdAbs) are particularly suited for this purpose due to their small size, high stability, and rapid and cost-effective production, making them ideal candidates for outbreak response. Developing flexible platforms capable of rapidly generating recombinant antibodies against new viral threats is essential to boost future pandemic response under a One Health perspective.

AIMS: This study aimed to develop and characterise sdAbs targeting SARS-CoV-2 RBD to block its interaction with the ACE2 and prevent viral entry. Using SARS-CoV-2 as a model, we established a versatile and rapid-response platform for generating therapeutic sdAbs adaptable to emerging viruses.

METHODOLOGY: A New Zealand White rabbit was immunised with the SARS-CoV-2 RBD from Delta variant and the antibody titre determined by ELISA. A rabbit-derived sdAb library was constructed by PCR and subjected to phage display selection. High-throughput screening was used to identify candidates with strong RBD binding and high expression levels. Selected sdAbs were produced in *E. coli* and tested for SARS-CoV-2 neutralisation using sVNT and pseudovirus-based assays. To enhance pharmacokinetic properties, the lead sdAb candidate was genetically fused to the albumin-binding domain (ABD) derived from *Streptococcus zooepidemicus* Zag protein, enabling binding to albumin and prolonging serum half-life.

RESULTS: The immunised rabbit developed a high antibody titre of 1:640.000 against the SARS-CoV-2 RBD. Four rounds of phage display panning enriched clones with increased RBD binding activity. The most promising clone, B3, showed strong RBD binding and high neutralising activity, achieving 94% inhibition against SARS-CoV-2 wild-type and 97% and 81% against Delta and Omicron variants, respectively. The sdAb B3 was subsequently fused to the ABD derived from the Zag protein. Expression and functional activity studies of the B3-ABD fusion are currently ongoing.

CONCLUSIONS: This study identified RBD-specific sdAbs capable of neutralising multiple SARS-CoV-2 variants. This platform offers a rapid and adaptable antibody development strategy for future pandemic threats.

ACKNOWLEDGMENTS/FUNDING: This work was funded by Program Gilead GÉNESE (Grant ID:13823) and Fundação para a Ciência e a Tecnologia (FCT) through 2020.08209.BD and PTDC/CVT-CVT/0149/2021 and supported by CIISA through projects UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS.

A SINGLE-DOMAIN ANTIBODY PLATFORM TARGETING SARS-CoV-2 AND FUTURE VIRAL THREATS

Isa Moutinho[1,2*]; Rafaela Marimon[1,2]; Mafalda Henriques; Ana Leonardo[1,2]; João Gonçalves [1,2]; Luís Tavares[1,2]; Frederico Aires-da-Silva[1,2]

[1] Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
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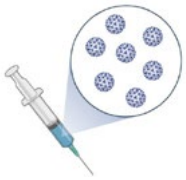
GILEAD fct Fundação para a Ciência e a Tecnologia

INTRODUCTION

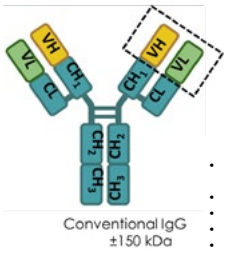
The COVID-19 pandemic, caused by SARS-CoV-2, exposed critical gaps in our capacity to respond rapidly to emerging viral threats. Although mass vaccination programmes were essential to control viral spread and disease severity, the emergence of immune-evasive variants highlighted the need for complementary strategies. In addition, new pathogens with pandemic potential continue to emerge, reinforcing the urgent need for better preparedness. SARS-CoV-2 enters host cells via its spike protein (S), particularly through its receptor-binding domain (RBD), which interacts with the ACE2 cell receptor. Targeting this interaction provides an opportunity to block infection at its earliest stage. Single-domain antibodies (sdAbs) are particularly suited for this purpose due to their small size, high stability, and rapid and cost-effective production, making them ideal candidates for outbreak response. Developing flexible platforms capable of rapidly generating recombinant antibodies against new viral threats is essential to boost future pandemic response under a One Health perspective.



Vaccination against COVID-19

Problems:
Immune-evasive variants

Alternative Approaches



Conventional Monoclonal Antibodies (mAbs):
Inaccessibility of hidden epitopes
Relatively high production costs

sdAbs

Advantages of sdAbs

- Lower size and access to hidden epitopes;
- Better tissue penetration;
- Higher stability;
- Lower production cost;
- Easily formulated to be administrated by inhalation;

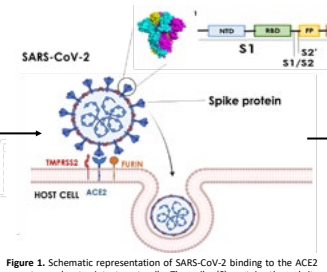


Figure 1. Schematic representation of SARS-CoV-2 binding to the ACE2 receptor and entry into target cells. The spike (S) protein, through its receptor-binding domain (RBD), mediates attachment to the host cell receptor ACE2, enabling membrane fusion and subsequent viral internalisation.

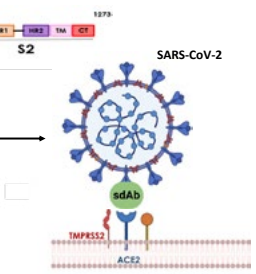


Figure 2. Inhibition of SARS-CoV-2 entry by blocking the RBD–ACE2 interaction. The single-domain antibody (sdAb) binds to the viral RBD, preventing its attachment to the ACE2 receptor on host cells and thereby blocking viral entry at the initial stage of infection.

Goals:

- **Develop sdAbs targeting SARS-CoV-2 RBD to block its interaction with the ACE2 and prevent viral entry.**
- **Establish a versatile and rapid-response platform for generating therapeutic sdAbs adaptable to emerging viruses.**

MATERIALS AND METHODS / RESULTS

I. Immunization and immune response validation

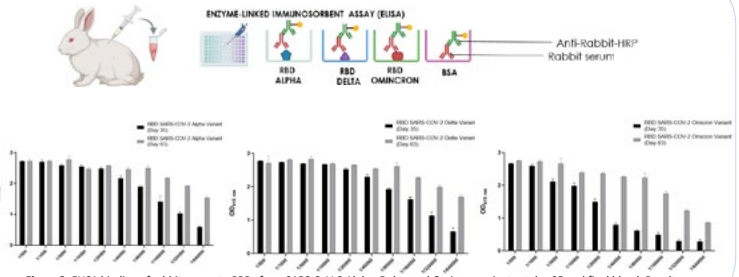
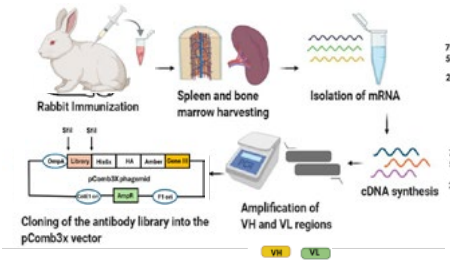


Figure 3: ELISA binding of rabbit serum to RBDs from SARS-CoV-2 Alpha, Delta, and Omicron variants at day 35 and final bleed. Results shown as OD at 415 nm.

II. Antibody library construction



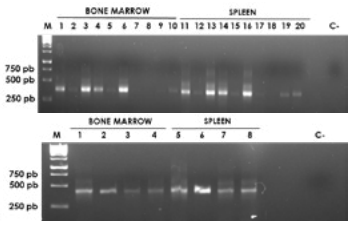


Figure 4: PCR amplification of VH (±400 bp) and VL (±350 bp) gene fragments from different sdAb families.

III. Selection of sdAbs against SARS-CoV-2

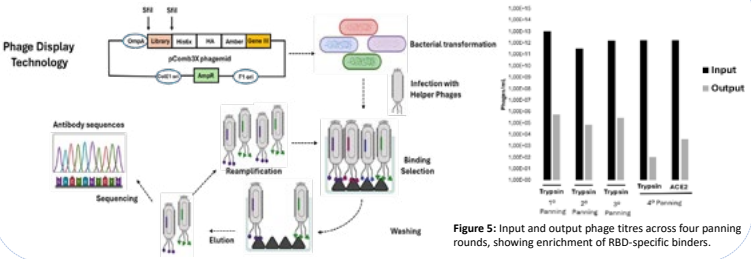


Figure 5: Input and output phage titres across four panning rounds, showing enrichment of RBD-specific binders.

IV. SARS-CoV-2 Binding Assays

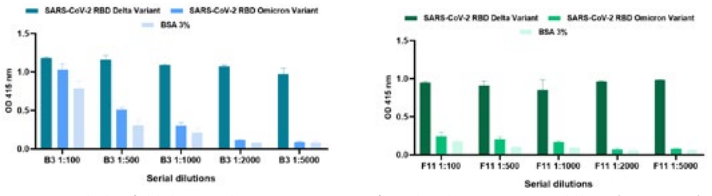


Figure 6. ELISA binding of sdAb clones B3 and F11 against SARS-CoV-2 RBD from Delta and Omicron variants. Serial dilutions (1:100 to 1:5000) were tested starting from 0.381 mg/ml. Binding to BSA was included as a control for non-specific interactions.

V. Surrogate virus neutralization test (sVNT)

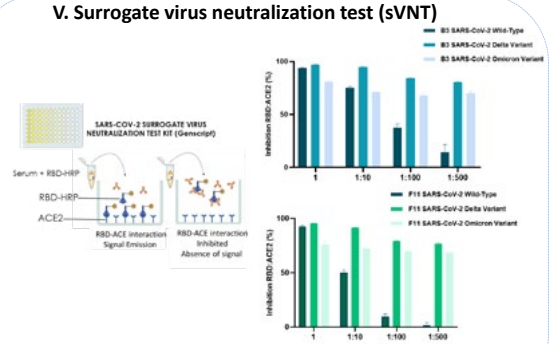


Figure 7. Neutralising activity of sdAb clones B3 and F11 assessed by surrogate virus neutralisation test (sVNT). Serial dilutions (1:10, 1:100, 1:500) were tested starting from 0.554 mg/mL. Percentage inhibition of RBD–ACE2 interaction was measured for each concentration.

VI. SARS-CoV-2 Pseudotyped neutralization assays

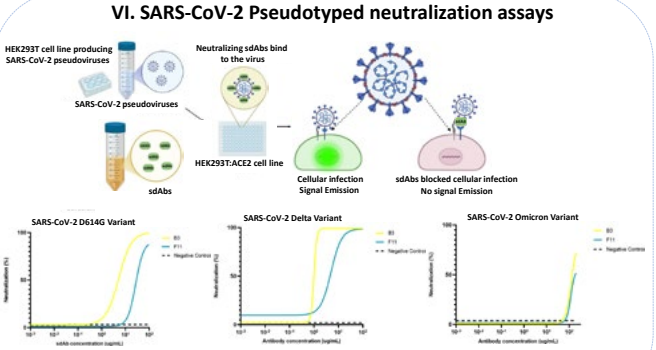
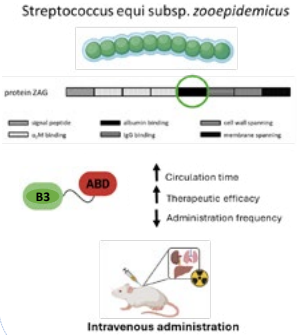


Figure 8. Pseudovirus neutralisation assay of sdAbs B3 and F11. Viral entry inhibition was assessed across a concentration range from 200 to 0.001 µg/mL. Blocking of cellular infection led to a reduction in luciferase signal emission. Results are expressed as relative luminescence units (RLU) converted to percentage inhibition.

Ongoing:



CONCLUSIONS

The immunised rabbit developed a high antibody titre of 1:640.000 against the SARS-CoV-2 RBD. Four rounds of phage display panning enriched clones with increased RBD binding activity. The most promising clone, B3, showed strong RBD binding and high neutralising activity, achieving 94% inhibition against SARS-CoV-2 wild-type and 97% and 81% against Delta and Omicron variants, respectively. To enhance pharmacokinetic properties, the sdAb B3 was genetically fused to the albumin-binding domain (ABD) derived from Streptococcus zooepidemicus Zag protein, enabling binding to albumin and prolonging serum half-life. Expression and functional activity studies of the B3-ABD fusion are currently ongoing. In summary, this study identified RBD-specific sdAbs capable of neutralising multiple SARS-CoV-2 variants. This platform offers a rapid and adaptable antibody development strategy for future pandemic threats.

ACKNOWLEDGMENTS

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PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT2.11

LT 2 Emergent Infectious Diseases and Zoonosis

VIRAL DIVERSITY IN PORTUGUESE BATS: A METAGENOMIC STUDY

Mahima Hemnani[1], Mustafa Karatas[2], Andreia V. S. Cruz[1], Priscilla Gomes da Silva[1,3], Gertrude Thompson[1,], Patrícia Poeta[4,5,6,7], Hugo Rebelo[8,], Jelle Matthijnssens[2], João R. Mesquita[1,6*]

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[2] KU Leuven, Department of Microbiology, Immunology, and Transplantation, Rega Institute, Laboratory of Viral Metagenomics, Leuven, Belgium
[3] Department of Protein Science, KTH Royal Institute of Technology, SciLifeLab, Stockholm, Sweden
[4] Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
[5] Associated Laboratory for Green Chemistry (LAQV), Chemistry Department, Faculty of Science and Technology, University NOVA of Lisbon, 2829-516 Caparica, Portugal
[6] Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), 1300-477 Lisboa, Portugal
[7] Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
[8] Instituto Politécnico de Setúbal, ESS, Campus da Estefanilha, Setúbal, Portugal

INTRODUCTION: Bats are highly diverse mammals and known reservoirs of numerous zoonotic viruses, including highly pathogenic ones like SARS-CoV-2. Their interactions with animals and humans through shared habitats and practices facilitate viral transmission, driving interest in their role in emerging infectious diseases.

AIMS: Assess the presence of coronaviruses (Covs) in Portuguese bats, explore viral diversity through metagenomics, and predict the affinity of CoV spike protein with the aminopeptidase N (APN) receptor of a bat, porcine and human.

METHODOLOGY: Ten bats (five *Myotis myotis* and five *Miniopterus schreibersii*) were captured at Mina da Nogueirinha cave in 2022 (Montemor-o-Novo, Central Portugal), and fecal samples, oral swabs, and anal swabs were collected (n=27). A Pan-CoV nested RT-PCR was used for initial screening, followed by viral metagenomic sequencing of all 7 fecal samples and one CoV-positive buccal swab. In silico protein docking studies were performed between a Portuguese bat CoV spike protein and APNs of bats, pigs, and humans.

RESULTS: Pan-CoV nested RT-PCR identified three positive samples: two fecal samples from distinct *M. myotis* and *M. schreibersii.* Metagenomic sequencing allowed us to determine two near complete CoV genomes, picornaviruses, adenovirus, and dependoparvovirus in fecal samples Protein docking predicted strong binding of this spike protein to bat, porcine, and human APN receptors.

CONCLUSIONS: This study reports the first near complete genome sequences of two members of the *Alphacoronavirus* genus from a Portuguese bat. The identification of other viral families highlights the diverse virome of these cave-dwelling bat species. Protein docking studies suggest a potential for cross-species transmission of this bat CoV between bats, porcines and humans.

ACKNOWLEDGMENTS/FUNDING: This work has been funded by Fundação para a Ciência e a Tecnologia (FCT), contract number 2021.09380.BD and project numbers UIDB/04750/2020, LA/P/0064/2020, UIDP/00772/2020 (Doi:10.54499/UIDB/00772/2020), and LA/P/0059/2020.

Metagenomic Analysis of Viral Diversity in Portuguese Bats

Mahima Hemnani[1], Mustafa Karatas[2], Andreia Cruz[1], Priscilla Gomes da Silva[1,3], Gertrude Thompson[1], Patrícia Poeta[4,5,6,7], Hugo Rebelo[8], Jelle Matthijnssens[2], João R. Mesquita[1,6*]

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INTRODUCTION

- Bats are a very diverse group of animals and can be found in attics, barns, unoccupied buildings, rock crevices, trees, and underground cavities.
- While they make critical and multivariate contributions to human well-being, they have also received much attention in recent years for their role as potential reservoirs of emerging viral

AIMS

Assess the presence of coronaviruses (Covs) in Portuguese bats, explore viral diversity through metagenomics, and predict the affinity of CoV spike protein with the aminopeptidase N (APN) receptor of a bat, porcine and human.

METHODOLOGY

- Ten bats (five *Myotis myotis* and five *Miniopterus schreibersii*) were captured at Mina da Nogueirinha cave in 2022 (Montemor-o-Novo, Central Portugal).
- Fecal samples, oral swabs, and anal swabs were collected (n=27).
- A **Pan-CoV nested RT-PCR** was used for initial screening, followed by **viral metagenomic sequencing** of all 7 fecal samples and one CoV-positive buccal swab. **In silico protein docking studies** were performed between a Portuguese bat CoV spike protein and APNs of bats, pigs, and humans.

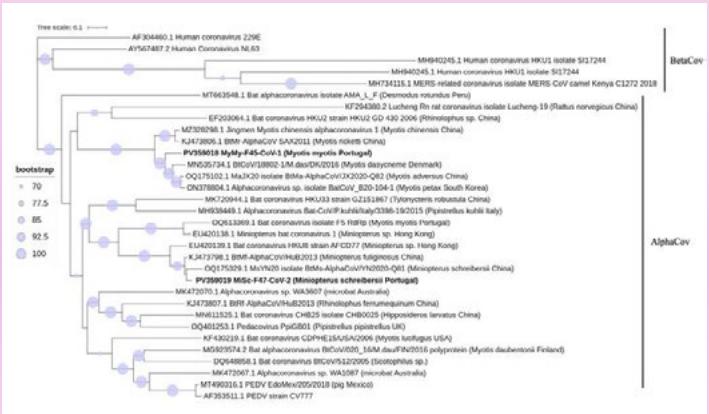
RESULTS

Pan-CoV nested RT-PCR

Sample ID	Species	Sample Type	Virus Identified
F47	*M. schreibersii*	Fecal	*Alphacoronavirus*
F52	*M. myotis*	Fecal	*Alphacoronavirus*
B51	*M. myotis*	Buccal swabs	*Alphacoronavirus*

Viral metagenomics

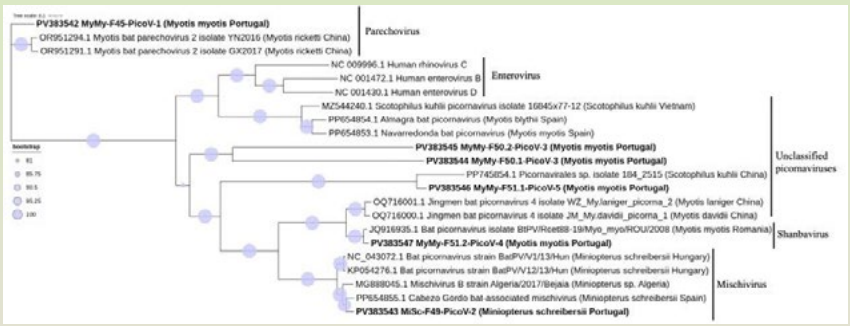
Coronavirus



- Two near complete CoV genomes were assembled
- One of the positive samples (F47) was also positive through PCR, while the other sample (F45) did not show any positive results for either method

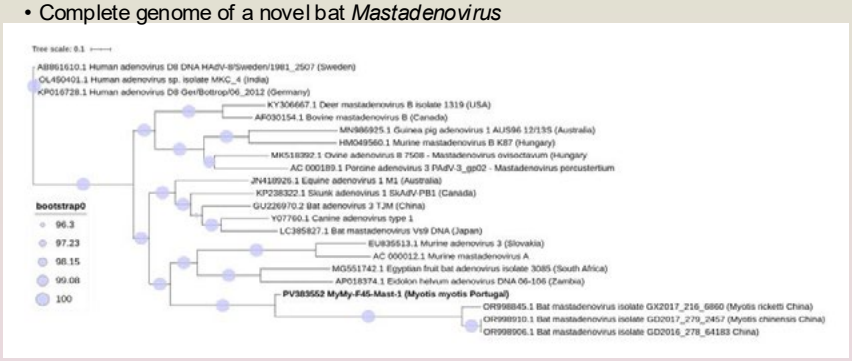
Picornavirus

- Identification of six highly divergent picornaviruses
- Viral strains show regional (Spain, Hungary) and distant evolutionary connections (China)



Mastadenovirus

- Complete genome of a novel bat *Mastadenovirus*



Dependoparvovirus

- We detected a member of the genus *Dependoparvovirus* in one bat sample.
- The contig consists of four partial sequences that exhibited no overlapping regions and were therefore considered distinct fragments.
- Due to the lack of overlap, we were unable to construct a reliable phylogenetic tree.

In silico protein docking studies

- Full spike sequence retrieved from F47 sample.
- CoV Spike-receptor binding determines host specificity & entry.
- Porcine APN Included: Pedacoviruses infect both bats & pigs
- Evolutionary Insight: PEDV & bat CoVs share a likely ancestor.
- Zoonotic Assessment: Human APN used to test theoretical human receptor binding.
- Goal: Evaluate cross-species potential & possible bat-to-pig-to-human transmission.

	APN		
	Bat	Porcine	Human
F47	-126.1 +/- 4.8	-109.0 +/- 4.4	-106.2 +/- 7.6
PEDV	-90.7 +/- 7.5	-82.8 +/- 2.2	-100.2 +/- 5.4

- **Lower values = stronger predicted binding**
- F47 spike shows strongest binding to bat APN
- PEDV spike protein demonstrated weaker binding to porcine APN
- **Implication**: F47 may have potential for cross-species infection

CONCLUSIONS

This study reports the first near complete genome sequences of two members of the *Alphacoronavirus* genus from a Portuguese bat. The identification of other viral families highlights the diverse virome of these cave-dwelling bat species. Protein docking studies suggest a potential for cross-species transmission of this bat CoV between bats, porcines and humans.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.12

LT 2 Emergent Infectious Diseases and Zoonosis

EFFECT OF ALGINATE COATINGS WITH LAUREL AND OLIVE PLANT EXTRACTS AGAINST *L. MONOCYTOGENES* AND *SALMONELLA* INOCULATED IN CHICKEN MEAT

Márcio Moura-Alves[1,2], Alexandra Esteves[1,2,3], Kamila Soares[1,2], José António Silva[1,2,3], Cristina Saraiva[1,2,3]

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INTRODUCTION: Chicken meat is highly perishable and often linked to foodborne illnesses. To control the microbial growth, new active packaging systems are emerging. The development of films and coatings has attracted considerable interest due to promising results in food preservation and its biodegradable, biocompatible, recyclable, and of renewable origin.

AIMS: Monitor the efficacy of sodium alginate (SA) coatings impregnated with laurel (LLE) and olive (OLE) leaves extracts to control the microbial growth of foodborne pathogens, namely *Listeria monocytogenes* and *Salmonella* Typhimurium inoculated in chicken meat.

METHODOLOGY: SA (1%) and glycerol (0.5 %) were mixed in distilled water overnight. Then, freeze-dried extracts obtained by ultrasonic-assisted extraction were dissolved in distilled water and added: LLE 2% and LLE 1% + OLE 1% (MIX). The inoculum was prepared by optical density at 600 nm. *Listeria monocytogenes* and *Salmonella Typhimurium* were mixed in equal proportions (1:1) at 1× 10^8 CFU/mL. Chicken breasts samples weighing 5.0 ± 0.1 g and approximately 0.5 cm thick, (surface 2.0 × 2.0 cm) were inoculated with 0.1 mL aliquots. After 20 min, the samples were coated by spraying and cross-linking was performed with $CaCl_2$. Coated and uncoated samples (control) were packed, stored at 4 ºC and analysed on day 0 (immediately), and on days 1, 3, 6 and 13 of storage. Successive decimal dilutions were performed followed by plating on Oxford and Hektoen with incubation at 37 °C for 48 h and 24 h, respectively. Results were expressed as log CFU/g.

RESULTS: *L. monocytogenes* counts ranged between 5.22 (LLE, day 0) and 8.7 log CFU/g (control, day 13). At day 0, LLE2% samples showed significantly lower counts than control samples (~0.6 log less). Over the storage period, the counts in LLE2% samples remained stable for longer (bacteriostatic effect). In the last day of storage, LLE2% and MIX counts were ~1 log lower than control samples. For *Salmonella*, counts ranged between 5.7 (LLE, day 13) and 6.4 (control, day 13). At day 0, the lowest counts were achieved by LLE2% (6.05 log CFU/g), ~0.4 log lower than control samples. All samples showed a slight decrease until day 3. By day 6-13 days, the counts increased for control samples and consistently decreased for coated samples, being statistically significant compared to day 0.

CONCLUSIONS: The incorporation of laurel and olive plant extracts into alginate-based coatings demonstrated substantial effectiveness in inhibiting microbial growth. The antimicrobial activity of the coatings appeared to follow a gradual pattern, likely attributable to the sustained and controlled release of bioactive compounds throughout the storage period.

ACKNOWLEDGMENTS/FUNDING: This work was supported by the projects UIDB/00772/2020 (doi:10.54499/UIDB/00772/2020) and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Márcio Moura-Alves would also like to thank the financial support by a Ph.D. fellowship UI/BD/150835/2021 supported by FCT (doi:10.54499/UI/BD/150835/2021). The authors would like to thank Ana Leite and Helena Santos for their valuable contributions.

EFFECT OF ALGINATE COATINGS WITH LAUREL AND OLIVE PLANT EXTRACTS AGAINST *L. MONOCYTOGENES* AND *SALMONELLA* INOCULATED IN CHICKEN MEAT

Márcio Moura-Alves[1,2], Alexandra Esteves[1,2,3], Kamila Soares[1,2], José A. Silva[1,2,3], Cristina Saraiva[1,2,3]

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[3] *Department of Veterinary Sciences, ECAV, UTAD, Vila Real, Portugal*

Introduction and Objetives

Chicken meat is highly perishable and often linked to foodborne illnesses. The development of films and coatings has attracted considerable interest due to promising results in food preservation. The aim of this study was to monitor the efficacy of sodium alginate (SA) coatings impregnated with laurel (LLE) and olive (OLE) leaves extracts to control the microbial growth of foodborne pathogens, namely *Listeria monocytogenes* and *Salmonella* Typhimurium inoculated in chicken meat.

Methodology

Plant extracts — Laurel and olive leaves

Ultrasound-assisted extraction (ethanol:water, 70:30 (v/v))

Solvent removal and lyophilization

Chicken breast samples (5 g)

Inoculation

- 0.1 mL at 1×10^8 CFU/mL
- *L. monocytogenes* and *S.* Typhimurium cocktail

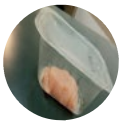
- LLE 2%;
- LLE 1% + OLE 1% (MIX)

Coating

- Sodium alginate (1%)
- Glycerol (0.5%)

Packaging:
Uncoated: polylactic acid and fossil-based
Coated: polylactic acid

Storage (4 ºC, 13 days)



L. monocytogenes and *Salmonella* enumeration

Results

Table 1 - Counts (mean ± standard deviation, log CFU/g) of *L. monocytogenes* and *S.* Typhimurium inoculated in chicken meat each treatment during storage time.

		Treatment				
		CNT	PLA	LLE	MIX	*P*
L. monocytogenes	0 d	5.79±0.12$^{a\ BC}$	5.82±0.13$^{a\ B}$	5.22±0.08$^{b\ B}$	5.40±0.13$^{ab\ B}$	***
	1 d	5.63±0.26$^{a\ C}$	5.91±0.03$^{a\ B}$	5.42±0.02$^{a\ B}$	5.23±0.44$^{a\ B}$	***
	3 d	5.42±0.14$^{a\ C}$	5.71±0.13$^{a\ B}$	5.36±0.21$^{a\ B}$	5.46±0.02$^{a\ B}$	***
	6 d	6.59±0.15$^{a\ B}$	6.32±0.26$^{a\ B}$	5.28±0.21$^{b\ B}$	5.92±0.11$^{ab\ B}$	***
	13 d	8.70±0.29$^{a\ A}$	8.52±0.28$^{a\ A}$	7.43±0.18$^{b\ A}$	7.71±0.30$^{ab\ A}$	***
	P	***	***	***	***	
S. Typhimurium	0 d	6.45±0.03^{a}	6.43±0.07^{a}	6.05±0.03$^{b\ A}$	6.29±0.13$^{ab\ A}$	**
	1 d	6.24±0.01	6.28±0.21	6.02±0.12^{AB}	5.99±0.23^{AB}	ns.
	3 d	6.09±0.13^{a}	6.04±0.02^{ab}	5.78±0.05$^{bc\ BC}$	5.65±0.08$^{c\ B}$	**
	6 d	6.37±0.20^{a}	6.24±0.08^{ab}	5.87±0.05$^{bc\ ABC}$	5.64±0.05$^{cd\ B}$	***
	13 d	6.41±0.17^{a}	6.10±0.12^{ab}	5.67±0.12$^{bc\ C}$	5.78±0.03$^{cd\ B}$	***
	P	ns.	ns.	**	*	

CNT: uncoated samples packed with polypropylene trays and film; PLA: uncoated samples packed with polylactic acid trays and film; LLE: coated with LLE 2% and packed with polylactic acid trays and film; MIX: coated with LLE 1% + OLE 1% and packed with polylactic acid trays and film. d: days; ns.: non-significant; For storage time (rows), means with different letters (lowercase) differ significantly, *P < 0.05, **P < 0.01, ***P < 0.001. For treatments (columns), means with different letters (uppercase) differ significantly, *P < 0.05, **P < 0.01, ***P < 0.001.

For *L. monocytogenes*, at day 0, LLE2% samples showed significantly lower counts than control samples. Over the storage period, the counts in LLE2% samples remained stable for longer (bacteriostatic effect). On the last day of storage, LLE2% and MIX counts were ~1 log lower than control samples. For *Salmonella,* the lowest counts on day 0 were achieved by LLE2%. All samples showed a slight decrease until day 3. From day 6-13, the counts increased in the control samples and decreased consistently in the coated samples, which was statistically significant compared to day 0.

Conclusions

The incorporation of laurel and olive plant extracts into alginate-based coatings demonstrated substantial effectiveness in inhibiting *L. monocytogenes* and *S.* Typhimurium growth.

The PLA packaging showed similar performance as conventional plastic. This finding suggests that bio-based packaging can provide a wider range of options for meat packaging.

Acknowledgments: This work was supported by the projects UIDB/00772/2020 (doi:10.54499/UIDB/00772/2020) and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Márcio Moura-Alves would also like to thank the financial support by a Ph.D. fellowship UI/BD/150835/2021 supported by FCT (doi:10.54499/UI/BD/150835/2021). The authors would like to thank Ana Leite and Helena Santos for their valuable contributions.

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.13

LT 2 Emergent Infectious Diseases and Zoonosis

COMBINING TLR2 WITH TLR4 OR TLR9 AGONISTS IN LIPOSOMAL FORMULATIONS TO FINE-TUNE VACCINE-INDUCED IMMUNE RESPONSES

Mariana Ferreira[1, 2*], Sara Zúquete[1, 2], Manuela C. Carvalheiro[3], Inês L. S. Delgado[1, 2], Ana Catarina Mendes[4], Sofia Nolasco[1, 2], Luís Graça[4], Maria L. Corvo[3], Alexandre Leitão[1, 2] Afonso P. Basto[1, 2]

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INTRODUCTION: Vaccines are essential tools for safeguarding human and animal health. However, developing safe and effective vaccines remains a challenge for many important diseases. Our group investigates how activation of Toll-like receptor 2 (TLR2), in combination with other innate stimuli (PAMPs), can shape long-lasting and tissue-targeted adaptive immunity for the development of better vaccines. We have screened a set of PAMP combinations to identify stimuli that promote germinal centre reactions – the basis for the production of long-lasting high-affinity antibodies – while inducing a polarised Th1 immune profile – required for the control of most intracellular pathogens. This screening identified TLR2+TLR4 and TLR2+TLR9 as promising stimuli and agonists of these receptors were successfully incorporated into liposomes together with the model antigen ovalbumin.

AIMS: This study aimed to evaluate the immunostimulatory potential of liposomes combining TLR2 with either TLR4 or TLR9 ligands to induce immune mechanisms that can improve vaccine efficacy.

METHODOLOGY: Dendritic cells were incubated with three concentrations of liposomes for 24 h. Maturation was assessed by flow cytometry analysis of CD40, CD80 and CD86 surface expression. TNF-α levels in the culture supernatant were measured by ELISA. Two liposomal formulations were tested *in vivo* in mice combining ovalbumin with a TLR2 agonist and either TLR4 or TLR9 agonists. Eleven days after primary immunisation, antigen-specific IgG isotypes in serum and IFN-γ levels in culture supernatants of *ex vivo* restimulated lymphocytes were evaluated by ELISA. Flow cytometry was used to assess Tfh and Tfr cells, as well as OVA-specific CD8+ T cells, in draining lymph nodes.

RESULTS: Both TLR2/TLR9 and TLR2/TLR4 formulations induced TNF-α production on stimulated dendritic cells at higher levels than single-PAMP liposomes and both upregulated CD40, CD80 and CD86 expression, although to different extents. *In vivo*, both formulations induced the differentiation of Tfh ($CXCR5^+PD1^+$), OVA-specific CD8+ and IFN-γ-producing cells in the draining lymph node, as well as serum anti-OVA IgG2c (Th1-related antibodies). Immunisation with TLR2/TLR9 ligands led to a higher percentage of Tfh and CD8+ T cells and increased IFN-γ production, whereas TLR2/TLR4 agonists induced higher IgG2c levels.

CONCLUSIONS: These results indicate that both liposomal formulations, albeit with slightly different properties, are promising adjuvant candidates to induce Th1 responses with high-affinity antibodies and support their progression to final preclinical testing. For this, we will incorporate a *Toxoplasma gondii* antigen into the liposomes and test them in a murine toxoplasmosis infection model.

ACKNOWLEDGMENTS/FUNDING: Fundação para a Ciência e Tecnologia (FCT; Portugal) through the project grants: PTDC/CVT-CVT/31840/2017; PTDC/CVT-CVT/4599/2021; 2022.04903.PTDC; UID/CVT/00276/2019, UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS). Fundación la Caixa (Spain) through the project HR22-00741.

Combining TLR2 with TLR4 and TLR9 agonists in liposomal formulations to fine-tune vaccine-induced immune responses

Mariana Ferreira[1, 2*], Sara Zúquete[1, 2], Manuela C. Carvalheiro[3], Inês L. S. Delgado[1, 2], Ana Catarina Mendes[4], Sofia Nolasco[1, 2], Luís Graça[4], Maria L. Corvo[3], Alexandre Leitão[1, 2] Afonso P. Basto[1, 2]

[1] CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, 1300-477, Lisbon, Portugal. [2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal. [3] FARM-ID - Faculty of Pharmacy Research and Development Association, 1649-003, Lisbon, Portugal. [4] GIMM - Gulbenkian Institute for Molecular Medicine, 1649-028 Lisbon, Portugal.

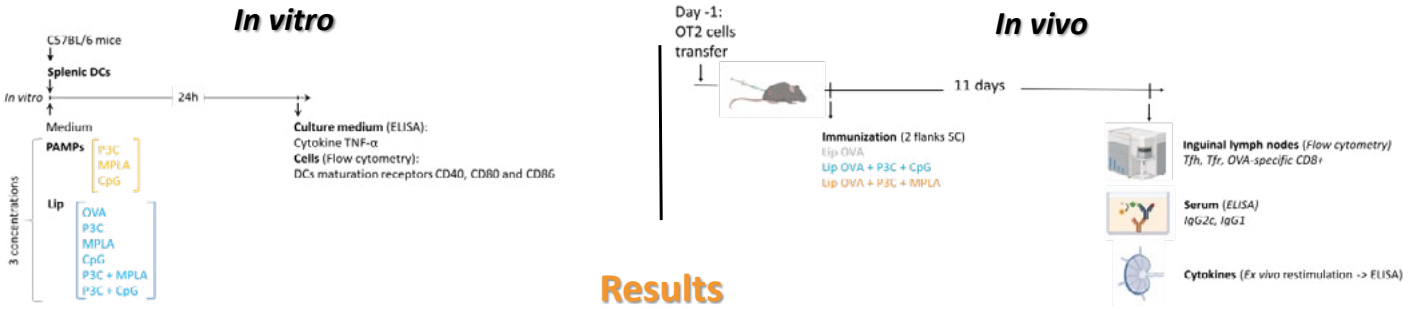
Introduction

Our group investigates how activation of Toll-like receptor 2 (TLR2), in combination with other innate stimuli (PAMPs), can shape long-lasting and tissue-targeted adaptive immunity for the development of better vaccines.
We have screened a set of PAMP combinations to identify stimuli that promote germinal centre reactions – the basis for the production of long-lasting high-affinity antibodies – while inducing a polarised Th1 immune profile – required for the control of most intracellular pathogens. This screening identified TLR2+TLR4 and TLR2+TLR9 as promising stimuli and agonists of these receptors were successfully incorporated into liposomes together with the model antigen ovalbumin.

Objective

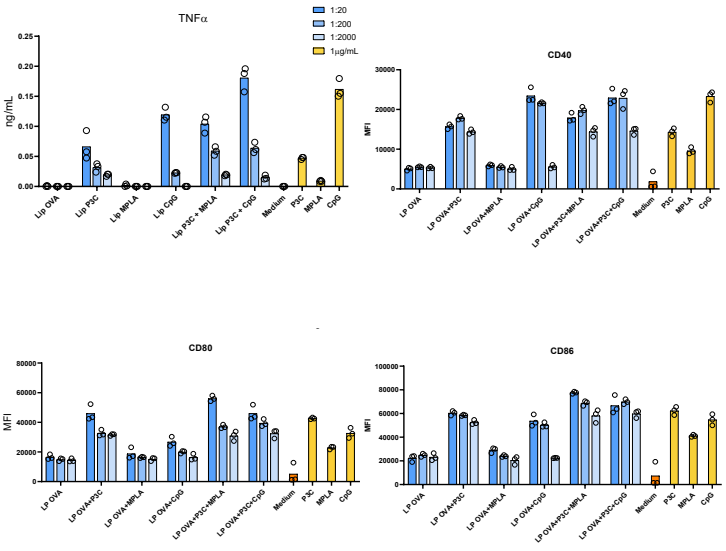
This study aimed to evaluate the immunostimulatory potential of liposomes combining TLR2 with either TLR4 or TLR9 ligands to induce immune mechanisms that can improve vaccine efficacy.

Methods

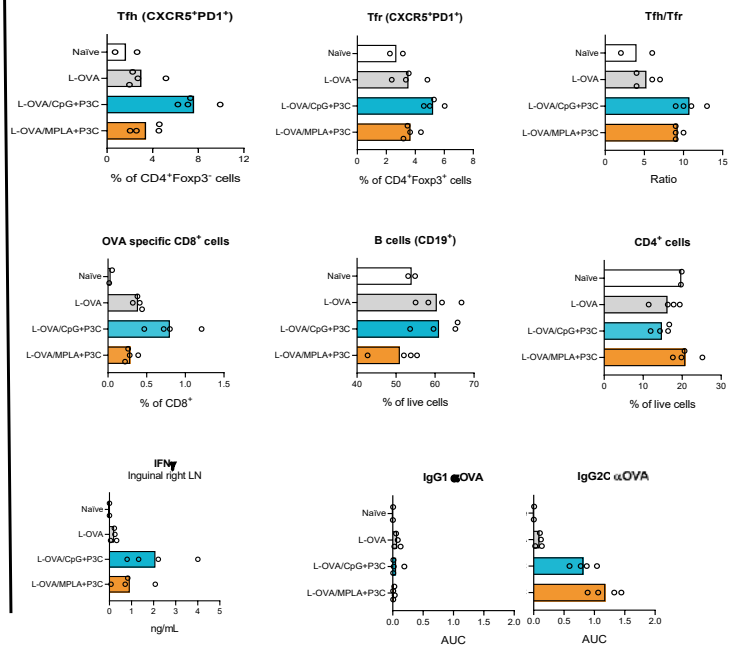


Results

- Both formulations induced TNF-α production on stimulated dendritic cells at higher levels than single-PAMP liposomes.
- Both upregulated CD40, CD80 and CD86 expression, although to different extents.



