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CIISA CONGRESS 2022

INNOVATION IN ANIMAL, AND VETERINARY BIOMEDICAL RESEARCH

LISBON
NOV 11 – 12
2022

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CIISA CONGRESS 2022

INNOVATION IN ANIMAL, VETERINARY AND BIOMEDICAL RESEARCH

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Welcome

Dear colleagues
On behalf of the CIISA´s Coordination Team and Congress Organizing Commitee, it is our pleasure to welcome you to the CIISA Congress 2022 under the theme “Innovation in Animal, Veterinary and Biomedical Research”.

The Plenary Sessions feature internationally renowned keynote speakers, most of whom have ongoing collaborations with CIISA’s scientists. One speaker for each of the four sessions that constitute the scientific programme - Biomedical Sciences and Biotechnology, Animal and Food Science, Animal Health and Clinical Research. In addition to these 4 talks, there will be an “out of the box” presentation by fellow veterinarian Diogo Guerra, addressing the art and science of scientific illustration.

As in previous editions, the Oral Communications and Poster Presentation Sessions intend to showcase ongoing research, in particular from more junior colleagues. To further motivate them, awards will be conferred to the best poster and oral presentations by MSc and PhD students.

Importantly, this is the first congress we organize since AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences, has been instituted and, therefore, it includes a relevant participation of our colleagues from CECA and CECAV, to whom we extend a very warm welcome and thank for joining us.

After two years of COVID pandemic and postponed meetings, we are hoping this will be an enjoyable and fruitful meeting, that will reinforce links within AL4AnimalS and yield good ideas for future collaborations.
We are looking forward to seeing you!

Best regards

António Duarte
CIISA Coordinator

Esmeralda Delgado
CIISA Congress Committee

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Esmeralda Delgado
Frederico Silva
Isabel Fonseca
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Scientific Commission

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Ana Colette Maurício, CECA
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Scientific Programme

11 NOV

08:30 **Registration and bag collection**

09:00 **Opening Ceremony**

09:30 **SESSION 1**
BIOMEDICAL SCIENCES AND BIOTECHNOLOGY
Chairpersons: Alexandre Leitão & António Duarte

ORAL COMMUNICATIONS I

Afonso Basto (CIISA)
Impact of aging on the CD4 T cell polarization and mucosal tropism imprinted by TLR2/4-activated dendritic cells

Ana André (CIISA)
A novel rabbit VL single-domain antibody with versatile conjugation properties against canine lymphoma

Juliana Santos (CECA)
RNA as a promising molecule to treat a rare neurodegenerative lysosomal storage disorder

Liliana Matos (CECA)
A personalized antisense oligonucleotide exon-skipping therapeutic approach for Mucolipidosis II

10:40 **Coffee Break**

11:10 **ORAL COMMUNICATIONS II**

Nuno Jordão (CIISA)
Functional impairment and antigen suppression of African Swine Fever virus ubiquitin-conjugating enzyme pI215L

Pedro Bule (CIISA)
Unconventional incorporation of the dual-binding mode in ruminal cellulosomes

Sofia Carvalho (CECA)
Modelling genetic disorders with an unexpected co-worker: the Tooth Fairy that gifts hope to both children and scientists

Elisabete Silva (CIISA)
Bovine Genital Campylobacteriosis: transcriptomic signatures of endometrial epithelial cells challenged with *Campylobacter fetus* subsp. venerealis

12:10 **Invited Speaker**
Diogo Guerra | DVM, Medical & Veterinary Illustrator

OUT OF BOX(PLOT): TIPS TO VISUALLY IMPROVE SCIENTIFIC GRAPHS

12:40 **Lunch and Poster Sessions 1 & 2**

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(1) iMM - Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon
(2) MARETEC - Marine, Environment & Technology Center, Instituto Superior Técnico, University of Lisbon

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14:30 **SESSION 2**
ANIMAL AND FOOD SCIENCE
Chairpersons: Cristina Guedes & Rui Bessa

Keynote Speaker: **Tiago Domingos** | MARETEC/LARSyS, IST, ULisboa (2)

THE ROLE OF RUMINANT LIVESTOCK IN A SUSTAINABLE FOOD SYSTEM: A PORTUGUESE PERSPECTIVE

15:10 **ORAL COMMUNICATIONS I**

Ana Vitor (CIISA)
Development of a novel coating material for rumen-protected lipid encapsulation using *Nannochloropsis oceanica* algaenan

Cátia Falcão Martins (CIISA)
Chlorella vulgaris as ingredient in piglet diets: digestibility, microbiota and intestinal morphology studies

Francisco Duarte (CIISA)
Effects of sunflower oil infusions of *Asparagopsis taxiformis* on in vitro rumen methane production

José Santos Silva (INIAV,CIISA)
Haylage-based diets as an alternative to concentrate-based diets for finishing of young-bulls

16:10 **Coffee Break**

16:40 **Keynote Speaker**
Luís Graça | IMM–FMUL (1)

REGULATION OF ANTIBODY PRODUCTION IN HEALTH AND DISEASE: VACCINATION AND AUTOIMMUNITY

17:20 **ORAL COMMUNICATIONS II**

Maria João Fradinho (CIISA)
Is *Nannochlopsis oceanica* a new source of omega-3 polyunsaturated fatty acids for horse diets?

Mónica Mendes Costa (CIISA)
Individual carbon-oxygen lyases (EC 4.2) as a solution to disrupt Laminaria digitata and Ulva lactuca recalcitrant cell walls

Paula Lopes (CIISA)
Tissue specific variation of aquaporins and cytokines transcriptional profile in piglets under LPS challenge

Susana Alves (CIISA)
Unraveling biomarkers of trans-10 shift in ruminants: a multi-Omics approachv

18:20 **End of Session**

19:30 **Departure for Congress Dinner**

20:00 **Congress Dinner - JOCKEY RESTAURANT - With DJ**

Scientific Programme

12 NOV

09:30 SESSION 3
ANIMAL HEALTH
Chairpersons: Isabel Pereira da Fonseca & José Correia Costa

Keynote Speaker: **Ana Oliveira** | CEB–UM (3)

BACTERIOPHAGES FOR ANIMALS: BREAKTHROUGHS AND CHALLENGES

10:10 ORAL COMMUNICATIONS I

Ana Sofia Soares (CECAV)
What lives in the fur of shelter animals? A mycological survey

Catarina Anastácio (CIISA)
Significance of uterine cytology macrophage counts on endometritis recovery in postpartum dairy cows

10:40 Coffee Break

11:10 ORAL COMMUNICATIONS II

Cláudia Istrate (CIISA)
Structural characterization of African Swine Fever Virus nucleoprotein p10 interaction with DNA