- Both formulations induced the differentiation of Tfh , OVA-specific CD8+ and IFN-γ-producing cells as well as serum anti-OVA IgG2c.
- Immunisation with TLR2/TLR9 ligands led to a higher percentage of Tfh and CD8+ T cells and increased IFN-γ production, whereas TLR2/TLR4 agonists induced higher IgG2c levels.



Conclusions

These results indicate that both liposomal formulations, albeit with slightly different properties, are promising adjuvant candidates to induce Th1 responses with high-affinity antibodies and support their progression to final preclinical testing. For this, we will incorporate a *Toxoplasma gondii* antigen into the liposomes and test them in a murine toxoplasmosis infection model.

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PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.14

LT 2 Emergent Infectious Diseases and Zoonosis

DIAGNOSTICS AND DIVERSITY: MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* SPP. IN REPTILES

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INTRODUCTION: *Cryptosporidium* spp. is a parasitic infection in reptiles, hard to diagnose and treat, posing risks when exposing new animals. Diagnosis often requires specialized tests such as PCR, immunological tests and faecal analysis. Despite challenges, accurate diagnosis is crucial for effective management in reptile veterinary practice.

AIMS: 1. Assess the efficacy of different diagnostic methods; 2. Determine genetic diversity of *Cryptosporidium* species in reptiles.

METHODOLOGY: Fourty three faecal samples from 14 reptile species were collected. Samples were filtered, and centrifuged and the sediment was used for DNA extraction and detection of *Cryptosporidium* spp. oocysts using modified Ziehl-Neelsen stain (MZN) and direct immunofluorescence assay antibody (DFA). The presence of *Cryptosporidium* spp. was also tested by a nested PCR amplification of an 840 bp fragment of the small subunit rRNA gene. Positive samples were sequenced to identify *Cryptosporidium* species.

RESULTS: Eighteen samples (41.9%) were positive for *Cryptosporidium* spp. Detection rates were 39.5% (PCR: n=17/43), 25.6% (MZN: n=11/43), and 14% (DFA: n=6/43). Six *Cryptosporidium* species were identified by sequencing: *C. ducismarci* (n=4) and *C. muris* (n=2) were detected by MZN and DFA, *C. testudinis* (n=2) and *C. serpentis* (n=6) by MZN. *C. tyzzeri* (n=2) and *C. ditrichi* (n=1) were not detected by MZN or DFA.

CONCLUSION: DFA was not effective for diagnosing *Cryptosporidium* spp. in reptiles. PCR was more effective than MZN for infection detection. The presence of rodent-associated *Cryptosporidium* species in reptiles suggests ingestion of contaminated prey or food and water.

ACKNOWLEDGEMENTS/FUNDING: This work is financed by national funds through FCT - Foundation for Science and Technology, I.P., within the scope of the project CIISA - UIDB/00276/2020 and Al4AnimalS - LA/P/0059/2020. Also, Mariana Louro holds the PhD Research Fellowships UI/BD/152818/2022 (funded by FCT).

Supported by LA/P/0059/2020

Diagnostics and Diversity: Molecular Characterization of *Cryptosporidium* spp. in Reptiles

Mariana Louro[1,2,*], Laura Hernández-Hurtado[3], João Antunes[1,2], Luís Madeira de Carvalho[1,2], Isabel Pereira da Fonseca[1,2] and Jacinto Gomes[1,2,3]

1. CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon.
2. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS).
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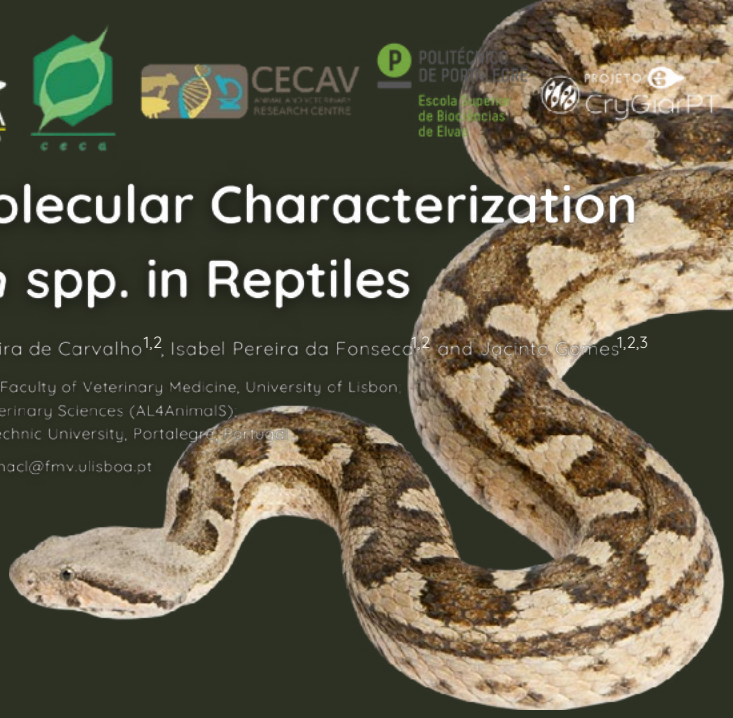


Introduction:

Cryptosporidium spp. causes a parasitic infection in reptiles, hard to diagnose and treat, posing risks when exposing new animals. Diagnosis often requires specialized tests such as PCR, immunological tests and fecal analysis.

Despite challenges, accurate diagnosis is crucial for effective management in reptile veterinary practice.

Objectives:

1. Assess the efficacy of different diagnostic methods for *Cryptosporidium* spp. in captive reptiles.
2. Determine genetic diversity of *Cryptosporidium* species in diferent reptiles.

Materials & Methods:

43 faecal samples
14 reptile species

Snakes

7 species
n=14

Tortoises

3 species
n=13

Lizards

4 species
n=16

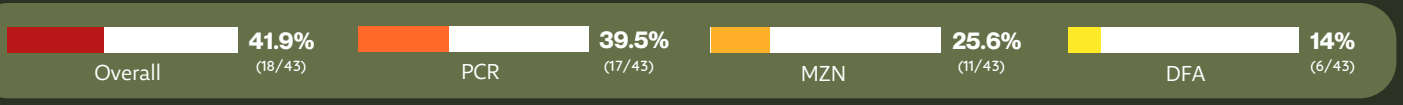
Analyzed by:

- Modified Ziehl-Nelseen stain (MZN)
- Direct immunofluorescence assay (DFA)
  (Merifluor ® Cryptosporidium/Giardia, Kit)
- Nested PCR amplification of an 840 bp fragment of the small subunit rRNA gene

Sequencing for *Cryptosporidium* species identification

Results:

Positives



Cryptosporidium species & hosts

Cryptosporidium species	Host species
Snakes	
C. serpentis (n=3) *	*Boa constrictor* * **
C. muris (n=2) *	*Ophiophagus hannah* *
C. tyzzeri (n=2) *	*Pantherophis guttatus* *
	Python regius *
	Sanzinia madagascariensis *
Tortoises	
C. ducismarci (n=4) *	*Testudo horsfieldii* ** *
C. testudinis (n=2) *	*Stigmochelys pardalis* ** *
Lizards	
C. serpentis (n=3) *	*Eublepharis macularius* *** *
C. ditrichi (n=1) *	

Detected by both MZN & DFA

C. ducismarci

- Found in tortoises and turtles
- Assymptomatic
- No Human cases worlwide

C. muris

- Found mostly in rodents
- Zoonotic
- Ingestion of contaminated mice?
- Environmental contamination?

Detected only by MZN

C. serpentis

- Found mostly in snakes
- Can cause severe gastritis
- Can also infect lizards and cause severe disease
- No Human cases worldwide

C. testudinis

- Found in tortoises and turtles
- Assymptomatic
- No Human cases worlwide

Not detected by MZN or DFA

C. ditrichi

- Found mostly in *Apodemus* spp.
- Zoonotic
- Environmental contamination?

C. tyzzeri

- Found mostly in rodents
- Zoonotic
- Ingestion of contaminated mice?
- Environmental contamination?

Repetitions in samples with high concentration of *Cryptosporidium* oocysts, confirm that *C. serpentis* is not detected using DFA.
C. serpentis, ***C. tyzzeri***, ***C. ducismarci*** and ***C. ditrichi***: first time reported in Portugal.

References & Poster Download

Conclusion:

- DFA was not effective for diagnosing *Cryptosporidium* spp. in reptiles.
- MZN was better than DFA but less sensitive than PCR.
- The presence of rodent-associated *Cryptosporidium* species in reptiles suggests ingestion of contaminated prey.
- Highlight the need to identify *Cryptosporidium* species in association with clinical signs, in reptile veterinary clinical pratice.

Acknowledgements: This work is financed by national funds through FCT - Foundation for Science and Technology, I.P., within the scope of the project CIISA - UIDB/00276/2020 and AI4AnimalS - LA/P/0059/2020. Also, Mariana Louro hold the PhD Research Fellowships UI/BD/152818/2022 (funded by FCT).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.15

LT 2 Emergent Infectious Diseases and Zoonosis

BAT-ASSOCIATED CORONAVIRUS FOUND IN PIGS: INSIGHTS INTO POTENTIAL CROSS-SPECIES TRANSMISSION

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INTRODUCTION: Coronaviruses (CoVs) are a diverse group of RNA viruses with zoonotic potential, often associated with cross-species transmission, particularly between wildlife and domestic animals. The Alphacoronavirus genus includes several strains identified in bats and swine, raising concerns about interspecies transmission and its implications for public health. Previous research has shown that in other parts of the world, such as China, Alphacoronaviruses found in pigs have originated from bats. In Europe, the potential for bat-to-swine CoV transmission remains underexplored, especially in regions with overlapping habitats and shared environmental resources.

AIMS: The aim of this study was to assess the presence of coronaviruses in farmed swine from northern Portugal and Spain and explore their potential phylogenetic relationship with bat-associated Alphacoronaviruses previously identified in the Iberian Peninsula.

METHODOLOGY: A total of 400 fecal samples were collected from pigs across multiple farms and screened using a pan-coronavirus nested RT-PCR assay. Positive samples were sequenced and analyzed using phylogenetic methods to determine genetic similarities with known CoV sequences from both swine and bat reservoirs.

RESULTS: Out of the 400 swine fecal samples analyzed, 18 (4.5%) tested positive for Alphacoronavirus. Sequence analysis revealed that these swine-derived CoVs shared high nucleotide identity, ranging from 95.40% to 99.76%, with Alphacoronaviruses previously detected in Iberian bat species and other European countries. Phylogenetic trees grouped the swine sequences closely with other previously isolated bat Alphacoronaviruses, suggesting a potential epidemiological link or shared evolutionary origin.

CONCLUSIONS: The genetic relationships between swine CoVs and bat-associated Alphacoronaviruses in Europe found in this study provide valuable insights. The high genetic similarity between swine and bat coronaviruses underscores the importance of conducting further research on the role of bats in the transmission dynamics and the zoonotic potential of Alphacoronaviruses. Broadening phylogenetic analyses and exploring recombination events could provide insights into the evolutionary trajectories of these viruses. Comparative research between European and Asian swine coronaviruses, including SADS-CoV, may reveal common patterns in their emergence and transmission.

In summary, our findings highlight the need for continuous surveillance and interdisciplinary research to better understand the dynamics of CoVs in wildlife and livestock. These efforts are essential for enhancing global health readiness and reducing the risks of future zoonotic outbreaks.

ACKNOWLEDGMENTS/FUNDING: Sérgio Santos-Silva thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of his Ph.D work under the scholarship 2021.09461.BD contract through the Maria de Sousa-2021 program. Andreia V. S. Cruz thanks Fundação para a Ciência e a Tecnologia (FCT – Portuguese Foundation for Science and Technology) for the financial support of her PhD work under the Maria de Sousa scholarship 2022.15408.BD.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.16

LT 2 Emergent Infectious Diseases and Zoonosis

MOLECULAR DETECTION OF CANINE GASTROINTESTINAL VIRUSES: A CALL FOR ONE HEALTH SURVEILLANCE

Sofia Sirage[1,2]; Miguel Maximino[1,2]; Joana F. Guerreiro[1,2]; Luís Tavares[1,2]; Virgílio Almeida[1,2]; Solange Gil[1,2,3];

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INTRODUCTION: Zoonotic diseases represent an increasing global health concern, as they can affect both animal and human populations. Gastrointestinal viral infections in domestic dogs are of relevance, as they contribute to significant morbidity and may pose zoonotic risks. Understanding the prevalence and dynamics of these infections is essential for effective control strategies.

OBJECTIVES: This study aimed to assess the prevalence of key gastrointestinal viral pathogens—Canine Circovirus, Astrovirus, Sapovirus, Rotavirus, Coronavirus, and Norovirus—in dogs using multiplex real-time PCR, to better understand their role in canine gastrointestinal disease.

METHODOLOGY: Samples were collected from 90 dogs admitted to the Isolation and Biological Containment Unit at the Hospital de Medicina Veterinária de Lisboa. Multiplex real-time PCR was employed to detect the presence of the selected gastrointestinal viruses, allowing for the simultaneous identification of multiple pathogens in canine biological samples.

RESULTS: The study revealed that 68.9% of the dogs tested positive for at least one gastrointestinal viral pathogen. Astrovirus was the most prevalent (25.6%), followed by Coronavirus (20.0%) and Norovirus (11.1%). Circovirus was found in 8.9% of the samples, while Sapovirus and Rotavirus were detected in 2.2% and 1.1% of samples, respectively. These results highlight Astrovirus, Coronavirus, and Norovirus as the most common pathogens in the studied population.

CONCLUSIONS: The findings emphasize the importance of ongoing viral surveillance and early detection through molecular diagnostics. They also underscore the need for adopting a One Health approach to control zoonotic diseases, as these gastrointestinal pathogens pose a potential risk to both animal and human health. Strengthening cross-sectoral collaboration is essential for effective disease prevention and control, enhancing surveillance, improving diagnostic capabilities, and developing robust strategies to mitigate the spread of zoonotic pathogens.

ACKNOWLEDGMENTS/FUNDING: This study was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Project UIDB/00276/2020 (FCT).

MOLECULAR DETECTION OF CANINE GASTROINTESTINAL VIRUSES: A CALL FOR ONE HEALTH SURVEILLANCE

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INTRODUCTION

Zoonotic diseases represent an increasing global health concern, as they pose a dual burden, to humans and animals. Gastrointestinal viral infections in domestic dogs are of relevance, as they contribute to significant morbidity and may pose zoonotic risks. Understanding the high prevalence and dynamics of these infections is essential for effective control strategies.

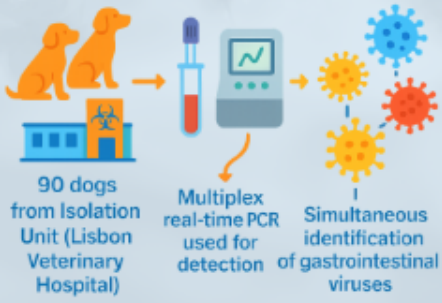
Source:World Health Organization(WHO)

OBJECTIVES

This study aimed to assess the prevalence of key gastrointestinal viral pathogens—Canine Circovirus, Astrovirus, Sapovirus, Rotavirus, Coronavirus, and Norovirus— to better understand their role in canine gastrointestinal disease.

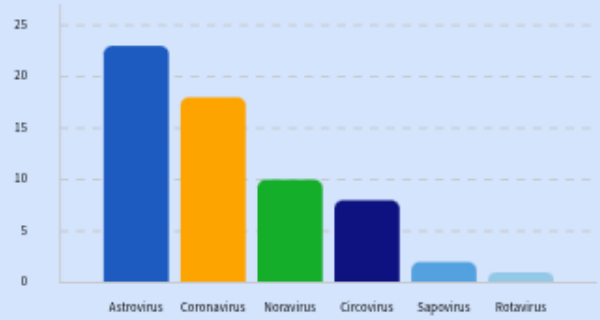
METHODOLOGY

Samples were collected from 90 dogs admitted to the Isolation and Biological Containment Unit at the Hospital de Medicina Veterinária de Lisboa. Multiplex real-time PCR was employed to detect the presence of the selected gastrointestinal viruses, allowing for the simultaneous identification of multiple pathogens in canine biological samples.



RESULTS

Sixty-two dogs (68.9%) tested positive for at least 1 gastrointestinal virus.



These results highlight Astrovirus, Coronavirus, and Norovirus as the most common pathogens in the studied population.

CONCLUSIONS

- Continuous viral surveillance is essential for early detection.
- Molecular diagnostics enable rapid and accurate pathogen identification.
- A One Health approach is critical for the control of zoonotic diseases.
- Canine gastrointestinal pathogens may pose a public health risk.
- Strengthening cross-sectoral collaboration is vital for effective disease prevention and control.
- Enhancing diagnostic capacity, surveillance systems, and response strategies is necessary to mitigate zoonotic pathogen spread.

FUNDING: This research was funded by the Portuguese Foundation for Science and Technology(FCT), under projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020(AL4AnimalS).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT2.33

LT 2 Emergent Infectious Diseases and Zoonosis

A DEEP LOOK AT CANINE MONOCYTE-DERIVED CELLS IN THE CONTEXT OF CANINE LEISHMANIOSIS: PHASE CONTRAST AND SCANNING ELECTRON MICROSCOPY

Marta Monteiro[1,2*], Graça Alexandre-Pires[1,2*], Cláudia Moreno[3], Armanda Rodrigues[3], Rui Ferreira R[4], Inês Cardoso[4], Telmo Nunes[5], Wilson Antunes[6], Rodolfo Leal[1, 2, 7], Gabriela Santos-Gomes[3], Isabel Pereira Da Fonseca[1, 2]

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INTRODUCTION: Monocyte-derived dendritic cells (moDC) have been studied in immune-precision research settings such as leishmaniosis, for their role as antigen-presenting cells specialised in stimulation of naïve T-cells. Their morphology can be analysed through microscopy, being phase-contrast (PCM) and scanning electron microscopy (SEM) two techniques which allow to see different aspects of cellular morphology.

AIMS: To explore the morphological features of canine monocyte-derived cells obtained by three cytokine cocktails and the effect of a proinflammatory stimulus (phorbol 12-myristate 13-acetate, PMA), by conducting PCM and SEM imaging techniques.

METHODOLOGY: PBMCs were obtained from blood of ten healthy dogs and differentiated into moDCs using three different protocols: cell culture medium (CCM) only (Protocol 1, P1); CCM and colony stimulating factor (CSF) (P2); and CCM, CSF and interleukin-4 (P3). At day 7, PMA was added to half of the cultures within each protocol. At day 8, cells were analysed by PCM and processed for SEM.

RESULTS: From P1 to P3, both PCM and SEM images revealed an increase in cell dimension and cytoplasmatic projections. Furthermore, PMA induced an increase in cell size, a more rounded and flattened shape and the reduction of the dendrites, compared with non-PMA-stimulated cells. While SEM gave a much higher resolution of the cell surface/topography, PCM allowed to observe the internal structures, showing an increase in granularity and in vacuole-like forms in PMA-stimulated cells.

CONCLUSIONS: This study allowed a complementary deeper understanding of the morphological features of monocyte-derived cells following different protocols. Protocol 3 led to the development of cells which were morphologically more compatible with moDCs, known to be important in the context of cell-based immune precision research studies. All cells showed morphological alterations upon a proinflammatory stimulus (PMA).

ACKNOWLEDGMENTS/FUNDING: This study was supported by FCT-Foundation for Science and Technology, through research grants UI/BD/152819/2022, PTDC/CVT CVT/0228/2020, UIDB/00276/2020-CIISA; LA/P/0059/2020-AL4AnimalS and 2022.00499.CEECIND/CP1725/CT0023.

A DEEP LOOK AT CANINE MONOCYTE-DERIVED CELLS IN THE CONTEXT OF CANINE LEISHMANIOSIS: PHASE CONTRAST AND SCANNING ELECTRON MICROSCOPY

Marta Monteiro[1,2*], Graça Alexandre-Pires[1,2*], Cláudia Moreno[3], Armanda Rodrigues[3], Rui Ferreira[4], Inês Cardoso[4], Telmo Nunes[5], Wilson Antunes[6], Rodolfo Leal[1, 2, 7], Gabriela Santos-Gomes[3], Isabel Pereira Da Fonseca[1, 2]

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INTRODUCTION & AIMS

- **Monocyte-derived dendritic cells** (moDC) have been studied in immune-precision research settings, including in the study of leishmaniosis. Their **morphology** can be analysed through microscopy, such as **phase-contrast** (PCM) **and scanning electron microscopy** (SEM).
- This work aimed **to explore the morphological features of canine monocyte-derived cells** obtained by **three cytokine cocktails**, and the effect of a **proinflammatory stimulus (PMA)**, by **conducting PCM and SEM imaging**.

METHODOLOGY

Day 0: Isolation of canine PBMCs (n=10)
Days 0 – 7: Differentiation into monocyte-derived cells, with:
P1: cell culture medium (CCM) only (RPMI, FBS, antibiotics);
P2: CCM and colony stimulating factor (CSF);
P3: CCM, CSF and interleukin-4
Day 7: Stimulation with PMA (24h)
Day 8: Morphological characterization (PCM and SEM)

RESULTS

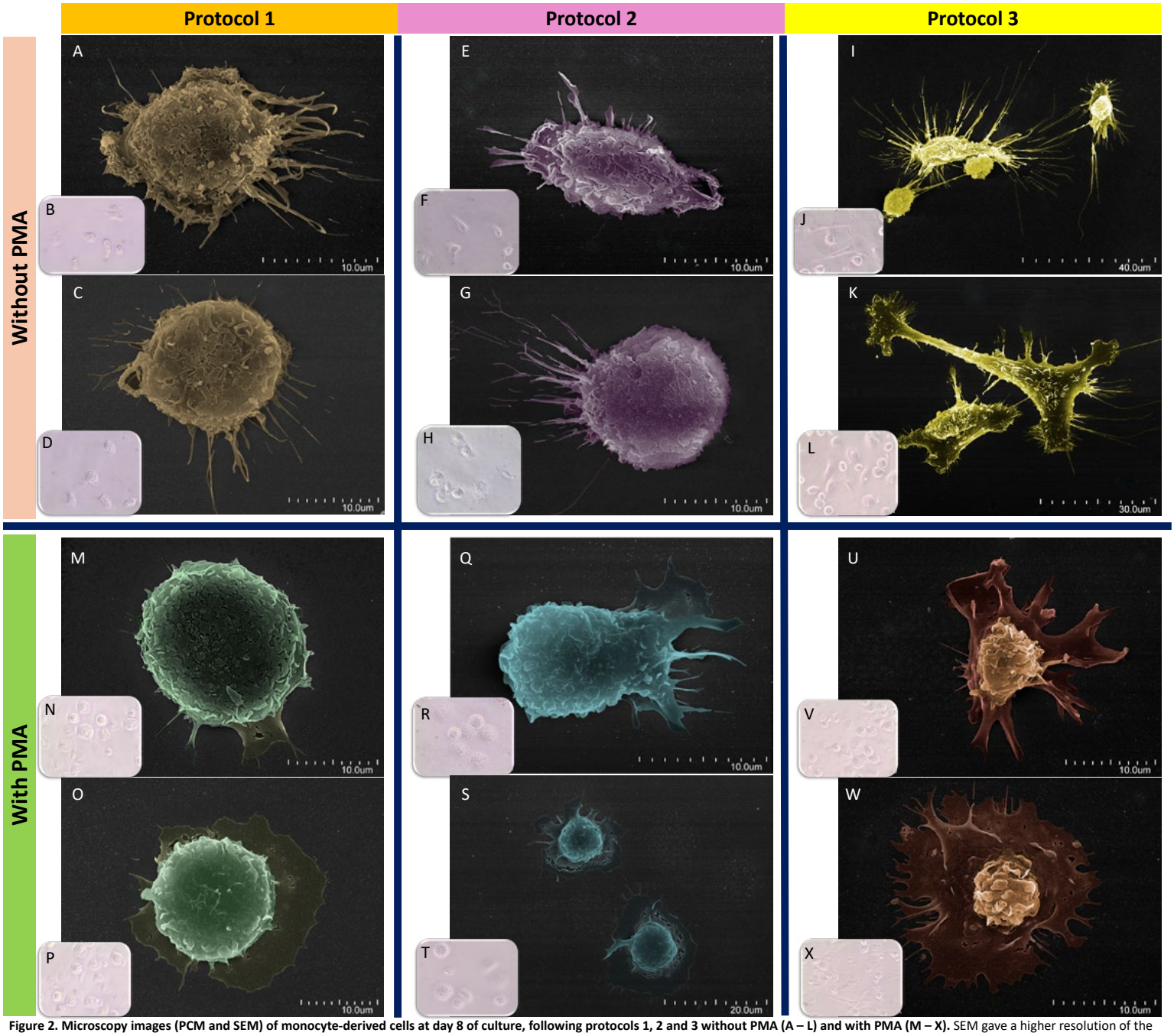

Figure 2. Microscopy images (PCM and SEM) of monocyte-derived cells at day 8 of culture, following protocols 1, 2 and 3 without PMA (A – L) and with PMA (M – X). SEM gave a higher resolution of the cell topography; PCM shows the internal structures, such as an increase in granularity and in vacuole-like forms in PMA-stimulated cells.

CONCLUSIONS

- Deeper understanding the **morphological features of monocyte-derived cells** following **three cytokine cocktails** and comparing **two microscopy techniques**.
- **Protocol 3** allowed the development of cells more compatible with **monocyte derived DCs.**
- All cells showed **morphological alterations** upon a proinflammatory stimulus (**PMA**).

ACKNOWLEDGMENTS/FUNDING

This study was supported by FCT-Foundation for Science and Technology, through research grants UI/BD/152819/2022, PTDC/CVT CVT/0228/2020, UIDB/00276/2020-CIISA; LA/P/0059/2020-AL4AnimalS and 2022.00499.CEECIND/CP1725/CT0023.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.10

LT 3 Comparative and Translational Medicine and Biotechnology

DESIALYLATION-BASED IMMUNOTHERAPY FOR FELINE MAMMARY CARCINOMA

Alexandra Couto Oliveira[1,2], Magda Ferreira[1,2], Margarida Ferreira-Silva[1,2] Afonso Basto[1,2], Joana Dias[1,2], Frederico Aires da Silva[1,2], Paula Videira[3], Inês Costa[1,4], Joana Brás[1,4], Carlos Fontes[1,4], Pedro Bule[1,2]

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INTRODUCTION: Despite the significant advances brought by immunotherapies in oncology, many patients remain unresponsive, highlighting the urgent need for novel therapeutic strategies. Aberrant glycosylation has emerged as a hallmark of malignancy, with the overexpression of sialic acid capped glycans identified as a key contributor to immune evasion. These tumour-associated glycans are recognized by sialic-acid-binding Ig-like lectins (Siglecs), expressed on immune cells, thereby activating inhibitory signalling pathways that suppress anti-tumour immunity. In human breast cancer (HBC), multiple Siglecs have been implicated in these immunosuppressive mechanisms. Given the similarities between human breast cancer and feline mammary carcinoma (FMC), the feline model is considered a powerful framework for the design of novel immunotherapeutic strategies.

AIMS: In this context, we aimed to develop a novel immunotherapeutic strategy based on a sialidase–single-chain variable fragment (scFv) antibody conjugate, designed to selectively promote tumour desialylation, with potential translational benefits for both human and feline species.

METHODOLOGY: A panel of putative neuraminidases was designed, expressed in *E. coli* and purified. The sialolytic activity was assessed using the fluorogenic substrate 4MU Neu5Ac, while cell surface sialic acid removal ability from tumour cells was evaluated through flow cytometry. The most promising sialidase, a feline neuraminidase (fNeu), was conjugated to a single-chain antibody targeting Trop2, a protein overexpressed in tumour cells. The resulting conjugate (fNeu-scFv) was tested for its ability to desialylate tumour cells and bind Trop2 using flow cytometry, fluorescence microscopy, and western blot.

RESULTS: The sialolytic activity of the fNeu-scFv conjugate was comparable to that of unconjugated fNeu, indicating that enzymatic function was preserved upon antibody linkage. Moreover, the conjugate exhibit Trop2-binding capability in both HBC and FMC cell lines.

CONCLUSIONS: These findings suggest that targeted tumour desialylation using a sialidase-scFv conjugate represents a promising immunotherapeutic approach. This strategy aligns with the “One Health” concept, emphasizing its translational potential. Future studies will evaluate the conjugate’s ability to enhance anti-tumour clearance of FMC cells by PBMCs and macrophages *in vitro*, as well as its efficacy in attenuating disease progression in murine models of FMC.

ACKNOWLEDGMENTS/FUNDING: This research was funded by the Portuguese Foundation for Science and Technology (FCT), under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS) and the PhD studentship UI/BD/153068/2022 (Alexandra Couto Oliveira). We acknowledge Agência Nacional de Inovação through grant LISBOA-01-0247-FEDER-047033 (Glycomed), the Gilead GÉNESE program through the project 17805, the project grant 2022.07903. PTDC and La Caixa through the Junior Leader Fellowship LCF/BQ/PR23/11980039.

DESIALYLATION-BASED IMMUNOTHERAPY FOR FELINE MAMMARY CARCINOMA

Alexandra Couto Oliveira[1,2], Magda Ferreira[1,2], Margarida Ferreira-Silva[1,2], Afonso Basto[1,2], Joana Dias[1,2], Frederico Aires da Silva[1,2], Paula Videira[3], Inês Costa[1,4], Joana Brás[1,4], Carlos Fontes[1,4], Pedro Bule[1,2]

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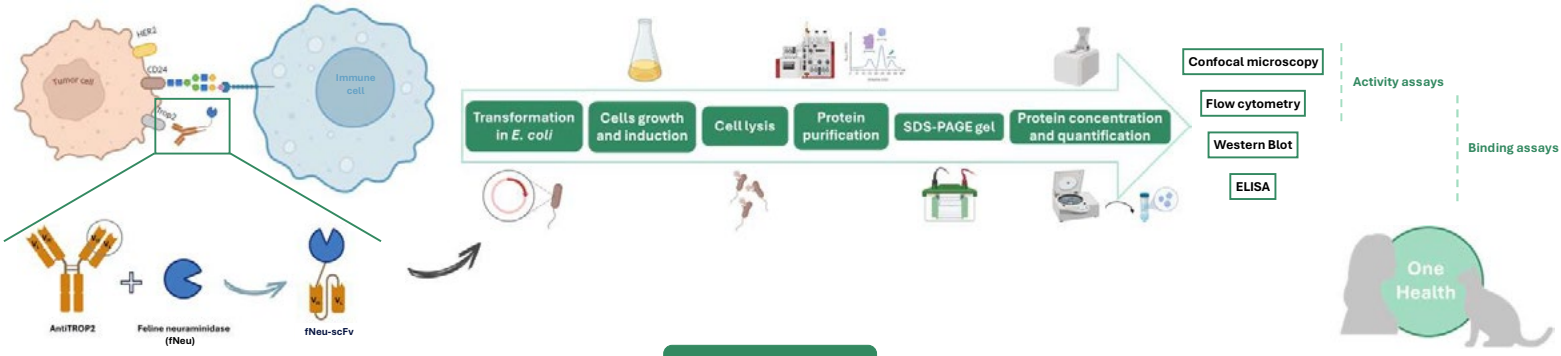
Background

Immunotherapies, particularly immune checkpoint inhibitors, have revolutionized cancer treatment. However, some patients fail to respond with many others relapsing, highlighting the urgent need for novel therapeutic strategies. Aberrant glycosylation in cancer has emerged as a key mediator of immune evasion, with the overexpression of sialic acid-capped glycans being a common cancer associated alteration. These tumour-associated glycans can engage sialic-acid-binding Ig-like lectins (Siglecs) expressed on immune cells. Some Siglecs, which contain cytosolic inhibitory motifs, suppress anti-tumour immunity when recognizing sialoglycans, as observed with Siglec-10 on tumour-associated macrophages. In human breast cancer (HBC), multiple Siglecs have been implicated in these immunosuppressive mechanisms. Given the similarities between HBC and feline mammary carcinoma (FMC), the feline model is considered a powerful framework for the design of novel immunotherapeutic strategies. Therefore, we sought to develop a new immunotherapy for FMC with potential translation to HBC, based on targeted tumour cell desialylation, using TROP2 as the molecular target and a feline neuraminidase.

Aims

- **Design of a sialidase – single chain fragment variable (scFv) antibody conjugate specific to TROP2;**
- **Assess the desialylation activity of the conjugate in feline and human mammary carcinoma cell lines;**
- **Evaluate the binding ability of the conjugate in feline and human mammary carcinoma cell lines.**



Results

Activity assay - Flow Cytometry

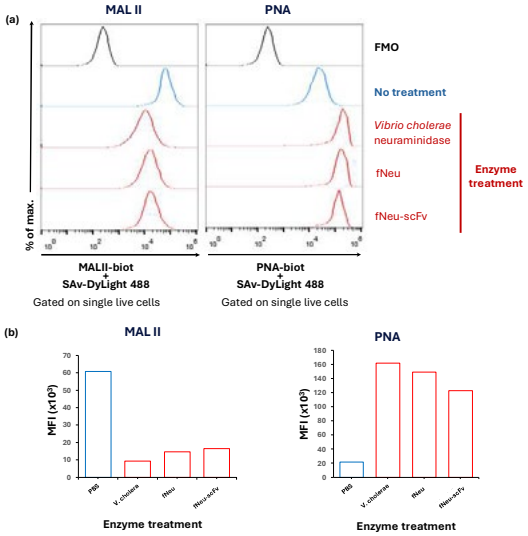


Figure 1. Flow cytometry assay to evaluate enzymes activity. (a) Fluorescence intensity of Streptavidin-DyLight 488 conjugated to biotinylated MAL II and PNA before and after treatment of **CAT-MT** cells with *Vibrio cholerae* neuraminidase, fNeu and fNeu-scFv; (b) Mean Fluorescence Intensity (MFI) after enzyme treatment of **CAT-MT** cells.

Activity assay - Confocal microscopy

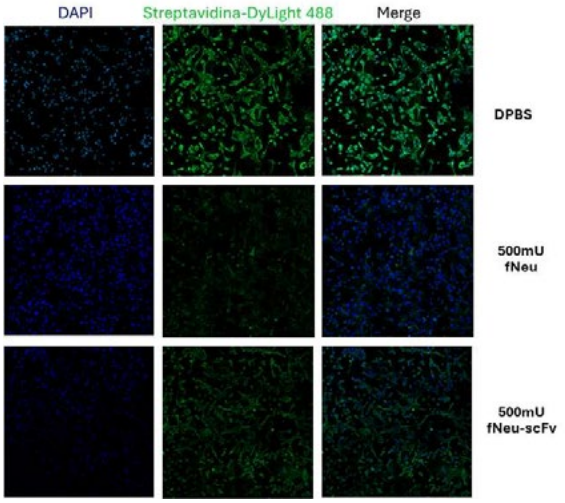


Figure 2. Visualization of fNeu and fNeu-scFv sialolytic activity on human breast cancer cell line. Confocal microscopy assay showing cell-surface sialic acid removal using 500mU fNeu and 500mU fNeu-scFv on **MDA-MB-231** cells. Cells were co-stained with Streptavidina-DyLight 488 and DAPI.

Binding assay - ELISA

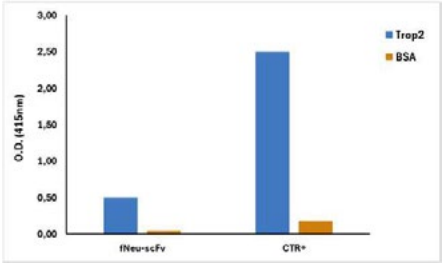
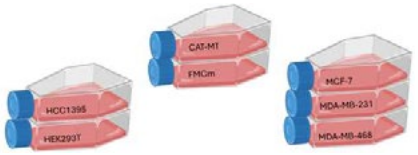
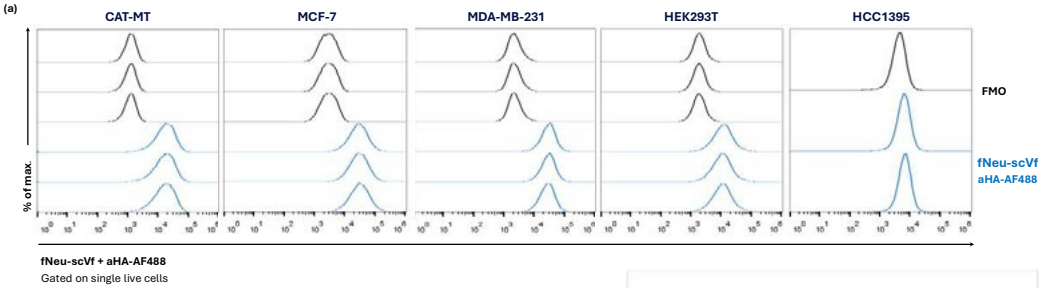


Figure 3. ELISA assay to assess the binding of fNeu-scFv conjugate to TROP2. A positive control (CTR+) containing anti-TROP2 polyclonal antibodies was included for comparative analysis with the conjugate.



Binding assay - Flow Cytometry



Binding assay - Western blot

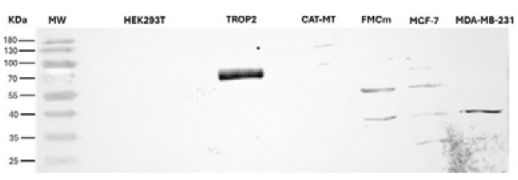


Figure 4. Western Blot analysis of fNeu-scFv binding to FMC cell lines and human cell lines extracts: CAT-MT, FMCm, MCF-7, MDA-MB-231 and HEK293T. A positive control, TROP2 protein with hFc (67KDa), was included for results validation.

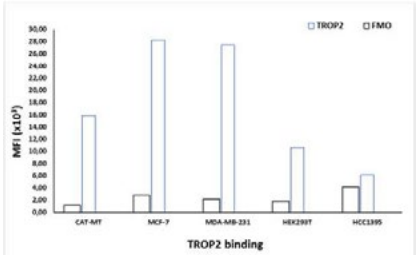


Figure 5. Evaluation of fNeu-scFv binding to FMC cell line and human cell lines. (a) Fluorescence intensity of Alexa Fluor 488 conjugated to HA tag antibody after incubation of **CAT-MT**, **MCF-7**, **MDA-MB-231**, **HEK293T** and **HCC1395** cells with fNeu-scFv; (b) MFI after incubation of **CAT-MT**, **MCF-7**, **MDA-MB-231**, **HEK293T** and **HCC1395** cells with fNeu-scFv.

Conclusions

- The fNeu-scFv conjugate retained desialylation activity comparable to that of unconjugated fNeu in both human and feline mammary carcinoma cell lines, suggesting that antibody conjugation did not compromise the enzyme's catalytic function.
- Binding experiments showed that fNeu-scFv conjugate binds to purified TROP2 protein and recognizes human and feline tumour cells.
- These findings point to the possibility of therapeutic applications across species, supporting the principles of the "One Health" concept.
- Further studies are required to determine whether the conjugate exhibits targeted tumour cell desialylation both in human and in feline tumour cells. Also, the conjugate's capacity to stimulate PBMC- and macrophage-mediated clearance of FMC cells *in vitro* will be explored, as well as its ability to reduce disease progression in mouse models of FMC.