Fábio Abade dos Santos (CIISA)
Iberian hare extinction: the foundation of a Breeding Centre in Portugal

Gonçalo Pereira (CIISA)
Circulating cell free miRNA profiles of postpartum dairy cows with subclinical endometritis

Joana Moreira da Silva (CIISA)
Carbapenemase-producing Enterobacterales clinical strains causing infections in cats and dogs

Juliana Menezes (CIISA)
Highest priority critically important antimicrobial resistance gene, mcr-1, shared within households, Lisbon, Portugal, 2018-2020

Patrícia Poeta (CECAV)
"One Health" approach to challenge antimicrobial resistance

12:40 Lunch and Poster Sessions 3 & 4

14:30 SESSION 4
CLINICAL RESEARCH
Chairperson: Esmeralda Delgado & Luís Lopes da Costa

Keynote Speaker: **Ana Colette Maurício** | CECA- ICBAS–UP (4)

NEUROMUSCULAR REGENERATION USING NEW CELL-BASED THERAPIES ASSOCIATED WITH BIODEGRADABLE BIOMATERIALS FOR VETERINARY AND HUMAN PATIENTS - ONE HEALTH PERSPECTIVE

15:10 ORAL COMMUNICATIONS I

Ana Reisinho (CIISA)
Encephalitozoon cuniculi infection in pet rabbits – could it be an issue?

Beatriz Silva (CIISA)
Nanoparticulate system carrying epoetin beta as a neuroprotective agent: topical ocular administration in glaucomatous rats

Gonçalo Santos (CIISA)
Biocompatibility and tolerance of the InEye® ophthalmic insert in rabbits

Joana Alpoim-Moreira (CIISA)
Decitabine effect on collagen type I and III in TGF-β1 treated mare endometrial fibroblasts

16:10 Coffee Break

16:40 ORAL COMMUNICATIONS II

Luís Maltez da Costa (CECAV)
Hip Dysplasia in a New Functional Rabbit Model: Computed Tomography Characterization

Mafalda Pires Gonçalves (CIISA)
Preliminary data on the owner's insights for an effective weight management programme in dogs and cats

Maria Teresa Prego (CIISA)
Plasma and urinary Normetanephrines – a promising biomarker for future diagnosis of feline pheochromocytoma

Vera Pessoa (CIISA)
Comparison of Skin Allergy tests, Intradermal tests and Skin Prick tests in the characterization of Insect Bite Hypersensitivity in a population of Lusitano horses

17:40 CIISA's Congress Awards

18:00 Closing Ceremony and Farewell

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(3) CEB - Centre of Biological Engineering, University of Minho School of Engineering
(4) CECA- Center of Animal Science Research, ICBAS - University of Porto School of Medicine and Biomedical Sciences

Keynote and Invited Speaker Bio & Talks

Luís Graça

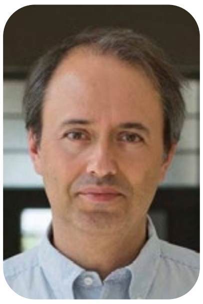
IMM–FMUL

Luís Graça has an MD from the University of Lisbon, Portugal, and a PhD in transplant immunology from Oxford, UK. He developed his post-doctoral research first in Oxford and later at the Institute for Child Health Research in Perth, Australia. He is currently Full Professor of Immunology and Vice-Dean at the University of Lisbon Medical School, directing a research group in Cellular Immunology at the Instituto de Medicina Molecular.
His most significant scientific contributions have been related to the fields of transplantation and autoimmunity. Graça worked on the regulation of antibody production following vaccination and strategies to overcome transplant rejection and the induction of immune tolerance in autoimmunity and allergy. Among these topics, he has been especially interested in the biology of different types of regulatory T cells, namely T follicular regulatory cells – a cell type he co-discovered.
Graça has collaborated with several artists, including a long-term relationship with Marta de Menezes (www.martademenezes.com), a pioneer in bioart, and possibly the artist with the mostdiverse approach within this field. Artworks developed by different artists in collaboration with the Graça Laboratory have been exhibited worldwide.
Graça is the chair of the National COVID-19 Vaccination Technical Advisory Group, past-president of the Portuguese Society for Immunology, and past-president of the Sociedade das Ciências Médicas de Lisboa, the oldest medical society in Europe (founded in 1822).

Affiliation: Instituto de Medicina Molecular, Faculdade de Medicina; Universidade de Lisboa, Lisbon, Portugal.
Website | Publications

Regulation of antibody production in health and disease: vaccination and autoimmunity

It has long been known that CD4 T cells are necessary for effective antibody responses, to provide help to B cells, triggering a germinal center (GC) reaction where affinity maturation and isotype switching occurs. However, the nature of the dedicated CD4 helper T cells, known today as T follicular helper (Tfh), was only recently described. A population of Foxp3+ regulatory cells with unique access to the follicle – T follicular regulatory (Tfr) cells – represent the counterpart of Tfh cells. In addition, Tfh cells also specialize according to the inflammatory environment, acquiring different properties under type-1 or type-2 conditions. The interplay between different specialized subsets of Tfh and Tfr cells can determine the outcome of humoral responses, namely in autoimmunity or following vaccination, offering an opportunity for therapeutic modulation.



Tiago Domingos

MARETEC/LARSyS, IST, ULisboa

Tiago Domingos has a “licenciatura” in Physical Technological Engineering (IST), a PhD in Environmental Engineering (IST) and is an associate professor in the Scientific Area of Environment and Energy, Department of Mechanical Engineering, IST. His research is based on an integrative view of sustainability, combining fundamental research, technological development and practical application, addressing, among others, topics such as the sustainability of agri-food systems and the evaluation of rural development policies.
He is president of MARETEC – Marine, Environment and Technology Centre, coordinator of the Master’s in Environmental Engineering and coordinator of IST-Ambiente, IST’s Environmental Science and Engineering Platform. He is the CEO and founder of Terraprima, considered IST Spin-Off and winner of the A World You Like with a Climate You Like Award, awarded by the European Commission in 2013 for the best climate solution in Europe.

The role of ruminant livestock in a sustainable food system: a Portuguese perspective

Ruminant livestock production is now taken as a main culprit in environmental pressures. This talk will strive towards a balanced assessment of its impacts and benefits, and will present ways forward in minimising the former and maximising the latter, taking Portugal as a main case study. It starts with the challenges of climate change, biodiversity conservation, perturbation of the nitrogen and phosphorus cycles and the increased competition for land, balancing its demand for food production, renewable energy (e.g., solar, wind, bioenergy), carbon sequestration and nature conservation. It addresses the critical role of nitrogen in a sustainable food system and the relative merits of Haber-Bosch vs. legume sources of nitrogen for the food system. Then it focuses on the special role of ruminants in a sustainable food system, due to their capacity to process human inedible (namely fiber-rich) feed transforming it into high nutritional value milk and meat for human consumption and nutrients for plant production , but with trade-offs, namely regarding higher methane emissions from less digestible feed. Based on this, it proposes a model for sustainable beef production in Mediterranean areas, covering the use of sown biodiverse legume-rich pastures, the integration of pastures and forestry (*montado*) and optimised intensive fattening including methane reducing feed additives.

Ana Oliveira

CEB-UM

Ana Oliveira has a degree in Zootechnical Engineering (2002, UTAD) and an EU degree in Engineering of Livestock Production (2001, CAH Dronten, NL). She got her MsC degree in Biotechnology (2004) and her PhD degree (2009) in Biological and Chemical Engineering, both by the University of Minho. AO is now a researcher at the Centre of Biological Engineering, from UM. During the PhD, she developed a therapeutic phage product to control Avian pathogenic E. coli in poultry, for the company (Controlvet SA) Since then, her main area of interest is phage Biotechnology applied to Animal Science. Since 2011 her research has been focused on the development of phage-based solutions to control a bacterial infection (AFB) affecting Apiculture. Besides, since 2017 she is leading another project, this time targeting post-weaning diarrhoea in swine

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Bacteriophages for animals: breakthroughs and challenges

Animal health has been threatened by the absence of responses to bacterial infections. The increasingly emerging problem of antibiotic resistance, together with the strict restrictions imposed by the EU on the use of antibiotics and with the lack of new active ingredients in the market are the basis for such a threat.

Biotechnology-derived solutions, in particular bacteriophages and their derived products are being considered valuable antimicrobial alternatives and their application in animal production constitutes an important strategy towards the reduction of antibiotics usage in agro-food, increasing animal productivity, and providing environmental protection.

Bacteriophages (phages) are naturally occurring predators of bacteria, ubiquitous in the environment, with high host specificity and harmless to animals. Several *in vivo* studies support the use of phages as an intervention strategy for reducing diverse bacterial infections in food-producing animals. The steps for the development of a new phage product for animals will be here presented, by using an example of application in the poultry industry, highly affected by the avian pathogenic Escherichia coli (APEC). In addition, main results from recent assays aiming at establishing proofs of concept of the efficacy of phage products to tackle other pathogens and animal hosts will be highlighted. For example, the control of American Foulbrood (caused by *Paenibacillus larvae*) in Beekeeping, and the action against *E. coli*-associated diarrhea (caused by enterotoxigenic E. coli), affecting young pigs will be explored.

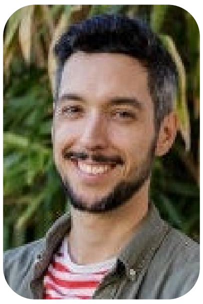
Ana Colette Maurício

CECA - ICBAS–UP

Ana Colette Maurício has a degree in Veterinary Medicine (1995), a PhD in Veterinary Sciences (1999, FMV-UL), and Habilitation in Veterinary Sciences (2011, ICBAS-UP). The PhD experimental work was developed at Instituto Gulbenkian Ciência (IGC), Freiburg Medicine Faculty, Germany and FCT-UNL. At the present, she is an Associated Professor with Habilitation, from the Veterinary Clinics Department (ICBAS-UP). She is a Member of the Scientific Council and of the Representatives Council (ICBAS-UP), Director of the Veterinary Sciences Doctoral Program (ICBAS-UP), Scientific Coordinator of Regenerative Medicine/ Experimental Surgery sub-unit (CECA-ICETA) and of Comparative and Translational Medicine and Biotechnology of the Laboratório Associado para Ciência Animal e Veterinária (Lab4AnimalS). For the past 15 years she coordinates a multidisciplinary research group of Experimental Surgery/Regenerative Medicine, developing biomaterials and cell-based therapies. She started working with embryonic stem cells obtained by somatic nuclear transfer for therapeutic use with Ian Wilmut´s group (Dolly's cloned sheep). Several relevant publications had been produced and conducted to the first Portuguese cloned animal and a PhD thesis. But ethical issues related to the collection and manipulation of human embryonic stem cells, even for therapeutic use is controversial. Therefore, a continued effort to identify and characterize novel stem cell populations appears critical for widespread clinical success. This effort implies in vitro studies, experimental surgery and in vivo testing, before the clinical trials and the compassive treatment in such clinical cases where the traditional and standard treatments failed. Her research groups works exactly in this direction, so she created a multidisciplinary team, including Veterinarians, Engineers, Medical Doctors that through Experimental Surgery have a crucial role in the development of new therapies, allowing a close share of knowledge between biomaterials design, development of cellular systems, and surgeons needs when related to specific clinical cases in the area of Human and veterinary Health. This group has several recent relevant publications in the research areas of tissue regeneration. In her laboratory have been working several PhD/Post-Doctoral/Master students from various countries who have acquired a high level of competence in the study of tissue regeneration. She is the supervisor of several PhD, Post-Doctoral/Master students (16 PhD thesis concluded), she is the co-author of several scientific articles and book chapters (h-index 25). She was the principal researcher of several national/international scientific projects. Editor of 4 international scientific books and inventor of 3 international patents.

Neuromuscular regeneration using new cell-based therapies associated with biodegradable biomaterials for Veterinary and Human patients - One Health perspective.

Considering the neuromuscular regeneration using novel therapies, Regenera® is a game-changer biopharmaceutical product dedicated to the development of advanced cell therapies for veterinary use. The first Regenera® product-ARTICEL® is a breakthrough since for the first time takes advantage of the synergistic activity of two different types of stem cells. Regenera®'s method is unconstrained by the existing bias towards the use of autologous cells from the adipose tissue. Instead, Regenera® has based itself on more than 15 years of research and experience in stem cell biology to develop proprietary technology to isolate the best indicated stem cells for Musculo-skeletal tissue regeneration. Unlike other stem cell therapies available, ARTICEL® is an off-the-shelf (allogeneic) product, readily available to be administered, thus taking advantage of early and appropriate windows of clinical opportunity. In the case of equestrian sports, horses are subject to extremely demanding training programs from very early ages. Such programs impose unnatural torque forces to joints that are still not fully developed. The resulting musculo-skeletal conditions account for up to 79% of lost training days, up to 33% of all missed competition tournaments, and are the main reason why the functional life of top recreational horses does not last over 4 years. Consequently, there is only a tight and fringed period of the animal's competitive life spans for owner's and investors to obtain their returns. ARTICEL® has proven to be able to bring otherwise lost-for-competition animals back to their highest performance, to prevent and to reduce lesion-driven stoppage times by 60%. Given the innovative character of its proprietary technology, Regenera® has the vision of becoming an international reference for the production and supply of innovative cell therapy products, not only for equines but also for canines and other species. Regenera® will support its activity on building intellectual property through open innovation involving strategic partnerships and operating with a One-Health initiative mindset. Regenera® will transfigure the way stem cell therapies performed by looking at treatments through a holistic approach.