Acknowledgments

This research was funded by the Portuguese Foundation for Science and Technology (FCT), under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS) and the PhD studentship UI/BD/153068/2022 (Alexandra Couto Oliveira). We acknowledge Agência Nacional de Inovação through grant LISBOA-01-0247-FEDER-047033 (Glycomed), the Gilead GÉNESE program through the project 17805 and the project grant 2022.07903.PTDC. The project that gave rise to these results received the support of a fellowship from "la Caixa" Foundation (ID 100010434), fellowship code: LCF/BQ/PR23/11980039.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.11

LT 3 Comparative and Translational Medicine and Biotechnology

ELECTRORETINOGRAPHY AS A MEASURE OF RETINAL TOLERANCE TO CONDITIONED MEDIA APPLICATION IN HEALTHY RATS

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INTRODUCTION: Glaucoma is one of the major causes of vision loss and irreversible blindness worldwide in both humans and dogs. This disease is caused by retinal ganglion cell death secondary to elevated intraocular pressure (IOP). Current treatments fail to prevent the disease progression, reinforcing the need for new therapeutic options. Cell-based therapies have shown retinal neuroprotective effects, and conditioned media (CM) from mesenchymal stem cells holds promise for ocular diseases; however, its safety profile remains poorly defined.

OBJECTIVES: This study aims to evaluate the retinal tolerance of CM from human umbilical cord mesenchymal stem cells when administered through intravitreal or subconjunctival routes in healthy rats. Retinal responses were monitored using a complete electroretinography (ERG) protocol before and after treatment, to determine the safest administration route for potential therapeutic use in experimental models of glaucoma.

METHODOLOGY: 24 Wistar Hannover albino rats were divided into 8 experimental groups with 3 rats each, based on CM' administration route (subconjunctival – SbC – or intravitreal – IVT –) and euthanasia time (5 or 28 days). Flash ERG was conducted on all animals twice: the first recording was performed before the CM administration, to ensure that only animals with healthy retinas were included in the study, and the second was performed prior to euthanasia. The ERG protocol followed established methodologies previously described in the literature, allowing the evaluation of rod and cone activity through measurements of a-wave and b-wave amplitudes (in µV) in response to light stimuli.

RESULTS: The results revealed that in general there were no statistically significant differences between the two routes of administration ($p > 0.05$). For the SbC route, most ERG parameters showed no statistical differences between groups, however, in three specific parameters: Photopic Luminescent Response (PLR) – wave a ($p = 0.0049$) and wave b ($p = 0.041$), and the Scotopic Luminescent Response – wave a ($p = 0.0238$), animals euthanized after 28 days displayed significantly higher amplitudes. For the IVT route, both the Photopic Flicker and the PLR showed significantly lower amplitudes at day 28 compared to day 5, in both waves a ($p < 0.001$) and b ($p = 0.035$).

CONCLUSIONS: This study provides evidence supporting that the subconjunctival route is the safest alternative option for CM administration in healthy retinas, comparing to the intravitreal route. The results demonstrate that while both routes can modulate retinal function, subconjunctival administration allows progressive recovery, whereas the intravitreal route exhibits a more prolonged impact. Notably, scotopic responses remained stable across timepoints, reinforcing the functional safety of both routes, with an advantage for the subconjunctival one.

ACKNOWLEDGMENTS/FUNDING: This work is supported by Fundação para a Ciência e Tecnologia (FCT) under Grant 2023.00544.BD, AL4AnimalS 2023 Internal Project (LT3.9) and the 2024 Research Grant from the European Society of Veterinary Ophthalmology (ESVO).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.12

LT 3 Comparative and Translational Medicine and Biotechnology

SHORT-TERM SAFETY OF TOPICAL AND ORAL CBD IN DOGS: A PRECLINICAL EVALUATION

Ana Filipa Bizarro[1,2,3]; Vanessa Schmidt[1,4]; Beatriz Fernandes[1,2,3]; Marta Pinto[1,2,3]; Hugo Pereira[1,2]; António Caldeira Mendes[1,2]; Joana Marto[3]; Ana Mafalda Lourenço[1,2].

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INTRODUCTION: Canine atopic dermatitis (CAD) affects approximately 20-30% of the canine population, significantly impairing their quality of life (QoL). Owing to the variability in treatment outcomes and a growing interest in alternative therapies, cannabidiol (CBD) has gained attention. Its claimed anti-inflammatory and antipruritic effects, as well as its involvement in skin homeostasis, suggest promise for dermatological use.

AIMS: This ethically approved study aimed to evaluate the safety of two novel CBD formulations – a topical ointment and an oral oil with defined concentrations – in healthy dogs.

METHODOLOGY: Twenty dogs were enrolled (n=10 per group), receiving one of the formulations for a duration of 14 days. The topical formulation was assessed based on localised cutaneous reactions such as erythema, alopecia, excoriations, papules, and scaling. The oral formulation was evaluated through clinical observation and haematological testing on days 0 and 14, analysed via repeated measures One-Way ANOVA.

RESULTS: Mild erythema (1/10) and papules on the ventral tail (1/10) were noted following ointment application. Administration of the oil resulted in transient vomiting (2/10), polyuria/polydipsia (1/10), mild somnolence (2/10), and enhanced mobility in two dogs (2/10). No dogs required discontinuation of the oral treatment. Biochemical analyses revealed a statistically significant rise in alkaline phosphatase (ALP) levels (from 50.3 to 302 U/L; p=0.02) in 9/10 dogs, although 4 of these remained within the reference range (10.6-100.7 U/L). Alanine aminotransferase (ALT) levels remained stable, and all dogs maintained good overall health.

CONCLUSIONS: Both CBD formulations were deemed safe for short-term use in healthy dogs. Nevertheless, liver enzyme monitoring is advisable during oral administration. Further clinical trials are underway to evaluate their efficacy in managing CAD.

ACKNOWLEDGMENTS/FUNDING: This study was funded by Foundation for Science and Technology, Portugal 2021.05986. BD PhD research grant to A. F. Bizarro; UID 04138 to Instituto de Investigação do Medicamento to iMed.ULisboa and CEECINST/00145/2018 to J. Marto; Training Research Grant 2022 by European Society of Veterinary Dermatology (ESVD); and UIDB/00276/2020 to Centre for Interdisciplinary Research in Animal Health. This work was also supported by Centre for Interdisciplinary Research in Animal Health and Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020).

Supported by LA/P/0059/2020

SHORT-TERM SAFETY OF TOPICAL AND ORAL CBD IN DOGS: A PRECLINICAL EVALUATION

ANA FILIPA BIZARRO[1,2,3], VANESSA MERTA SCHMIDT[1,4], BEATRIZ FERNANDES[1,2,3], MARTA PINTO[1,2,3], HUGO PEREIRA[1,2], ANTÓNIO CALDEIRA MENDES[1,2], JOANA MARTO[3], ANA MAFALDA LOURENÇO[1,2]

1 – CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal
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Introduction

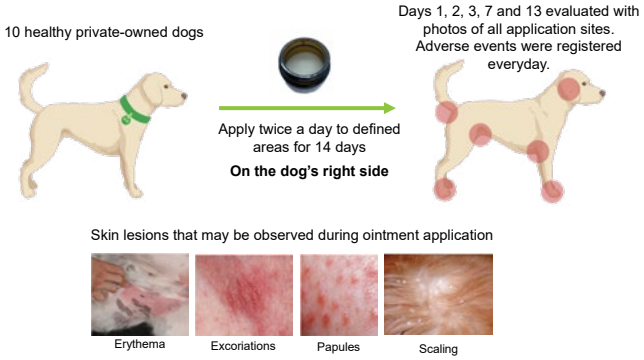
Canine atopic dermatitis (CAD) affects 20-30% of dogs, significantly reducing their quality of life (QoL). Due to variability in treatment responses and increasing demand for alternative therapies, interest in cannabidiol (CBD) has grown. **CBD's potential anti-inflammatory and antipruritic properties**, along with its role in skin homeostasis, suggests potential for dermatological applications.

Objectives

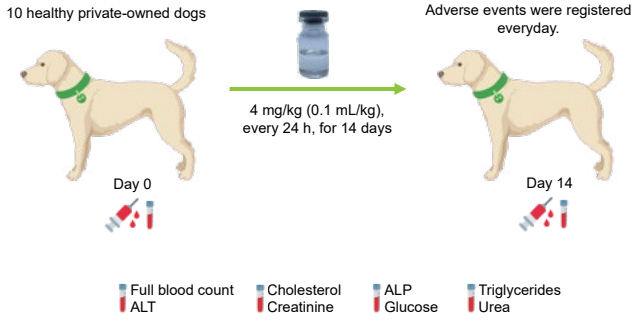
This ethically approved study evaluated the **safety of two newly developed CBD formulations** – an ointment for topical application and an oil for oral administration with known concentrations – in healthy dogs.

Methods

1 CBD ointment safety



2 CBD oil safety



Results

1 CBD ointment safety

Mild erythema on the right ear pinna (fig. 4) and right axilla (fig. 5) (1/10) were observed on Day 2. **Papules** on the ventral tail (1/10) were observed on Day 14 (fig. 6).

Figures 1 and 2 (left ear pinna and axilla) and Figure 3 (ventral tail on Day 9) are presented as controls for comparison, as no product was applied to the left side and no cutaneous lesions were observed at the ventral tail on Day 9.

The observed lesions resolved spontaneously without the need for any intervention.

No dogs required discontinuation of the topical treatment.

2 CBD oil safety

Observed adverse events

Transient vomiting (2/10)
Polyuria/polydipsia (1/10)
Mild somnolence (2/10)
Improved mobility (2/10)

No need to stop oil administration.

Platelets ↓
p = 0.019
Lower platelet counts were accompanied by reports indicating the presence of platelet aggregates.

Eosinophils ↑
p = 0.020
1 dog with eosinophilia on Day 0 and Day 14.
Remaining dogs with values within the reference range.

Alkaline phosphatase (ALP) ↑
p = 0.022
9/10 with increased levels of ALP.
4/9 remained within the reference range (10.6-100.7 UI/L).
Max. 302.00 UI/L

The most notable finding was an **increase in ALP levels** in most dogs (9/10). Among these, 4 remained within the reference range. This rise in ALP was not accompanied by an increase in alanine ALT, which remained stable throughout the study. Therefore, it is unlikely that the ALP elevation is indicative of hepatopathy. A plausible explanation is that the increase in ALP may be linked to the metabolism of CBD via the cytochrome P450 pathway, as observed with other drugs metabolised by the same enzyme system, such as phenobarbital. All dogs remained in good overall health during the study.

Conclusions

Both formulations were considered **safe for short-term use**, though liver enzyme monitoring is recommended with oral administration. Ongoing clinical trials will assess their efficacy in CAD treatment.

Acknowledgments

Centre for Interdisciplinary Research in Animal Health (CIISA) and Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS).
Foundation for Science and Technology (FCT): 2021.05986.BD PhD research grant to Ana Filipa Bizarro; UID 04138 - Instituto de Investigação do Medicamento to iMed.ULisboa and CEECINST/00145/2018 to J. Marto; UIDB/00276/2020 to CIISA; LA/P/0059/2020 to AL4AnimalS.
European Society of Veterinary Dermatology: Training Research Grant 2022.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT3.13

LT 3 Comparative and Translational Medicine and Biotechnology

OVARIAN METASTASES IN A QUEEN WITH SYNCHRONOUS MAMMARY AND ENDOMETRIAL ADENOCARCINOMA

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INTRODUCTION: Mammary gland tumours are among the most common neoplasms in cats, with 80–90% classified as malignant and exhibiting high metastatic potential. Metastases typically affect the lungs and pleura. The spread of this disease to the reproductive organs is rarely described in veterinary literature. In entire animals affected by mammary tumours, ovariohysterectomy is often performed concurrently with mastectomy or nodulectomy. However, the reproductive organs are not routinely submitted for histopathological examination unless they display gross changes. Feline endometrial adenocarcinoma (FEA) is a malignant epithelial neoplasm which, over the past ten years, has been recognised as more common in cats than previously reported, particularly in middle-aged to geriatric queens.

OBJECTIVES: This report aims to describe a case of ovarian metastasis in a female cat with synchronous mammary and endometrial adenocarcinoma.

METHODOLOGY: An entire adult European Shorthair cat underwent simultaneous nodulectomy, oophorectomy and hysterectomy due to the presence of mammary masses. Tissues from the mammary glands, ovaries and uterus were submitted for histopathological examination to LHAP-UTAD.

RESULTS: The mammary gland exhibited a broad and ulcerated invasive carcinoma with extensive necrosis, foci of calcification, moderate to marked cytological atypia, focal areas of squamous (epidermoid) differentiation, and regions showing intracystic papillary and tubulopapillary patterns. The tumour invaded the dermis and extended into the underlying musculature, provoking a marked desmoplastic stromal reaction. Numerous neoplastic cells were observed in perivascular locations, and a severe neutrophilic infiltrate was also present. The uterine horn showed areas of endometrial atrophy alongside extensive neoplastic proliferation occupying nearly the entire lumen, with a solid cribriform pattern and a pronounced desmoplastic stromal reaction. Invasion of the serosa and severe neoplastic embolism were also noted. The ovaries contained corpora lutea and follicles. One ovary exhibited marked ectasia of medullary lymphatic vessels. Two metastatic foci were observed adjacent to the ovary.

CONCLUSIONS: The morphology of the ovarian lesions raised questions regarding the origin of the metastases. Based on their histological characteristics, it was suspected that the metastases observed at the ovarian periphery originated from the mammary carcinoma; however, an endometrial origin could not be excluded. This case report highlights the potential for metastasis of feline mammary carcinoma to the reproductive tract, an uncommon occurrence that should be considered during diagnostic evaluation and treatment planning. The findings emphasise the importance of submitting all excised reproductive tissues for histopathological examination in cats diagnosed with mammary tumours. Further research is warranted to elucidate the mechanisms of tumour dissemination and its clinical significance.

fct Fundação para a Ciência e a Tecnologia

OVARIAN METASTASES IN A QUEEN WITH SYNCHRONOUS MAMMARY AND ENDOMETRIAL ADENOCARCINOMA

ANA JORDÃO[1,2,3]; FERNANDA SEIXAS[1,2]; ELISABETE FREIRE[1,2]; RITA PAYAN-CARREIRA[4]; HUGO VILHENA[1,2,3,5]; MARIA DOS ANJOS PIRES[1,2]

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INTRODUCTION

- ✓ Mammary gland tumours are common in cats and are often malignant with a high risk of metastasis, though involvement of reproductive organs is rarely reported
- ✓ Feline endometrial adenocarcinoma (FEA), a malignant tumour, has become more frequently recognised in the past decade, especially in older female cats

AIMS

- ✓ This report aims to describe a case of ovarian metastasis in a female cat with synchronous mammary and endometrial adenocarcinoma

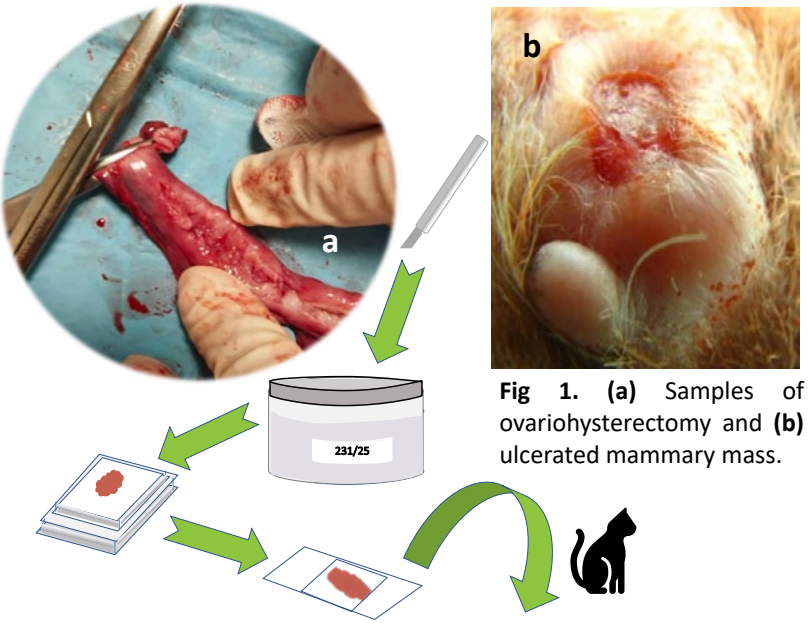

Fig 1. (a) Samples of ovariohysterectomy and **(b)** ulcerated mammary mass.

METHODOLOGY

- ✓ An entire adult cat underwent simultaneous nodulectomy, oophorectomy and hysterectomy due to the presence of mammary masses
- ✓ Tissues from the mammary glands, ovaries and uterus were submitted for histopathological examination to LHAP-UTAD

RESULTS

- ✓ The mammary gland exhibited a broad and ulcerated invasive carcinoma with extensive necrosis
- ✓ The uterine horn showed areas of endometrial atrophy alongside extensive neoplastic proliferation
- ✓ Two metastatic foci were observed adjacent to the ovary

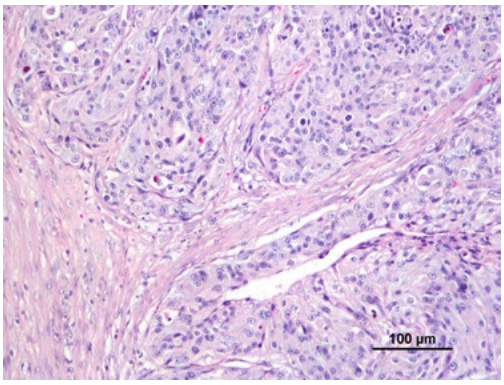

Fig 2. Uterus showing endometrial adenocarcinoma. Neoplastic cells presented increased nucleus/cytoplasm ratio, evident nucleoli, and eosinophilic cytoplasm. H&E.

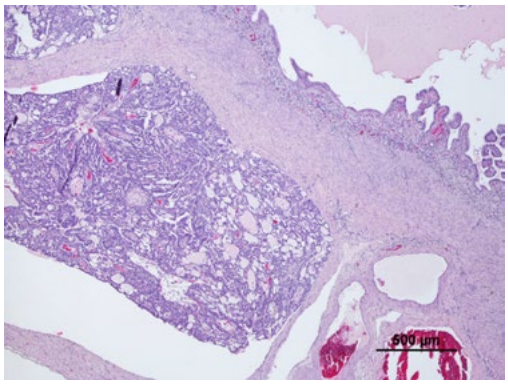

Fig 3. Uterus showing endometrial atrophy and a large metastasis, likely of mammary gland origin, within the lymphatic vessels of the myometrium. H&E.

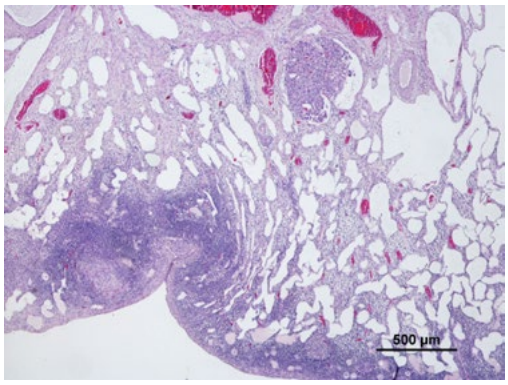

Fig 4. Ovary with metastatic neoplasm, most likely of mammary origin. This ovary also shows lymphatic vessel ectasia. H&E.

CONCLUSIONS

- ✓ The morphology of the ovarian lesions raised questions regarding the origin of the metastases; based on their histological characteristics, it was suspected that the metastases observed at the ovarian periphery originated from the mammary carcinoma; however, an endometrial origin could not be excluded
- ✓ This case report highlights the potential for **metastasis of feline mammary carcinoma to the reproductive tract**, an uncommon occurrence that should be considered during diagnostic evaluation and treatment planning
- ✓ The findings emphasise the importance of **submitting all excised reproductive tissues for histopathological examination in cats diagnosed with mammary tumours**

Acknowledgments/funding: This work was funded by Foundation for Science and Technology (FCT), under the projects UIDB/00772/2020 (CECAV, doi:10.54499/UIDB/00772/2020) LA/P/0059/2020 (AL4AnimalS).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.14

LT 3 Comparative and Translational Medicine and Biotechnology

ADVANCED BIOMATERIALS FOR BONE REGENERATION: COMBINING COMPOSITES AND BIOACTIVE HYDROGELS

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INTRODUCTION: Bone tissue regeneration continues to represent a major clinical challenge due to the inherent complexity of replicating the natural bone environment. The development of biomaterials capable of providing both mechanical support and biological cues is essential for the success of bone tissue engineering strategies. In this context, combining biodegradable polymers with bioactive hydrogels loaded with stem cells emerges as a promising approach to promote effective regeneration of critical-sized bone defects.

AIMS: The aim of this study was to develop a hybrid scaffold with high physical stability and bioactivity for bone tissue regeneration.

METHODOLOGY: Bioinks with hDPSCs, alginate, nHA, and collagen were rheologically characterized. Swelling assays evaluated water uptake. Hybrid PCL/nHA scaffolds were tested *in vitro* for cytocompatibility and osteogenic differentiation, and *in vivo* for biocompatibility and tissue integration in a rat model.

RESULTS: Crosslinked hydrogels showed solid-like behavior with stable rheological properties. Collagen and nHA enhanced swelling, promoting cell proliferation. LIVE/DEAD assays confirmed hDPSC viability. Hybrid scaffolds improved cell viability and osteogenic differentiation versus controls. *In vivo*, scaffolds showed minimal inflammation and effective tissue integration.

CONCLUSIONS: The hybrid scaffold developed, combining the mechanical robustness of PCL and nHA with the bioactivity of an alginate-nHA-Col hydrogel loaded with hDPSCs, demonstrated excellent cytocompatibility, promoted osteogenic differentiation, and achieved effective in vivo tissue integration. These findings suggest that this hybrid scaffold represents a promising approach for clinical applications in bone repair and regeneration, providing an advanced platform for treating critical-sized bone defects and non-union fractures.

ACKNOWLEDGMENTS/FUNDING: Ana Catarina Sousa (SFRH/BD/146689/2019), Bruna Lopes (2021.05265.BD), Patrícia Sousa (2023.00246.BD), Alícia Moreira (2023.00544.BD) and acknowledge Fundação para a Ciência e Tecnologia (FCT), for financial support. Rui Alvites acknowledges the CECA, UP, and FCT for the funding and accessibility of all technical, structural, and human resources necessary for the development of this work. The work was supported through the project UIDB/00211/2020 funded by FCT/MCTES, national funds.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.16

LT 3 Comparative and Translational Medicine and Biotechnology

ADIPOKINES AND MACROPHAGE INTERPLAY IN COW POSTPARTUM SUBCLINICAL ENDOMETRITIS

Catarina Anastácio[1,2], Gonçalo Pereira[1,2], Elisabete Silva[1,2], Joëlle Dupont[3], Patrice Humblot[4], Luís Lopes-da-Costa[1,2]

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INTRODUCTION: Adipokines are molecules linked to inflammation, regulating leucocyte chemotaxis and macrophage polarization. Macrophages are involved in inflammation resolution and display the surface APJ and CMKLR1 receptors for the anti-inflammatory Apelin and the pro-inflammatory Chemerin, respectively. In bovine endometrium, Chemerin content was related to persistence of subclinical endometritis (SCE) in a previous work. However, the role of Apelin in endometrial inflammation remains unknown.

AIMS: This study aimed to evaluate the endometrial presence of Apelin and its concentrations in plasma and uterine fluid in postpartum dairy cows, and relate them with uterine macrophage phenotype in the pathogenesis of SCE.

METHODOLOGY: Based on uterine cytology at 21 and 42 days postpartum (DPP), dairy cows (n=30) with the strict endometrial phenotypes of healthy (H, n=10), spontaneously recovered from SCE (SCE-R, n=10) and persistent SCE (SCE-P, n=10) were allocated to the study. A blood sample was collected at 10, 21 and 42 DPP and uterine fluid was recovered at 21 DPP for measurement of Adipokines (Chemerin and Apelin) concentrations by ELISA. The protein content of macrophage phenotype biomarkers was quantified by Western Blot in the pellet of uterine fluid sample. An endometrial biopsy was taken at 42 DPP for detection of Apelin by immunohistochemistry. Comparisons between groups were evaluated by one-way ANOVA and Spearman correlations assessed associations with macrophage phenotype and Adipokines concentrations.

RESULTS: Plasma concentrations of Apelin at 10 DPP were higher in SCE-R than in H cows ($P< 0.05$), and at 42 DPP, Apelin and Chemerin concentrations were higher in SCE-P than in H cows ($P< 0.01$). At 21 DPP, uterine fluid concentrations of Apelin were higher in SCE-R than in H cows ($P< 0.05$), whereas Chemerin concentrations were higher in SCE-P than in H cows ($P< 0.01$). Western Blot analysis showed that CD163 protein bands (marker of macrophage M2 phenotype) had higher intensity in SCE-R than in H and SCE-P cows ($P< 0.05$). M2 phenotype protein abundance was correlated with plasma Apelin at 10 DPP ($r= 0.453$; $P< 0.05$). In immunohistochemistry at 42 DPP, Apelin was mainly detected in glandular epithelial cells, and SCE-R cows showed stronger staining in both glandular and stromal cells than H cows.

CONCLUSIONS: Results evidenced that Apelin is produced in endometrium, and that plasma and uterine fluid concentrations in early postpartum are associated to spontaneous recovery of SCE and high abundance of macrophage M2 protein. High concentrations in later postpartum are associated to a persistent endometritis. Apelin and Chemerin emerge as potential early postpartum non-invasive diagnostic markers of the recovery/persistence of SCE.

AKNOWLEDGMENTS: Funded by Fundação para a Ciência e a Tecnologia (FCT), project PTDC/CVT-CVT/6932/2020, CIISA Project UIDB/00276/2020 and AL4AnimalS project LA/P/0059/2020. FCT funded CA scholarship (UI/BD/153069/2022).

ADIPOKINES AND MACROPHAGE INTERPLAY IN COW POSTPARTUM SUBCLINICAL ENDOMETRITIS

Anastácio C[1,2], Pereira G[1,2], Silva E[1,2], Dupont J[3], Humblot P[4], Lopes-da-Costa L[1,2]

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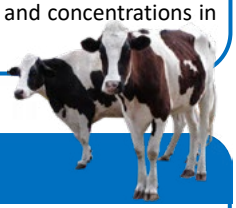
Supported by LA/P/0059/2020

INTRODUCTION

Adipokines are molecules linked to inflammation, regulating leucocyte chemotaxis and macrophage polarization. Macrophages are involved in inflammation resolution and display the surface APJ and CMKLR1 receptors for the anti-inflammatory Apelin and the pro-inflammatory Chemerin, respectively. In bovine endometrium, Chemerin content was related to persistence of subclinical endometritis (**SCE**) in a previous work. However, the role of Apelin in endometrial inflammation remains unknown. This study aimed to evaluate the endometrial presence of Apelin and concentrations in plasma and uterine fluid in postpartum dairy cows, and relate them with uterine macrophage phenotype in pathogenesis of SCE.



MATERIALS AND METHODS

Based on uterine cytology at 21 and 42 days postpartum (DPP), dairy cows (n=30) with the endometrial phenotypes of healthy (H, n=10), spontaneously recovered from SCE (SCER, n=10) and persistent SCE (SCEP, n=10) were allocated to the study (**Fig. 1**).

A blood sample was collected at 10, 21 and 42 DPP and uterine fluid was recovered at 21 DPP for measurement of Adipokines (Chemerin and Apelin) concentrations by ELISA. The protein content of macrophage phenotype biomarkers was quantified by Western Blot in the pellet of uterine fluid sample. An endometrial biopsy was taken at 42 DPP for detection of Apelin and macrophages by immunohistochemistry.

Comparisons between groups were evaluated by one-way ANOVA and Spearman correlations assessed associations.

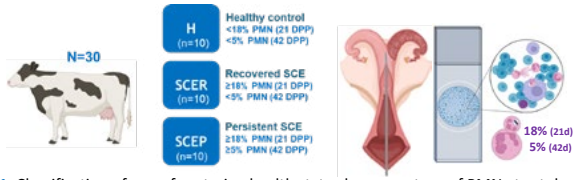


Fig. 1: Classification of cows for uterine health status by percentage of PMN at cytology

RESULTS

Plasma concentrations of Apelin at 10 DPP were higher in SCER than in H cows ($P< 0.05$). At 42 DPP, Apelin and Chemerin concentrations were higher in SCEP than in H cows ($P< 0.01$) (**Fig. 2**).

At 21 DPP, uterine fluid concentrations of Apelin were higher in SCER than in H cows ($P< 0.05$), whereas Chemerin concentrations were higher in SCEP comparing to H cows ($P< 0.01$) (**Fig. 3**).

Western Blot analysis at 21 DPP showed that CD163 protein bands (marker of macrophage M2 phenotype) had higher intensity in SCER than in H and SCEP cows ($P< 0.05$) (**Fig. 4A**) and was correlated with plasma Apelin at 10 DPP (r= 0.453; $P< 0.05$). In biopsies at 42 DPP, counts of M2 macrophages were higher in SCEP than in SCER cows ($P< 0.05$) (**Fig. 4B**), and were negatively correlated with uterine fluid Apelin at 21 DPP (r= – 0.529; $P<0.05$) (**Fig. 5**).

In immunohistochemistry at 42 DPP, Apelin was mainly detected in glandular epithelial cells (GL), and SCER cows showed stronger staining in both glandular and stromal cells (ST) than H cows. SCEP cows showed a strong staining in luminal epithelium cells (LE) (**Fig. 6**).

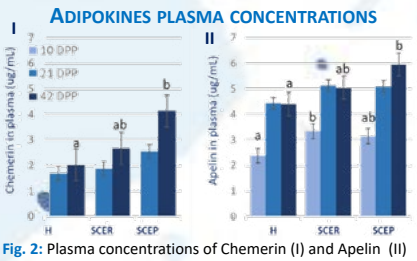


Fig. 2: Plasma concentrations of Chemerin (I) and Apelin (II)

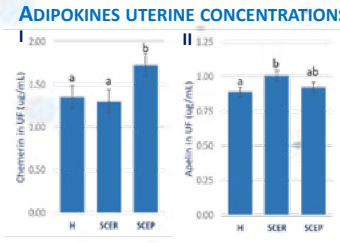


Fig. 3: Uterine Chemerin (I) and Apelin (II)

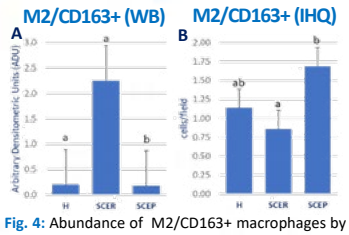


Fig. 4: Abundance of M2/CD163+ macrophages by Western blot (**A**) and Immunohistochemistry (**B**)

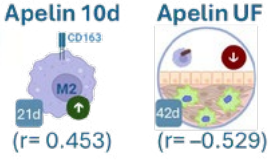


Fig. 5: Significant Spearman correlations between M2 and apelin concentrations

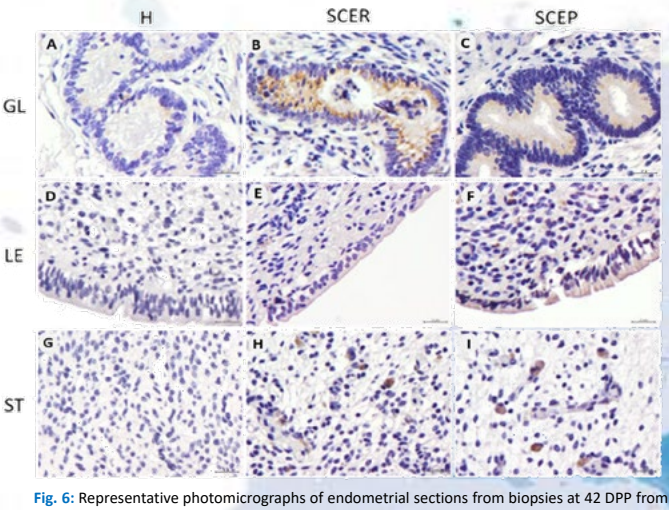


Fig. 6: Representative photomicrographs of endometrial sections from biopsies at 42 DPP from a subset of H, SCER and SCEP groups with immunostaining for apelin at 400x magnification

CONCLUSION

Results evidenced that Apelin is produced in endometrium, and that plasma and uterine fluid concentrations in early postpartum are associated to spontaneous recovery of SCE and high abundance of macrophage M2 protein. High concentrations in later postpartum are associated to a persistent endometritis. Apelin and Chemerin emerge as potential early postpartum non-invasive diagnostic markers of the recovery/persistence of SCE.

ACKNOWLEDGMENTS: Funded by FCT – PTDC/CVT-CVT/6932/2020; CIISA – UIDB/00276/2020; AL4AnimalS – LA/P/0059/2020. FCT funded CA scholarship (UI/BD/153069/2022).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.17

LT 3 Comparative and Translational Medicine and Biotechnology

BIODEGRADABLE POLYMERS FOR THE FABRICATION OF FELINE URETERAL STENTS: MECHANICAL AND TOPOGRAPHICAL CHARACTERIZATION IN AN FELINE ARTIFICIAL URINE MODEL

Daiana Cardoso[1,2,3,4]; Alexandre Barros[5]; André Meneses[1,2]; Margarida Pacheco[5]; João Requicha[3,4,6]

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[6] Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal.

INTRODUCTION: Ureteral obstructions are a significant clinical challenge in feline medicine, commonly resulting from urolithiasis, strictures, or neoplasia. Current treatment options—including non-biodegradable stents, subcutaneous ureteral bypass systems, and ureterotomy— present notable limitations. The development of biodegradable ureteral stents offers a promising alternative by potentially reducing the need for secondary interventions and minimizing long-term complications. Their success depends on selecting polymers that exhibit controlled degradation behavior and adequate mechanical stability.

OBJECTIVE: To evaluate the degradation behavior and surface morphological changes of three absorbable polymers—Poly(glycolide-co-trimethylene carbonate-co-epsilon-caprolactone) (PGTC), Poly-p-dioxanone (PDO), and Poly(glycolide-co-epsilon-caprolactone) (PGC)—under simulated feline urinary flow system, assessing their suitability for biodegradable ureteral stents.

METHODOLOGY: A dynamic in vitro model mimicking ureteral flow was developed to expose polymeric samples to artificial feline urine over 8 weeks. Mass loss, tensile strain, and qualitative macroscopic changes were evaluated at regular intervals. Surface morphology was assessed by scanning electron microscopy (SEM) at baseline, week 2, and day 42 at x100 and x300 magnifications.

RESULTS: PGC exhibited complete degradation by week 6, with SEM showing early fissuring and extensive delamination at that time. PDO maintained gross structural integrity throughout the study period, with no significant surface damage on SEM. PGTC showed a gradual degradation pattern, with SEM revealing micron-scale globules and the formation of surface cracks by week 6.

CONCLUSION: Each polymer displayed distinct degradation profiles and surface morphological features. PGC, with early fissuring and extensive delamination, appears suitable for short-term stenting due to its rapid resorption, while PDO offers prolonged structural integrity, supporting potential use in long-term applications. PGTC exhibited a balanced degradation rate with preserved surface morphology, making it a promising candidate for intermediate-term stenting.

ACKNOWLEDGMENTS: This research was supported by FCT through the projects UIDB/00772/2020 (CECAV, doi:10.54499/UIDB/00772/2020), LA/P/0059/2020 (AL4AnimalS), POCI-01-0145-FEDER-007440. FCT supported Daiana R. Cardoso through a PhD Scholarship (2024.06631.BDANA) and João F. Requicha by the Scientific Employment Stimulus - Institutional Call (CEECINS/00127/2018).

BIODEGRADABLE POLYMERS FOR THE FABRICATION OF FELINE URETERAL STENTS:

MECHANICAL AND TOPOGRAPHICAL CHARACTERIZATION IN AN FELINE ARTIFICIAL URINE MODEL

Daiana R. Cardoso[1,2,3,4], Alexandre Barros[5], André Meneses[1,2], Margarida Pacheco[5], João F. Requicha [3,4,6]

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INTRODUCTION

Ureteral obstructions are a significant challenge in feline medicine, commonly resulting from urolithiasis, strictures or neoplasia (Vrijsen *et al*., 2021). Current treatment options, including non-biodegradable ureteral stents, subcutaneous ureteral bypass systems, and ureterotomy, present notable limitations (Wormser *et al*., 2016). The development of biodegradable stents offers a promising alternative by potentially reducing the need for secondary interventions and minimizing long-term complications. Their success depends on selecting polymers that exhibit controlled degradation behavior and adequate mechanical stability.

MATERIAL AND METHODS

A dynamic *in vitro* model reproducing ureteral flow was developed to expose polymers samples to artificial feline urine over 8 weeks. The tested polymers included:

PGC, poly(glycolide-co-epsilon-caprolactone); **PDO,** poly-p-dioxanone; and **PGTC,** poly(glycolide-co-trimethylene carbonate -co- epsilon - caprolactone). Tensile properties was performed at weeks 0, 4, 6 and 7 and surface scanning electron microscopy (SEM) at day 1, week 2, and week 6 at x100 and x300 magnifications.

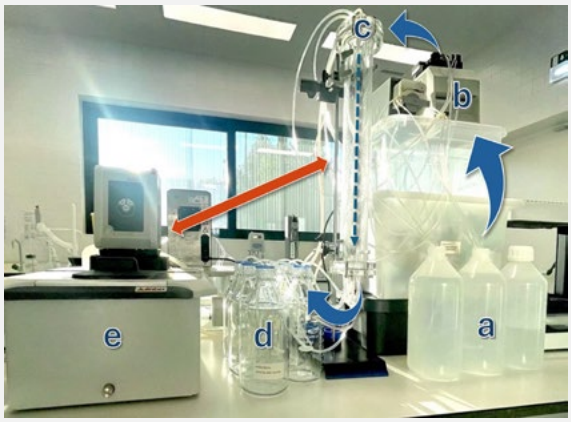

Figure 1. Dynamic experimental system setup. **a.** Artificial urine reservoir; **b.** Peristaltic pumps; **c.** vessel with the tubes containing each sample; **d.** Flasks collecting the artificial urine after passing through the samples; **e.** Thermostat to maintain 38ºC in cylindrical holder with the tubes containing the samples. The orange arrow represents the water circulation between the thermostat and the holder; the blue arrows indicate the flow of the artificial urine.

RESULTS

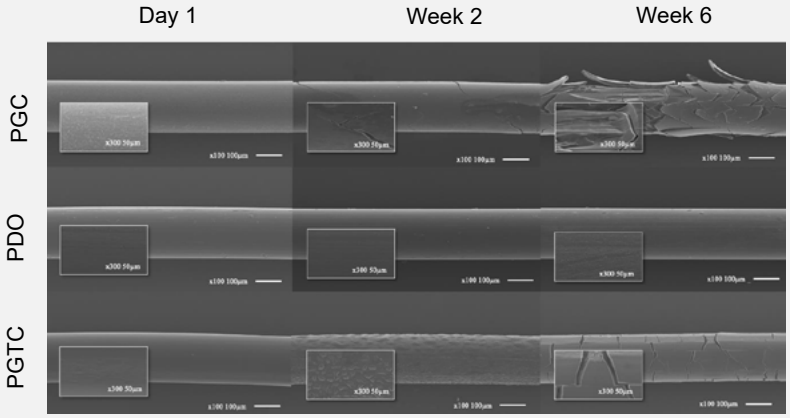

Figure 2. Images obtaining by scanning electron microscopy. **PGC** revealed early fissuring and extensive delamination by week 6; **PDO** maintained gross integrity throughout, with no significant surface damage; **PGTC** showed a gradual degradation pattern, with SEM revealing micron-scale globules and the formation of surface cracks by week 6.