Diogo Guerra

DVM, Medical & Veterinary Illustrator

Diogo Guerra is a medical illustrator with an atypical background. He completed a Masters in Veterinary Medicine at the University of Lisbon, and a doktorarbeit (dr med vet) in Veterinary Parasitology at the University of Zurich, Switzerland. He currently works as a freelance medical illustrator and dataviz consultant, after taking several courses in scientific illustration, painting and data visualization. He was part of the graphic department of the VetSuisse Faculty in Zurich (VETCOM) between 2014–2015, and worked as medical illustrator at the medical communications agency Medicalwriters.com between 2018–2022.

His medical illustration portfolio combines his scientific background with the passion to visualize complex ideas – from creating anatomical and cellular illustrations for Human and Veterinary Medicine textbooks and articles, to collaborating with pharmaceutical companies, designing and optimising scientific graphs on the development and efficacy of new drugs. He also teaches workshops on visual science communication to doctors and researchers.

Portfolio at www.diogoguerra.com

Out of the Box(plot): Tips to visually improve scientific graphs

In an age of information explosion, scientists have increased pressure and responsibility to ensure clear communication with peers and the general public. However, principles of effective science visual communication are oftentimes absent from scientific curricula. The main goal of this very practical session is to furnish attendees with easy tools to rapidly improve the way they communicate their research findings. We will go through different case studies and discuss (1) easy tips to visually optimize common graph types; (2) effective yet less-known graphical alternatives; (3) topics on how to make graphs more accessible.

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ORAL COMMUNICATIONS

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SESSION 2
ANIMAL AND FOOD SCIENCE

SESSION 3
ANIMAL HEALTH

SESSION 4
CLINICAL RESEARCH

ORAL COMMUNICATIONS

SESSION 1
BIOMEDICAL SCIENCES AND BIOTECHNOLOGY

O001 Afonso P. Basto[1,2], Sara Zúquete[1,2], Inês L. S. Delgado[1,2], Maria Teresa Rosa[1,3], Ana Catarina Mendes[4,5], Dulce Santos[1], Sofia Nolasco[1,2], Luís Graça[4,5], Alexandre Leitão[1,2]
Impact of aging on the CD4 T cell polarization and mucosal tropism imprinted by TLR2/4-activated dendritic cells

O002 Ana S. André[1,2], Joana N.R. Dias[1,2], Sandra I. Aguiar[1,2], Sara Nogueira[1,2], Isa Moutinho[1,2], Joana Carvalho[3], João António[3], Marco Cavaco[4], Vera Neves[4], Soraia Oliveira[4], Lurdes Gano[5], João D.G. Correia[5], Belmira Carrapiço[1,2], Berta São Braz[1,2], Pedro Góis[3], Miguel Castanho[4,] João Gonçalves[3], Solange Gil[1,2], Luís Tavares[1,2], Frederico Aires-da-Silva[1,2]
A novel rabbit VL single-domain antibody with versatile conjugation properties against canine lymphoma

O003 Marta F Silva[1,2], Andreia J Amaral[1,2], Gonçalo Pereira[1,2], Patrícia Diniz[1,2], Luísa Mateus[1,2], Luís Lopes-da-Costa[1,2], Elisabete Silva[1,2]
Bovine Genital Campylobacteriosis: transcriptomic signatures of endometrial epithelial cells challenged with Campylobacter fetus subsp. Venerealis

O004 Juliana I. Santos[1,2,3,4], Mariana Gonçalves[1,5], Liliana Matos[1,3,4], Paulo Gaspar[6], Mª João Pires[5], Paula A. Oliveira[5], Mª João Prata[2,7], Mª Francisca Coutinho[1,3,4], Sandra Alves[1,3,4]
RNA as a promising molecule to treat a rare neurodegenerative lysosomal storage disorder

O005 Liliana Matos[a,b,c*], Mariana Gonçalves[a,d*], Juliana I. Santos[a,b,c,e], Maria Francisca Coutinho[a,b,c], Maria João Prata[e,f], Maryam Omidi[g], Sandra Pohl[g], Sandra Alves[a,b,c]
A personalized antisense oligonucleotide exon-skipping therapeutic approach for Mucolipidosis II

O006 Nuno Jordão[1,2], Fernando Boinas[1,2], Carlos Martins[1,2], Fernando Ferreira[1,2]*
Functional impairment and antigen suppression of African Swine Fever virus ubiquitin-conjugating enzyme pI215L

O007 Marlene Duarte[1,2], Victor D. Alves[1,2], Catarina Caseiro[1,2], Luís M. A. Ferreira[1,2], Ana Luísa Carvalho[3,4], Shabir Najmudin[5], Edward A. Bayer[6], Carlos MGA Fontes[7], Pedro Bule[1,2,*]
Unconventional incorporation of the dual-binding mode in ruminal cellulosomes

O008 Sofia Carvalho[1,2,3,4], Juliana Inês Santos[1,3,4,5], Luciana Moreira[1,3,4], Paulo Gaspar[6], Mariana Gonçalves[1], Liliana Matos[1,3,4], Marisa Encarnação[1,3,4], Diogo Ribeiro[1,3,4], Ana Joana Duarte[1,3,4], Maria João Prata[6], Maria Francisca Coutinho[1,3,4], Sandra Alves[1,2]
Modelling genetic disorders with an unexpected co-worker: the Tooth Fairy that gifts hope to both children and scientists

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SESSION 2
ANIMAL AND FOOD SCIENCE

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Development of a novel coating material for rumen-protected lipid encapsulation using Nannochloropsis oceanica algaenan

O010 Cátia F. Martins[1,2,3], P. Trevisi[4], D. Coelho[1,2], F. Correa[4], D.M. Ribeiro[3], M. Pinho[1,2], A.M. Almeida[3], J.P.B. Freire[3], J.A.M. Prates[1,2]
Chlorella vulgaris as ingredient in piglet diets: digestibility, microbiota and intestinal morphology studies

O011 F. Sena[1,2], P.V. Portugal[3], M.T. Dentinho[2,3]; K. Paulos[3], C. Costa[3], D.M. Soares[1,2,4], A. Oliveira[6], H. Ramos [6]; S.P. Alves[1,2,5], R.J.B. Bessa [1,2,5] and J. Santos-Silva [2,3,5*]
Effects Of Sunflower Oil Infusions Of Asparagopsis taxiformis On In Vitro Rumen Methane Production

O012 Santos-Silva[abi] J.; A.E. Francisco[abi]; A.V. Portugal[a], M. T. Dentinho[ab], A. Barradas[c]; A. J. Rodrigues[d]; E. Jerónimo[ef]; S. P. Alves[bi]; N.R. Rodrigues[g], R. F. M. Teixeira[gh]; T. Domingos [gh]; R. J. B. Bessa[bi]
Haylage-based diets as an alternative to concentrate-based diets for finishing of young-bulls

O013 M.J. Fradinho[a,b], A.B. Rodrigues[a], R.M. Caldeira[a,b], G. Ferreira-Dias[a,b], J.L. Silva[c], S.P. Alves[a,b] & R.J.B. Bessa[a,b]
Is Nannochlopsis oceanica a new source of omega-3 polyunsaturated fatty acids for horse diets?