Maximum Tensile Strain (%)			
Weeks	PGC	PDO	PGTC
0	36,00 ± 5,88	40,36 ± 4,66	25,54 ± 20,40
4	19,13 ± 5,41	4 ± 6,68	34,53 ± 4,03
6		5,2 ± 15,55	18,72 ± 6,10
7		4,7 ± 1,70	7,88 ± 1,80

Table 1. Results of mechanical tests. **PGC** exhibited a rapid decline in tensile strain , becoming untestable after 4 weeks due to its loss of structural integrity, making it impossible to handle; **PDO** started with the higher strain but remained more stable over time; **PGTC** began with a lower strain but declined consistently. PDO retained its load-bearing ability longer than the other materials.

CONCLUSION

PGC showed the fastest degradation and rapid decline in tensile strain, potentially offering short-term support but insufficient for the full ureteral healing period. **PDO** maintained structural integrity throughout, consistent with its slow resorption, making it suitable for long-term use such as malignant obstructions. However, its marked loss of strain led to brittleness, which may limit its performance in dynamic environments. **PGTC** presented an intermediate profile with progressive degradation and steady strain reduction, indicating predictable and resilient support, especially in cases involving ureteral trauma. SEM analysis revealed early fissuring and delamination in **PGC**, indicating aggressive hydrolysis. **PDO** surfaces remained mostly intact, reflecting stability and slow degradation. **PGTC** showed gradual changes—globule formation and cracking—without full delamination, suggesting a controlled, two-phase resorption pattern aligned with previous copolyester studies.

References | Vrijsen, E., Devriendt, N., Mortier, F., Stock, E., Van Goethem, B., & de Rooster, H. (2021). Complications and survival after subcutaneous ureteral bypass device placement in 24 cats: a retrospective study (2016–2019). Journal of Feline Medicine and Surgery, 23(8), 759–769. Wormser, C., Clarke, D. L., & Aronson, L. R. (2016). Outcomes of ureteral surgery and ureteral stenting in cats: 117 cases (2006–2014). Journal of the American Veterinary Medical Association, 248(5), 518–525.

Acknowledgments | This research was supported by FCT through the projects UIDB/00772/2020 (CECAV, doi: 10.54499/UIDB/00772/2020) and LA/P/0059/2020 (AL4AnimalS). FCT supported Daiana R. Cardoso through a PhD Scholarship (2024.06631.BDANA) and João F. Requicha by the Scientific Employment Stimulus - Institutional Call (CEECINS/00127/2018).

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.18

LT 3 Comparative and Translational Medicine and Biotechnology

CHITOSAN AND HYALURONIC ACID NANOPARTICULATE SYSTEM DEVELOPMENT FOR ENHANCED OCULAR DELIVERY OF DORZOLAMIDE

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[3] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal;

INTRODUCTION: Glaucoma is a chronic, non-curable neurodegenerative disease and the leading cause of irreversible blindness worldwide. It is characterized by progressive loss of retinal ganglion cells (RGCs), often associated with elevated intraocular pressure (IOP), the most significant modifiable risk factor. While the clinical management of glaucoma remains centered on IOP reduction, the incorporation of neuroprotective strategies, such as erythropoietin (EPO), has gained growing scientific interest. Previous studies demonstrated that chitosan/hyaluronic acid (CS/HA) nanoparticles (NPs) enabled the safe *in vivo* delivery of EPO to the posterior segment of the eye. Additionally, functionalization with the cell-penetrating peptide P28 significantly enhanced EPO's *ex vivo* permeation, with highest permeability observed through the conjunctiva. Based on these findings, we now developed CS/HA NPs encapsulating dorzolamide (DRZ) without P28, aiming to integrate IOP-lowering and neuroprotective strategies in a single topical platform.

AIMS: This study aimed to formulate and characterize DRZ-loaded CS/HA NPs and evaluate their *in vitro* and *ex vivo* ocular permeation as a basis for co-delivery with EPO in glaucoma therapy.

METHODOLOGY: DRZ-loaded NPs were prepared using ionic gelation with CS and HA. The resulting formulations were characterized in terms of particle size, zeta potential (ZP), polydispersity index (PDI), entrapment efficiency (EE%), and drug loading capacity. *In vitro* permeation was tested using polyethersulfone (PES) membranes to mimic ocular barriers and *ex vivo* permeation was evaluated using porcine conjunctiva, sclera, and cornea, obtained from freshly excised eyes. A validated high-performance liquid chromatography (HPLC) method was developed for quantitative drug analysis.

RESULTS: The DRZ-loaded CS/HA NPs exhibited a uniform size (277.4 ± 2.6 nm), negative surface charge (-11.3 ± 0.4 mV), and a low PI (0.123 ± 0.018). EE% (20–26% for inputs ≤1600 µg/mL) enabled high encapsulation (331.6 ± 31.4 µg/mL). *In vitro* release assays demonstrated slower diffusion compared to free drug, indicating a controlled release profile. *Ex vivo* studies revealed rapid onset of permeation (lag time <0.03 h) and high cumulative delivery through conjunctiva (118 ± 8 µg/cm²), cornea (67 ± 15 µg/cm²), and sclera (36 ± 16 µg/cm²) over 6 hours. The conjunctiva exhibited the highest flux (27.3 ± 1.9 µg/cm²/h) and Papp (95 ± 7 × 10^{-7} cm/s), followed by the sclera and cornea, confirming tissue-specific permeation consistent with their anatomical properties.

CONCLUSION: These results validate the CS/HA system's potential for sustained and efficient ocular delivery of hydrophilic small molecules such as DRZ and support its future application in a co-delivery system for glaucoma therapy integrating both IOP reduction and neuroprotection.

ACKNOWLEDGMENTS/FUNDING: The authors thank the Fundação para a Ciência e Tecnologia (FCT), Portugal for the financial support: projects UIDB/04138/2020 and UIDP/04138/2020 (iMed.Ulisboa), UIDB/00276/2020 (CIISA/FMV), LA/P/0059/2020- AL4AnimalS, L. Gonçalves Principal Researcher grant (CEECIND/03143/2017), Gonçalo Santos acknowledges FCT/MCTES for the PhD studentship 2023: UIDB/00276/2020.

CHITOSAN AND HYALURONIC ACID NANOPARTICULATE SYSTEM DEVELOPMENT FOR ENHANCED OCULAR DELIVERY OF DORZOLAMIDE

Gonçalo Santos [1,2,3]; Berta S. Braz [2,3]; Esmeralda Delgado [2,3]; Lídia Gonçalves [1]

1 - Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal;
2 - CIISA - Centro de Investigação Interdisciplinar em Saúde Animal, Faculty of Veterinary Medicine, Universidade de Lisboa;
3 - Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal;

One nanosystem. Dual action. An innovation in glaucoma therapy!

1 Introduction

Glaucoma is a chronic, non-curable neurodegenerative disease and **the leading cause of irreversible blindness worldwide.**

- **Intraocular pressure (IOP)** is the most significant modifiable risk factor - **Dorzolamide (DRZ).**
- **Neuroprotective strategies** have gained growing scientific interest - **Erythropoietin (EPO).**
- **Less than 5%** of the topically administered dose reaches the inner ocular tissues.

2 Aims

Develop and characterize **a novel CH/HA nanoparticulate system** for **DRZ delivery** and assess its **ocular permeation.**

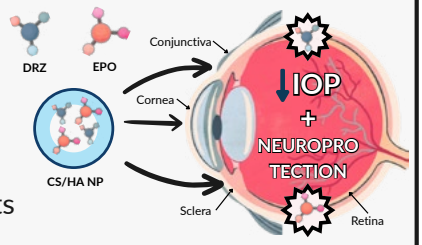

Figure 1. CS/HA Dual-Drug Nanoparticulate Delivery System for Glaucoma Therapy

3 Methodology

NPs preparation and charaterization

- Ionic gelation method: **Chitosan** (CH) and **Hyaluronic Acid** (HA)
- Particle size, zeta potential (ZP) and polydispersity index (PI)
- Entrapment efficiency (EE%) and drug loading capacity

Evaluation

- In vitro
- Ex vivo

Analytical Method

- **High-performance liquid chromatography (HPLC)** development and validation

4 Results

- Size: 277.4 ± 2.6 nm
- ZP: -11.3 ± 0.4 mV
- PI: 0.123 ± 0.018
- **EE%: 20–26%**

In Vitro Permeation

- Controlled release profile

Ex vivo Permeation

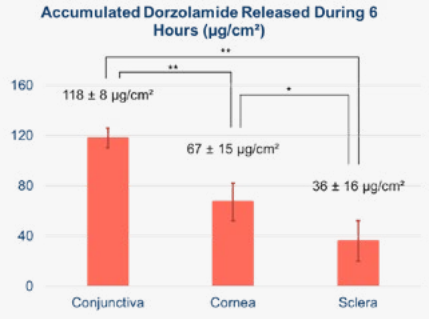

Figure 2. Ex vivo permeation of dorzolamide over 6 h across ocular tissues. Conjunctiva showed the highest accumulation (118 ± 8 µg/cm²), followed by cornea (67 ± 15) and sclera (36 ± 16) (n = 3). *p < 0.05, **p < 0.001.

Table 1. Ex vivo permeation parameters of dorzolamide through ocular membranes. (n = 3).

Membrane	Flux (µg/cm²/h)	Papp (×10⁻⁷ cm/s)	Lag time (h)
Conjuntiva	**27.3 ± 1.9 ****	**95 ± 7 ****	0,187 ± 0,37
Cornea	3.7 ± 0.4	13 ± 1	**0,025 ± 0,06 ***
Sclera	6.2 ± 3.2	22 ± 12	0,86 ± 0,36

**p < 0.001 for conjunctiva vs. all tissues; *p < 0.05 for cornea vs. sclera.

5 Conclusion

The CS/HA nanosystem **pioneers** sustained ocular delivery of **dorzolamide**, establishing a robust platform for **next-generation dual-action glaucoma therapies** that synergize **IOP reduction with neuroprotection.**

Acknowledgments/funding
The authors thank the Fundação para a Ciência e Tecnologia (FCT), Portugal for the financial support: UID 04138 - Instituto de Investigação do Medicamento, UIDB/00276/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.19

LT 3 Comparative and Translational Medicine and Biotechnology

GLOBAL PROFILING OF TESTICULAR AND EPIDYDIMAL SPERM PROTEOMES: PROTEIN MARKERS OF SPERM POST-TESTICULAR MATURATION

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[2] AL4AnimalS – Laboratório Associado para a Ciência Animal e Veterinária, Lisbon, Portugal
[3] Department of Medical Sciences, Institute of Biomedicine (iBiMED), University of Aveiro, Portugal

INTRODUCTION: Mammalian spermatogenesis involves a series of events during which germ cells differentiate into immature sperm cells. Following spermatogenesis in the seminiferous tubules, sperm undergo post-testicular maturation where they suffer extensive remodelling of their proteome culminating in the acquisition of forward motility and fertilizing ability. Despite the advances, the proteomic landscape of mammalian testicular and epididymal sperm cells remains largely uncharacterized.

OBJECTIVES: Shotgun proteomics was used to compare proteomes of bull testicular, caput and cauda epididymal spermatozoa. This is crucial for understanding the physiological mechanisms that lead to sperm competence.

METHODOLOGY: Reproductive tracts from 5 bulls (ages 15 to 20 months, crossbred) were obtained in a local abattoir. Testis and epididymis were isolated, and sperm cells were collected from the testes, caput and cauda epididymis by flushing with saline solution. After somatic cell lysis and protein extraction, sperm proteomes were analysed by LC-MS/MS. DiaNN algorithm validated peptide/protein identification (FDR<1%). Enrichment analysis was performed using DAVID with significance assessed by Benjamini-Hochberg method with a FDR q-value of 5%. Differentially expressed proteins (DEPs) were statistically tested using the package 'limma' considering a FDR<5% and $-2 \geq Log_2FC \geq 2$.

RESULTS: A total of 2,305 Testicular, 2,554 Caput and 2,038 Cauda Sperm proteins were quantified. Of these, 702, 483 and 314 proteins were identified as specific to Testicular, Caput and Cauda Sperm, respectively, and 1,106 proteins were common to all sperm populations. Functional analysis revealed enriched Biological Processes (adj p value<0.05%) in Testicular sperm related to RNA processing and splicing, essential for germ cell differentiation. DEPs analysis showed upregulated proteins in the Caput and Cauda sperm (vs Testicular), mainly related to flagellum function, motility and fertilization. Enrichment analysis of the three populations showed that sperm's energy metabolism changes along the maturation process, becoming increasingly dependent on oxidative phosphorylation and less dependent on glycolysis, with mature sperm equipped for both. The *in silico* analysis using GO annotations identified 36 proteins involved in acrosome reaction and capacitation processes, 93 proteins in sperm motility, 64 proteins in the fertilization process and 50 with roles in embryo development. Some of these proteins are conserved across species, and were identified in mouse, ram and pig sperm epididymal proteomes. This includes 13 proteins related to capacitation, 18 to motility, 19 to fertilization and 2 related to embryo development.

CONCLUSIONS: The sperm proteomes characterized in this study enhanced our understanding of sperm cell biology and highlighted the extensive proteomic changes occurring during post-testicular maturation involved in the acquisition of sperm competence.

FUNDING: Funded by FCT projects (UIDB/00276/2020-CIISA; LA/P/0059/2020-AL4AnimalS). FCT funded IL (UIDB/00276/2020) and ES (https://doi.org/10.54499/CEECINST/00140/2021/CP2807/CT0001).

GLOBAL PROFILING OF TESTICULAR AND EPIDYDIMAL SPERM PROTEOMES: PROTEIN MARKERS OF SPERM POST-TESTICULAR MATURATION

Inês Leites[1,2]; Patrícia Diniz[1,2]; Margarida Fardilha[3]; Joana Santiago[3]; Luís Lopes da Costa[1,2]; Elisabete Silva[1,2]

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[2] AL4AnimalS – Laboratório Associado para a Ciência Animal e Veterinária, Lisbon, Portugal

[3] Department of Medical Sciences, Institute of Biomedicine (iBiMED), University of Aveiro, Portugal

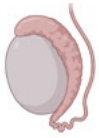
1 INTRODUCTION

Mammalian spermatogenesis involves a series of events during which germ cells differentiate into sperm cells. Following spermatogenesis in the seminiferous tubules, sperm undergo **post-testicular maturation** where they suffer extensive remodelling of their proteome culminating in the acquisition of **forward motility** and **fertilizing ability**. Despite the advances, the proteomic landscape of mammalian testicular and epididymal sperm cells remains largely uncharacterized.

Aims: Shotgun proteomics was used to compare proteomes of bull testicular, caput and cauda epididymal spermatozoa. This is crucial to identify the physiological mechanisms and proteins that regulate the acquisition of sperm competence.

2 METHODOLOGY

5 crossbred bulls (age 15 to 20 months)



Testis and epididymis (**caput** and **cauda**)

Spermatozoa recovered by flushing technique

Shotgun proteomics (LC-MS/MS)

Protein Extraction

Somatic Cell Lysis and Red Blood Cell lysis

DATA ANALYSIS: DiaNN algorithm validated peptide/protein identification (FDR<1%). Enrichment analysis was performed using DAVID (v2025_1) with significance assessed by Benjamini-Hochberg method with a FDR q-value of 5%. Differentially expressed proteins (DEPs) were statistically tested using the package 'limma' considering a FDR<5% and $-2 \geq \text{Log}_2\text{FC} \geq 2$.

3 RESULTS and DISCUSSION

A total of 2,305 Testicular (TestSperm), 2,554 Caput (CaputSperm) and 2,038 Cauda Sperm (CaudaSperm) proteins were quantified. Of these, 702, 483 and 314 proteins were identified as specific to Testicular, Caput and Cauda Sperm, respectively, and 1,106 proteins were common to all sperm populations (Figure 1).

Functional analysis showed that the most enriched process in **TestSperm** were related to RNA splicing. Post-transcriptional regulatory mechanisms, such as **mRNA splicing** and **alternative splicing**, are present in the male testis and play a major role in the regulation of spermatogenesis, particularly in the cell transition to meiosis (Figure 2).

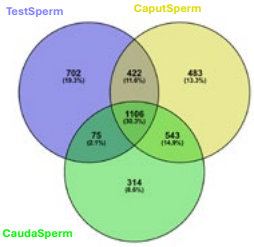
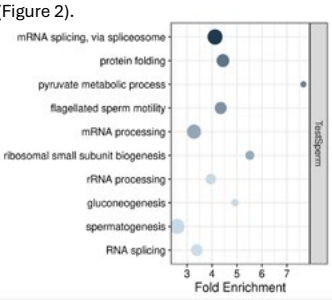
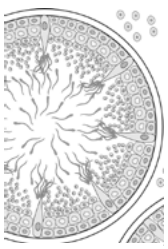


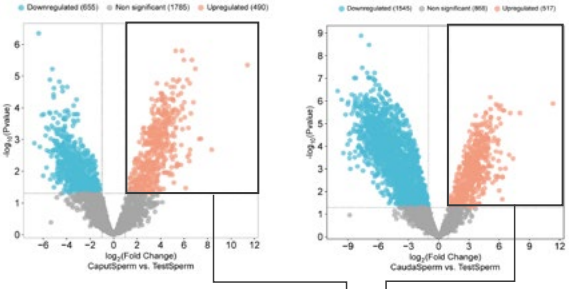
Figure 1 – Venn diagram depicting the shared and unique number of proteins within the three spermatozoa samples



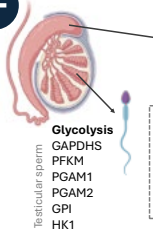


RBM5
SRSF2
BCAS2
SNRPA1

Figure 2 – Enrichment analysis of the TestSperm population. Graphic representation of the 10 most enriched Biological Processes GO terms (adjusted p value<0.05). Representation of proteins related to RNA splicing in bull germline cells.



Upregulated proteins in the CaputSperm and CaudaSperm (vs TestSperm) were mainly related to **flagellum function**, **motility** and **fertilization** (biding to zona pellucida), consistent with the progression of post-testicular maturation.



Sperm mitochondria seemed to become progressively activated along the maturation process becoming fully functional in mature sperm. This provides the **tyrosine phosphorylation** machinery required for **capacitation**-related events.

Enrichment analysis of the three populations showed that sperm's energy metabolism changes along the maturation process, becoming increasingly dependent on oxidative phosphorylation (OXPHOS) and less dependent on glycolysis, with mature sperm equipped for both.

An *in silico* analysis using GO annotations from ID Mapping (Uniprot) and QUICKGO (EMBL-EBI) for Biological Processes in Bos taurus allowed for the identification bull testicular and epididymal sperm proteins related to specific sperm functions.

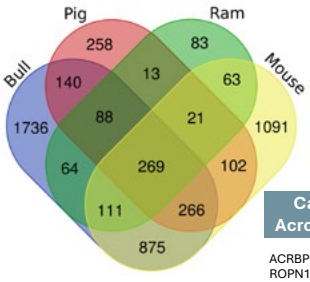
Motility
36 proteins

Capacitation
Acrosome reaction
93 proteins

Fertilization
64 proteins

Embryo development
50 proteins

Further analysis showed that **269** bull epididymal sperm proteins were also found in **mouse**, **pig** and **ram epididymal sperm proteomes**. Proteins represented in Figure 3 were identified as being conserved between these species and possibly playing roles in essential sperm functions.



Sperm motility

TEKT3, LRRC23, TEKT1, CCDC39, TPPP2, ROPN1, TEKT2, CCDC40, SPAG6, IQUB, ROPN1L, SORD, SPEF2, TEX101, ENKUR, EFHC1, LDHC, TSSK4

Fertilization

SPACA3, ACRBP, CCT4, CCT2, ACTL9 ACTL7C, SPESP1, MFGE8, VDAC2, ACTL7A, CCT5, LYZL4, ZPBP, TEX101, PARK7, SPA17, ZPBP2, TCP1, SPAM1, TSSK4

Capacitation + Acrosome reaction

ACRBP, ROPN1, SPACA1, ACTL9, ACTL7C, SPESP1, IQCF1, DLD, ACTL7A, ZPBP, ROPN1L, ACR, PRKACA, ZPBP2

Embryo development

SLC25A20, SOD1

Figure 3 – Venn diagram represents shared proteins between epididymal sperm proteomes of mouse, pig, ram and bull (our data). Graphical representation of conserved proteins that have evidence of participating in sperm functions acquired through the process of post-testicular maturation.

4 CONCLUSIONS

The sperm proteomes characterized in this study enhanced our understanding of sperm cell biology and highlighted the extensive proteomic changes occurring during post-testicular maturation involved in the acquisition of sperm competence.

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REFERENCES

Aitken RJ, Nixon B, Lin M, Koppers AJ, Lee YH, Baker MA. Proteomic changes in mammalian spermatozoa during epididymal maturation. Asian J Androl. 2007 Jul;9(4):554-64. doi: 10.1111/j.1745-7262.2007.00280.x. PMID: 17589795.

Yuan S, Zheng H, Zheng Z, Yan W (2013) Proteomic Analyses Reveal a Role of Cytoplasmic Droplets as an Energy Source during Epididymal Sperm Maturation. PLoS ONE 8(10): e77466. https://doi.org/10.1371/journal.pone.0077466

Amaral A. Energy metabolism in mammalian sperm motility. WIREs Mech Dis. 2022 Sep;14(5):e1569. doi: 10.1002/wsbm.1569. Epub 2022 Jun 9. PMID: 35680646.

Legrand JND, Hobbs RM. RNA processing in the male germline: Mechanisms and implications for fertility. Semin Cell Dev Biol. 2018 Jul;79:80-91. doi: 10.1016/j.semcdb.2017.10.006. Epub 2017 Oct 20. PMID: 29024760.

Elliott DJ, Grellscheid SN. Alternative RNA splicing regulation in the testis. Reproduction. 2006 Dec;132(6):811-9. doi: 10.1530/REP-06-0147. PMID: 17127741.

Abruzzese, G. A., Sanchez-Rodriguez, A., & Roldan, E. R. S. (2024). Sperm Metabolism. Molecular Reproduction and Development, 91(10), e23772. https://doi.org/10.1002/mrd.23772

Aitken, R. J., Nixon, B., Lin, M., Koppers, A. J., Lee, Y. H., & Baker, M. A. (2007). Proteomic changes in mammalian spermatozoa during epididymal maturation. Asian Journal of Andrology, 9(4), 554–564. https://doi.org/10.1111/j.1745-7262.2007.00280.x

Caballero, J., Frenette, G., & Sullivan, R. (2011). Post Testicular Sperm Maturational Changes in the Bull: Important Role of the Epididymosomes and Prostasomes. Veterinary Medicine International, 2011, 1–13. https://doi.org/10.4061/2011/757194

Castillo, J., De La Iglesia, A., Leiva, M., Jodar, M., & Oliva, R. (2023). Proteomics of human spermatozoa. Human Reproduction, 38(12), 2312–2320. https://doi.org/10.1093/humrep/dead170

Dacheux, J.-L., Belleannée, C., Guyonnet, B., Labas, V., Teixeira-Gomes, A.-P., Ecroyd, H., Druart, X., Gatti, J.-L., & Dacheux, F. (2012). The contribution of proteomics to understanding epididymal maturation of mammalian spermatozoa. Systems Biology in Reproductive Medicine, 58(4), 197–210. https://doi.org/10.3109/19396368.2012.663233

Karoii, D. H., Baghaei, H., Abroudi, A. S., Djamali, M., Mahforoozmahallehi, Z. H., Azizi, H., & Skutella, T. (n.d.). OPEN Alteration of the metabolite. Scientific Reports.

Licatalosi, D. D., & Darnell, R. B. (2010). RNA processing and its regulation: Global insights into biological networks. Nature Reviews Genetics, 11(1), 75–87. https://doi.org/10.1038/nrg2673

Park, Y.-J., & Pang, M.-G. (2021). Mitochondrial Functionality in Male Fertility: From Spermatogenesis to Fertilization. Antioxidants, 10(1), 98. https://doi.org/10.3390/antiox10010098

Plaza Davila, M., Martin Muñoz, P., Tapia, J. A., Ortega Ferrusola, C., Balao Da Silva C, C., & Peña, F. J. (2015). Inhibition of Mitochondrial Complex I Leads to Decreased Motility and Membrane Integrity Related to Increased Hydrogen Peroxide and Reduced ATP Production, while the Inhibition of Glycolysis Has Less Impact on Sperm Motility. PLOS ONE, 10(9), e0138777. https://doi.org/10.1371/journal.pone.0138777

Samanta, L., Swain, N., Ayaz, A., Venugopal, V., & Agarwal, A. (2016). Post-Translational Modifications in sperm Proteome: The Chemistry of Proteome diversifications in the Pathophysiology of male factor infertility. Biochimica et Biophysica Acta (BBA) - General Subjects, 1860(7), 1450–1465. https://doi.org/10.1016/j.bbagen.2016.04.001

Skerget, S., Rosenow, M. A., Petritis, K., & Karr, T. L. (2015). Sperm Proteome Maturation in the Mouse Epididymis. PLOS ONE, 10(11), e0140650. https://doi.org/10.1371/journal.pone.0140650

Skerrett-Byrne, D. A., Anderson, A. L., Bromfield, E. G., Beinsteln, I. R., Mulhall, J. E., Schjenken, J. E., Dun, M. D., Humphrey, S. J., & Nixon, B. (2022). Global profiling of the proteomic changes associated with the post-testicular maturation of mouse spermatozoa. Cell Reports, 41(7), 111655. https://doi.org/10.1016/j.celrep.2022.111655

Zhou, W., De Iuliis, G. N., Dun, M. D., & Nixon, B. (2018). Characteristics of the Epididymal Luminal Environment Responsible for Sperm Maturation and Storage. Frontiers in Endocrinology, 9, 59. https://doi.org/10.3389/fendo.2018.00059

Zigo, M., Kerns, K., & Sutovsky, P. (2023). The Ubiquitin-Proteasome System Participates in Sperm Surface Subproteome Remodeling during Boar Sperm Capacitation. Biomolecules, 13(6), 996. https://doi.org/10.3390/biom13060996

Zoca, S. M., Northrop-Albrecht, E. J., Walker, J. A., Cushman, R. A., & Perry, G. A. (2022). Proteomic analyses identify differences between bovine epididymal and ejaculated spermatozoa that contribute to longevity. Theriogenology, 184, 51–60. https://doi.org/10.1016/j.theriogenology.2022.02.021

B.T. Sherman, M. Hao, J. Qiu, X. Jiao, M.W. Baseler, H.C. Lane, T. Imamichi and W. Chang. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Research. 23 March 2022. doi:10.1093/nar/gkac194.[PubMed]

Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009;4(1):44-57. [PubMed]

Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G, Wu S, Wang Y. SRplot: A free online platform for data visualization and graphing. PLoS One. 2023 Nov 9;18(11):e0294236. doi: 10.1371/journal.pone.0294236. PMID: 37943830.

Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html

4 list venn diagram was performed using: https://bioinformatics.psb.ugent.be/webtools/Venn/

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT3.20

LT 3 Comparative and Translational Medicine and Biotechnology

CANNABINOID RECEPTORS CB1 AND CB2 IN CANINE AMELANOTIC ORAL MELANOMAS: A SPONTANEOUS MODEL TO EXPLORE TRANSLATIONAL TARGETS IN IMMUNO-ONCOLOGY

Karla Pinto[1,2,3], Inês Faria[1], Nuno Lima[2,3,4], Joaquim Henriques[5,6,7], Pedro Faísca[2,6,8] , João Requicha[1,2,3], Maria dos Anjos Pires[1,2,3]

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INTRODUCTION: Cannabinoid receptors 1 (CB1R) and 2 (CB2R) are integral components of the endocannabinoid system and play a crucial role in modulating immune response and tumor progression. In human oncology, CB2R has been implicated in influencing the tumor microenvironment and immune cell infiltration, suggesting its potential as a therapeutic target. Canine oral melanomas, particularly the amelanotic types, are highly aggressive and share biological similarities with human mucosal melanomas, making them valuable spontaneous models for translational research.

AIMS: To assess the immunohistochemical expression of CB1R and CB2R in canine amelanotic oral melanomas

METHODOLOGY: Twenty samples of canine oral amelanotic melanoma were analyzed. Indirect immunohistochemistry was performed using anti-CB1R (Origen®) and anti-CB2R (Abcam®) antibodies, diluted 1:100 and 1:200, respectively. Inflammatory cells and mucosa epithelium served as internal positive controls. Receptor expression in neoplastic cells was semi-quantitatively scored on a scale from 0 (negative) to 3 (strong). Three independent observers (KP, IF and MAP) performed the scoring in a blinded manner.

RESULTS: CB1R expression was absent or minimal (<10% of tumor cells) in 62% of cases. Conversely, CB2R exhibited moderate and diffuse expression (score 2) in all samples. All controls were positive.

CONCLUSIONS: The moderate expression of CB2R and minimal presence of CB1R in neoplastic cells, and strong positivity in inflammatory cells, suggest a significant role for CB2R in modulating the tumor immune microenvironment. These findings support the potential of CB2R as an immunotherapeutic target and reinforce the relevance of canine models in translational oncology research. To our knowledge, this is the first study to investigate the expression of cannabinoid receptors CB1R and CB2R specifically in canine amelanotic oral melanomas.

Further research is warranted to elucidate the functional role of CB2R in tumor-immune interactions, particularly its correlation with immune cell infiltration and clinically relevant outcomes such as tumor progression, therapeutic response, and overall survival.

ACKNOWLEDGEMENTS/FUNDING: This work was funded by Fundação para a Ciência e a Tecnologia, under the projects UIDB/00772/2020 (CECAV, doi:10.54499/UIDB/00772/2020), LA/P/0059/2020 (AL4AnimalS), POCI-01-0145-FEDER-007440, UIDB/04539/2020 and UIDP/04539/2020, and under the Scientific Employment Stimulus - Institutional Call - CEECINS/00127/2018 (J.F. Requicha).

CANNABINOID RECEPTORS CB1 AND CB2 IN CANINE AMELANOTIC ORAL MELANOMAS: A SPONTANEOUS MODEL TO EXPLORE TRANSLATIONAL TARGETS IN IMMUNO-ONCOLOGY

Karla Pinto[1,2,3], Inês Faria[1], Nuno Lima[2,3,4], Joaquim Henriques[5,6,7], Pedro Faísca[2,6,8], João Requicha[1,2,3] and Maria dos Anjos Pires[1,2,3]

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INTRODUCTION

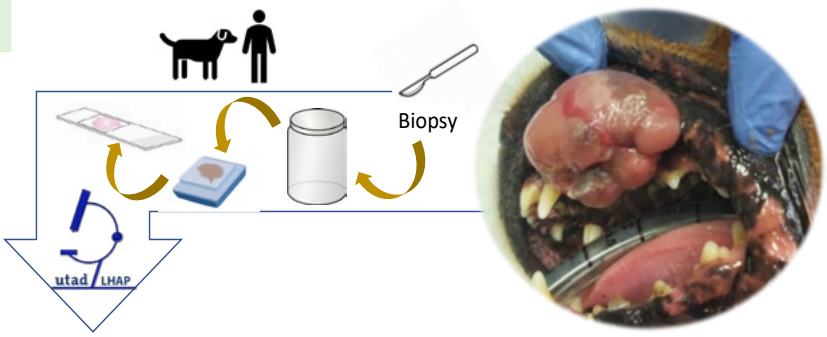
Cannabinoid receptors 1 (CB1R) and 2 (CB2R) are integral components of the endocannabinoid system[1] and play a crucial role in modulating immune response and tumor progression[2]. In human oncology, CB2R has been implicated in influencing the tumor microenvironment and immune cell infiltration, suggesting its potential as a therapeutic target[2,3]. Canine oral melanomas, particularly the amelanotic types, are highly aggressive and share biological similarities with human mucosal melanomas, making them valuable spontaneous models for translational research.

AIMS

To assess the immunohistochemical expression of **CB1R** and **CB2R** in canine amelanotic oral melanomas.

METHODOLOGY

Twenty samples of canine oral amelanotic melanoma were analyzed. Indirect immunohistochemistry was performed using anti-CB1R (Origen®) and anti-CB2R (Abcam®) antibodies, diluted 1:100 and 1:200, respectively. Inflammatory cells and mucosa epithelium served as internal positive controls. Receptor expression in neoplastic cells was semi-quantitatively scored on a scale from 0 (negative) to 3 (strong). Three independent observers (KP, IF and MAP) performed the scoring in a blinded manner.

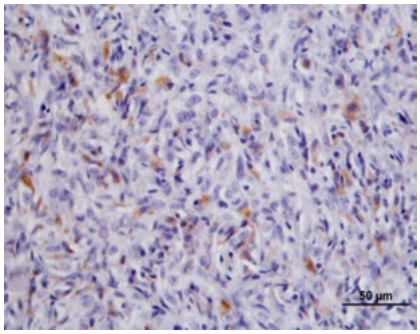


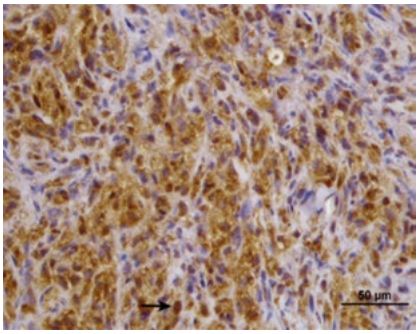
RESULTS

CB1R expression was absent or minimal (<10% of tumor cells) in 62% of cases. Conversely, **CB2R** exhibited moderate and diffuse expression (score 2) in all analyzed samples. All controls were positive.

Cannabinoid receptor 1

CB1R marked less that 10% of tumor cells, that present brown positivity of the cytoplasm. Gill's hematoxylin counterstained.





Cannabinoid receptor 2

CB2R with diffuse expression (score 2) of the neoplastic cells, which present cytoplasmic, and sometimes nuclear (arrow) positivity. The stroma cells are negative. Gill's hematoxylin counterstained.

CONCLUSIONS

The moderate expression of CB2R and minimal presence of CB1R in neoplastic cells, and strong positivity in inflammatory cells, suggest a significant role for CB2R in modulating the tumor immune microenvironment. These findings support the **potential of CB2R as an immunotherapeutic target** and reinforce the relevance of canine models in translational oncology research. To our knowledge, this is the first study to investigate the expression of cannabinoid receptors CB1R and CB2R specifically in canine amelanotic oral melanomas.

Further research is warranted to elucidate the functional role of CB2R in tumor-immune interactions, particularly its correlation with immune cell infiltration and clinically relevant outcomes such as tumor progression, therapeutic response, and overall survival.

REFERENCES

1 - Silver, R. J. (2019). The endocannabinoid system of animals. Animals, 9(9), 686. **2 -** Blázquez, C., Carracedo, A., Barrado, L., Real, P. J., Fernández-Luna, J. L., Velasco, G., Malumbres, M., & Guzmán, M. (2006). Cannabinoid receptors as novel targets for the treatment of melanoma. FASEB Journal, 20(14), 2633–2635. **3 -** Zhao, Z., Yang, J., Zhao, H., Fang, X., & Li, H. (2012). Cannabinoid receptor 2 is upregulated in melanoma. Journal of Cancer Research and Therapeutics, 8(4), 549.

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PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.21

LT 3 Comparative and Translational Medicine and Biotechnology

IS EXON SKIPPING THE KEY TO CORRECT N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE DEFICIENCY? AN ANTISENSE OLIGONUCLEOTIDE THERAPEUTIC APPROACH

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[§] These authors contributed equally to this work.

INTRODUCTION: Mucolipidosis II (ML II) is a Lysosomal Storage Disorder caused by N-acetylglucosamine-1-phosphotransferase (GlcNAc-PT) deficiency, which impairs the trafficking of lysosomal hydrolases. Of all ML II mutations, c.3503_3504delTC in *GNPTAB* exon 19 is the most frequent, making it a good target for a personalized therapy. Here, we explored an innovative therapeutic strategy based on the use of antisense oligonucleotides (ASOs). Previously, in ML II patients' fibroblasts, we tested ASOs to induce exon 19 skipping in pre-mRNA, successfully generating an in-frame mRNA.

AIMS: Now, our aim is to determine whether this in-frame transcript leads to increased GlcNAc-PT levels improving ML II cellular phenotype.

METHODOLOGY: First, the GlcNAc-PT activity was measured in fibroblasts by a radioactive assay, but activity levels were similar in ML II and control fibroblasts (treated and non-treated) showing that the assay is not proper to measure endogenous levels. To overcome this, we designed 3 constructs: a WT (full *GNPTAB* cDNA), a del_ex19 (without the exon 19) and a mutant (with the mutation c.3503_3504delTC) that were transfected in HEK293T cells. Then GlcNAc-PT expression was analyzed by Western Blot (WB).

Additionally, we have measured the activity of several lysosomal hydrolases and evaluated the expression of α-galactosidase A (α-Gal) by WB after ASO treatment of control and patient cells.

To further help in the validation of this therapy we are also generating a novel GlcNAc-PT antibody in rabbits.

RESULTS: Our results demonstrated that HEK293T cells were able to express all the constructs. The WB of both WT and del_ex19 constructs showed bands corresponding to the α/β precursor. However, only the WT construct expressed the β subunit, suggesting that there is no GlcNAc-PT activity in the absence of exon 19. As expected, the c.3503_3504delTC construct showed no expression, with no detectable α/β precursor band.

We also observed a slight increase in the activity of various lysosomal hydrolases in ML II fibroblasts treated with the ASO, particularly 24h and 48h post-treatment. However, only the values relatively to the α-Gal were statistically significant, but the WB analysis using an antibody against this enzyme did not detect any band in ASO-treated ML II fibroblasts.We also developed a novel antibody for GlcNAc-PT. Preliminary results revealed a band corresponding to the β-subunit in both control and ML II patient fibroblasts (unexpected), but in overexpression assays both WT and del_ex19 constructs presentedα/β precursor bands. So, further assays are needed to assess their specificity

CONCLUSION: Our ASO-based approach effectively promotes the skipping of exon 19. However, this strategy, as far as we have been able to prove, is not able to restore any GlcNAc-PT enzymatic activity. Further validation, including Co-localization studies are planned to clarify these findings.

fct Fundação para a Ciência e a Tecnologia

Is exon skipping the key to correct N-acetylglucosamine-1-phosphotransferase deficiency ? An antisense oligonucleotide therapeutic approach

Mariana Gonçalves[1,2,3,4*]; Luciana Moreira [1,2,3]; Marisa Encarnação[1,2,3]; Paulo Gaspar[5]; Juliana Inês Santos[1,2,3,6]; Maria Francisca Coutinho[1,2,3]; Maria João Prata[6,7]; Maryam Omidi[8]; Sandra Pohl[8]; Frederico Silva[3,9]; Paula Oliveira[4]; Liliana Matos[1,2,3§] ; Sandra Alves[1,2,3§]

[1] Research and Development Unit, Department of Human Genetics, National Health Institute Dr. Ricardo Jorge, Porto, Portugal;[2] Center for the Study of Animal Science, University of Porto, Portugal; [3] Associate Laboratory for Animal and Veterinary Sciences, Faculty of Veterinary Medicine, University of Lisbon, Portugal; [4] Center for the Research and Technology of Agro-environmental and Biological Sciences/ University of Trás-os-Montes and Alto Douro, Vila Real, Portugal;[5] Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, National Health Institute Dr. Ricardo Jorge, Porto, Portugal;[6] Biology Department, Faculty of Sciences, University of Porto, Portugal; [7] i3S – Health Research and Innovation Institute, University of Porto, Portugal;[8] Department of Osteology and Biomechanics, University Medical Center Hamburg – Eppendorf, Germany;[9] Microbiology and Immunology Lab, Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal.§ These authors contributed equally to this work.

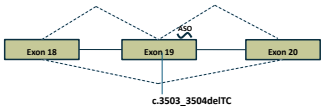
Background

The N-acetylglucosamine-1-phosphotransferase (GlcNAc-PT) is an α2β2γ2 hexameric complex.

The absence of an active GlcNAc-PT, impairs the proper targeting of almost all lysosomal enzymes to the lysosome, leading to a very severe disease - Mucolipidosis type II (ML II) [1].

The ML II most frequent mutation worldwide is c.3503_3504delTC, a dinucleotide deletion in exon 19.

Our group used antisense oligonucleotides (ASOs) to induce exon 19 skipping thus circumventing the effect of the c.3503_3504delTC mutation, having obtained the *in vitro* proof-of-concept on its efficacy at mRNA level[2].



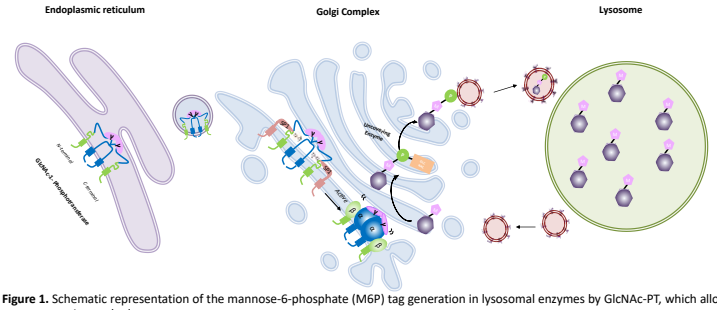


Figure 1. Schematic representation of the mannose-6-phosphate (M6P) tag generation in lysosomal enzymes by GlcNAc-PT, which allows their proper targeting to the lysosome.

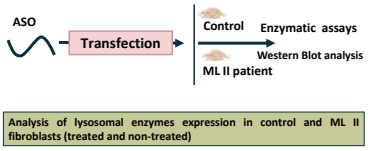
Aims

To further validate our ASO approach at protein level, the major aims of this work were:

1. To measure and compare the activity and expression levels of several lysosomal hydrolases in ASO treated and untreated ML II fibroblasts
2. To design WT & mutant *GNPTAB* constructs and transfect them in HEK293T cells to analyse the GlcNAc-PT activity under overexpression
3. To develop a novel antibody for GlcNAc-PT β subunit once presently there is no good antibody available for this enzyme

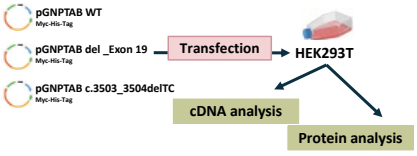
Methodology

Lysosomal Enzymes Expression Studies

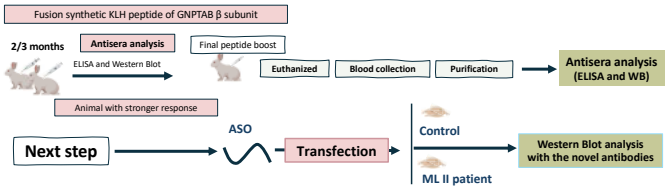


Analysis of lysosomal enzymes expression in control and ML II fibroblasts (treated and non-treated)

Analysis of the GlcNAc-PT Activity Under Overexpression

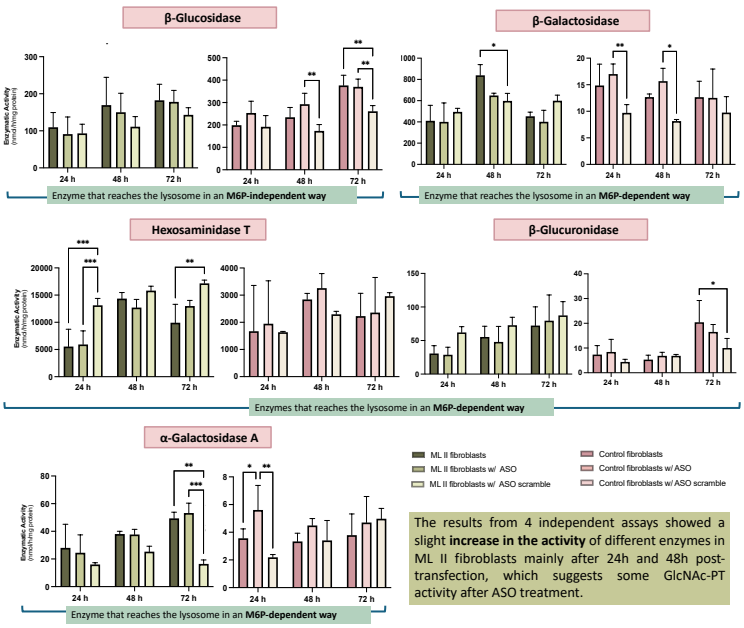


GlcNAc-1-phosphotransferase antibody production and analysis



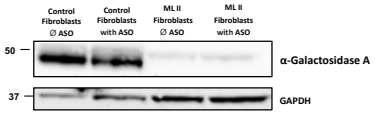
Results and Discussion

Lysosomal Enzymes Activity Studies



The results from 4 independent assays showed a slight **increase in the activity** of different enzymes in ML II fibroblasts mainly after 24h and 48h post-transfection, which suggests some GlcNAc-PT activity after ASO treatment.

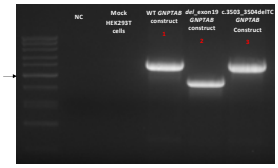
Western Blot analysis after transfection of WT and ML II fibroblasts with the ASO



The WB analysis with alpha-galactosidase (α-Gal) antibody was performed in control and ML II fibroblasts after transfection with the candidate ASO. Although only the values relatively to the α-Gal were statistically significant in enzyme assays, after WB analysis using an antibody against this enzyme, we did not observe any recovery of ML II fibroblasts treated with our ASO.

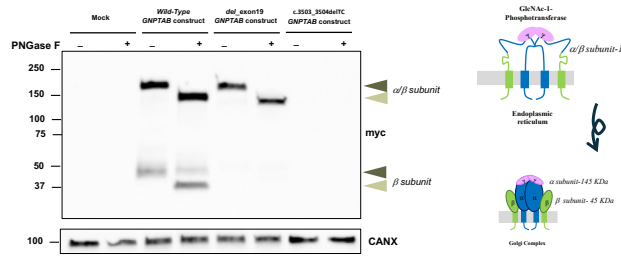
Analysis of the GlcNAc-PT Activity Under Overexpression

RT-PCR after transfection of WT, del_exon19 and c.3503_3504delTC constructs in HEK293T (24h)



The expected patterns were observed after transfection in HEK293T cells: (1) a full-length transcript with all *GNPTAB* exons in WT, (2) a transcript with the *GNPTAB* exon 19 deletion and (3) a transcript with the deletion of a dinucleotide TC in exon 19 (aiming to represent the most frequent mutation in ML II)

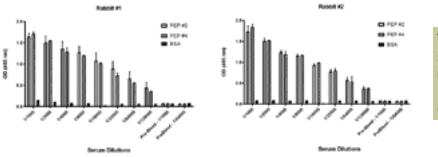
Western Blot analysis after WT or del_exon19 *GNPTAB* constructs overexpression (24h)



Extracts of HEK293T cells overexpressing *GNPTAB* WT full-length myc-6His, *GNPTAB* del_exon19 myc-6His and *GNPTAB* c.3503_3504delTC myc-6His constructs were incubated with (+) or without (-) PNGase F, and the WB analysis was performed with a myc-tag antibody. **PNGase F cleaves all N-linked oligosaccharides that are added to glycoproteins** (as the GlcNAc-PT) during their trafficking from the endoplasmic reticulum to the Golgi complex. So, the **observed shift in the weight of the α/β precursor bands (lane 4 & 6)** upon treatment with PNGase F, shows that the non-treated samples are N-glycosylated being **the myc-6His-tagged GlcNAc-PT full-length protein.**
Regarding the cleaved β-subunit expression, only the WT construct expressed the β subunit, suggesting that there is no GlcNAc-PT activity in the absence of exon 19. As expected, the c.3503_3504delTC construct showed no expression, with no detectable α/β precursor band (lane 7 & lane 8).

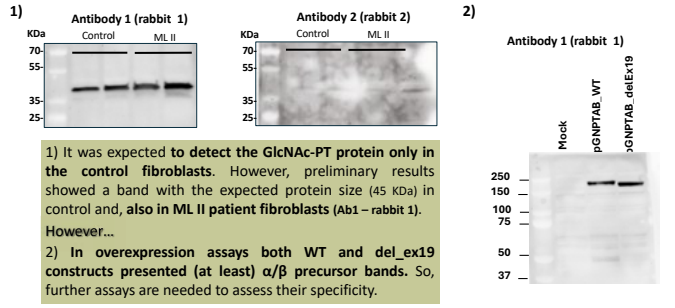
GlcNac-1-phosphotransferase Antibody Production And Analysis

Antibodies production



The **ELISA titration** of the **rabbits serum** showed that they produced a **strong immune response** after **GlcNAc-PT β-subunit** peptides injection – two different antibodies were obtained.

Western Blot analysis with both GNPTAB β subunit antibodies



1) It was expected **to detect the GlcNAc-PT protein only in the control fibroblasts**. However, preliminary results showed a band with the expected protein size (45 KDa) in control and, **also in ML II patient fibroblasts (Ab1 – rabbit 1).**

However…

2) **In overexpression assays both WT and del_ex19 constructs presented (at least) α/β precursor bands.** So, further assays are needed to assess their specificity.

Conclusion:

In summary, we developed molecular and biochemical approaches to indirectly evaluate at protein level the feasibility of our ASO exon-skipping approach to treat ML II patients with exon 19 mutations.
However, this strategy, as far as we have been able to prove, it is not able to restore any GlcNAc-PT enzymatic activity. Further validation, including Co-localization studies are planned to clarify these findings.

References:

[1] Coutinho MF, Prata MJ, Alves S. Mol Genet Metab. 2012;105(4): 542-550.

[2] Matos L, Vilela R, Rocha M, et al. Hum Gene Ther, 2020, 31(13-14):775-783

Funding:

AL4A-PROJ-LT3.5

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.22

LT 3 Comparative and Translational Medicine and Biotechnology

IMMUNE LANDSCAPES OF THE CANINE ORAL MUCOSA: OPTIMISING TARGETED ORO-MUCOSAL IMMUNOTHERAPY

Marta Pinto[1,2,3]; Solange Gil[1,2]; Margarida Venâncio[4]; Ana Bizarro Camões[1,2,3]; Beatriz Fernandes[1,2,3]; Hugo Pereira[1,2]; Joana Marto[3]; Ana Mafalda Lourenço[1,2]

[1] Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
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INTRODUCTION: Oro-mucosal immunotherapy (OMIT) represents a non-invasive, user-friendly, home-administered method for inducing allergen-specific immune tolerance, leveraging the unique immunological properties of the oral mucosa. Although OMIT holds promise, its clinical effectiveness remains uncertain. In humans, the mucosal site where allergens are applied may influence treatment outcomes, with Langerhans cells (LCs) being key for immune modulation and contributing to efficacy, while mast cells (MCs) are associated with local adverse reactions. However, the regional distribution of these cell types within the canine oral cavity has yet to be characterised, leaving unresolved the question of the most suitable site for OMIT administration in dogs.

AIMS: This prospective *ex vivo* study investigated LCs and MCs densities and distribution across distinct regions of the canine oral mucosa, with the goal of identifying the region with highest LCs and lowest MCs density, thereby informing optimal OMIT application.

METHODOLOGY: Oro-mucosal 8-mm punch biopsies were collected post-mortem from the buccal, vestibular, sublingual, palatine, and gingival mucosa of six dogs. Samples underwent CD1a immunohistochemistry for LCs detection and 0.5% toluidine blue staining for MCs identification. Cellular densities were analysed by two independent investigators using microscopy at 400x for LCs and 200x for MCs. Only positively stained cells exhibiting proper morphology were recorded and expressed as cells/mm^2.

RESULTS: The buccal mucosa exhibited the highest LCs density (8.2 ± 4.06 cells/mm2), significantly surpassing the vestibular (3.7 ± 2.65 cells/mm2; p=0.043), sublingual (3.6 ± 1.30 cells/mm2; p=0.039), and palatine (0.6 ± 0.93 cells/mm2; p=0.00028) mucosa. Conversely, MCs density did not differ significantly among mucosal sites (p=0.379), although the sublingual mucosa showed the highest MCs density (44.8 ± 18.34 cells/mm2) and the gingival the lowest (31.1 ± 8.57 cells/mm2).

CONCLUSIONS: The buccal mucosa emerged as the most favourable site for OMIT application, distinguished by high LCs density, highlighting a previously unexplored advantage. This study provides novel insights into the immune landscapes of the canine oral mucosa and highlights a promising factor for optimising OMIT, potentially guiding future targeted application strategies.

ACKNOWLEDGMENTS/FUNDING: This study was funded by Foundation for Science and Technology (FCT), Portugal, within the framework of EXPL/CVT-CVT/1458/2021 exploratory research project, UI/BD/153072/2022 PhD research grant to M. Pinto, CEECINST/00145/2018 to J. Marto, UID04138 to Research Institute for Medicines, and UIDB/00276/2020 to Centre for Interdisciplinary Research in Animal Health (CIISA). This work was also supported by Centre for Interdisciplinary Research in Animal Health (CIISA) and Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020).

Supported by LA/P/0059/2020

IMMUNE LANDSCAPES OF THE CANINE ORAL MUCOSA: OPTIMISING TARGETED ORO-MUCOSAL IMMUNOTHERAPY

MARTA PINTO[1,2,3], SOLANGE GIL[1,2], MARGARIDA VENÂNCIO[4], ANA BIZARRO CAMÕES[1,2,3], BEATRIZ FERNANDES[1,2,3], HUGO PEREIRA[1,2], JOANA MARTO[3], ANA MAFALDA LOURENÇO[1,2]

1 Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal
2 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
3 Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal
4 Faculty of Veterinary Medicine, Universidade de Lisboa, Portugal

mpinto@fmv.ulisboa.pt

Background

Oro-mucosal immunotherapy (OMIT) is a non-invasive, user-friendly, home-administered method for inducing allergen-specific immune tolerance, leveraging the unique immunological properties of the oral mucosa. Although OMIT holds promise, its clinical effectiveness in dogs is still uncertain.

In humans, the allergen application site within the oral mucosa may influence treatment outcomes, with Langerhans cells being key for immune modulation and contributing to efficacy, while mast cells are associated with local adverse reactions.

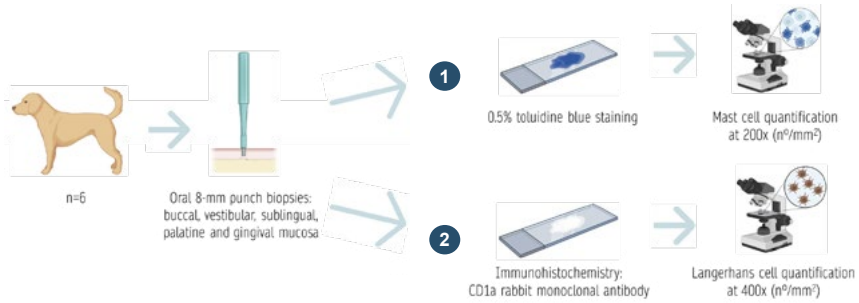
The distribution of Langerhans and mast cells in the canine oral mucosa remains unexplored, and the ideal site for OMIT application in dogs has yet to be identified.

Objectives

To investigate Langerhans and mast cell densities and distribution across distinct regions of the canine oral mucosa, aiming to identify the site with the highest Langerhans cell and lowest mast cell densities, thereby informing optimal OMIT application.

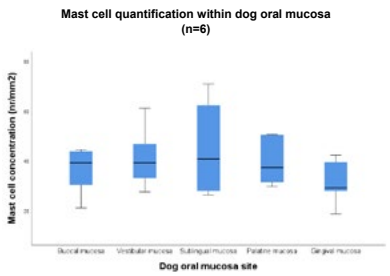
Methodology

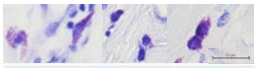
- Oro-mucosal punch biopsies (8 mm) collection post-mortem from six dogs at buccal, vestibular, sublingual, palatine, and gingival sites.
- Mast cell identification by 0.5% toluidine blue staining and microscopy at 200x magnification.
- Langerhans cell detection by CD1a immunohistochemistry and microscopy at 400x magnification.
- Cell density evaluation by two independent investigators; only positively stained cells with proper morphology were recorded as cells/mm2.



Results

1 Mast cell distribution and quantification in canine oral mucosa:

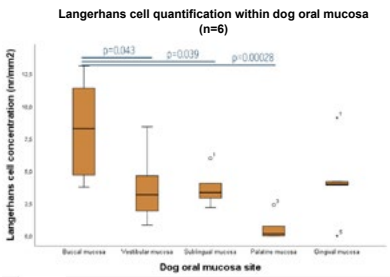




	Mean (nr/mm²)	Standard deviation	p-value
Buccal	36.3	9.02	0.379
Vestibular	41.2	12.00	
Sublingual	44.8	18.34	
Palatine	39.5	9.77	
Gingival	31.1	8.57	

Statistical analysis: oneway ANOVA (IBM SPSS Statistics®, 29.0 (241), Armonk, New York, USA).

2 Langerhans cell distribution and quantification in canine oral mucosa:



	Mean (nr/mm²)	Standard deviation	p-value
Buccal	8.2	4.06	0.00096
Vestibular	3.7	2.65	
Sublingual	3.6	1.30	
Palatine	0.6	0.93	
Gingival	4.2	2.89	

Statistical analysis: oneway ANOVA, tukey post hoc tests (IBM SPSS Statistics®, 29.0 (241), Armonk, New York, USA).

Conclusion

The buccal mucosa emerged as the most favourable site for OMIT application, distinguished by high Langerhans cell density, highlighting a previously unexplored advantage.

This study provides novel insights into the immune landscapes of the canine oral mucosa and highlights a promising factor for optimising OMIT, potentially guiding future targeted application strategies.

Acknowledgments

- Centre for Interdisciplinary Research in Animal Health (CIISA) and Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS).
- Foundation for Science and Technology (FCT) [EXPL/CVT-CVT/1458/2021 exploratory research project; UI/BD/153072/2022 PhD research grant to Marta Pinto; UIDB/00276/2020 to CIISA; LA/P/0059/2020 to AL4AnimalS].

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD
LT3.23

LT 3 Comparative and Translational Medicine and Biotechnology

CANINE MELANOMA AND FOCAL ADHESION KINASE-TARGETED STRATEGIES IN VETERINARY ONCOLOGY

Marta A. L. Silva[1], Bárbara Carreira[2], Rita Acúrcio[2], Marta B. Afonso[2], Nuno Leal[1], Helena Florindo[2], Lisa Mestrinho[1,3]

[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon. Lisbon, Portugal.
[2] Research Institute for Medicines (iMed. ULisboa), Faculty of Pharmacy, University of Lisbon. Lisbon, Portugal.
[3] Laboratório Associado para a Ciência Animal e Veterinária (AL4AnimalS).

INTRODUCTION: Canine melanoma (CM) exhibits aggressive biological behavior and affected dogs have a post-surgical survival time of less than one year. Given the conserved molecular pathways between canine and human melanoma, insights gained from this study may support the development of therapies with focal adhesion kinase inhibitors (FAKi) for human melanoma patients. Focal adhesion kinase (FAK) is a protein critical for several cellular processes, including cell survival, invasion, and migration. FAK is associated with advanced stages of cancer development, the presence of metastatic cells, and their increased mobility. Currently, there are no studies in veterinary research that investigated FAK expression in CM, neither the benefits of FAKi for these patients.

AIMS: This project aims to assess FAK expression in CM and test the potential of FAKi using *in vitro* and *ex vivo* models of CM.

METHODOLOGY: FAK expression in CM was evaluated using molecular and fluorescent methods. The impact of three different FAKi on CM was assessed through cell metabolic activity and migration assays using the TLM-1 cell line.

RESULTS: It was found that FAK expression is higher in CM compared to healthy tissues. Mirroring findings in human melanoma, it reinforces FAK's role as a potential therapeutic target. The tested FAKi were able to reduce TLM-1 cell function and viability and appeared to delay cell migration capacity. The observed impact of FAKi on cell viability and migration suggests a conserved mechanism that may be exploited in future translational studies.

CONCLUSIONS: Further studies involving canine DC and primary T-cell cultures, alongside co-cultures with CM spheroids 3D model, will contribute to examining the benefits of this dual approach for treatment, enhancing its relevance and facilitating comparisons with human cases in support of a One Health strategy for broader benefit.

ACKNOWLEDGMENTS: This research work was funded by nacional funds through *Fundação para a Ciência e Tecnologia* (FCT), I.P., (UIDB/00276/2020) and *Centro de Investigação Interdisciplinar em Sanidade Animal* (CIISA); and by international funds through Foundation for Veterinary Dentistry (Awarded Grants & Scholarships 2022) and La Caixa Foundation (LCF/PR/HR24/52440018).

Canine Melanoma and Focal Adhesion Kinase-targeted Strategies in Veterinary Oncology

Marta A. L. Silva,[1] Bárbara Carreira,[2] Rita Acúrcio,[2] Marta B. Afonso,[2] Nuno Leal,[1] Helena Florindo,[2] Lisa Mestrinho[1,3]

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Introduction

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Aims

This project aims to assess FAK expression in CM and test the potential of FAKi using in vitro and ex vivo models of CM.

Methodology

FAK expression in CM was evaluated using molecular and fluorescent methods. The impact of three different FAKi on CM was assessed through cell metabolic activity and migration assays using the TLM-1 cell line.

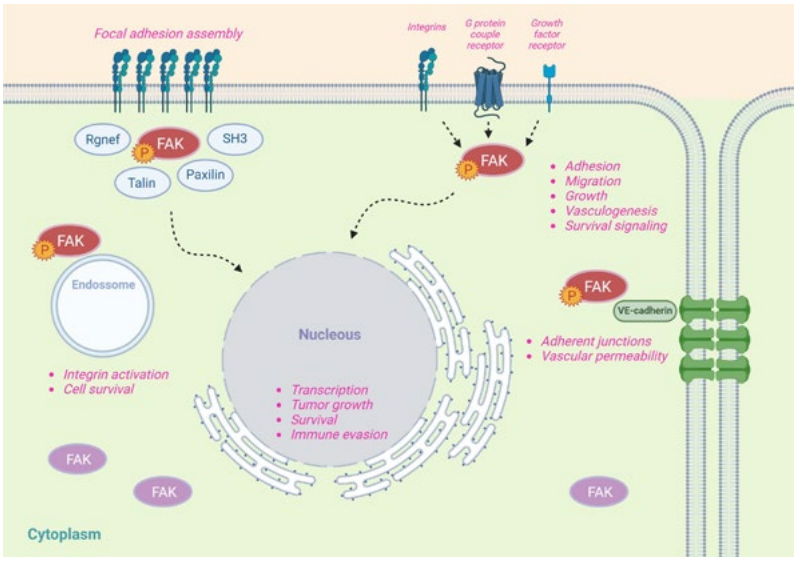
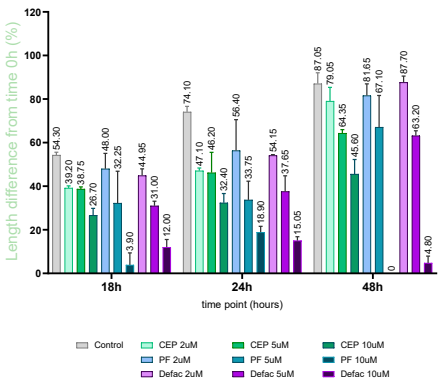


Fig. 1. Focal adhesion kinase pathways and functions.

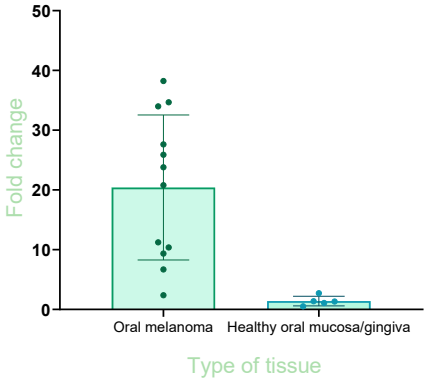
Results

It was found that FAK expression is higher in CM compared to healthy tissues. Mirroring findings in human melanoma, it reinforces FAK's role as a potential therapeutic target. The tested FAKi were able to reduce TLM-1 cell function and viability and appeared to delay cell migration capacity. The observed impact of FAKi on cell viability and migration suggests a conserved mechanism that may be exploited in future translational studies.

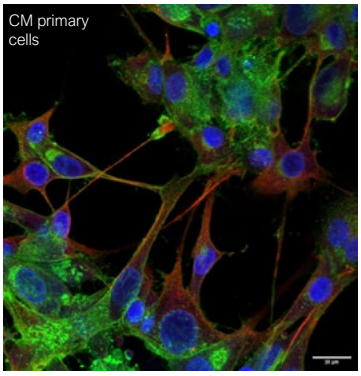
Migration assays



FAK expression by PCR



Confocal microscopy



Cell membrane Nucleus Phospho-FAK

Conclusions

Further studies involving canine DC and primary T-cell cultures, alongside co-cultures with CM spheroids 3D model, will contribute to examining the benefits of this dual approach for treatment, enhancing its relevance and facilitating comparisons with human cases in support of a One Health strategy for broader benefit.

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Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

LT 3 Comparative and Translational Medicine and Biotechnology

PhD LT3.24

3D BIOPRINTING OF A SECRETOME-INFUSED CONTACT LENS FOR CORNEAL WOUND HEALING

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[2] Faculdade de Ciências da Universidade do Porto (FCUP), Porto, Portugal
[3] Escola Superior de Saúde do Politécnico do Porto (E2S), Porto, Portugal
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[5] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Faculdade de Medicina Veterinária (FMV), Universidade de Lisboa (UL), Lisboa, Portugal
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* Martim Silva, Maria Pereira and Ana Catarina Sousa contributed equally and should all be considered as first authors.

INTRODUCTION: Nowadays, people live much longer, and as a result, age-related degenerative diseases, such as corneal disorders, are more common. These conditions often lead to complications such as corneal opacity, neovascularization, and significant vision loss. Current treatment therapeutic approaches — such as topical eye drops and corneal transplantation — are limited by low regenerative capacity, poor bioavailability, scarcity of donor tissues, and the risk of immune rejection.

AIMS: This study aims to develop a bioactive, bioprinted contact lens composed of polyvinyl alcohol (PVA), hyaluronic acid (HA), and conditioned medium derived from umbilical cord mesenchymal stem/stromal cells (UC-MSCs), which contains the secretome. The purpose is to provide an effective alternative to conventional therapies by supporting corneal healing and regeneration.

METHODOLOGY: The proposed contact lens is designed to deliver bioactive molecules in a sustained manner via the secretome, while HA incorporation aims to replicate the hydration and mechanical properties of the native cornea, ensuring comfort and biological functionality. Using the BioX bioprinter, a transparent and flexible lens will be fabricated by printing a customized bioink and stabilizing it through a hybrid crosslinking strategy that combine ionic crosslinking and freeze-thaw cycling. Comprehensive characterization of the final hydrogel material will be performed through physicochemical tests. These analyses include Fourier Transform Infrared Spectroscopy (FTIR), for chemical characterization, Scanning Electron Microscopy (SEM) for morphological assessment, and swelling and degradation assays to evaluate hydrogel behavior under physiological conditions. Furthermore, cytocompatibility and regenerative potential will be evaluated via *in vitro* assays, including Presto Blue™, Live/Dead viability assay, and scratch assay for cell migration.

CONCLUSIONS: This study proposes a novel, non-invasive therapeutic approach to enhance corneal wound healing, reduce fibrosis, and improve visual outcomes. Future work will include *in vivo* validation in rat model to confirm the efficacy and biocompatibility of the bioactive lens system.

ACKNOWLEDGMENTS/FUNDING: Martim Silva and Maria Pereira acknowledge the Faculdade de Ciências da Universidade do Porto (FCUP) and the Escola Superior de Saúde do Politécnico do Porto (E2S), respectively, for providing the knowledge, technical support, infrastructure, and human resources essential for the development of this work. Rui Alvites acknowledges the CECA, UP, and FCT for the funding and accessibility of all technical, structural, and human resources necessary for the development of this work. The work was supported through the project UIDB/00211/2020 funded by FCT/MCTES, national funds.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT 3 Comparative and Translational Medicine and Biotechnology

LT3.25

COMPARISON OF NGF AND RECEPTORS GENE TRANSCRIPTION IN EQUINE ENDOMETRIUM

Nélio Galrito-Cebola[1,2,3,4]; Marta Cerveira-Pinto [1,2]; Anna Wójtowicz [5]; Graça Ferreira-Dias [1,2]; Andreia J. Amaral[1,2,6]; Ana Amaral [1,2,7]; Anna Szóstek-Mioduchowska [5]

[1] Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal
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[4] Institute for Research and Advanced Training, University of Évora, Évora, Portugal
[5] Institute of Animal Reproduction and Food Research, Polish Academy of Science, Olsztyn, Poland
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INTRODUCTION: Nerve Growth Factor (NGF) is a neurotrophin with both neuronal and non-neuronal functions. It is considered a pleiotropic molecule, as it regulates multiple physiological processes, including the estrous cycle, embryo differentiation, nerve growth, extracellular matrix remodeling, wound healing, and tissue regeneration, among others. Its actions are mediated by two cell surface receptors, expressed on epithelial, endothelial, stromal, and immune cells: the tyrosine kinase receptor (TRKA) and the p75 neurotrophin receptor (p75NTR). NGF binds with high affinity to TRKA, which mediates its main trophic and proliferative effects. In contrast, the p75NTR has low affinity for NGF but plays a modulatory role, influencing cellular outcomes such as survival, apoptosis, and axonal growth. However, the mRNA expression patterns of *NGF* and its receptors in equine endometrium during the follicular phase (FP) and mid-luteal phase (MLP) have not yet been described.

AIMS: The aim was to characterize *NGF, TRKA* and *p75NTR* mRNA expression in equine endometrium of category I of histopathological classification (healthy endometrium) in FP and MLP of the estrous cycle.

METHODOLOGY: Endometria from 20 mares (n=10 from FP and n=10 from MLP) were collected *post-mortem*. After mRNA extraction, *NGF, TRKA* and *p75NTR* mRNA expression was assessed by qPCR using *RPL32* as reference gene. Data were analyzed by unpaired t-test in GraphPad PRISM.

RESULTS: The *NGF* mRNA levels were upregulated in FP compared to MLP ($p<0.001$). The transcription of *TRKA* increased in FP endometria relative to MLP ($p<0.05$), whereas *p75NTR* mRNA transcription increased in MLP related to FP ($p<0.01$).

CONCLUSION: The present findings suggest that NGF signaling in the equine endometrium is dynamically regulated across the estrous cycle, with a potential change in receptor-mediated actions depending on the hormonal influence. The upregulation of *NGF* and *TRKA* mRNA transcription during the FP may indicate a predominance of trophic and proliferative signaling under estrogen influence, which may support endometrial proliferation and receptivity. In contrast, the increased mRNA transcription of *p75NTR* in MLP may suggest a possible role in modulating cell survival, remodeling, or immune responses under progesterone influence.

AKNOWLEDGMENTS/FUNDING: FCT projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), and 2022.07400.PTDC (10.54499/2022.07400.PTDC) and PhD scholarship 2023.04679.BD (10.54499/2023.04679.BD). Poland NAWA Project BPN/BPT/2021/1/00026/U/00001 and IAR&FR project FBW/8/2023.

COMPARISON OF NGF AND RECEPTORS GENE TRANSCRIPTION IN EQUINE ENDOMETRIUM

Nélio Galrito-Cebola[1,2,3,4], Marta Cerveira-Pinto[1,2], Anna Wójtowicz[5], Graça Ferreira-Dias[1,2], Andreia J. Amaral[1,2,6], Ana Amaral[1,2,7], Anna Szóstek-Mioduchowska[5]

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4th Annual Meeting of the Associate Laboratory for Animal and Veterinary Science, May 15th-16th, 2025, Lisboa, Portugal

BACKGROUNG

- Nerve growth factor (NGF) is a neurotrophin involved in nerve growth, tissue repair, estrous cycle regulation, extracellular matrix remodelling, and is associated with inflammation and fibrosis.
- It signals via two receptors:
  - Tyrosine kinase receptor (TRKA) - high-affinity, mediates trophic/proliferative effects.
  - p75 neurotrophin receptor (p75NTR) - low-affinity, modulates survival, apoptosis, and axonal growth.
- The mRNA expression of NGF and its receptors in equine endometrium during the follicular (FP) and mid-luteal (MLP) phases remains unexplored.

This study aimed to characterize *NGF*, *TRKA* and *p75NTR* mRNA expression in equine endometrium of category I of histopathological classification (healthy endometrium) in FP and MLP of the estrous cycle.

METHODOLOGY

Endometria from mares at FP and MLP → Kenney and Doig's grading (I; n=10/cycle phase):

- *NGF, TRKA* and *p75NTR* transcription levels → RT-qPCR

RESULTS

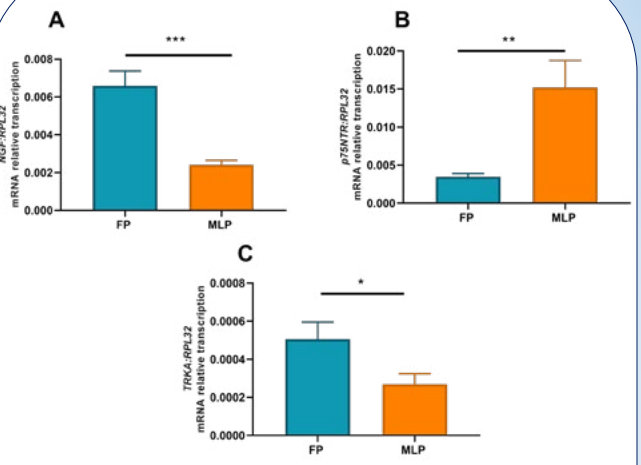

Figure 1 – *NGF* (A), *p75NTR* (B) and *TRKA* (C) mRNA transcription levels in follicular (FP) and mid-luteal (MLP) phases from category I of Kenney and Doig. Data were analyzed by unpaired t-test and expressed as mean ± SEM. Asterisks designate statistical differences between categories (*p<0.05; **p<0.01; ***p<0.001).

- *NGF* mRNA levels were upregulated in FP compared to MLP (p<0.001). The transcription of *TRKA* increased in FP endometria relative to MLP (p<0.05), whereas *p75NTR* mRNA transcription increased in MLP related to FP (p<0.01).

CONCLUSIONS

- The present findings suggest that NGF signaling in the equine endometrium is dynamically regulated across the estrous cycle, with a potential change in receptor-mediated actions depending on the hormonal influence.
- The upregulation of *NGF* and *TRKA* mRNA transcription during the FP may indicate a predominance of trophic and proliferative signaling under estrogen influence, which may support endometrial proliferation and receptivity.
- In contrast, the increased mRNA transcription of *p75NTR* in MLP may suggest a possible role in modulating cell survival, remodeling, or immune responses under progesterone influence.

FUNDING:

This research was funded by Portuguese Foundation for Science and Technology (FCT), under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS), and 2022.07400.PTDC (http://doi.org/10.54499/2022.07400.PTDC); MCP was funded by a doctoral fellowship from FCT under grant 2023.04679.BD (https://doi.org/10.54499/2023.04679.BD).

Supported by LA/P/0059/2020

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.26

LT 3 Comparative and Translational Medicine and Biotechnology

IMMUNOHISTOCHEMICAL CHARACTERIZATION AND LOCALIZATION 11 β – HYDROXYSTEROID DEHYDROGENASE TYPE 2 IN THE FELINE KIDNEY AND ADRENAL GLAND – IS THERE A LINK TO CHRONIC KIDNEY DISEASE?

Patricia Lunet Marques[1,2], Rute Noiva[1,2], Sandra Carvalho[1,2], Beatriz Azevedo[1,2], Monique van Wolferen[3], Luísa Mateus[1,2], Sara Galac[3], Rodolfo Oliveira Leal[1,2]

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INTRODUCTION: Mineralocorticoid receptor (MCR) selectivity for aldosterone is thought to be protected by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts active glucocorticoids into inactive 11-keto analogues, like cortisone. This suggests cortisol may act as a MCR agonist in certain tissues or under specific conditions. While 11β-HSD2 expression has been detected in feline kidneys, its location and implications in chronic kidney disease (CKD) have not yet been described.

AIMS: This study aims to characterize the distribution patterns of 11β-HSD2 expression in healthy feline kidneys and adrenal glands, as well as compare these to those of cats with diagnosed CKD.

METHODOLOGY: A cross-sectional study was conducted including tissue samples obtained from a total of 11 cats recruited at a veterinary teaching hospital. Two cats had clinically normal kidney function prior to euthanasia for reasons unrelated to renal or endocrine disease. The other 9 cats had been previously diagnosed with CKD. Owners consented to the use of tissue collected post-mortem. Tissues were collected within 24 hours of death, fixed in buffered formalin and embedded in paraffin. Immunohistochemistry was performed on 3 µm-thick sections, using a commercially available antibody (RRID AB_2642555). A previously validated scoring system was used to assign a final score (FS) based on staining intensity and prevalence in several nephron segments, such as the convoluted tubules (CT) and the loop of Henle (LH); in the adrenal gland, the zonas glomerulosa (ZG), fasciculata (ZF), reticularis (ZR), and medulla were evaluated. Serum creatinine concentration was also assessed for correlation with the FS.

RESULTS: Immunohistochemical staining for 11β-HSD2 in healthy kidneys revealed moderate to strong expression of 11β-HSD2 in the epithelial lining of the distal CT and LH. Staining was cytoplasmic, diffuse to granular, often concentrated around the nuclear envelope and in the perinuclear rough endoplasmic reticulum area. Occasionally, there was mild granular cytoplasmic staining of the proximal CT. In the adrenal gland, 11β-HSD2 expression was limited to the cortex; it was inconsistent and mild in the ZG and progressively stronger toward the ZR. Staining was cytoplasmic, granular to diffuse. In the kidney, the CKD group had a lower FS in every location analyzed. Conversely, in the adrenal cortex, the CKD group had higher FS in the ZG and the ZF. Both groups had the same FS in the ZR and the medulla. However, no significant difference ($p > 0.05$) was identified between groups in any part of the kidney or the adrenal gland, probably due to the low number of healthy participants. Serum creatinine correlated positively with the FS in the ZF ($p = 0.003$, $r = 0.853$).

CONCLUSIONS: This preliminary analysis shows, for the first time, the immunolocalization of 11β-HSD2 in the feline kidney. It also suggests 11β-HSD2 may play a role in the pathophysiology of feline CKD.

ACKNOWLEDGMENTS/FUNDING: This project was funded by the Morris Animal Foundation Grant D24FE-816, FCT IP grant UIDB/00276/2020 and by LA/P/0059/2020—AL4AnimalS.