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SESSION 1
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Chairpersons: Alexandre Leitão & António Duarte

Impact of aging on the CD4 T cell polarization and mucosal tropism imprinted by TLR2/4-activated dendritic cells

Afonso P. Basto[1,2], Sara Zúquete[1,2], Inês L. S. Delgado[1,2], Maria Teresa Rosa[1,3], Ana Catarina Mendes[4,5], Dulce Santos[1], Sofia Nolasco[1,2], Luís Graça[4,5], Alexandre Leitão[1,2]

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Introduction: Toll-like receptor (TLR)2 activation induces aldehyde dehydrogenase (ALDH) enzymes on non-mucosal dendritic cells (DCs) equipping them with the machinery needed to metabolize vitamin A into all-trans retinoic acid, a property usually restricted to mucosal DCs. During T cell priming, retinoic acid produced by TLR2-activated DCs induces the expression of mucosal-homing molecules (α4β7 and CCR9) in the activated T cells. Recently, we found that the simultaneous activation of non-mucosal DCs through TLR2 and TLR4 maintains such capacity while reinforcing the polarization of primed CD4 T cells towards Th1. These results suggest that the inclusion of TLR2/TLR4 agonists in non-mucosal vaccines could promote the gut localization of Th1 cells. However, in older individuals this is difficult to predict since both DCs and T cells have been shown to have altered functions with aging.

Materials & methods: Here, we used a murine model to evaluate how aging simultaneously impact the lymphocyte polarization and the mucosal tropism imprinted by TLR-activated DCs, assessed upon DC-T cell cocultures by multiplex or ELISA cytokine assays and by flow cytometry.

Results: We found that the cytokines produced by TLR-activated DCs, as well as the polarization profile of primed CD4 T cells and the DC-imprinted expression of mucosal homing receptors, is altered with age, and that this age-related effect varies depending on the TLR stimulus. Importantly, this impact occurs despite equivalent levels of TLR2 expression and a similar TLR-induced ALDH activity in the DCs isolated from animals of different age.

Discussion: These results emphasize the importance of considering the age-related DC dysfunctions when developing novel vaccine strategies that rely on modulation of the DC-T cell crosstalk through PRR activation.

Support/interest disclosure: FCT-Fundação para a Ciência e Tecnologia, I.P. (Portugal) projects PTDC/CVT-CVT/31840/2017; PTDC/CVT-CVT/4599/2021; UIDB/00276/2020 (CIISA), LA/P/0056/2020 (AL4AnimalS).

Keywords: TOLL-LIKE RECEPTORS; DENDRITIC CELLS; MUCOSA; VACCINES; AGING.

O 002

A novel rabbit VL single-domain antibody with versatile conjugation properties against canine lymphoma

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Introduction: Among mAb-based therapies, antibody-drug conjugates (ADCs) and immunotoxins are considered one of the most promising strategies, combining tumor selectivity with the cytotoxic potency of small molecules. Thus, by exploiting the properties of rabbit-derived single domains (sdAbs), we aimed to develop a new generation of highly selective and potent ADC and immunotoxin for cancer treatment.

Material and Methods: A rabbit was immunized with canine primary non-Hodgkin's lymphoma (cNHL) cells. An immune library was constructed, and in vitro and in vivo phage display was performed on a cNHL xenograft model. ELISA was performed, and C5 was selected as the most promising candidate. C5 binding and internalization in cNHL cells was confirmed by flow cytometry and immunofluorescence. After conjugation to SN-38 via diazaborine and PE-38 via a peptide linker, the cytotoxicity of C5-DAB-SN-38 and C5-PE38 on cNHL cells was confirmed. TopoI activity and inhibition of protein synthesis was evaluated for C5-DAB-SN-38 and C5-PE38, respectively. Biodistribution was performed on a xenograft model of cNHL.

Results: A panel of highly specific V_L sdAbs was identified. C5 was chosen to be conjugated with SN-38 and PE38 using the free exposed Cys80, resulting in stable ADC and immunotoxin. C5-DAB-SN-38 and C5-PE38 exhibited a potent anti-tumoral effect on cNHL cells and inhibited the DNA-TOPOI and protein synthesis, respectively. Moreover, both molecules demonstrated a specific tumor uptake of 1.5% and 2% for C5-DAB-SN-38 and C5-PE38, respectively.

Discussion: These results validated two promising sdAb-based targeted therapies using the same sdAb, contributing to the development of a promising therapy for cNHL.

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Keywords: THERAPEUTIC ANTIBODIES, ANTIBODY-DRUG CONJUGATE; IMMUNOTOXIN; CANINE LYMPHOMA

Bovine Genital Campylobacteriosis: transcriptomic signatures of endometrial epithelial cells challenged with Campylobacter fetus subsp. Venerealis

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Introduction: Bovine Genital Campylobacteriosis (BGC) is a venereal disease of cattle caused by *Campylobacter fetus* subsp. venerealis (*Cfv*). Infection of females causes infertility, namely embryo mortality and early abortion. Despite the worldwide distribution and economic impact, BGC pathogenesis remains elusive. As bovine endometrial epithelial cells (bEECs) are involved in embryo implantation and pregnancy maintenance, this study evaluated their response to *Cfv* infection.

Material & Methods: To simulate natural infection during luteal phase, in vitro cultures of bEECs were assembled from 4 healthy luteal phase uteri, exposed to 5 ng/µL of progesterone for 48 hours before co-culture with *Cfv*. For co-culture, bEECs with 90% confluence and >93% purity were challenged (6h) with *Cfv* or non-stimulated (control). Total RNA was extracted and analyzed by RNA sequencing.

Results: Overall, 142 differentially expressed genes (DEGs) (106 upregulated, 36 downregulated) were identified. Analysis of gene set enrichment of DEGs revealed significant enrichment for 32 gene ontology (GO) terms for biological processes and 14 functional pathways (13 upregulated, 1 downregulated). Most significant GO terms are related to endometritis, including inflammation, immune response, chemotaxis and G-protein coupled receptor signaling pathways ($p < 0.001$). DEGs implicated in pregnancy (*CCL2, CCL4, CCL20, CXCL2, PTGES*) and implantation (*CCL2, CX3CL1, CCL4, VEGFA, CSF3, TNFS11, ACOD1, SLC2A3, PFKB3, PTGES*) were among the most upregulated (>3 fold), as well as among the most downregulated (>2.2 fold; *ATOH8, LRRTM4, NOG, CSF1R, ADRA1B*).

Discussion: In conclusion, this study shows that Cfv infection deregulates the transcriptomic profile of endometrial epithelial cells, which may lead to endometritis and adversely affect implantation and pregnancy maintenance, the known consequences of BGC.

Support/interest disclosure: This study was supported by Fundação para a Ciência e a Tecnologia (FCT) and Fundo Europeu de Desenvolvimento Regional (FEDER), under the project PTDC/CVT-CVT/30145/2017. This study was also supported by Centro de Investigação Interdisciplinar em Sanidade Animal - CIISA (Project UIDB/00276/2020, funded by FCT) and by the Associate Laboratory for Animal and Veterinary Science (LA/P/0059/2020 - AL4AnimalS). ES was funded by FCT (DL 57/2016/CP1438/CT0001). AJA was funded by H2020 project BovREG (815668) and by FCT 2021.02058.CEECIND. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Keywords: BOVINE GENITAL CAMPYLOBACTERIOSIS; Campylobacter fetus subsp. venerealis; BOVINE ENDOMETRIAL EPITHELIAL CEELS; RNA SEQUENCING

RNA as a promising molecule to treat a rare neurodegenerative lysosomal storage disorder

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The RNA molecule is a specific and essential regulator of gene expression. In our lab, we are addressing the potential of different RNA-based drugs to correct/ameliorate the sub-cellular phenotype of a group of genetic diseases caused by lysosomal dysfunction. Our major efforts are focused on those presenting a predominant neurological phenotype, such as the Sanfilippo syndrome. Briefly, two major research lines are being pursued: the first is a mutation-specific approach to correct abnormal splicing; the second depends upon selective downregulation of one gene involved in the biosynthesis of the substrate that accumulates in this pathology. For the splicing correction approach, we are using U1snRNA to restore the splicing defect caused by the *HGSNAT* mutation c.234+1G>A. We demonstrated in vitro that a modified U1snRNA vector designed to improve the definition of *HGSNAT* exon 2 could partially restore its normal splicing (Matos et al., 2014 DOI: 10.1186/s13023-014-0180-y). Preliminary assessments in mice transiently expressing the human defect have also been performed. Future analysis will rely on AAV-mediated gene transfer techniques.

For the substrate reduction approach, we are using siRNAs. By acting over a specific biosynthethic cascade, siRNAs promote an overall decrease of the accumulating substrate. So far, we have already tested this approach in patients' fibroblasts and observed a high inhibition of the target mRNA. Overall, there are substantial differences between these two approaches but they also face common challenges and show equally promising results.

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Keywords: RNA; LYSOSOMAL STORAGE DISORDERS; SANFILIPPO SYNDROME; U1snRNA; siRNAs

O 005

A personalized antisense oligonucleotide exon-skipping therapeutic approach for Mucolipidosis II

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Introduction/Background: Mucolipidosis II (MLII) is a Lysosomal Storage Disorder caused by the deficiency of the enzyme GlcNAc-1-phosphotransferase, which is responsible for the Mannose-6-Phosphate marker addition to lysosomal enzymes. Of all MLII mutations, the 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) for MLII. Previously, on MLII patients' fibroblasts, ASOs were used to skip exon 19 of the GNPTAB pre-mRNA, successfully resulting in the production of an in-frame mRNA. Now, our aim is to analyze if these results are transcribed to the enzymatic and cellular phenotype level.

Materials & Methods/Results: The recovery of GlcNAc-1-phosphotransferase activity after ASO transfection in fibroblasts was measured through a radioactive assay. However, the MLII fibroblasts enzyme activity was similar to that of control fibroblasts, even without any treatment, showing that the assay is not specific for this analysis. So, now we obtained a human WT GNPTAB cDNA plasmid which will be used to generate a mutant construct without the exon 19. After their transfection in Hep3B/HEK293T cells the GlcNAc-1-phosphotransferase expression and activity will be analyzed. Furthermore, the lysosomal storage levels and the activity of some lysosomal hydrolases in control and patients' fibroblasts after ASO treatment will be assessed.

Discussion: The mentioned assays will allow to analyze the feasibility of our approach for the treatment of MLII patients with exon 19 mutations. The results obtained so far will be presented.

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Keywords: LYSOSOMAL STORAGE DISORDERS; MUCOLIPIDOSIS II; ANTISENSE OLIGONUCLEOTIDES; EXON-SKIPPING; RNA-BASED THERAPIES

Functional impairment and antigen suppression of African Swine Fever virus ubiquitin-conjugating enzyme pI215L

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Background: African Swine Fever (ASF) is an infectious viral disease that affects domestic pigs and wild boars, with high mortality and transmission capacity but absent of any treatment. Given its resurgence in Europe and Asia, ASF represents a serious threat to the farming industry and the development of preventive or remedial therapeutics is of the utmost interest. pI215L is an ubiquitin conjugating enzyme encoded by the virus, which has been previously suggested to have an essential role in the cycle of the viral infection. Site-specific mutagenesis of this protein could then provide valuable information regarding the pathophysiology of the disease.

Methods and Results: Multi-residue mutants of pI215L were designed and characterized by functional assays in order to assess its enzymatic activity, compared to the wild-type form. Several mutations capable of a 50 to 90% decrease of activity were found. Furthermore, an ELISA screening using pI215L peptide libraries allowed the identification of determinant antigens related to the immune response triggered by ASF, as well as two key mutations able to reduce significantly the peptide affinity to immunoglobulins associated with the viral infection.

Discussion: A combination of the obtained mutations is planned to be incorporated into the NHV/P68 strain of African Swine Fever virus, through homologous recombination, to further investigate the role of pI215L in the viral infection cycle of ASF.

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Keywords: AFRICAN SWINE FEVER; pI215L; LIVE ATTENUATED VACCINE; DIVA

Unconventional incorporation of the dual-binding mode in ruminal cellulosomes

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Introduction: The cellulosome is an elaborate multi-enzyme structure secreted by many anaerobic microorganisms for the degradation of lignocellulosic substrates. It is composed of multiple pieces assembled through high-affinity interactions between enzyme-borne dockerin (Doc) modules and repeated cohesin (Coh) modules present in non-catalytic scaffoldins. The cellulosome of the ruminal bacterium *Ruminococcus flavefaciens* is one of the most intricate described to date and its elaborate architecture is assembled exclusively through single-binding-mode Coh-Doc interactions. However, a set of R. flavefaciens Docs exhibits certain features associated with the classic dual-binding mode.