IMMUNOHISTOCHEMICAL CHARACTERIZATION AND LOCALIZATION OF 11 β – HYDROXYSTEROID DEHYDROGENASE TYPE 2 IN THE FELINE KIDNEY AND ADRENAL GLAND – IS THERE A LINK TO CHRONIC KIDNEY DISEASE?

Patricia Lunet Marques[1,2], Rute Noiva[1,2], Sandra Carvalho[1,2], Beatriz Azevedo[1,2], Monique van Wolferen[3], Luísa Mateus[1,2], Sara Galac[3], Rodolfo Oliveira Leal[1,2]
[1]CIISA – Centre for Interdisciplinary Research in Animal Health, FMV-UL, Lisbon, Portugal
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Supported by LA/P/0059/2020

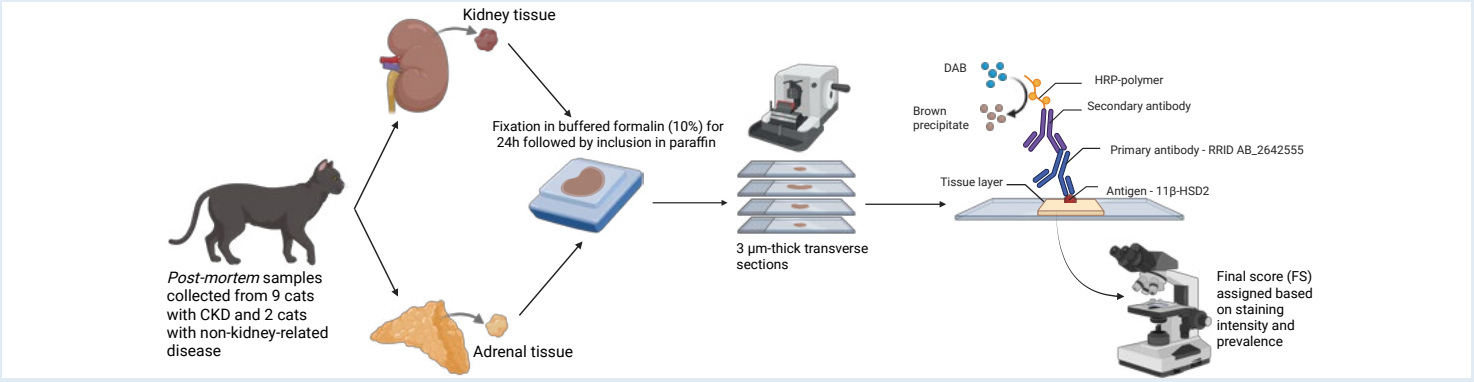
INTRODUCTION

- Mineralocorticoid receptor (MCR) selectivity for aldosterone is thought to be protected by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2).
- 11β-HSD2 converts active glucocorticoids into inactive 11-keto analogues, like cortisone.
- Cortisol may act as a MCR agonist in certain tissues or under specific conditions, including 11β-HSD2 dysfunction or depletion.
- While 11β-HSD2 expression has been detected in feline kidneys, its location and implications in chronic kidney disease (CKD) have not yet been described.

OBJECTIVES

- Characterize the distribution patterns of 11β-HSD2 expression in healthy feline kidneys and adrenal glands.
- Compare 11β-HSD2 staining in healthy cats and cats with CKD.
- Correlate FS with circulating creatinine values

METHODS



RESULTS

Immunohistochemical staining for 11β-HSD2 in healthy kidneys revealed moderate to strong expression of 11β-HSD2 in the epithelial lining of the distal convoluted tubule (DCT) and loop of Henle (LH).

Occasionally, there was mild granular cytoplasmic staining of the proximal convoluted tubule (PCT) (figure 1).

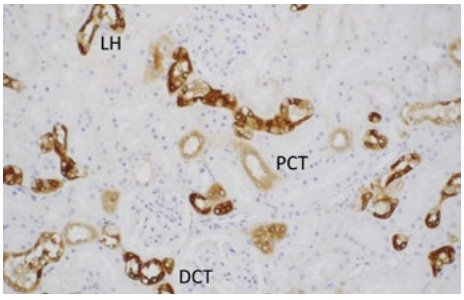


Figure 1 - Cortical 11β-HSD2 staining in a healthy kidney (200x magnification).

Staining was cytoplasmic, diffuse to granular, often concentrated around the nuclear envelope and in the perinuclear rough endoplasmic reticulum area (figure 2).

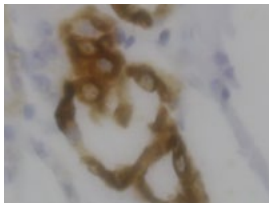


Figure 2 - Cortical 11β-HSD2 staining in the DCT of healthy kidney (500x magnification).

In the kidney, the CKD group had a lower FS in every location (figures 3 and 4).

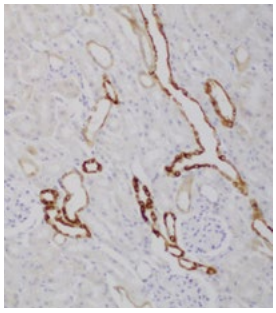


Figure 3 - Cortical 11β-HSD2 staining in a healthy kidney (100x magnification).

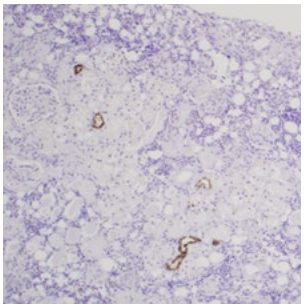


Figure 4 - Cortical 11β-HSD2 staining in a kidney from a cat with CKD (100x magnification).

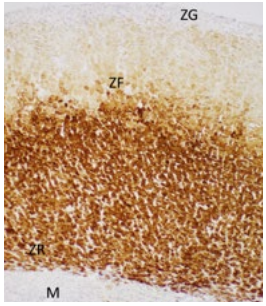


Figure 5 - Adrenal 11β-HSD2 staining from a cat with healthy kidneys (100x magnification).

In the adrenal gland, 11β-HSD2 expression was limited to the cortex; it was inconsistent and mild in the zona glomerulosa (ZG) and progressively stronger toward the zona reticularis (ZR) (figure 5).

Staining was cytoplasmic, granular to diffuse.

The CKD group had higher FS in the ZG and the zona fasciculata (ZF). Both groups had the same FS in the ZR and the medulla (M).

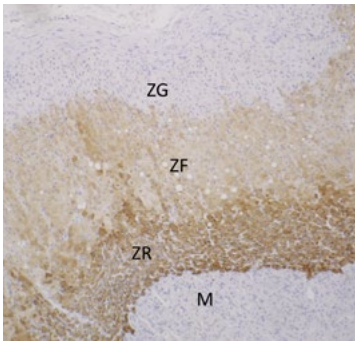


Figure 6 - Adrenal 11β-HSD2 staining from a cat with CKD (100x magnification).

In the adrenal cortex, the CKD group had higher FS in the ZG and the ZF. Both groups had the same FS in the ZR and the medulla (figure 6).

No significant difference ($p > 0.05$) was identified between groups in any part of the kidney or the adrenal gland, probably due to the low number of healthy participants.

On the other hand, circulating serum creatinine correlated positively with the FS in the ZF ($p = 0.003$, $r = 0.853$) but not in any other zone of the adrenal gland or part of the kidney.

CONCLUSION

This preliminary analysis shows, for the first time, the immunolocalization of 11β-HSD2 in the feline kidney. It also suggests 11β-HSD2 may play a role in the pathophysiology of feline CKD.

ACKNOWLEDGEMENTS

This project was funded by the Morris Animal Foundation Grant D24FE-816.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.27

LT 3 Comparative and Translational Medicine and Biotechnology

LYMPHOCYTE PROFILE OF HEALTHY CATS: PRELIMINARY RESULTS OF A FLOW CYTOMETRY STUDY

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INTRODUCTION: The paucity of studies on the immunophenotyping of peripheral blood leukocytes in healthy cats is notable, and databases on their immune systems remain scarce due to species-specific characteristics.

AIMS: To characterise the peripheral blood lymphocytes in healthy cats and to relate them with sex, vaccination status and life stages.

METHODOLOGY: Animals were recruited during admission to elective surgery at the University Veterinary Hospital of Coimbra. Cats were excluded if they any clinical signal of disease were observed, if they are seropositive for FIV and FeLV, and if abnormalities were detected in the pre-anaesthetic blood analysis. Leukocytes were labelled with mouse anti-dog CD18, mouse anti-dog CD21, rat anti-mouse CD45R (tube 1) and mouse anti-cat CD4, CD5 and CD8 (tube 2) antibodies and analysed using flow cytometry (FC).

RESULTS: Thirty-one healthy cats, 15 kitten (<1 yo) and 17 young adult (1-6 yo), 18 female and 14 males from different breeds (Domestic Shorthair, 81.3%; Persa, Siamese and Domestic Longhair, 6.25% each) were selected. FC revealed no significant variations in total leukocytes, and differential blood cell counts between the vaccination status, sex, and age parameters. Minor discrepancies were observed in leukocytes, and both B and T lymphocyte subsets (P>0.05). In addition, a T-cell subpopulation with low CD8 expression was identified ($CD8^{lo}$).

CONCLUSIONS: These findings suggest that the feline immune response exhibits minimal variation based on the studied variables indicating no substantial immunosenescence evidence. In healthy animals $CD8^{lo}$ T-cells may include regulatory $CD8^+$ T cells (Tregs) with immunosuppressive functions, immature thymic $CD8^+$ T cells, chronically activated exhausted $CD8^+$ T cells, or memory $CD8^+$ T cells with lower CD8 expression than naïve or effector cells. Further research is required to understand the impact of age on the immune system, involving a greater number of animals and life stages.

ACKNOWLEDGEMENTS/FUNDING: This work was funded by Fundação para a Ciência e a Tecnologia, under the projects UIDB/00772/2020 (CECAV, doi:10.54499/UIDB/00772/2020), LA/P/0059/2020 (AL4AnimalS), POCI-01-0145-FEDER-007440, UIDB/04539/2020 and UIDP/04539/2020, and under the Scientific Employment Stimulus - Institutional Call - CEECINS/00127/2018 (J.F. Requicha).

LYMPHOCYTE PROFILE OF HEALTHY CATS: PRELIMINARY RESULTS OF A FLOW CYTOMETRY STUDY

RAFAEL SIMÕES LOPES[1,2,3*], PEDRO PIRES CARVALHO[2,4], MARIA DOS ANJOS PIRES[1,3], PAULO RODRIGUES- SANTOS[5,6], EDUARDO COSTA[2,7,8], JOÃO FILIPE REQUICHA[1,3]

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INTRODUCTION

The paucity of studies on the immunophenotyping of peripheral blood leukocytes in healthy cats is notable, and databases on their immune systems remain scarce due to species-specific characteristics.

AIMS

This work aims to characterise the peripheral blood lymphocytes in healthy cats and to relate them with sex, immunization status and life stages.

MATERIALS & METHODS

Animals

32 Healthy Cats

♂ N=14 ♀ N=18

N=15 | **Kitten** (< 1 year)
N=17 | **Young-adult** (1-6 year)

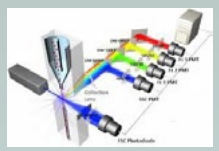
N=6 | **Vaccinated** (FHV-1, FCV, FPV)
N=26 | **Non-Vaccinated**

Inclusion criteria

- No clinical signs of illness
- Seronegative for FIV and FeLV
- No abnormalities identified in summary blood analysis

Immunophenotyping

Flow cytometry



- Fluorochrome-conjugated mAbs (Bio-Rad®)
- 3-colour mAb mixes (2 tubes)
- BD FACSCanto II flow cytometer

Data analysis

- FlowJo™ v10.10.0 (BD Biosciences)

Statistical analysis

- GraphPad Prism™ v10.4.2 software

Tube 1

RPE **CD5** (Total T cells)
FITC **CD4** (Helper T cells)
AF647 **CD8** (Cytotoxic T cells)

Tube 2

AF647 **CD18** (Pan leukocytes)
FITC **CD45R** (T, B and NK cells)
RPE **CD21** (B lymphocytes)

RESULTS

Contour plots of flow cytometric analysis and gating strategy of peripheral blood cells

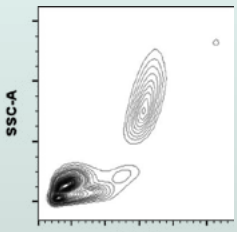


Figure 1 - Forward (FSC-A) versus side scatter (SSC-A) for the evaluation of cell size and granularity, respectively.

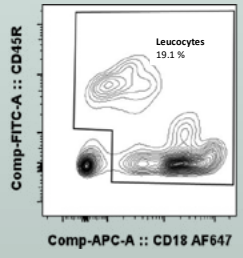


Figure 2 - Leucocyte gating for CD45R+ CD18+ cells.

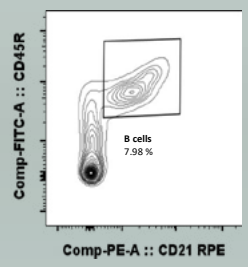


Figure 3 - Gating for CD45R+ CD21+ B cells.

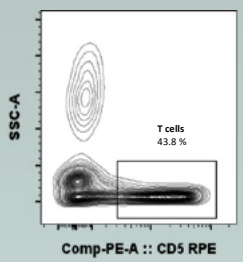


Figure 4 - Gating for CD5+ T cells within the lymphocyte granularity (SSC-A).

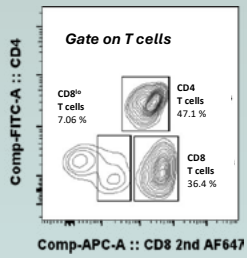


Figure 5 - On gated T cells (fig. 4), identification of CD4, CD8 T cells and CD8^{lo} T cells.

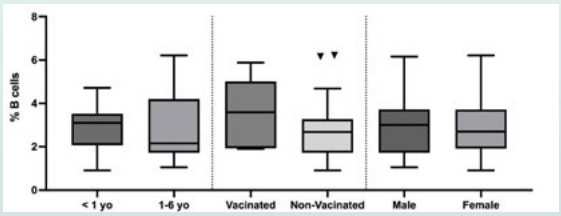


Figure 6 - Relative frequencies of B cells from total lymphocytes according to age, immunization status and sex (p>0.05).

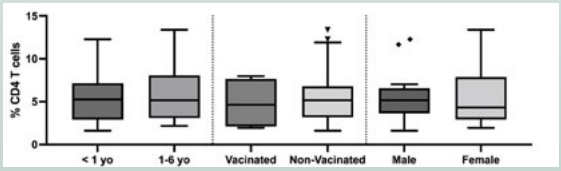


Figure 7 - Relative frequencies of Helper T-cells (CD5+CD4+) according to age, immunization status and sex (p>0.05).

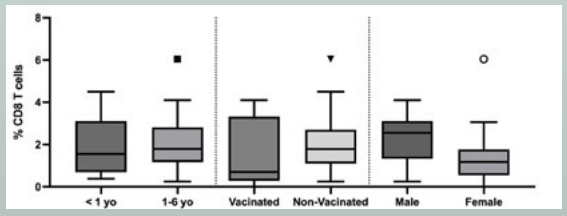


Figure 8 - Relative frequencies of Cytotoxic T-cells (CD5+CD8+) according to age (p>0.05), immunization status (p>0.05) and sex (p=0.05).

No significant differences were observed between:

- Age, immunization status and sex for B cells (p>0.05)
- Age, immunization status and sex for CD5+CD4+ T cells (p>0.05)
- Age and immunization status (p>0.05) and sex (p=0.05) for CD5+CD8+ T cells

CONCLUSIONS

- No evidence of **immunosenescence** were detected between groups
- **Females** may have **low** expression of **CD8 T-cells**
- Need for **high number** of animals in each group to validate the data obtained
- Need for longitudinal studies to assess **aging effects**
- A T-cell subpopulation with **low CD8 expression** was identified (CD8^{lo})
- The presence of **CD8^{lo}** T-cells may hint at **regulatory** or **memory** T-cell mechanisms

AL4ANIMALS 4th Annual Meeting of the Associate Laboratory for Animal and Veterinary

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PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD

LT 3 Comparative and Translational Medicine and Biotechnology

LT3.28

GRAPE STEM POLYPHENOL EXTRACTS ON PROSTATE CANCER CELL MODULATION

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INTRODUCTION: Prostate cancer (PCa) is the leading cause of cancer-related morbidity and mortality worldwide. PCa conventional therapies cause significant side effects and resistance. Consequently, there is growing interest in green, targeted therapies using natural compounds. Isolated polyphenols have demonstrated potential in cancer research due to their anti-inflammatory, antioxidant, and antiproliferative activities. Our group has previously characterized grape stems from the Douro Region as a unique source of phenolic compounds, distinguished by the high abundance and presence of specific phenolics within this natural matrix. However, the biological effects of grape stem polyphenol extract on PCa cells is still unknown.

AIMS: Extraction and characterization of grape stem varieties harvested in the Douro region and evaluation of their antioxidant capacity and effects on inflammation modulation and PCa cells.

METHODOLOGY: Polyphenols from Tinta Amarela, Sousão, Touriga Franca, and Touriga Nacional were extracted and characterized. The variety with higher phenolic content and antioxidant capacity was selected to evaluate its potential on immune cell modulation, PCa viability, reactive oxygen species (ROS) production, cell cycle and cell death.

RESULTS: Among the varieties, Touriga Nacional exhibited the highest phenolic content and antioxidant activity. This extract inhibited the production of interleukin (IL)-1β and IL-6 in stimulated macrophages, suggesting suppression of inflammation and inflammasome formation. In PCa cells, the extract reduced reactive oxygen species (ROS), induced morphological changes, caused G0/G1 cell cycle arrest, and increased necrosis, while having no impact on a non-cancerous prostate cell line.

CONCLUSIONS: This pilot study highlights the potential of valorising winery by-products within a circular economy framework, while exploring innovative co-adjuvant therapies for PCa.

ACKNOWLEDGMENTS/FUNDING: This work was supported by National Funds by FCT–Portuguese Foundation for Science and Technology, Ph.D. grant 2023.02608.BD and the projects UIDB/04033/2020 (https://doi.org/10.54499/UIDB/04033/2020), LA/P/0126/2020 (https://doi.org/10.54499/LA/P/0126/2020), and the strategic funding of the UIDB/04469/2020 unit. The authors acknowledge CITAB–UTAD for providing the necessary conditions for the development of this work and Rozès winery industry for supplying the grape stem varieties used in this study.

PAINÉIS CIENTÍFICOS A CONCURSO

DOUTORAMENTOS

PhD LT3.29

LT 3 Comparative and Translational Medicine and Biotechnology

SILANIZED HYALURONIC ACID HYDROGELS FOR ADVANCED SKIN THERAPY

Cristiana Oliveira[1], Diana Sousa[1], Rui Rodrigues[1,2], José António Teixeira[1,2], Juan Luis Paris[3], Claudia M. Botelho[4]

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INTRODUCTION: A person's appearance, particularly their skin, is crucial in society as it reflects overall health and vitality. Skin disorders, due to their visibility, can significantly impact daily life and mental well-being. This societal emphasis has long driven people to find ways to care for and enhance their skin.

AIM: Develop silanized hyaluronic acid hydrogels with easily mouldable properties for biomedical applications, advancing the field with innovative therapeutic possibilities.

METHODOLOGY: To develop silanized hyaluronic acid hydrogels with tuneable properties, hyaluronic acid (HA) was chemically modified using 3-aminopropyltriethoxysilane (APTES) as a crosslinker and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM) as a coupling agent. HA was dissolved in MES buffer and reacted with DMTMM followed by APTES at varying molar ratios to produce hydrogels with different degrees of crosslinking. After synthesis, the hydrogels were purified using Amicon filters and crosslinked by incubation at 37 °C for 24 hours. Physico-chemical characterization included ATR-FTIR spectroscopy to confirm chemical modification and crosslinking efficiency. Swelling behaviour was analysed by immersing the hydrogels in ultrapure water and measuring mass gain over time. Rheological properties were assessed with a rotational rheometer, evaluating viscosity and shear-thinning behaviour. Mechanical properties such as firmness, consistency, cohesiveness, and work of cohesion were determined using texture profile analysis. For biological evaluation, the two most promising hydrogel formulations were selected for in vitro testing using L929 fibroblasts and HaCaT keratinocytes. Cell viability was measured using the resazurin reduction assay after 24 and 48 hours. A scratch assay was performed to evaluate the influence of hydrogels on cell migration. Reactive oxygen species (ROS) levels were assessed with and without oxidative stress using a commercial fluorescence-based assay. All experiments were conducted in triplicate to ensure statistical reliability.

RESULTS: Four Si-HA hydrogels were synthesized with varying APTES concentrations. Hydrogel 4 showed poor crosslinking due to APTES self-condensation. ATR-FTIR confirmed successful modification in hydrogels 1–3. Swelling decreased with higher crosslinking, and rheology revealed shear-thinning behaviour, with hydrogel 3 having the highest viscosity. Mechanical tests showed increased cohesiveness with higher APTES, but excessive crosslinking reduced firmness. Hydrogels 2 and 3 were selected for biological testing. Both showed no cytotoxicity after 24 hours, though hydrogel 3 slightly reduced L929 cell viability at 48 hours. Neither hydrogel significantly affected cell migration or ROS levels. Overall, hydrogels 2 and 3 demonstrated suitable physical and biological properties, highlighting their potential for customizable skin therapies.

CONCLUSIONS: These findings demonstrate the potential of silanized hyaluronic acid hydrogels as customizable platforms for skin therapy and biomedical applications, paving the way for future integration with bioactive agents for enhanced therapeutic effects.

PAINÉIS CIENTÍFICOS
LINHAS TEMÁTICAS
MESTRADOS

LT 2
Emergent Infectious Diseases and Zoonosis

MSc LT2.01	First Detection of Rickettsia Helvetica and Rickettsia Conorii Subsp. Raoultii in Ixodes Ricinus Ticks Collected from Domestic Dogs in Luxembourg
MSc LT2.02	Detection of Cryptosporidium Spp. in Bile of Small Ruminants
MSc LT2.03	Antimicrobial Activity of Electrolyzed Water Against Foodborne Path ogens
MSc LT2.04	Screening for the Ciliate Buxtonella Sulcata in Free-Ranging Dairy Cattle on Terceira Island, Azores Archipelago
MSc LT2.06	Molecular Prevalence of Equine Piroplasms in Horses in Mainland Portugal – Preliminary Results
MSc LT2.07	Targeted-Amplicon NGS for Blastocystis Sp. in Shepherd Dogs of Portugal Discriminates Co-Colonization with Multiple Zoonotic Subtypes

LT 3
Comparative and Translational Medicine and Biotechnology

MSc LT3.01	Can Urothelial Carcinomas in Dogs' Benefit from the use of Toceranib Phosphate (Palladia®) as the First Therapeutic Option?
MSc LT3.02	Changes in the Endometrial Proteome Induced by Eosinophils in the Jenny
MSc LT3.03	Ctrl+Alt+Degenerate: Creating Cell Models for Sanfilippo Syndrome
MSc LT3.04	Retrospective Evaluation of Toceranib Phosphate Use in Cats With Solid Tumours
MSc LT3.05	Development of novel immunotherapies for the treatment of canine lymphoma based on immunotoxins derived from Pseudomonas aeruginosa and Corynebacterium diphtheriae
MSc LT3.06	Development of Novel Immunotoxins Using Trastuzumab-Derived Single-Chain Variable Fragments for Breast Cancer Treatment
MSc LT3.07	Case Series of Diopathic Chronic Kidney Disease in Cats: A Retrospective Study
MSc LT3.08	Precision Medicine for Canine Lymphoma: Identification of Novel and Potent Cytotoxic Compounds for Conjugation into an Antibody-Drug Conjugate
MSc LT3.09	Evaluation of PD-L2 as a serological biomarker of feline mammary carcinoma

PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT1

LT1.10

Green Animal Production

BETTER UNDERSTANDING THE LARGE GAME MEAT CHAIN IN PORTUGAL

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INTRODUCTION: Sanitary assessment of the entire wild large game meat chain ("forest-to-fork") is crucial. This approach integrates three main phases: (a) pre-harvest (live populations), where the importance of the large game populations' health management should be focused on control and monitoring risk practices; (b) harvest (hunted animals), where the initial examination of the hunted animals, hygienically and effectively performed, represents an important source on populations health status and a capital tool to mitigate the risk of disease transmission to animals and Human; (c) post-harvest (game meat): which includes carcass handling practices and meat consumption habits, both with relevance on the human exposure to zoonosis.

AIMS: This work aims to characterize the entire wild large game chain with a mental map correlation.

METHODOLOGY: A mental map exercise was done. Presenting several recent and relevant scientific results for the various phases of the large game chain, a mental design was drawn up and presented to stakeholders.

RESULTS: There is evident interdependence between the various phases of the hunting chain, with both a positive and negative causal relationship existing between the various facts. An integrated sanitary assessment provides a complete understanding of the entire chain, allowing a more effective control and mitigation strategy to be implemented in order to reduce the risk to animal and human health.

CONCLUSIONS: As the main conclusions, based on the results achieved in this mental map exercise, it is important to emphasize that the wild large game meat production chain requires more attentive veterinary intervention. Under the motto of "Prevention is better than cure", to minimize the risk for animal and human health along this chain, an integrated sanitary assessment approach is crucial, which success depends on the collaboration of all-important stakeholders, like academia, veterinarians, Competent Authorities, game managers, and hunters.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT1

LT1.11

Green Animal Production

EXTRUDED AND ENZYME-ENHANCED *LIMNOSPIRA PLATENSIS* AS A SUSTAINABLE PARTIAL REPLACEMENT FOR SOYBEAN MEAL: EFFECTS ON BROILER PERFORMANCE, MEAT PHYSICOCHEMISTRY, AND NUTRITIONAL PROFILE

Joana Ferreira[1,2]; Mónica Costa[1,2]; Maria Spínola[1,2]; José Pestana[1,2]; Cátia Martins[3,4]; Cristina Mateus[1,2]; Daniela Carvalho[3,4]; Ana Mendes[1,2,3,4]; Madalena Lordelo[3,4]; José Prates[1,2]

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INTRODUCTION: The escalating demand for food and feed calls for sustainable protein sources to replace soybean meal, thereby bolstering food security and minimizing environmental footprint. Microalgae, rich in protein, essential amino acids and health-promoting pigments, represent a promising alternative, although their rigid cell walls can limit nutrient bioavailability.

AIMS: This study evaluates the effects of including 15% Spirulina (*Limnospira platensis, L. platensis*), either extruded or not, in broiler diets, with and without a multi-enzyme cocktail, on growth performance and meat nutrient deposition.

METHODOLOGY: A total of 120 male broiler chicks were initially reared on a standard maize–soybean meal diet until day 14. From day 14 to day 35, birds were randomly assigned to one of four dietary treatments (n = 10 birds per treatment): control diet based on soybean meal-maize (CTR), control diet incorporated with 15% *L. platensis* (LP), control diet incorporated with 15% extruded *L. platensis* (LPE) *and* control diet incorporated with 15% *L. platensis* plus 0.21% of a commercial enzyme mixture (LPM). During the trial, growth performance was monitored. Breast meat was collected *post-mortem* and analysed for meat quality.

RESULTS: All *L. platensis*-incorporated diets, regardless of processing or enzyme addition, induced a significant rise in feed-conversion ratio *versus* the control ($P < 0.001$). At 24 h *post-mortem,* breast muscle from *L. platensis*-fed birds exhibited a lower pH ($P = 0.001$) and a higher b* value, reflecting increased yellowness, than muscle from control birds ($P < 0.001$). Nutritionally, inclusion of *L. platensis* markedly increased total carotenoid deposition and raised both n-3 PUFA ($P < 0.001$) and SFA contents ($P < 0.001$), while reducing n-6 PUFA levels ($P < 0.001$). These compositional shifts yielded significantly improved n-6/n-3 and PUFA/SFA ratios compared to the control group ($P < 0.001$).

CONCLUSIONS: Extruding *L. platensis* and adding a multi-enzyme cocktail restores nutrient utilisation to control levels despite a slight increase in feed conversion ratio, while significantly enhancing breast-meat quality, higher carotenoids, more n-3 PUFA and improved n-6/n-3 and PUFA/SFA ratios. These findings support *L. platensis* processed as a viable, sustainable partial replacement for soybean meal in broiler diets.

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PREDICTION OF BEEF AND PORK QUALITY BY COMPUTER VISION AND PHYSICOCHEMICAL ANALYSIS

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INTRODUCTION: In beef, a common quality defect is DFD (Dark, Firm, and Dry) condition, linked to stress that reduces muscle glycogen and maintains a high pH, while pork is more prone to PSE (Pale, Soft, and Exudative), which results in a rapid pH drop caused by stress and genetic factors, leading to protein denaturation, increased exudation and light scattering. Traditional quality assessments are often destructive, slow, and subjective. As an alternative, computer vision systems (CVS) have emerged as promising tools in slaughterhouses, offering rapid, objective, and non-destructive evaluation of meat quality attributes. Studies have demonstrated that CVS can effectively detect conditions like DFD and PSE by analysing colour parameters (L*, a*, b*) and correlating them with pH levels, enabling real-time quality control and reducing reliance on labour-intensive methods.

AIMS: The aim of this study is to develop a model applicable in slaughterhouses to estimate the quality of beef and pork using a computer vision system.

METHODOLOGY: The *longissimus thoracis et lumborum* (LTL) muscle of male bovine and swine carcasses was used to assess meat quality 24h *post-mortem*. The pH at 24h *pm* was measured directly on the meat. The colour coordinates (L*, a*, b*, C* and h) were measured after 1h of blooming using a Minolta CR-400 colorimeter. Red colour intensity was evaluated on a 1–7 sensory scale. Drip loss (after 3 days *pm* at 4 °C) and cooking loss (after 6 days *pm*, cooked at 80 °C until 71 °C internally) were assessed under controlled conditions. Shear force was determined with a Warner-Bratzler probe on a TA.XT plus texturometer. Samples were photographed using a Sony α58 camera under controlled lighting. The image analysis in MATLAB provided L^*_{image}, a^*_{image}, and b^*_{image} values. Data were used to train (75%) and test (25%) a predictive model in XLSTAT.

RESULTS: In beef, significant correlations were observed between the image data obtained by CVS (L^*_{image}, a^*_{image}, b^*_{image}) and various meat quality attributes, including pH_{24h} (negative correlations), and L*, a*, b*, C*, h, drip loss, cooking loss and sensory colour (positive correlations). In pork, the colour traits obtained by CVS showed significant positive correlations not only with each other, but also with the pH and h; and sensory colour only correlating with a^*_{image}, suggesting that the red colour captured by image may influence the visual perception to some extent.

CONCLUSIONS: Computer vision-based image analysis estimated successfully both the quality of DFD and normal beef and PSE and normal pork, based on colour information. This technique is promising compared to conventional methods since it is nondestructive, fast, and easily adaptable to slaughterhouses.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT1

Green Animal Production

LT1.13

INSIGHTS OF 15 % DIETARY INCORPORATION OF *LIMNOSPIRA PLATENSIS* IN BROILER CHICKENS' DIETS ON BLOOD COUNT, PLASMA METABOLITES, AND HEPATIC LIPID COMPOSITION

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INTRODUCTION: Soybean meal is widely used in poultry diets as a primary protein source, but its long-term sustainability is challenged by environmental and economic concerns. In response, alternative feed ingredients are being explored, such as microalgae, particularly *Limnospira platensis* (Spirulina). Nevertheless, their rigid cell wall and protein-pigment complexes limit digestibility in poultry. Pretreatments can partially break down the cell wall, but nutrient accessibility remains challenging.

AIMS: To evaluate the impact of 15% dietary inclusion of Spirulina as a core feed ingredient in broiler chicken's diets, focusing on blood cellular components, systemic metabolites and hepatic lipid composition.

METHODOLOGY: From days 14 to 35 of age, 120 broiler chickens were randomly distributed into four dietary treatments: a maize and soybean meal-based diet (CTR), a 15% Spirulina diet (SP), a 15% extruded Spirulina diet (SPE), and a 15% Spirulina diet with an enzyme blend (0.20% porcine pancreatin + 0.01% lysozyme) (SPM).

RESULTS: The haematological analysis revealed no significant differences ($p > 0.050$) in blood cell counts across treatments, suggesting that high Spirulina inclusion maintains haematological balance. The systemic metabolic assessment indicated an enhanced antioxidant capacity in birds fed Spirulina diets ($p < 0.001$), pointing toward a potential reduction in oxidative stress. However, there was a detrimental impact on final body weight and feed conversion ratio (both $p < 0.001$), in Spirulina treatments. Regarding hepatic composition, birds fed SPE and SPM diets showed a notable increase in *n*-3 fatty acids ($p < 0.001$), leading to an improved *n*-6/*n*-3 polyunsaturated fatty acids ratio ($p < 0.001$). Despite this positive shift, a reduction in total hepatic lipids ($p = 0.003$) was observed without a significant change in cholesterol levels.

CONCLUSIONS: These findings suggest that high Spirulina inclusion benefits broiler health and liver lipid profile but reduces growth performance, highlighting the need for further exploration into the optimal inclusion levels, processing methods and potential enzymatic enhancements of Spirulina in broiler diets.

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USING LASER-LIGHT BACKSCATTERING TO EVALUATE MEAT QUALITY ATTRIBUTES ON LONGISSIMUS LUMBORUM SAMPLES OF AROUQUESA MALE STEERS

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INTRODUCTION: Meat quality assessment is vital in the industry processing operations, chain commercialisation and consumer satisfaction. In this regard, an accurate meat quality evaluation is essential for ensuring its correct valorisation. Traditional methods of meat quality evaluation often require destructive sampling, expensive and polluting reagents and time-consuming laboratory analyses. Unlike other analytical methods that may require reagents, laser-light backscattering is a non-invasive and non-contact technique that does not require adding chemicals or reagents to the sample. This technique relies on the interaction of laser light with the meat sample, measuring the scattering patterns to extract information about various quality traits.

AIMS: A trial was performed to evaluate a flexible l, low-cost laser light backscattering imaging system (LBIS) for non-destructively assessing meat quality attributes of *Longissimus lumborum* muscle (LM) samples of Arouquesa steers.

METHODOLOGY: Six quality traits (pH, colour, sarcomere length, exudative losses, cooking losses and shear force) were measured in 14 LM samples obtained from Arouquesa male steers with 273±43 kg live weight and 149±25 kg carcass weight. The LBIS comprised of three parts: (1) a green laser diode operating the wavelength of 532 nm, with a power of 5 mW and a beam size of 1 mm; (2) an Olympus EM-5 digital photo camera equipped with an an OM macro lens 60mm with a circular polarizing filter with a 16 Megapixel sensor, mounted vertically on a support, at a distance of 30 cm from the LM samples; and (3) a Macro Olympus OM T28 Double Flash with polarizing filters on both heads. The laser was positioned at an angle of 75°, and the LM samples were placed over a black background. The entire process was carried out in a constant standard light and constant camera position. The laser backscattering images (LBI) show radial symmetry at the point where the laser light is emitted. The centre area in the LBI is bright white compared to others. In this work, three distinctive circular patterns were considered. Image processing analysis was carried out using Fiji (ImageJ 1.49u software). The area, major and minor axes were obtained for each distinctive circular pattern. Correlation analysis was performed between backscattering dimensional attributes (BDA) and LM quality traits.

RESULTS: A negative correlation was observed between pH at 24 hours post-mortem and all BDA, especially with area (r = -0.559 to -0.742). Cooking loss showed positive correlations with all BDA (ranging from 0.659 to 0.833). Finally, shear force, an indicator of meat tenderness, showed moderate positive correlations with BDA (r = 0.258 to 0.523).

CONCLUSIONS: This study suggests that laser-light backscattering could effectively assess meat quality traits. Future research should explore additional image analysis methods and involve larger sample sizes to enhance the findings.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT1

Green Animal Production

LT1.15

FARM TO FORK: HOW BREED AND CERTIFICATION INFLUENCE BEEF'S MICROELEMENTS AND VITAMINS

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INTRODUCTION: Throughout human evolution, meat has been a key source of essential nutrients, contributing to a healthy, balanced diet. Beef provides highly bioavailable vitamins and trace elements (Pereira & Vicente, 2013). In Portugal, beef production involves diverse cattle breeds and systems, including certification schemes such as organic (BIO) and Protected Designation of Origin (PDO), both contributing to native breed preservation.

AIMS: This study evaluated the effects of breed (Cachena, Maronesa) and certification system (BIO, PDO) on concentrations of key microelements (Fe, Zn, Cu, Mn) and B-complex vitamins (B1, B2, B3, B5, B6, B9, B12).

METHODOLOGY: Thirty-five bullocks (14 ± 2 months) were studied: 18 Cachena (8 BIO, 10 PDO) and 17 Maronesa (7 BIO, 10 PDO). Sirloin samples were collected. Microelements were quantified in freeze-dried, digested samples by ICP-OES (iCAP 7200). B vitamins were extracted by enzymatic hydrolysis and analyzed by HPLC-DAD. Statistical analysis was performed using GLM in SAS, considering breed, certification, and their interaction.

RESULTS: Certification system had no significant effect ($P>0.05$) on vitamin or microelement concentrations. In contrast, breed significantly influenced ($P<0.05$) Fe, Cu, B1, B9, and B12 levels. Maronesa beef had higher Fe (+16.4%) and Cu (+6.2%) concentrations, while Cachena beef showed higher B1 (+58.1%), B9 (+27.9%), and B12 (+14.7%) levels. Compared with feedlot beef (Mortensen, 2021), both native breeds exhibited higher amounts of microelements and B-complex vitamins.

CONCLUSION: Breed significantly affected half of the microelements and three B vitamins, while certification system showed no impact. Beef from Cachena and Maronesa under BIO and PDO schemes contains appreciable amounts of essential elements.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT1

LT1.16

Green Animal Production

DEVELOPING AND TESTING A WELFARE ASSESSMENT FRAMEWORK FOR STABLED HORSES IN PORTUGAL – PROOF OF CONCEPT

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INTRODUCTION: Most horses in Portugal are stabled in conditions lacking welfare monitoring. Despite rising concerns over equine well-being, national regulations emphasise resource-based provisions and overlook ethological and emotional needs. This PhD project addresses the lack of data on horse welfare in Portuguese equestrian centres by proposing an approach combining the AWIN protocol, additional behavioural and physiological indicators, and stakeholder perceptions.

AIMS: The main objectives are to: 1) map stable designs and management practices in Portuguese equestrian centres; 2) assess the welfare of stabled horses using animal-based indicators, including hair cortisol concentration (HCC), behavioural markers, and insect load; and 3) evaluate perceptions of horse owners and centre staff for future education and legislation.

METHODOLOGY: Eighteen federated equestrian centres were randomly selected to ensure geographical representation across mainland Portugal. The welfare assessment protocol includes: a) application of the AWIN framework; b) HCC analysis using LC-HRMS/MS to assess chronic stress; c) behavioural observations (behavioural stereotypies, withdrawn posture, time budget); d) environmental assessment on insect load. This will be complemented with the perceptions of staff and owners through focus groups and online surveys. Construct validity and inter-observer reliability will be evaluated. A pilot study at an equestrian centre in the district of Monção with 5 stabled horses was conducted in August 2024 to refine indicators.

RESULTS: Initial observations during the pilot study highlighted the need for practical and context-specific indicators. Horses were individually stalled, fed at chest-level corner mangers and bedded in wood shavings. Several methodological challenges emerged: the high frequency of insect load indicators - skin shivering and tail swishing - made them impractical to quantify reliably. Sticky trap deployment raised logistical issues Thus, visible skin lesions and periocular insects were used as proxy indicators (thresholds under revision). Lighting conditions varied, requiring standardised inspection tools. Indicators such as vaginal prolapse and discharge were simplified due to impracticality in field settings. A simplified ethogram was developed for student observers to ensure standardised data collection. This visit confirmed the need to streamline the welfare protocol to ensure feasibility in field conditions, particularly regarding insect-related discomfort and behavioural observations.