Materials & methods: To investigate the dual-binding mode in ruminal cellulosomes, we have solved the structures of two Coh-Doc complexes through X-ray crystallography, one involving the Doc of a monovalent adaptor scaffoldin without specificity switch (DocScaH) and another involving an unusual truncated dockerin with a single calcium-binding repeat. The interactions were then further characterized through site-directed mutagenesis and isothermal titration calorimetry.

Results: Unlike previously characterized *R. flavefaciens* Docs, DocScaH interacts with its cognate Cohs in a dual-binding mode. Furthermore, the truncated Docs can interact with three distinct conformations, including one with two Docs binding to a single Coh, a feature never previously reported. We were also able to improve Coh-Doc affinity through structure-informed protein engineering.

Discussion: These results suggest the existence of adaptor scaffoldins with the sole purpose of improving cellulosomal spatial conformation and of naturally occurring atypical dockerins with distinct binding mechanisms. We have also shown that Coh-Doc affinity can be manipulated through rational design, a key feature for the design of affinity-based technologies using tailored Coh-Doc interactions.

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Keywords: CELULLOSOME, COHESIN, DOCKERIN, PROTEIN-PROTEIN COMPLEXES, Ruminococcus flavefaciens

Modelling genetic disorders with an unexpected co-worker: the Tooth Fairy that gifts hope to both children and scientists

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In vitro models represent a gateway to better disease knowledge in both pathophysiology and future therapeutics development. Besides several years of investigation in this field, the best approach to achieve a greater disease model appears to be patient-derived cells. Still, not every cell recapitulates the disease-relevant features, especially when we want to address systems with hard access as the brain and the skeletal, for example.

Those two systems are among those strongly affected in our target disorders: the Mucopolysaccharidoses (MPSs). Characterized by a metabolic and inherited pattern, this is a subgroup of rare, life-threatening diseases belonging to the larger Lysosomal Storage Diseases (LSDs) family. Quite remarkably, none of these systems is reachable by the currently available therapies. Thus, even treated patients still develop neurological and skeletal symptoms.

Our main goal is to establish an innovative non-invasive method to generate disease-relevant cell models from stem cells from deciduous (baby) teeth (SHED) and differentiate them into MPS-target cell lines.

So far, we have already implemented and optimized the protocol for collection, isolation, establishment and cryopreservation of those stem cells. Then, our rationale is simple: for those obtained from MPS patients suffering from multisystemic disease with musculoskeletal alterations, we are using a chondrogenesis differentiation protocol. For those derived from patients with neurological pathology, we will establish mixed neuronal/glial cultures.

This represents a total innovation in the MPS world, and hopefully, the established cells will provide valuable understanding of the cellular and molecular processes of these diseases.

Support/interest disclosure: This work is partially supported by the Portuguese Society for Metabolic Disorders (SPDM - Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018;2019DGH1629/SPDM2018I&D), Sanfilippo Children's Foundation (2019DGH1656/SCF2019I&D), FCT (EXPL/BTM-SAL/0659/2021), Center for the Study of Animal Science (UIDB/00211/2020), and Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020).

Keywords: IN VITRO MODELS; BRAIN AND SKELETAL SYSTEM; MUCOPOLYSACCHARIDOSES; SHEDs.

CIISA CONGRESS 2022

INNOVATION IN ANIMAL, VETERINARY AND BIOMEDICAL RESEARCH

LISBON NOV 11 – 12 2022

ORAL COMMUNICATIONS

SESSION 2
ANIMAL AND FOOD SCIENCE

Chairpersons: Cristina Guedes & Rui Bessa

Development of a novel coating material for rumen-protected lipid encapsulation using *Nannochloropsis oceanica* algaenan

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Introduction: Long-chain polyunsaturated omega-3 fatty acids (*n*-3 LC PUFA) are biohydrogenated (BH) by ruminal microorganisms, originating fatty acids (FA) with lower nutritional value, limiting the *n*-3 LC PUFA deposited in tissues. Eicosapentaenoic acid (EPA) is one of the most health-beneficial *n*-3 LC PUFA, therefore there is an interest in enhancing its content in meat, using rumen-protecting techniques to overcome ruminal BH. *Nannochloropsis oceanica* is a marine microalga enriched in EPA that has a cell wall with an outer layer containing algaenans. Algaenans are candidates for ruminal lipid protection technologies due to strong chemical and microbiological resistance. The aim of this work was to develop a rumen-protecting technique for *n*-3 LC PUFA using algaenan.

Materials & methods: Algaenan was obtained from freeze-dried *Nannochloropsis* biomass after removal of lipids and carbohydrates. The residue containing algaenan was incorporated (0.3% w/v) in emulsions with agar-agar, alginate and chitosan and capsules were formulated to encapsulate 20% (w/v) omega-3 PUFA-rich oil. The capsules were incubated with rumen inoculum in an *in vitro* batch system under anaerobiosis for 24 h.

Results: The percentage of mass lost averaged 10% at 24 h. Higher EPA protection was achieved with the alginate coating (98%), followed by the alginate-chitosan (96%) and agar-agar (90%). EPA protection at 24 h was 88% in the capsules not containing algaenan and 95% (+7%) in the capsules containing algaenan.

Discussion: Algaenan addition improved EPA protection in 2% in alginate-chitosan and agar-agar and in 10% in alginate capsules, revealing its potential as rumen protection technology.

Support disclosure: This work was financed by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., within the scope of the projects PTDC/CAL-ZOO/29654/2017, UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4Animals).

Keywords: RUMEN; EPA; *NANNOCHLOROPSIS OCEANICA*; ALGAENAN

Chlorella vulgaris as ingredient in piglet diets: digestibility, microbiota and intestinal morphology studies

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Background: Microalgae have been referred for their interesting nutritive value, free use of agricultural fields and ability to sequester carbon. Moreover, its high cost and the presence of a recalcitrant cell wall have made its feeding use challenger. Our aim was to study the effect of 5% *Chlorella vulgaris* (CH) microalga incorporation in the diet of weaned piglets, individually or combined with two carbohydrases, on nutrient digestibility, gut morphology, fermentation products and microbiota.

Materials & methods: Forty-four male piglets with 11.2 ± 0.46 kg of live weight were used and allocated to 1 of 4 dietary treatments: basal diet (cereal and soybean meal) (control, n = 11); control diet with 5% of CH instead soybean meal (CH, n = 10); CH diet supplemented with 0.005% of Rovabio® Excel AP (CH+R, n = 10); CH diet supplemented with 0.01% of a recombinant 4-carbohydrase mixture (CH+M, n = 11). After 21 days of trial, the animals were slaughtered and the samples of interest collected.