CONCLUSIONS: This research aims to deliver a validated, practical welfare assessment protocol for stabled horses in Portugal. The pilot enabled refinement of the assessment tools and provided support for the soundness of the methodology. Simplified context-specific indicators are essential for reliable field application. Data collected will provide the groundwork for evidence-based legislative reform and stakeholder education.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT1

LT1.17

Green Animal Production

FERMENTATION-DERIVED PEPTIDES AS ANTIBIOTIC ALTERNATIVES FOR SUSTAINABLE ANIMAL HEALTH

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INTRODUCTION: Reducing antibiotic use in livestock is a global priority to mitigate antimicrobial resistance and protect human, animal, and environmental health. Bioactive peptides generated through microbial fermentation are emerging as sustainable alternatives, offering antibacterial and immunomodulatory effects aligned with the One Health approach. In intensive production systems, maintaining intestinal health without antibiotics is crucial to ensure animal performance and food safety. **AIMS:** This study aimed to characterize a novel fermentative hydrolysis process using a *Bacillaceae* species (identity withheld for confidentiality), capable of producing peptides with antibacterial and anti-inflammatory activities, as well as to assess its impact on the gut microbiome.

METHODOLOGY: Fermentation was carried out using the selected *Bacillaceae* strain in the presence of milk for 48 hours. After fermentation, coagulated proteins were removed by filtration, and peptides were isolated from the soluble fraction by ultrafiltration. Antibacterial activity was tested against *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* using broth microdilution assays, and minimum inhibitory concentrations (MICs) were calculated. Anti-inflammatory potential was assessed *in vitro* by quantifying its effect on matrix metalloproteinase-9 (MMP-9), TNF-α, and interleukin (IL) levels. To evaluate microbiota modulation, fecal samples from treated and untreated mice were collected, and 16S rRNA gene sequencing was performed.

RESULTS: The peptides demonstrated dose-dependent antibacterial activity, with MICs ranging from 3 to 6 µg/mL. *In vitro*, it significantly reduced MMP-9, TNF-α, and IL expression in a dose-dependent manner, confirming its anti-inflammatory potential. *In vivo*, gut microbiome analysis showed a beneficial shift in microbial composition, including increased abundance of health-associated taxa and enhanced microbial diversity.

CONCLUSIONS: These novel peptides are resistant to digestive degradation and demonstrate a dual bioactivity profile, simultaneously inhibiting pathogenic bacterial growth and inflammatory mediators such as MMP-9, TNF-α, and interleukins. These effects contribute to improved gut health, intestinal resilience, and nutrient absorption — all essential for optimal animal growth and productivity, particularly where gastrointestinal disturbances can impair performance. Its duplibiotic effect supports its development as a feed additive or therapeutic tool, offering a sustainable and effective alternative to antibiotics. This aligns with the goals of antibiotic alternatives, improved animal welfare, and the broader One Health strategy.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

Emergent Infectious Diseases and Zoonosis

LT2.05

VECTOR-BORNE PATHOGENS IN STRAY DOGS FROM SÃO VICENTE (CAPE VERDE) – AN ONGOING MOLECULAR STUDY

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INTRODUCTION: Close contact between dogs and human populations increases the risk of transmission of vector-borne zoonotic pathogens. Microorganisms such as Anaplasma spp., Ehrlichia spp., Borrelia spp., Bartonella spp., and Rickettsia spp. can cause several diseases in both dogs and humans. Identifying these agents supports veterinary care and strengthens epidemiological surveillance under a One Health perspective. To date, limited data are available on vector-borne pathogens in dogs from São Vicente Island, Cape Verde.

OBJECTIVES: To identify vector-borne pathogens in stray dogs from São Vicente Island using morphological and molecular approaches.

METHODOLOGY: During a trap-neuter-return (TNR) campaign conducted in March 2024, blood samples (collected for clinical purposes) and ectoparasites were obtained from 20 stray dogs in São Vicente Island. DNA was extracted from the collected blood and from individual ectoparasites using commercial kits. Ectoparasites were morphologically identified using taxonomic keys. Molecular detection of Anaplasma/Ehrlichia spp., Bartonella spp., Borrelia spp., and Rickettsia spp. was performed by PCR.

RESULTS: Ectoparasites were detected in 80.0% (16/20) of dogs, from which a total of 47 ectoparasites were collected, namely 38 hard ticks (Rhipicephalus spp.), five fleas (one Echidnophaga gallinacea and four Ctenocephalides felis), and four lice (Heterodoxus spiniger). Molecular screening revealed Rickettsia spp. DNA in 10 (26.3%) ticks and all (100%) fleas, Anaplasma/Ehrlichia spp. DNA in five (13.2%) ticks, and Borrelia spp. DNA in two (5.3%) ticks. Additionally, Anaplasma/Ehrlichia spp. DNA was detected in the blood of 45.0% (9/20) of the dogs.

CONCLUSIONS: This study provides new molecular data on ectoparasites and vector-borne pathogens in stray dogs from São Vicente Island. Preliminary results indicate the presence of potentially zoonotic agents, which will be further characterised at the species level through phylogenetic analysis. These findings support the need for continued surveillance and integrated One Health approaches.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

LT2.18

Emergent Infectious Diseases and Zoonosis

OCCURRENCE OF MUCOR SPP. IN WILD AND DOMESTIC CARNIVORES: A ONE HEALTH PERSPECTIVE

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INTRODUCTION: Fungi of the order Mucorales, particularly species of the genera Absidia, Mucor and Rhizopus, are recognized as causative agents of mucormycosis, a life-threatening opportunistic infection characterized by aggressive tissue invasion. The recent rise in mucormycosis cases during the COVID-19 pandemic, often linked to comorbid conditions such as diabetes mellitus, has renewed interest in understanding environmental and animal reservoirs of Mucorales. Given the close interactions between humans, domestic animals, and wildlife, animals may act as asymptomatic carriers, contributing to the environmental load of pathogenic fungi. Investigating fungal colonization in animals is, therefore, essential to anticipate potential zoonotic risks within a One Health framework.

AIMS: This study aimed to evaluate the occurrence of Mucor spp. in the fur of wild and domestic carnivores in Portugal.

METHODOLOGY: Samples were collected from 143 animals, including 5 ginetas (Genetta genetta, Viverridae family), 53 foxes (Vulpes vulpes, Canidae family), 63 dogs (Canis lupus familiaris, Canidae family), 3 badgers (Meles meles, Mustelidae family), and 19 Egyptian mongooses (Herpestes ichneumon, Herpestidae family). The Mackenzie technique was used for fur sampling, and specimens were processed at the Medical Microbiology Laboratory, UTAD. Fungal isolation involved direct microscopic examination and culture on Sabouraud Dextrose Agar and Potato Dextrose Agar at 25°C and 37°C for 3 to 7 days.

RESULTS: Out of the 143 animals sampled, the isolation of Mucor varied significantly among host species. In ginetas, Mucor was isolated in 20.0% (1/5) of the samples. Among foxes, a high occurrence was observed, with Mucor isolated in 60.4% (32/53) of the cases. Dogs showed a lower occurrence, with Mucor isolated in 17.5% (11/63) of samples. No Mucor isolates were detected in badgers (0/3). In Egyptian mongooses, the occurrence was 36.8% (7/19).

CONCLUSIONS: The detection of Mucor spp. in the fur of both wild and domestic animals emphasizes their potential role as environmental carriers of opportunistic fungi. These findings reinforce the importance of a One Health approach, recognizing that environmental, animal, and human health are interconnected. Monitoring fungal colonization in animals is essential to understand potential transmission pathways and to anticipate emerging fungal threats to public health.

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STRAY CATS AND PUBLIC HEALTH: ZOONOTIC RISKS AND ENVIRONMENTAL CONTAMINATION FROM A ONE HEALTH PERSPECTIVE

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INTRODUCTION: Stray cats are frequently infected with gastrointestinal and pulmonary parasites due to their exposure to contaminated environments and lack of veterinary care. Several of these parasites have zoonotic potential, representing a risk to public health. In Portugal, data on the parasitic burden of stray cats remain scarce.

OBJECTIVES: To estimate the prevalence of gastrointestinal and pulmonary parasites in stray cats across various municipalities in mainland Portugal, and to assess the potential zoonotic relevance.

METHODOLOGY: Between September and December 2024, 105 fecal samples were collected from stray cat colonies under trap-neuter-return (TNR) programs in the municipalities of Benavente, Foz Côa, Sintra, Amadora, Alcácer do Sal, and Régua. Samples were analyzed using modified Willis flotation, Baermann, and sedimentation techniques to detect gastrointestinal and pulmonary parasites.

RESULTS: Gastrointestinal and/or pulmonary parasites were detected in 89.5% (89/105) of the sampled cats. The most prevalent was Toxocara cati (55.2%), followed by Ancylostomadidae (31.4%), Cystoisospora spp. (23.8%), Aelurostrongylus abstrusus (11.4%), and Taeniidae (7.6%), Dipylidium caninum was identified in 5.7% of samples, Tritrichomonas foetus in 4.8%, and both Capillaria aerophila and Giardia duodenalis in 1.9%. Parasitic coinfections were observed in 44.8% of the cats.

CONCLUSION: The high prevalence of endoparasites observed among stray cats colonies underscores the sanitary relevance of these animals as hosts of zoonotic pathogens. The detection of parasites with zoonotic potential, namely Toxocara cati, Ancylostomatidae, and Giardia duodenalis, highlights the need for integrated parasite control strategies, with a focus on environmental surveillance and public health. These findings reinforce the relevance of a One Health approach to reduce the risk of parasite transmission and promote safer human–animal–environment interactions.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

LT2.20

Emergent Infectious Diseases and Zoonosis

CHARACTERIZATION OF A NOVEL AFRICAN SWINE FEVER VIRUS (ASFV) MODULATOR OF CYTOKINE RESPONSES

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INTRODUCTION: The African swine fever virus (ASFV) has now become a worldwide threat for which the only realistic control strategy is the development of a vaccine for immediate global use. Due to the acute nature of the infection and the complexity of the protective porcine anti-ASFV response, development of a virus vaccine attenuated by the deletion of virus host evasion gene(s) inhibiting innate immunity presents a practical solution, and which would stimulate both cellular and serological immunity. ASFV exhibits a specific tropism for macrophages, which secrete multiple cytokines controlling inflammation and the subsequent adaptive immune response. Through bioinformatic analysis, we have identified a Src Homology 2 (SH2) domain in the ASFV protein DP146L, a member of the multigene family (MGF) 100. Importantly, SH2 domains interact with phosphotyrosine residues which, through reversible tyrosine phosphorylation of intracellular proteins, play a key role in signal transduction pathways stimulated by extracellular ligands such as growth factors and cytokines.

AIMS: To evaluate the impact of DP146L expression on host signal transduction pathways, to characterise the role of the putative SH2 domain in the viral protein function, and to identify its putative interacting partner(s).

METHODOLOGY: Protein structure homology modelling (Swiss-Model) was used to identify residues exposed at the DP146L putative SH2 domain. DP146L constructs containing mutations in these residues were synthesised and tested for their ability to inhibit TNFα and IFNα signalling using luciferase reporter assays. Identification of DP146L host targets was done by BioID (proximity-dependent biotin identification) using cells transfected with DP146L or a control empty plasmid. Biotinylated proteins were captured by standard affinity methods. Protein identification and quantitation was performed by label free mass spectrometry. Perseus software was used to identify host proteins enriched in the DP146L samples as compared to the negative control samples.

RESULTS: We showed that the DP146L protein inhibits TNFα and IFNα induced signalling in transfected cells, suggesting a role for DP146L as a modulator of host innate immune responses. Surprisingly, none of the mutations in the putative SH2 domain reverted the effect of DP146L. Using BioID, we identified a DEAD-box RNA helicase (DDX) as a possible host target for this viral protein.

CONCLUSIONS: Our data indicates that DP146L inhibits host responses to TNFα and IFNα but does not support a role for an interaction with tyrosine phosphorylated proteins, as predicted from the presence of a SH2 domain, in this inhibition. The interaction with a DDX helicase is being further explored as this family of proteins plays an important role in the host innate antiviral immunity.

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CLAUDIN AND RAB PROTEINS ARE KEY MOLECULAR COMPONENTS INVOLVED IN COCCIDIOSIS RESISTANCE IN PORTUGUESE MERINO SHEEP

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INTRODUCTION: Coccidiosis is a parasitic disease caused by protozoa of the genus Eimeria. Although coccidial infection is often asymptomatic in sheep, clinical and subclinical forms of the disease can result in considerable production losses, mainly among young lambs. Current control of coccidiosis in sheep mainly relies on anticoccidial drugs. However, emergence of drug-resistant strains has reduced treatment efficacy and capacity to control outbreaks. Studies aiming to identify genetic markers for use in selection programs towards increasing genetic resistance to coccidiosis are lacking and have yet to be performed in Portuguese Merino sheep.

AIMS: The purpose of this study was to identify genomic regions associated with resistance to coccidiosis by conducting a genome-wide association study (GWAS) in Portuguese Merino sheep.

METHODOLOGY: From an initial population of 1,022 sheep having known phenotypic characteristics, 206 and 202 distinct animals were genotyped using 50K and 600K Single Nucleotide Polymorphism (SNP) arrays, respectively. Once the 50K array was imputed using the 600K as reference, an association analysis was performed using GCTA for faecal oocyst counts.

RESULTS: We identified 12 SNPs significantly associated with resistance by using a chromosome-wide significance threshold. The significant SNPs were related to Ccser1, Thsd4, Eci1, Tnfrsf12a, Znf200, Chrm3 and Slc20a2 genes. In addition, we identified 80 candidate genes located in the proximity of the significant SNPs using predefined confidence regions (100 Kb upstream and downstream). The GeneMANIA Cytoscape plugin was used to construct a network with the most related genes to the 80 candidate genes. The functional analysis of the network revealed a significant enrichment in in relation to transport vesicles.

CONCLUSIONS: Given the role that extracellular vesicles play in parasite-host interactions, these results suggest existence of reliable markers associated with resistance to coccidiosis. These markers should be explored in future studies to further validate their use in marker assisted selection, with the goal of enhancing sustainability of the breed conservation-management program.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

LT2.22

Emergent Infectious Diseases and Zoonosis

AVIAN ORTHOAVULAVIRUS 1 IN EURASIAN COLLARED DOVES IN THE NORTH OF PORTUGAL

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INTRODUCTION: Newcastle disease (ND) is a highly contagious and economically significant viral disease affecting a broad spectrum of avian species, with particular impact on domestic poultry. The etiological agent, avian orthoavulavirus 1 (AOAV-1), belonging to the Paramyxoviridae family, is responsible for outbreaks that can lead to high morbidity and mortality rates. Within the European Union, and specifically in Portugal, the relevance of Newcastle disease is underscored by its potential to disrupt poultry production, compromise animal welfare, and impose considerable trade restrictions. The strategic importance of Portugal in the European poultry market further accentuates the need for robust surveillance and control measures. Newcatsle disease is of mandatory reporting by the Direção Geral de Alimentação e Veterinária (DGAV) and the World Organization for Animal Health (WOAH) framing it as a public health concern.

AIMS: This study focuses on an outbreak of deaths in eurasian collared doves (Streptopelia decaocto) reported by a wildlife rehabilitation center in northern Portugal with unspecific clinical signs. Affected birds exhibited sudden death as the main clinical manifestation. Necropsy findings revealed notable brain hemorrhage and/or congestion. The aim of this study was to confirm and characterize the etiological agent responsible for the outbreak.

METHODOLOGY: Brain samples were collected from affected eurasian collared doves received by the wildlife rehabilitaion center. Pathological examination revealed non-suppurative encephalitis with neuronal degeneration, accompanied by alterations in the spleen and joints.

Sequence-Independent Single-Primer Amplification (SISPA) was employed to enrich viral nucleic acids and reduce host genetic material prior to analysis by Next Generation Sequencing (NGS) using the Oxford Nanopore platform. Reads were taxonomically classified using Kraken2 against a prebuilt viral refseq database, and sequences assigned to AOAV-1 were subsequently mapped to a reference genome with Minimap2 for confirmation and analysis.

RESULTS: The presence of Avian Orthoavulavirus 1 (AOAV-1) was confirmed, and a partial genome sequence was successfully recovered, with an average sequencing depth of approximately 20× in the region corresponding to the matrix (M) gene.

CONCLUSIONS: Confirmation of AOAV-1 in the sampled eurasian collared doves highlights the relevance of wildlife surveillance for early detection of potential spillover events. Further characterization of key genes, such as the Matrix (M) and Fusion (F) genes, is recommended to enhance understanding of viral pathogenicity and epidemiology.

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SEROPREVALENCE AND RISK FACTORS OF COXIELLA BURNETII EXPOSURE IN PORTUGUESE VETERINARIANS

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INTRODUCTION: Coxiella burnetii, the causative agent of Q fever, poses significant occupational risks to veterinarians due to frequent contact with livestock. Despite its zoonotic potential and environmental resilience, data on seroprevalence and associated risk factors among Portuguese veterinarians remain limited. This study aimed to assess C. burnetii seropositivity and identify protective practices in this high-risk group.

OBJECTIVES: The primary objectives were to (1) compare C. burnetii seroprevalence between veterinarians and a matched control group and (2) evaluate demographic, occupational, and biosecurity factors influencing infection risk.

METHODOLOGY: A cross-sectional study analysed 276 serum samples (92 veterinarians, 184 controls) collected in Portugal in 2024. IgG antibodies were detected via ELISA. Univariate and multivariate logistic regression assessed associations between seropositivity and variables such as geographic region, biosecurity practices (e.g., glove use, isolation of aborting animals), and occupational exposure.

RESULTS: Seroprevalence was significantly higher in veterinarians (33.7%) than controls (17.39%; OR: 2.41, 95% CI: 1.37–4.26, p = 0.0023). Univariate analysis identified elevated risk in the Northern region (p = 0.03), though this was non-significant after adjustment (aOR: 2.10, p = 0.07). Protective measures significantly reduced seropositivity: isolating aborting animals (aOR: 0.35, p = 0.03) and glove use during sample collection (aOR: 0.28, p = 0.009). Including Q fever in abortion diagnostics also lowered risk (aOR: 0.40, p = 0.04).

CONCLUSIONS: Portuguese veterinarians face elevated C. burnetii exposure, underscoring occupational hazards. Adherence to biosecurity measures—particularly PPE use and animal isolation—mitigates infection risk. These findings advocate for strengthened preventive strategies, including targeted training and vaccination programmes, to safeguard veterinary professionals and public health.

EMERGENCE OF CANDIDA (CLAVISPORA) LUSITANIAE IN VETERINARY MEDICINE: A CASE FROM A CANINE EAR CANAL

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INTRODUCTION: Candida (Clavispora) lusitaniae is a rare, emerging non-albicans Candida species capable of causing life-threatening invasive infections, particularly in immunocompromised hosts. It has the potential to spread within hospital environments and is notable for its ability to rapidly develop antifungal drug resistance, including multidrug resistance. While predominantly associated with human infections, its occurrence in veterinary settings remains poorly characterized.

AIMS: This study aimed to characterize yeast isolates recovered from the ear canals of dogs and cats, through molecular methods.

METHODOLOGY: Twenty yeast isolates obtained from the ear canals of dogs and cats, previously identified using phenotypic methods based on macro- and micromorphological characteristics, were subjected to molecular analysis. PCR amplification targeting the D1/D2 region of the 26S rDNA gene was performed, followed by DNA sequencing using the Sanger method to achieve precise genotypic identification. The obtained sequences were compared with reference sequences available in the NCBI database to confirm the identity.

RESULTS: Candida (Clavispora) lusitaniae was detected by genotypic method in two isolates from the same dog.

CONCLUSIONS: This study reports the identification of Candida (Clavispora) lusitaniae from a canine source, highlighting the importance of molecular techniques for accurate yeast identification in veterinary samples. Further studies are needed to assess the clinical significance, and potential zoonotic implications of such findings.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

Emergent Infectious Diseases and Zoonosis

LT2.25

MOLECULAR DETECTION OF MALASSEZIA PACHYDERMATIS FROM THE EXTERNAL EAR CANAL OF DOGS AND CATS

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INTRODUCTION: Malassezia spp. is a normal yeast of the skin and external ear canal microbiota, and it is commonly linked to cases of otitis externa. In humans, Malassezia spp. can cause various skin conditions and systemic infections, including fungemia. Due to their zoonotic potential, Malassezia spp. are of significant concern in public health, as multiple species can infect both animals and humans. AIMS: This study investigated the presence of Malassezia pachydermatis in samples collected from the ears of dogs and cats.

METHODOLOGY: A total of 47 swab samples were obtained from 18 dogs and 29 cats, Sampling was performed using phenotypic identification based on macro- and micro-morphological characters. Polymerase chain reaction (PCR) amplification of D1/D2 region of the 26S rDNAgene, followed by DNA sequencing using Sanger method were employed. The resulting sequences were subjected to comparative analysis against reference sequences deposited in the NCBI to achieve taxonomic classification.

RESULTS: Malassezia pachydermatis was detected in 9 animals (19.2%; 95% CI: 9.2-33.3%). Occurrence in dogs was 38.9% (95% CI: 17.3-64.3%) and in cats it was 6.9% (95% CI: 0.9-22.8%).

CONCLUSIONS: Malassezia pachydermatis was identified in a significant proportion of the sampled animals, particularly in dogs. These findings highlight the importance of recognizing M. pachydermatis as a potential zoonotic agent and emphasize the need for accurate diagnostic methods to monitor and control its spread among companion animals and humans.

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HISTOPLASMA CAPSULATUM IN BAT SPECIES IN PORTUGAL

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INTRODUCTION: Histoplasmosis is a systemic infection caused by *H. capsulatum*, a dimorphic fungus found worldwide. It alternates between a mold phase in the environment, producing microconidia, and a yeast phase within the host, where it survives in macrophages. This dimorphism and cell wall composition are key to its virulence. Based on genetic analysis, it has been classified into four species.

In immunocompetent individuals, infection is often asymptomatic but can be severe in those with compromised immunity, such as HIV/AIDS patients. Symptoms include fever, chest discomfort, and cough. While endemic in the Americas, Africa, and Asia, it is rare in Europe, usually as an imported disease.

Bats are important carriers of *H. capsulatum*, spreading the fungus through their guano. Bats play a crucial role in histoplasmosis epidemiology as both reservoirs and vectors. The disease is contracted by inhaling spores from contaminated environments, leading to outbreaks among those exposed to bat feces.

AIMS: This study is the first to investigate the presence of *H. capsulatum* in guano from bat species in Portugal, addressing the lack of research on Iberian bats despite their known role in the epidemiology of histoplasmosis.

METHODOLOGY: The study collected 285 guano samples from 33 locations in Portugal between 2014 and 2018. Bats were captured using mist nets or harp traps, and guano pellets were gathered and stored at −20°C for later analysis. Bat species were identified visually, and species confirmation was done using DNA extracted from wing punches. The DNA extraction followed a custom protocol, and PCR amplification was performed to detect *H. capsulatum*. The nested PCR used specific primers to amplify a 210 bp gene fragment. Positive control was included using a synthetic oligonucleotide. The PCR products were analyzed by gel electrophoresis and sequenced using the Sanger method, with sequences compared to the NCBI database.

RESULTS: In this study, a total of 285 stool samples were collected between 2024 and 2018 from bats in seven regions of Portugal. From the visual identification followed by COI DNA barcode analysis, it was determined that bats belonged to 22 species from four families (Vespertilionidae, Rhinolophidae, Molossidae, and Miniopteridae). In the screening of the total 285 guano samples for *H. capsulatum* by nested PCR, none were shown to be positive.

CONCLUSIONS: This study found no *H. capsulatum* in 285 bat guano samples from Portugal, possibly due to low pathogen levels or PCR limitations. These results align with the rarity of detection in Europe, while tropical regions report higher prevalence. Further research with more sensitive methods is needed, and monitoring cave ecosystems could help detect future outbreaks.

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DETECTION OF ANTIBIOTIC-RESISTANT ENTEROCOCCUS SPP. IN CHICKENS FOR CONSUMPTION: IMPLICATIONS FOR FOOD SAFETY

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INTRODUCTION: Chicken meat is one of the most widely consumed foods globally. To meet demand, poultry production often employs antibiotics, contributing to the selection of antibiotic-resistant bacteria, especially in the intestinal microbiota of poultry. In food microbiology, Enterococcus spp. are commonly used as indicators of antimicrobial resistance due to their role as intestinal commensals and their potential for transmission to humans through the handling or consumption of contaminated poultry. The presence of these resistant microorganisms in chicken meat compromises food safety, highlighting the need for enhanced control and surveillance measures across the production chain.

AIMS: This study aimed to isolate Enterococcus spp. from broiler chickens and evaluate their antimicrobial resistance profiles to assess the potential public health risks associated with contaminated poultry meat.

METHODOLOGY: A total of 170 samples were obtained from the cloaca of chickens intended for human consumption. Enterococcus spp. were isolated on Slanetz-Bartley agar, and antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method, according to CLSI guidelines (2024). The isolates were tested against ampicillin, vancomycin, teicoplanin, erythromycin, chloramphenicol, linezolid, quinupristin-dalfopristin, imipenem, tetracycline, and ciprofloxacin.

RESULTS: Of the 170 samples, 86 were identified as Enterococcus spp. Among these, 88% were resistant to tetracycline, 65% to erythromycin, 31% to imipenem, 30% to ciprofloxacin, 24% to quinupristin-dalfopristin, and 1% each to chloramphenicol and vancomycin.

CONCLUSIONS: Chickens destined for human consumption are a significant source of Enterococcus spp. resistant to multiple antibiotics. These results highlight the importance of closely monitoring antibiotic use in poultry production and adopting precautionary measures when handling and consuming chicken meat. Effective strategies are urgently needed to control the spread of antimicrobial resistance and safeguard public health and food safety.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

Emergent Infectious Diseases and Zoonosis

LT2.28

EVALUATION OF LEPTOSPIRA SPP. IN HEALTHY HORSES: RISK FACTORS AND MANAGEMENT PRACTICES IN LISBON AREA, PORTUGAL

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INTRODUCTION: Leptospirosis is a bacterial disease caused by spirochetes belonging to the genus Leptospira. It is recognized by the World Health Organization (WHO) as a globally important zoonosis. In addition to humans, it also affects domestic animals, primarily through contact with the urine of infected animals, such as rodents, or contaminated environments.

In horses, Leptospira spp. infections are predominantly asymptomatic; however, clinical cases involving recurrent uveitis and renal disease have been reported.

AIMS: This study aimed to evaluate the presence of Leptospira spp. in clinically healthy horses and identify the risk factors associated with infection, including management practices, exposure to stagnant water, types of water sources, and rodent control.

METHOD: Between 2022-2023, blood samples were collected from 34 clinically healthy horses in the Lisbon and Santarém regions. Detection of Leptospira spp. was performed by qPCR. In addition, a questionnaire was performed to identify potential risk factors for leptospirosis, including disease awareness, vaccination status, rodent control, and recent clinical signs. Horse owners were also asked about exposure to stagnant water, water sources, and feed storage conditions.

Ethical approval for this study was granted by the Ethics and Animal Welfare Committee of the Faculty of Veterinary Medicine, Lusófona University - Lisbon University Centre.

RESULTS: The majority of horse owners were aware of the existence of leptospirosis; however, only one owner recognized it as a zoonotic disease. Regarding vaccination, none of the horses in this study had been vaccinated against Leptospira spp. As for housing, 52.9% (n=19/34) of the horses were kept in paddocks. Concerning exposure to stagnant water, 88.2% (n=30/34) of the horses had no access to ponds or lakes. For their water supply, 55.9% (n=19/34) of the horses drank from automatic drinkers, while 44.1% (n=15/34) used buckets. Feed storage practices varied, with 52.9% (n=18/34) of the horses having feed stored in enclosed facilities, while 47.1% (n=16/34) had feed stored in exposed areas. Notably, there was no reported contact with rodents or wildlife in any of the facilities. Additionally, 88.2% (n=30/34) of the facilities had rodent control measures implemented. Recurrent uveitis was reported in 11.8% (n=4/34), with 25% (n=1/4) affected in the right eye, 25% (n=1/4) in the left eye, and 50% (n=2/4) in both eyes.

Leptospira spp. was not detected in any of the blood samples from the horses included in this study.

CONCLUSIONS: This study found that measures such as limiting access to stagnant water, using rodent control methods, and storing feed in closed facilities were already implemented on most of horse establishments visited, reducing the risk of Leptospira spp. in horses. However, knowledge about the zoonotic nature of the disease was almost absent. Therefore, it is crucial to raise awareness among owners, caretakers, and handlers about the associated health risks and the precautions necessary when handling infected animals.

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MICROBIAL CONTAMINATION ASSESSMENT OF DIFFERENT FOOD CONTACT SURFACES IN UNIVERSITY CANTEENS AND CAFES

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INTRODUCTION: Food safety and ensuring the hygienic conditions of food contact surfaces is essential to prevent food-borne illnesses, especially in collective catering environments such as university canteens and cafés.

AIMS: This study assessed the microbial contamination on food contact surfaces in four university canteens and seven cafes. Seven distinct zones were examined: kitchen, pastry, food preparation, refrigeration, non-food preparation, self-service, and dining room.

METHODOLOGY: The analysis focused on mesophilic microorganisms and Enterobacteriaceae on countertops, and mesophilic microorganisms, Enterobacteriaceae, Staphylococcus aureus, and Escherichia coli on food handlers' hands, following ISO standard methods.

RESULTS: Variations in microbial contamination were observed across different surfaces. Canteens exhibited higher contamination levels for mesophilic microorganisms (1.91 log CFU/cm²) compared to cafes (1.36 log CFU/cm²), showing a difference of 0.55 log CFU/cm². Countertops had an average of 1.81 log CFU/cm², slightly lower than food handlers' hands, which averaged 1.97 log CFU/cm². Enterobacteriaceae contamination levels were higher on the hands with an average of 0.68 log CFU/cm², the left hand showing greater contamination (0.81 log CFU/cm²) than the right hand (0.55 log CFU/cm). Countertops averaged 0.58 log CFU/cm² for Enterobacteriaceae. For S. aureus, contamination levels were 0.39 log CFU/cm² on the right hand and 0.32 log CFU/cm² on the left, with an average of 0.36 log CFU/cm².

No significative differences were observed between zones, types of establishments, or surface types regarding microbial contamination levels.

CONCLUSION: The results indicate that food handlers' hands exhibited higher contamination levels than countertops in both canteens and university cafes. Canteens showed greater contamination than cafes, and the variability across zones highlights the need for enhanced hygiene practices, particularly in high-risk areas such as food handling.

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SUSCEPTIBILITY OF SALMONELLA ISOLATED FROM SLAUGHTERED PIGS TO A QUATERNARY AMMONIUM COMPOUND BIOCIDE

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INTRODUCTION: Salmonella is one of the major foodborne pathogens causing gastro-intestinal infections in humans and pork is among its main food vehicles. Abattoirs represent a crucial stage for its control, namely by implementing good hygiene practices and effective cleaning and disinfection programmes. However, decreased susceptibility to biocides can lead to Salmonella environmental persistence.

AIMS: The present study aimed to assess the resistance of Salmonella isolated from pigs slaughtered in a Portuguese abattoir to a commercially available Quaternary Ammonium Compound (QAC) biocide (Suma Bac D10®) used in the agri-food industry.

METHODOLOGY: Forty-four Salmonella isolates previously characterized were studied for the presence of quaternary ammonium compound (QAC) biocide resistance genes (qacEΔ1, qacE and qacF/H/I complex) by PCR. The susceptibility to different concentrations of the QAC biocide, namely to working concentrations according to the manufacturers' instructions, was evaluated at three levels of organic matter (absent, low and high), according to the EN 1656:2009, using 12 selected isolates. Efflux pump activity was studied using the Ethidium Bromide-Agar cartwheel method, by exposing the isolates to different Ethidium Bromide concentrations.

RESULTS: The presence of the evaluated resistance genes was diverse among the isolates included in the study. Of the 44 isolates, 7 (15.6%) harboured both qacEΔ1 and the qacF/H/I complex simultaneously, 7 (15.6%) only qacEΔ1, 6 (13.3%) only qacF/H/I complex and none the qacE gene. The differences in the combination of resistance genes detected among isolates were also present in those belonging to the same serotype. Regarding the susceptibility assay for the QAC biocide, the Minimum inhibitory Concentration (MIC) obtained toward all the 12 selected isolates was the lowest of the concentration tested (0.100 %), in the assays with no organic matter, with low level of organic matter and with a high level of organic matter. Additionally, this concentration (0.100 %) had a bactericidal effect, also corresponding to the Minimum Bactericidal Concentration (MBC). The evaluation of the efflux pump activity did not reveal function increase in any of the 12 isolates at none of the Ethidium Bromide concentrations tested, when compared with the assay controls.

CONCLUSIONS: Despite the presence of some QAC resistance genes, the isolates' susceptibility to the QAC biocide did not seem to be affected, with a registered bactericidal concentration, in in vitro conditions, 10x lower than the concentration indicated by the manufacturer, and which was not influenced by the presence of organic matter.

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MOLECULAR INSIGHTS INTO ASPERGILLUS SPP. IN DOMESTIC AND WILD MAMMALS

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INTRODUCTION: Aspergillus spp. are opportunistic fungi with significant implications in the One Health approach, affecting human, animal, and environmental health.

AIMS: To investigate the occurrence of Aspergillus spp. in different mammalian species using conventional and molecular techniques, and to assess the genetic diversity among isolates through PCR-based methods, contributing to a better understanding of their epidemiology within the One Health framework.

METHODOLOGY: Samples were collected from 147 animals, including a mara (Dolichotis patagonum), a panthera (Panthera pardus), 53 foxes (Vulpes vulpes), 73 dogs (Canis lupus familiaris), 19 Egyptian mongooses (Herpestes ichneumon). The Mackenzie technique was used for fur sampling, and specimens were processed according to routine methodologies. Ten Aspergillus spp. isolates previously obtained from dogs were analyzed through molecular techniques. DNA was extracted from all isolates and subjected to PCR amplification targeting the β-tubulin gene using primers BT2a and BT2b. The amplified products were then analyzed by PCR–RFLP. Additionally, genetic diversity was assessed using PCR-ISSR with eight different primers. A presence/absence matrix was constructed based on the resulting banding patterns, and a dendrogram was generated to evaluate the genetic relationships among the isolates.

RESULTS: In foxes, Aspergillus was isolated in 30.2% (16/53) of the samples. No Aspergillus isolates were detected in Egyptian mongooses (0/19). In dogs, the occurrence was 24.7% (18/73). Aspergillus was also isolated in the samples from both the mara (1/1) and the leopard (1/1). PCR–ISSR analysis with eight primers revealed a polymorphism percentage of 91.11%, with three primers (UBC 835, UBC 864, and UBC 889) exhibiting 100% of polymorphism.

CONCLUSIONS: The results highlight the occurrence of Aspergillus across diverse mammalian species and reveal a high level of genetic diversity among isolates. These findings reinforce the relevance of continuous monitoring and molecular characterization of Aspergillus spp., contributing to a better understanding of their epidemiological dynamics within the One Health context.

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VIRAL PATHOGEN DETECTION IN BÍSARO PIGS AND WILD BOARS THROUGH MOLECULAR SCREENING

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INTRODUCTION: Zoonotic and infectious diseases represent a central topic in the One Health concept. Pigs are important reservoirs and intermediate hosts of pathogenic microorganisms, including zoonotic viruses. Swine outdoor farming systems are a perfect setting for pathogens circulation due to the potential for interspecies communication and environmental proximity with wild animals, representing a potential pathway for respiratory infection transmission to domestic pigs.

AIMS: Our main goal was to evaluate the presence of Influenza A and B virus, Porcine Respiratory Coronavirus, Japanese Encephalitis Virus, Porcine Reproductive and Respiratory Syndrome Virus, Porcine Circovirus and Pseudorabies Virus, in nasal samples collected from domestic pigs bred in outdoor systems (Bísaro breed) and in wild boars.

METHODOLOGY: Nasal samples from Bísaro pigs (BP) and wild boars (WB) were collected using sterile swabs (VTM®). Nucleic acids were extracted from each swab using a commercial kit from MGI, and their concentration and purity were assessed using Qubit™ High Sensitivity assays and NanoDrop™ spectrophotometry. Tetraplex RT-qPCR assay for detection of Pseudorabies virus, Porcine Circovirus, Porcine Reproductive and Respiratory Syndrome Virus was optimized and validated for sensitivity and specificity. Individual qPCR protocols were performed for detection of Influenza, Coronavirus and Japanese Encephalitis Virus.

RESULTS: A total of 62 nasal swabs were collected, 30 from BP and 32 from WB. Each RT-qPCR assay demonstrated 100% specificity and was sensitive to as few as 10 genome copies/µL for each viral target. All samples were tested in triplicate and yielded negative results for all viruses screened.

CONCLUSIONS: Several pathogens can circulate between animals and humans, posing a significant threat to global health. Viruses, in particular, have historically been associated with multiple pandemics, highlighting the importance of viral surveillance in animal populations. Although no relevant viruses were detected in the sampled animals, continuous surveillance remains crucial for identifying emerging viral threats and enhancing biosecurity measures in outdoor farming systems.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

Emergent Infectious Diseases and Zoonosis

LT2.34

BEHIND THE SNEEZE: ARE FELINE RESPIRATORY VIRUSES HIDING DEEPER INFECTIONS?

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INTRODUCTION: Feline respiratory viruses, including Feline Calicivirus (FCV) and Feline Herpesvirus (FHV), are common causes of upper respiratory disease in cats. Retroviral infections such as Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV), due to their immunosuppressive effects, may predispose infected cats to secondary viral infections.

AIMS: This study investigates whether cats diagnosed with FCV or FHV are more likely to be concurrently infected with FIV or FeLV.

METHODOLOGY: Biological samples were collected from 150 cats admitted to the Biological Isolation and Containment Unit of the Teaching Hospital, Faculty of Veterinary Medicine, University of Lisbon. Oral and conjunctival swabs were tested for FCV and FHV using multiplex real-time PCR, while retroviral status was determined by ELISA (Viracheck® and Vetline®). Associations between respiratory and retroviral infections were assessed using Chi-squared tests.

RESULTS: Twenty cats were FCV-positive, 18 were FHV-positive, 25 were FIV-positive, and 28 were FeLV-positive. Retroviral results were unavailable for a small number of cases (FeLV: 4; FIV: 6). Chi-squared analysis revealed a statistically significant association between FCV and FIV infection ($p = 0.0002$), suggesting that FCV-positive cats are more likely to be FIV-positive. A borderline association was observed between FCV and FeLV ($p = 0.0503$). No significant associations were identified between FHV and retroviral infections.

CONCLUSIONS: These findings raise the possibility that FCV infection—particularly in hospitalized cats—may signal underlying retroviral infection. Screening for FIV and FeLV in cats with respiratory signs may support earlier diagnosis and guide more effective clinical management.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT2

Emergent Infectious Diseases and Zoonosis

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CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN BACTERIAL ISOLATES FROM AURICULAR SWABS OF COMPANION ANIMALS (2023–2024)

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INTRODUCTION: Antimicrobial resistance (AMR) is an increasing threat to both animal and human health and is a key element of the One Health concept. Dogs and cats can serve as reservoirs for resistant bacteria with zoonotic potential. Otitis externa is a common condition in veterinary practice and is often associated with opportunistic pathogens such as Staphylococcus pseudointermedius, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.

AIMS: To characterize bacterial agents isolated from ear swabs of companion animals and to determine their antimicrobial resistance profiles over a two-year period (2023–2024).

METHODOLOGY: Fifty-eight bacterial isolates were obtained from dogs and cats presenting clinical signs of otitis externa. Samples were collected at the Santarém Veterinary Hospital using ear swabs and processed by a veterinary diagnostic laboratory. Bacterial identification was performed using conventional microbiological methods. Antimicrobial susceptibility testing was conducted using the VITEK automated system.

RESULTS: S. pseudointermedius was the most frequently isolated species, followed by S. aureus, P. aeruginosa, E. coli, Streptococcus canis, and Bordetella bronchiseptica. P. aeruginosa isolates showed high resistance to multiple β-lactams, including cefovecin and ceftiofur, as well as fluoroquinolones. S. pseudointermedius strains exhibited resistance to clindamycin, doxycycline, and trimethoprim-sulfamethoxazole. Multidrug resistance (MDR) profiles were identified in several strains, particularly in P. aeruginosa and S. pseudointermedius, with some isolates resistant to more than 10 antibiotics. Differences in resistance patterns were observed between the two years and among animal species.

CONCLUSIONS: These findings emphasize the importance of a diagnostic-based clinical approach, including culture and susceptibility testing, in the treatment of otitis infections in companion animals. The high prevalence of multidrug-resistant isolates raises concerns about the potential failure of commonly used antimicrobial therapies. Implementing routine microbiological diagnostics and regularly reviewing empirical treatment protocols are essential steps toward ensuring therapeutic efficacy and limiting the spread of antimicrobial resistance in veterinary settings.

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ISOLATION OF CLADOSPORIUM SPP. FROM THE NASAL MUCOSA OF HUMANS AND THEIR DOGS – A ONE HEALTH APPROACH

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INTRODUCTION: Cladosporium spp. are commonly encountered on all kinds of plant, fungal, and other debris, and they are often isolated from soil, food, paint, textiles, and from the air. This genus has been associated with allergic rhinitis, localized lesions, disseminated infections, subcutaneous keratomycosis, hypersensitivity pneumonitis, pulmonary fungus ball, intrabronchial lesions, and hemorrhagic pneumonia.

AIMS: The aim of this study was the identification and characterization of Cladosporium spp. isolated from the nasal mucous membrane of humans and pets in a way to establish a relationship.

METHODOLOGY: Samples were collected with a swab from the nasal mucosa of 46 human volunteers and 13 dogs, after Ethical approval. Routine microbiological culturing techniques were used for fungal isolation. Morphological identification was carried out based on macroscopic and microscopic characteristics. Molecular identification was performed on selected isolates through DNA extraction, followed by PCR amplification and sequencing of specific genetic markers.

RESULTS: From a total of 59 nasal mucosa samples, 380 fungal isolates were obtained, comprising clinically relevant molds. Cladosporium spp. were the most frequently isolated fungi, detected in 73.9% of human samples and 61.5% of dog samples. In this study, the occurrence of Cladosporium spp. growth was higher in individuals who claimed not to have allergies compared to those who claimed they had allergies ($p = 0.014$).

CONCLUSIONS: The high prevalence of Cladosporium spp. in human and dog nasal samples suggests a shared environmental exposure and highlights the potential role of this genus in colonization processes of the nasal mucosa. Molecular identification techniques proved essential to confirm morphological findings and may contribute to a better understanding of the epidemiology of fungal colonization in different hosts.

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LT2

Emergent Infectious Diseases and Zoonosis

LT2.37

PREVALENCE OF ANTIMICROBIAL RESISTANCE GENES IN SMALL RUMINANT FARMS FROM CENTRAL PORTUGAL

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INTRODUCTION: Antimicrobial resistance (AMR) poses a major health threat, with small ruminant farms potentially acting as ARG reservoirs. Data from Portugal's extensive farming systems are limited.

AIM: Assess the occurrence and diversity of ARGs in sheep and goat farms in central Portugal, focusing on tetracyclines, β-lactams, macrolides, and sulphonamides.

METHODS: Environmental samples (n=65) from 14 central Portugal municipalities were analysed via SYBR Green qPCR for 10 ARGs after DNA extraction.

RESULTS: ARGs were found in 83.1% of samples; 55.6% had ≥3 ARG classes. β-lactamases were most common, followed by sulphonamides, tetracyclines and finally macrolides

CONCLUSION: Central Portugal's small ruminant farms are potential significant ARG reservoirs, underscoring the need for targeted monitoring in extensive farming systems.

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LT2

Emergent Infectious Diseases and Zoonosis

LT2.38

MULTIDRUG-RESISTANCE OF STAPHYLOCOCCUS AUREUS ISOLATED FROM FRESH POULTRY MEATS

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INTRODUCTION: Antimicrobial resistance is a growing public health concern, with an increasing number of bacterial strains showing multidrug-resistant profiles. Staphylococcus aureus is a foodborne pathogen that can contaminate meat and meat products, potentially leading to foodborne outbreaks. In Portugal, S. aureus toxins are the primary cause of concern, occurring mainly in canteens, workplaces, and schools.

AIMS: This study aims to provide an overview of multidrug-resistant S. aureus isolated from fresh meat poultry samples collected from retail markets and supermarkets in Portugal.

METHODOLOGY: Ten grams of each sample were weighed and mixed with 90 ml of Chapman broth and homogenized for 60 seconds in a stomacher (Lab Blender, UK). Following incubation, 0.1 ml of the appropriate dilution was plated onto Baird-Parker agar (BPA) (Biolife, Monza, Italy) supplemented with egg yolk tellurite emulsion (VWR, Leuven, Belgium). The plates were incubated aerobically at 37°C for 24-48 hours. The confirmation of coagulase-positive Staphylococci strains was carried out using the coagulase test with rabbit plasma and fibrinogen. Presumptive S. aureus were confirmed by the automated VITEK® 2, and antimicrobial resistance was determined using the Kirby–Bauer disk diffusion method.

RESULTS: Out of 40.3% of presumptive Staphylococcus aureus isolates, 34.7% were confirmed as coagulase-positive S. aureus. Among these, 18.1% exhibited a multidrug-resistance profile, with 4.2% showing resistance to at least four different classes of antibiotics (penicillins, tetracyclines, macrolides and lincosamides).

CONCLUSIONS: A high frequency of S. aureus (34.7%) was detected in fresh poultry meat intended for human consumption, also showing a diverse range of antimicrobial resistance. These findings suggest that livestock animals may serve as a significant reservoir of antimicrobial resistance genes. Ongoing surveillance of S. aureus strains in livestock is crucial for assessing the public health risks associated with this foodborne pathogen and for tracking the evolution of its antimicrobial resistance profile.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT3

Comparative and Translational Medicine and Biotechnology

LT3.30

ISOLATION, CULTURE, AND REGENERATION ASSESSMENT OF PORCINE CORNEAS IN AN EX VIVO MODEL FOR OCULAR WOUND HEALING

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INTRODUCTION: Corneal diseases represent a prevalent clinical concern in veterinary ophthalmology. Despite advances in current therapies, severe corneal ulcers remain challenging to treat, highlighting the urgent need for innovative and effective therapies. A cornea ex vivo model intends to mimic in vivo conditions, allowing studies of cellular behaviour during wound healing, drug efficacy and toxicology. Porcine corneas are particularly useful due to their anatomical similarity to human corneas, ease acquisition from slaughterhouses, and the potential to isolate multiple ocular cell types, rendering them an important study model.

AIMS: In this study, we assessed an ex vivo porcine cornea culture model to evaluate the regeneration of corneal defects in culture medium supplemented with FBS and dextran under controlled conditions.

METHODOLOGY: Fresh pig eyeballs (n=6) obtained from the slaughterhouse (Seara S.A.) were, immediately after animal bleeding, enucleated and transported in individual containers to ICBAS-UP. All extraocular muscles, connective tissue and adipose tissue were removed. The eyeballs were decontaminated and abundantly rinsed. A 4 mm biopsy punch was used to create a central stromal corneal defect. The corneal defect was further dissected and removed. The cornea was then excised using an 8 mm biopsy punch and placed in a 12-well plate, divided into three groups: group A) DMEM with 10% FBS, group B) DMEM with 6% dextran, and group C) DMEM with 6% dextran and 10% FBS. The corneas were cultured at 37°C with 5% of CO2 for 14 days, fully immersed, in 1 mL of culture medium. Corneal regeneration was assessed by measuring the ulcer area at various time points over 14 days, photographing them using a stereomicroscope and ImageJ to quantify the wound area. This approach had the goal of calculating the re-epithelialization rate.

RESULTS: The defect edges of the regenerating epithelium surface were visible, and wound healing was observed over 14 days, with the ulcer size decreasing in all groups. After 14 days the wound size varied between groups. Group A showed a complete regeneration, with a wound healing rate (WHR) of 0,041 mm2/h. Group B showed partial healing, with a WHR of 0,035 mm2/h. Group C exhibited the slowest healing, with a WHR of 0,006 mm2/h. The highest healing rates were observed within the first 48h of the trial, a decline being observed over time, with rates slowing by days 8 to 14.

CONCLUSIONS: Groups A and B exhibited faster wound healing rates, as well as better wound resolution after 14 days, compared to group C. Due to the exploratory nature of this technique, the small number of corneas used did not allow for an in-depth statistical treatment of the data obtained, and as such, the existence of statistical differences between the groups was not confirmed. Future improvements could include methods to fixate the corneas to prevent damage and movement, facilitating better observation and evaluation of ulcer margins surface. A greater number of samples in each group may help confirm the presence of effective statistical differences.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT3

Comparative and Translational Medicine and Biotechnology

LT3.31

ALDICARB POISONING IN ANIMALS: CONCERNS ABOUT INCREASING OF POSITIVE CASES IDENTIFIED IN A VETERINARY TOXICOLOGY LABORATORY

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INTRODUCTION: Aldicarb, previously used as a pesticide, has been banned in Portugal since 2007. The substance was prohibited due to its high toxicity and significant risks to human and animal health. Despite the ban, cases of aldicarb poisoning - commonly known as "chumbinho" - are still reported. Poisoning in wild and domestic animals is a growing concern, posing serious risks to animal health and public safety.

AIMS: The purpose of this study is to analyze the increase in aldicarb poisoning among wild and domestic animals, based on all the suspected poisoning cases received by the Laboratory of Pharmacology and Toxicology of FMV-ULisboa (LFT-FMV-ULisboa) over the past years.

METHODOLOGY: A retrospective review of suspected poisoning cases from 2014 to 2025 analyzed by the Laboratory of Pharmacology and Toxicology of FMV-ULisboa was conducted. Biological samples, mainly including gastric content and liver, from suspected poisoning cases from the southern regions of Portugal were analyzed. Thin-layer chromatography (TLC) was performed using standard procedures and specific reagents for carbamate detection.

RESULTS: From 2014 to 2025, a total of 314 cases were identified as positive for toxic substances, including 66 from wild animals, 146 from domestic animals, and 90 from baits. Among those 314 cases, the presence of aldicarb was identified in 56, indicating it as the probable cause of poisoning. In the current year, the presence of aldicarb has already been reported in 12 cases, all of which involved wild animals such as buzzards, except for one case in a dog. Several baits containing aldicarb were also identified, highlighting a significant increase in cases. Furthermore, these cases have consistently been reported in the southern regions of Portugal, particularly in Alentejo and Algarve.

CONCLUSIONS: Since aldicarb has been banned in Portugal for many years, the recent increase in positive cases has been unexpected. It is crucial to investigate how and where aldicarb is still being accessed in order to implement preventive measures and reduce the incidence of poisoning cases. The rise in aldicarb poisoning cases demonstrates the urgent need for stricter control over the illegal distribution and use of toxic pesticides.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT3

Comparative and Translational Medicine and Biotechnology

LT3.32

IN VITRO ASSESSMENT OF HUMAN DENTAL PULP STEM CELLS ON CHITOSAN-BASED NERVE CONDUITS

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INTRODUCTION: Peripheral nerve injuries (PNI) are prevalent clinical challenges due to the limited regenerative capacity of the peripheral nervous system, particularly in extensive lesions. Human dental pulp stem cells (hDPSCs) and their conditioned medium (CM) have emerged as promising therapeutic approaches owing to their neuroregenerative properties. Developing bioengineered solutions, such as chitosan-based nerve conduits (Reaxon®), combined with stem cell therapy, could provide effective alternatives for nerve repair in both human and veterinary medicine, within the One Health framework.

AIMS: This study aimed to evaluate the cytocompatibility of hDPSCs with chitosan nerve conduits and to characterize the biological profile of the hDPSC-conditioned medium for potential application in peripheral nerve regeneration.

METHODOLOGY: hDPSCs were isolated and cultured, and CM was collected at passages P4 and P7. The CM was analyzed for the presence of 48 cytokines using Luminex multiplex technology. Cytocompatibility between hDPSCs and Reaxon® nerve conduits was assessed through viability assays (PrestoBlue™), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS). Additionally, the neurogenic differentiation potential of hDPSCs was evaluated by inducing differentiation and observing morphological changes characteristic of neuronal lineage.

RESULTS: Analysis of the CM revealed 30 detectable biomarkers. CM collected at passage P4 showed higher concentrations of anti-inflammatory and neurotrophic factors, while passage P7 CM exhibited increased levels of immune recruitment markers. Cytocompatibility assays confirmed that Reaxon® conduits supported hDPSC viability and proliferation without cytotoxic effects, according to ISO standards. SEM and EDS analyses demonstrated successful adhesion and proliferation of hDPSCs on the conduit surface. Neurogenic differentiation assays showed morphological changes compatible with neuronal lineage commitment, supporting the therapeutic potential of hDPSCs.

CONCLUSIONS: hDPSCs demonstrated excellent cytocompatibility with chitosan nerve conduits and exhibited neurogenic differentiation capabilities. The CM, particularly from early passages, displayed a favorable biomarker profile for nerve regeneration. These results reinforce the potential of combining hDPSCs and bioengineered conduits as a novel regenerative therapy for peripheral nerve injuries, promoting translational applications across human and veterinary medicine under a One Health approach.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

LT3

Comparative and Translational Medicine and Biotechnology

LT3.33

THE IMPACT OF IONS ON HUMAN BONE MARROW MESENCHYMAL STEM CELL DIFFERENTIANTION AND THE ACTIVITY OF MONOCYTE-DERIVED OSTEOCLASTS.

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INTRODUCTION: Bone remodelling is a dynamic and tightly regulated process involving osteoblast-mediated bone formation and osteoclast-driven bone resorption. This process is influenced by various factors, including the cellular microenvironment and the presence of specific ions such as Ca^{2+}, Mg^{2+}, Na^{+}, F^{-}, and Si^{4+}.

AIM: Assess the effect of ion concentration on human bone marrow-derived mesenchymal stem cells (MSCs) differentiation and human monocyte-derived osteoclasts the activity.

METHODOLOGY: Bone marrow (BM) aspirate was collected during orthopaedic surgery from a 50-year-old patient at Addenbrooke's Hospital, following all ethical guidelines and informed consent procedures. Mononuclear cells were isolated using Histopaque®, and resuspended in culture medium consisting of α-MEM supplemented with 20% (v/v) foetal calf serum, 1% (v/v) L-glutamine-penicillin-streptomycin, and 30 µg/mL vitamin C. Bone-derived cells were obtained from the femoral head of a 65-year-old patient undergoing orthopaedic surgery at the same hospital, also under approved ethical procedures. Trabecular bone spicules were cut, washed in PBS, and subjected to an enzymatic digestion process. The resulting pellet was resuspended in McCoy's medium supplemented with 10% (v/v) Human AB Serum, 1% (v/v) L-glutamine-penicillin-streptomycin, and 30 µg/mL vitamin C. Cells were seeded and cultured overnight in full medium before being exposed to the different ionic compounds. After 7 days, MSC cultures were immunolabeled for STRO-1 (early osteoblast marker) and alkaline phosphatase (ALP, a marker of mature osteoblasts). Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of healthy male and female donors, as previously described. The cells were seeded onto BD Biocoat™ Osteologic™ Multitest slides and, after 6 days, exposed to the soluble ionic compounds. At days 7 and 12, the culture medium was removed, and calcium phosphate resorption was visualized using the Von Kossa staining method.

RESULTS: Low concentrations of Ca^{2+} and Mg^{2+} promoted MSC differentiation along the osteoblast lineage. NaF and NaCl, used as ionic sources, did not significantly suppressed osteoclast activity. $Si(OH)_4$ (as a source of Si^{4+}) showed a dual, concentration-dependent effect: inhibiting osteoclast activity at lower concentrations while enhancing osteoblast differentiation at higher levels. High concentrations of NaF and NaCl were inhibitory to both osteoblast and osteoclast function, underlining the critical role of ion dosage in the cellular microenvironment.

CONCLUSIONS: These findings highlight the potential of modulating ion concentrations as a strategy to influence bone remodelling. This has significant implications for the development of therapeutic biomaterials in regenerative medicine. Tailoring the ion release profiles of such biomaterials could aid in the management of bone-related conditions including osteoporosis, fractures, and bone defects.

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LT3

Comparative and Translational Medicine and Biotechnology

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APPLICATION OF ALLOGENEIC SYNOVIAL MEMBRANE MESENCHYMAL STEM CELLS IN DOGS WITH SEVERE OSTEOARTHRITIS

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease, common in human and veterinary medicine, which is based on complex mechanisms of interaction between the environment and catabolic injury, leading to pain and motor disability. OA presents clinical signs in 80% of geriatric dogs and all therapeutic approaches aim to maintain range of motion (ROM) and slow disease progression.

AIMS: This prospective observational pilot study aims to verify the tolerability, efficacy, repeatability and safety of a functional rehabilitation multimodal protocol with regenerative medicine through cell-based therapies administration (Regenera® Combicell).

METHODOLOGY: Eight dogs aged 18 years or older, weighing 55 kg or more and body condition 5 (1-9) were admitted, regardless of sex and breed. All had severe chronic pain (grade 3 or more), grade 5 lameness, radiographic examination classified as grade 3 or higher, and osteophytosis of grade 2 or more. At admission (T0), 10 million allogeneic synovial membrane mesenchymal stem cells were administered intra-articularly, followed by shock waves (E6, 600-1000 pulses/joint) and class IV laser therapy (940 nm, 9-12 J, 5 days). All were evaluated according to the University of Colorado Pain Scale, Lameness Scale, ROM and muscle mass measurements at T0, T1 (7d), T2 (14d), F1 (30d), F2(90d), F3 (180d), F4 (270d) and F5 (365d).

RESULTS: The weight distribution had a mean of 35.63 kg and a mean age of 9.5 years. 75% (6/8) were females and of these, 33.3% were German Shepherds (2/6). Also 75% (6/8) had OA in the hip joint with 100% (8/8) of severe pain at T0. This pain assessment revealed a decrease in pain level, particularly in F5 (365d), with only one dog experiencing mild pain. All dogs showed severe lameness at T0, which decreased to mild lameness at F5 (365 days) in 4 dogs. Regarding the ROM measurements in the hip and knee joints, there was a clear improvement from T0 to T1. Muscle mass was maintained until T2, with improvement throughout the study. No systemic nor local adverse symptoms were observed after the cell-based therapies nor during the follow-up period. No side effects were observed during the study, such as infection or skin allergies. Our results are in agreement with, confirming the effects of stem cells for at least 1 year. Regarding the safety of intra-articular administration of allogeneic mesenchymal stem cells, it is also in agreement with different authors. The prolonged effect of this multimodal protocol promoting functional capacity is the same as that practiced in human medicine.

CONCLUSIONS: This multimodal protocol was effective, tolerable, repeatable and safe, showing a positive solution with long-term effects of 1 year, allowing improvements in pain and lameness scores. Further studies are needed in a clinical setting.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

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EFFECTS OF NOTCH AND WNT BLOCKADES AT THE EARLY EMBRYONIC STAGE ON LATE PLACENTA

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INTRODUCTION: Embryonic development depends on cellular proliferation and differentiation controlled by a network of signaling pathways, including Notch and Wnt. Previous work evidenced that early embryonic Notch and/or Wnt blockades imbalance blastocyst cell populations and affect fetal weight, with Notch blockade producing lighter foetuses compared to Wnt blockade. Stereological analysis of the placentas showed that Notch and Wnt signaling blockade at the first cellular differentiation affected also affected placental development.

AIMS: Our objective was to evaluate if Notch and/or Wnt blockades at an early embryonic development (blastocysts) affected gene transcription of members of the Notch pathway in the resulting late placenta. The influence of Notch and/or Wnt blockades in blastocysts in genes transcription of key development and metabolic pathways that where shown to be involved in placental function and deregulated in placental diseases, were also assessed.

METHODOLOGY: Embryos (8-16 cells) were in vitro cultured for 1.5 days with Notch (DAPT) and/or Wnt (DKK1) signaling inhibitors and transferred to pseudo-pregnant females. Eighteen-day old hemi-placentas were collected, and after RNA extraction, qRT-PCR was used to assess relative transcription of Notch pathway members (Notch1, Notch2, Notch3, Dll4, Jag1, Jag2, Hes1) and of genes connected to placental angiogenesis (VEGF-R1, VEGF-R2, VEGFa, VEGFc, CD31) and protein and glucose metabolism (Prl6a1, Prl7a1, Grb10, Igf2, Glut1, Glut3, Slc38a1, Slc38a2, Slc38a4).

Results: Gene transcription analysis showed that Notch blockade significantly decreased Hes1 ($p < 0.05$) transcription compared to all other treatment groups. Notch blockade tended to decrease Grb10 transcription compared to Wnt blockade ($p < 0.1$). Double blockade significantly decreased glucose transporter Glut1 compared to Notch blockade ($p < 0.05$).

CONCLUSION: We reported a carryover effect of Notch blockade in early embryo development in one of the effectors of this pathway in late gestation, which could have implications in the encountered phenotypes. Notch blockade decrease of Grb10 transcription contradicts previous findings which report heavier foetuses as a result Grb10 deficiency. Finally, the decrease in transcription of glucose transporter Glut1 in the double blockade could imply a reduced energetic efflux to foetal circulation. An analysis of specific compartments shown to be affected in the stereological analysis should be further explored, as some of the effects may not been detected by transcriptional analysis of the whole placenta. Although these results do not reveal an obvious phenotypic deviation from control, they prompt for a difference in metabolic status influenced by early signaling blockades.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

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CHANGES IN THE ENDOMETRIAL PROTEOME INDUCED BY ENDOMETRITIS AND ENDOMETROSIS IN THE MARE

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INTRODUCTION: In the mare, endometritis and endometrosis are major causes of subfertility/infertility. Transient breeding-induced endometritis is a physiological mechanism used to remove bacteria and non-viable sperm from the uterus. Nearly 30% of mares fail to resolve this within the first 48 hours after insemination and develop persistent post-breeding endometritis. This condition may lead to endometrosis, characterized by excessive fibrosis. Molecular mechanisms involved in equine endometritis and endometrial fibrosis (endometrosis) are not fully understood.

AIMS: This study investigated the proteomic changes induced by endometritis or endometrosis, in order to identify biological processes (BP) and pathways involved, and putative protein biomarkers.

METHODOLOGY: Endometrial biopsies form cyclic mares were histologically classified (Kenney and Doig's) and grouped: category IIA (n=5), category IIA with endometritis (IIA-E; n=4), category IIB (n=5), and category IIB with endometritis (IIB-E; n=5). Endometrial proteome was assessed by liquid chromatography-mass spectrometry (LC-MS/MS). Enrichment analysis was done with DAVID software, and BP identified by QIAGEN Ingenuity Pathway Analysis. Differentially expressed proteins (DEPs) were statistically tested considering a P-value< 0.05 and -1≥Log2FC≥1.

RESULTS: Different proteins (2,464) were quantified and 2,367 were common to all endometria. DEPs analysis showed 100 proteins differentially expressed in IIB vs. IIA endometria, involved in cell growth, structure organization and apoptotic process. Also, 115 proteins were differentially expressed in IIA-E vs. IIA endometria, related to actin filament organization, gene expression and immune response. In IIB-E vs. IIB endometria, 94 proteins were differentially expressed, related to DNA replication, immune response and apoptotic process. Five proteins related to inflammation (SAA1, MYO1B, QSOX1, ITGB2, DIAPH1) were unique to endometritis (IIA-E and IIB-E). Eight proteins related to fibrosis regulation (PLG, GST, SUSD2, MCM5; GPX8, AHNAK2, SDC4, NRP1) were found in IIB and IIB-E endometria, but not in IIA and IIA-E.

CONCLUSIONS: In endometritis, the pathways are related to gene expression and DNA replication, and 3 proteins have been identified for the first time (QSOX1, ITGB2, DIAPH1), and should be further evaluated as potential biomarkers for endometritis. In endometrial fibrosis, the pathways are related to oxidative stress response, while 7 proteins have been identified for the first time (PLG, SUSD2, MCM5, GPX8, AHNAK2, SDC4, NRP1), and could be tested as potential biomarkers for endometrial fibrosis progression.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

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METABOLIC ROLES OF SEMAPHORIN 4B/ADAM17/iRHOM2 PATHWAY

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INTRODUCTION: The metalloprotease ADAM17 (also called TACE) plays fundamental roles during development and promotes homeostasis and multiple inflammatory diseases and cancer by shedding key signalling molecules from the cell surface. Its importance in inflammation and growth control is well documented, while little is known about the role of ADAM17 and its regulator iRhom2 in metabolic homeostasis.

AIMS: The purpose of this study was to determine the impact of the sheddase ADAM17/TACE and of its modulator iRhom2 in the pathophysiology of obesity and in adipocyte homeostasis.

METHODOLOGY: We challenged controls versus iRhom2 global KO or adipocyte specific ADAM17 KO mice to positive energy balance by chronic exposure to a high fat diet, and then compared their metabolic phenotypes. We also carried out ex vivo assays with primary and immortalized mouse brown and beige adipocytes to establish the autonomy of the effect of loss of iRhom2 and ADAM17 on differentiation, thermogenesis and lipolysis.

RESULTS: We found that iRhom2 KO and adipocyte-specific ADAM17 KO mice are less prone to HFD-induced obesity and metabolic alterations (e.g., insulin resistance, hepatosteatosis, dyslipidemia). These mice had a hypermetabolic phenotype, with increased non shivering thermogenesis and body energy expenditure. In addition, we found a new ADAM17 dependent adipokine called Semaphorin 4B that controls adipocyte differentiation, thermogenesis and lipolysis.

CONCLUSIONS: Our findings identify a novel iRhom2/ADAM17-dependent axis, regulated by beta-adrenoceptors and mediated by the ADAM17-cleaved form of Semaphorin 4B, that modulates energy balance in adipocytes by inhibiting adipocyte differentiation, thermogenesis and lipid catabolism.

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PAR-1 EXPRESSION IN EQUINE ENDOMETRIUM FROM MID-LUTEAL PHASE IN DIFFERENT CATEGORIES OF HISTOPATHOLOGICAL CLASSIFICATION

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INTRODUCTION: Protease-activated receptors (PARs) are a class of G protein-coupled receptors that become active upon cleavage by proteolytic enzymes and are implicated in various inflammation-associated diseases. PAR-1, a thrombin receptor, has been reported to stimulate fibroblast proliferation and the synthesis of extracellular matrix components in renal tissue. Given the association of endometrosis, which is a chronic degenerative condition of the equine endometrium, with persistent inflammation and fibrotic processes, PAR-1 may play a contributory role in its pathogenesis. The Kenney and Doig classification categorize equine endometrium into category I (normal), IIA (inflammation, and/or light fibrosis), IIB (moderate fibrosis and inflammation), and III (severe fibrosis and glandular degeneration).

AIMS: The aim was to characterize PAR-1 mRNA expression and protein relative abundance in endometria of different categories of histopathological classification in the mid-luteal phase of the estrous cycle. Data were analyzed by one-way ANOVA in GraphPad PRISM.

METHODOLOGY: Uteri from 40 diestrus mares were collected post-mortem and endometria classified according to Kenney and Doig system (Category I, IIA, IIB, III; n=10 each). Samples were kept in RNAlater for mRNA and protein extraction. PAR-1 gene transcription was assessed by qPCR using RPL32 as the reference gene, and protein relative abundance was evaluated by Western blot with stain-free normalization and anti-PAR-1 antibody.

RESULTS: The PAR-1 mRNA transcription was upregulated in category I, compared to categories IIB and III endometria ($p<0.05$). The protein relative abundance of the mature PAR-1 isoform (~50kDa) was increased in categories IIB and III, with respect to category I ($p<0.05$ and $p<0.01$, respectively). The relative abundance of the cleaved PAR-1 isoform (~46kDa) was higher in category IIA compared to categories I and III ($p < 0.01$), and in category IIB compared to I and III endometria ($p < 0.001$).

CONCLUSION: The results indicate that PAR-1 expression and activation vary according to the severity of endometrial alterations, with increased transcription in healthy endometrium (category I), while the mature protein and its cleaved form accumulate mainly in the altered categories (IIA, IIB, III). These patterns suggest a possible dynamic role for PAR-1 in the progression of endometrial changes, potentially contributing to both the initiation and progression of fibrosis.

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ENHANCING WOUND HEALING: THE THERAPEUTIC POTENTIAL OF HONEY, GELLAN GUM, AND HYALURONIC ACID

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INTRODUCTION: Chronic wounds are a growing health concern, placing a significant burden on healthcare systems. Aims: This study aimed to develop new therapeutic options, based on honey, gellan gum, and hyaluronic acid for the treatment of chronic wounds at all stages.

METHODOLOGY: The formulations were designed to promote healing and improve outcomes in wound regeneration. After development, the formulations underwent in vitro cytocompatibility testing to ensure their safety. To further assess biocompatibility, an ovine wound model was employed, where full-thickness excisional wounds were treated with three different formulations: gellan gum and honey sponges (GG-HNY), gellan gum, honey, and hyaluronic acid sponges (GG-HA-HNY), and a honey-based cream (cream FB002). Over 30 days, daily evaluations, including visual inspection and wound scoring, were performed to monitor the healing process. After the treatment period, tissue samples were collected for histological analysis.

RESULTS: Macroscopic examination showed that all therapeutic groups supported wound closure, with reductions in lesion size, disappearance of granulation tissue, and formation of scar tissue observed across all groups. By day 30, substantial wound closure was achieved in all groups, with no significant differences in healing progress. However, the group treated with cream FB002 demonstrated an advanced stage of healing. Histopathological analysis, conducted according to ISO standards, revealed that the GG-HA-HNY formulation had the lowest ISO score, indicating minimal inflammation and reactivity, which aligned with the in vitro cytocompatibility findings.

CONCLUSIONS: The results suggest that GG-HA-HNY supports an optimal wound healing environment. This study offers valuable insights into wound regeneration and potential advancements in chronic wound treatment.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

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MODULATION OF FATTY ACIDS DEPOSITION ACROSS TARGET TISSUES IN ALZHEIMER'S DISEASE 5xFAD MICE FED SUSTAINABLE DHA-ENRICHED DIETS

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INTRODUCTION: As a major public health concern, Alzheimer's disease (AD) is a chronic neurodegenerative disorder and the leading cause of dementia worldwide, accounting for 60-70% of cases, with no cure to date. This neurological condition is characterised by a progressive decline in memory, cognitive abilities, and behaviour, which ultimately results on impaired daily activities and social functioning. AIMS: Assuming that n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), in particular docosahexaenoic acid (DHA, 22:6 n-3) is a safe and inexpensive link to a healthier long-life, this study aims to explore the potential of novel and sustainable non-fish sources within a blue biotechnology framework for human dietary supplementation.

METHODOLOGY: 40 five-week-old 5×FAD male mice, one of the most commonly used and commercially available AD mouse model, were assigned to 5 body weight-matched dietary groups with 8 mice each and fed isocaloric diets based on AIN-93M standard chow for rodents during 6 months. Each diet, except the control feed (non-supplemented group, control), enclosed a modified lipid fraction supplemented with 2% of: 1) linseed oil (LSO, rich in α-linolenic acid, 18:3 n-3 the precursor of n-3 LCPUFA pathway, the negative control); 2) cod liver oil (fish oil, FO rich in both DHA and eicosapentaenoic acid (EPA, 20:5n-3), the positive control); 3) Schizochytrium sp. microalga oil (Schizo) with 40% of DHA; 4) commercial DHASCO oil (DHASCO) with 70% of DHA. We investigated the impact of different dietary n-3 LCPUFA formulations by targeting brain DHA enrichment and key metabolic tissues, such as liver and also faeces by gas chromatography. RESULTS: Liver, brain, and faeces fatty acid profile were largely influenced by DHA-enriched diets. In the liver, total PUFA were increased in mice fed DHASCO oil relative to control and FO ($P=0.001$) diets, mainly due to changes observed for n-3 PUFA ($P<0.001$), in particular for DHA ($P<0.001$), which stands for almost the totality of hepatic n-3 PUFA identified. In the brain, n-3 PUFA sum ($P<0.001$) was higher for Schizo and DHASCO oils, intermediate for FO, and lower for control and LSO diets reflecting the variations observed for DHA ($P<0.001$), which represented, once more, almost the totality of n-3 PUFA identified in mice brain. On the contrary, total n-6 PUFA were reduced in the brain from mice fed LSO and DHA-enriched oils compared to the reference ($P<0.001$). In the faeces, the sum of PUFA ($P<0.001$) as well as n-3 PUFA ($P<0.001$) and n-6 PUFA ($P=0.022$), were consistently increased in mice fed DHASCO oil diet and a similar pattern of variation was observed for the n-3/n-6 ratio ($P<0.001$).

CONCLUSIONS: Our data show the beneficial modulation of DHA-enriched diets on PUFA incorporation, in particular DHA across key metabolic tissues in a traditional AD mouse model. These promising findings open new avenues for further studies focused on the beneficial effects of DHA derived from sustainable and underexploited Schizochytrium sp. microalga in the prevention of AD.

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PAINÉIS CIENTÍFICOS LINHAS TEMÁTICAS

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Comparative and Translational Medicine and Biotechnology

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DEVELOPMENT OF DEGRADER-ANTIBODY CONJUGATES FOR THE TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER USING FELINE MAMMARY CARCINOMA AS A COMPARATIVE ONCOLOGY MODEL

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INTRODUCTION: Triple-negative breast cancer (TNBC) is a highly aggressive subtype, lacking ER, PR, and HER-2 receptors, making it unresponsive to hormone and HER-2-targeted therapies. Consequently, TNBC is treated with chemotherapy and radiation, often leading to early relapse, resistance, and severe side effects due to lack of specificity. PROteolysis-TArgeting Chimeras (PROTACs) are emerging small molecules that promote degradation of disease-related proteins, including those previously deemed "undruggable". Despite their potential, PROTACs face challenges such as off-target effects, limited tissue penetration and suboptimal PK. To enhance specificity, degrader-antibody conjugates (DACs) have been explored, combining PROTAC cytotoxicity with antibody selectivity. Single-domain antibodies (sdAbs) are especially promising for DAC development due to their small size, stability and tumor penetration. Feline mammary carcinoma (FMC) shares many features with human TNBC, such as high malignancy and frequent triple-negativity. Both species may benefit from targeting TROP-2, a protein overexpressed in TNBC and FMC. Thus, FMC represents a valuable preclinical model for developing a TROP-2-targeting DAC with potential applications in both feline and human medicine.

AIMS: This study aims to develop a TROP-2-targeting DAC for TNBC using a comparative oncology approach by: Validating TROP-2 in FMC cell lines and tissue samples; Selecting anti-TROP-2 sdAbs by Phage Display; Identifying a PROTAC that targets a key intracellular protein in TNBC via HTS; Conjugating the selected sdAb with the PROTAC to develop a DAC.

METHODOLOGY: TROP-2 expression in FMC cell lines was assessed by Western Blot (WB), Flow Cytometry (FC), and Confocal Microscopy (CM). A rabbit was immunized with TROP-2, and a diverse sdAb library was constructed and subjected to Phage Display, including in vitro and in vivo selections optimized for internalization. A PROTAC library is being screened using an HTS platform to identify the most specific candidates for conjugation. The DAC will be developed by linking the selected sdAb to the PROTAC via the cysteine residue in the sdAb scaffold. The DAC will then be characterized for stability, antigen binding, internalization and activity.

RESULTS: All five FMC cell lines tested showed TROP-2 expression by WB, FC, and CM. Serum from the immunized rabbit specifically recognized TNBC and FMC cells. The sdAb library was successfully generated and demonstrated functional diversity. Two promising PROTACs candidates with cytotoxicity activity against FMC cells were identified.

CONCLUSIONS: Our results support TROP-2 as a viable target for DAC development in TNBC. FMC proves to be a relevant comparative oncology model. Ongoing work focuses on selecting a specific anti-TROP-2 sdAb and conjugating it with a PROTAC to develop a specific potent DAC capable of eliminating TNBC and FMC cells while sparing healthy tissues, benefiting both feline and human patients.

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THE CONTRIBUTION OF THE SCIENCE MANAGEMENT AND FUNDING OFFICE TO THE AL4ANIMALS COMMUNITY

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The AL4AnimalS Associate Laboratory aggregates the three Portuguese research centres dedicated to veterinary and animal science. The AL has more than 140 integrated members, most of whom teach and supervise students, and also has organisational functions in addition to research activities. The need to increase funding and diversify funding sources in order to boost the research effort is evident, but it is hampered by the strong competition for funding. Moreover, veterinary science is a field of knowledge that may not fit entirely into strictly agricultural or health topics, hindering access to funding under some calls. A clear need was identified for support in finding national and international funding opportunities that fit with the broad range of topics studied by AL4AnimalS researchers.

These premises underpinned the need for a scientific management and funding office to support AL4AnimalS researchers in screening funding opportunities to apply for and networking platforms to establish bridges of knowledge and contacts. Also, very important was the need to increase the visibility and image of AL4AninalS. The second major objective set for the office was to communicate the science produced, its benefits and impacts in science and society, both within the AL community and beyond.

The first steps consisted of mapping the research interests of all the researchers at the AL4AnimalS centres and building a database of scientific topics developed and potential services and infrastructures. This information will also feed the institutional website under development. In addition, funding opportunities were selected, mainly from European funds and disseminated through the AL community via Coordinators of the centres.

The funding schemes available are scattered in different frameworks. HORIZON Europe funding can be centred on the profile and career path of the researcher (Pilar I); on the excellence in research, both fundamental and applied, spread across six areas of knowledge and often requiring a multi-actor approach so that results reach the end-users (Pilar II); or geared towards a high level of innovation, the market and industry (Pilar III). European funding can also be accessed through co-financed Joint Undertakings, public-private Partnerships and Missions that design and structure their Annual Work Programme with research priorities on their field of activity. In addition, there is funding from European Development, Social and Maritime Funds, available through the Interreg (4), Portugal 2030 and Regional Funds programmes. The science management and funding office communicates funding opportunities, forwards specific calls for proposals to AL4AnimalS researchers whenever there is a match between expertise and the topics of the call, and provides support during the pre-award phase of applications.

As future activities, the office will continue to work closely with researchers, will analyse project proposals from the two last years that were ineligible and not funded to collect the main reasons for failure and work together with the researchers to ensure that there are no ineligible proposals and to improve funding success based on a broader knowledge of the criteria and reasoning of evaluators for classifying proposals. Collaboration between researchers and the science and funding office is key to increasing the visibility and networking of AL4AnimalS, disseminating the results and impact of the science produced, widening access to funding opportunities and improving the success rate of funding.

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