Results: CH had no effect on growth performance. In turn, digestibility of nutritional fractions was negatively affected by its inclusion. Parallelly, the viscosity of duodenum plus jejunum contents increased in all groups fed with microalga. Although, the compensatory mechanisms were denotated by intestinal morphology (higher duodenum villus height in CH groups) and microbiota (higher abundance of genera *Colidextribacter*, *Oscillospira* and *Lactobacillus* in CH groups).

Discussion: Following our line of investigation, these results indicate the viability of using CH by swine and transform the idea of a functional ingredient into feedstuff.

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Keywords: PIGLETS; *Chlorella vulgaris*; ENZYMES; DIGESTIBILITY; MICROBIOTA

Effects Of Sunflower Oil Infusions Of *Asparagopsis taxiformis* On *In Vitro* Rumen Methane Production

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Introduction: Supplementation with the red macroalga *Asparagopsis taxiformis* (*AT*) of ruminant diets is a promising strategy to reduce methane (CH_4) emissions from rumen. This effect is attributed to halogenated compounds, particularly bromoform ($CHBr_3$), a volatile compound. Immersion in *AT* biomass oil is effective in stabilising $CHBr_3$. The main objective of this study was to verify the effectiveness of sunflower oil naturally enriched in *AT* halogenated compounds in reducing CH_4 emissions.

Material & Methods: Six levels of $CHBr_3$ (0, 25, 50, 75, 100 and 150 ⌠g $CHBr_3$/g feed dry matter (DM)), included in 60 ⌠L of Bromoil were evaluated *in vitro*, using an ANKOM^{RF} gas production system, through 5 runs, with two replicates in each run. To perform the 48-hour incubations, rumen inocula were collected after the slaughter of young-bulls reared on the same farm and 1 g DM of a Total Mixed Ration was used as the feed sample. Total gas and CH_4 production, organic matter (OM) degradability and volatile fatty acids (VFA) proportion were determined.

Results: Total gas production was not affected by treatments but CH_4 emissions decreased in 50 % and 86 % with the treatments 100 and 150, respectively. The degradability of OM and Total VFA were not affected by treatments but the acetate to propionate ratio was 20 % and 25 % lower for treatments 100 and 150, respectively. Discussion: These results demonstrate that oil immersions of *AT* can be effective in reducing CH_4 emissions and need to be confirmed in *in vivo* trial.

Support/interest disclosure:Work funded by PRR-C05-i03-I-000027-LA3.1, GEEBovMit, and by Portuguese Foundation for Science and Technology (FCT) grants UIDB/00276/2020 (CIISA), LA/P/0059/2020(AL4AnimalS) and a PhD studentship to F. Sena (UI/BD/152817/2022).

Keywords: *ASPARAGOPSIS TAXIFORMIS*; OIL; METHANE; *IN VITRO*

Haylage-based diets as an alternative to concentrate-based diets for finishing of young-bulls

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Concentrate-based diets are frequently used for finishing of young-bulls. Diets based on high quality forage can provide a sustainable alternative, reducing the competition with humans for food, the national reliance on imported raw materials and the carbon footprint. Sixteen crossbred Limousine x Alentejana young-bulls, individually housed, were assigned to two diets during 64 days. The control diet (C) was based on concentrate (Biona Diobife Top2 GRA, DeHeus, Cartaxo) and the Total Mixed Ration diet (TMR), contained 54% dry matter (DM) of biodiverse haylage (SpeedMix, Fertiprado, Vaiamonte), 36% DM concentrate and 10% DM sunflower seed. The diets had 16% DM of crude protein, were offered *ad libitum*. Intake was controlled daily and live weight fortnightly. Methane production was individually measured, using a GreenFeed unit (C-Lock, Rapid City, SD, USA). A Life Cycle Analysis was conducted to estimate the carbon footprint during the finishing period. Daily weight gain was similar among diets (1718 g/day). Dry matter intake was 9% higher with TMR, corresponding to higher fat and fibre and lower starch intakes. Dry matter conversion ratio was 20% higher for TMR but feeding cost reported to live weight gain was similar between diets. Concentrate consumption was 46% lower and methane production was 75% higher with TMR, resulting in a carbon footprint per kg liveweight gain similar among diets. Haylage-based diets did not compromise growth performance or meat quality, improved nutritional value of fat and had no impact both on feeding costs and on the carbon footprint during the finishing phase of young-bulls.

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Keywords: BEEF; FORAGE-BASED DIETS; MEAT QUALITY; CARBON FOOTPRINT; GREENFEED

Is *Nannochlopsis oceanica* a new source of omega-3 polyunsaturated fatty acids for horse diets?

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Introduction: Marine microalgae have recently been used in human and animal feeding as a source of n-3 PUFA, such as EPA. As *Nannochloropsis oceanica* has a high content of EPA, it was hypothesized that the inclusion of this microalga in horse diets would increase EPA concentration in blood plasma and erythrocyte membrane lipids.

Material & Methods: Eight Lusitano horses were blocked considering age, live weight, and sex in two groups of 4 animals, randomly assigned to two treatments: CON (control – basal diet) and ALG (basal diet + 200 g of microalgae, which provided an average amount of 4.8 g of EPA/day). The feeding trial consisted of an adaptation period of 28 days, where the ALG group was fed increasing amounts of the microalgae, followed by 28 days on feed, and a final 56-day washout period. Blood and fecal samples were periodically collected to determine fatty acid composition by gas chromatography.

Results: EPA concentration in plasma was higher in the ALG than in the CON animals (1.71 *vs* 0.06%; $P<0.001$). This fatty acid was not detected in red blood cells of CON animals, whereas it was present in ALG group, reaching the highest value (1.2% of total fatty acids) at the end of the feeding trial. During the washout period, EPA remains in red blood cells until day 98, contrasting with the fast decline observed in plasma.

Discussion: The obtained results are innovative and encourage the pursuit of further investigation regarding the benefits of this microalga as an alternative source of EPA in horse diets.

Support/interest disclosure: Projects CIISA UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS.

Keywords: *Nannochlopsis oceanica*, EICOSAPENTAENOIC ACID, MICROALGAE, HORSE DIETS.

Individual carbon-oxygen lyases (EC 4.2) as a solution to disrupt *Laminaria digitata* and *Ulva lactuca* recalcitrant cell walls

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Background: Macroalgae are composed by bioactive and nutritive compounds, which makes them potential feedstuffs for monogastric animals. However, their recalcitrant cell walls compromises alga utilization. This study aimed to evaluate the ability of pre-selected carbohydrases to disrupt *L. digitata* and *U. lactuca* cell walls and release nutritional compounds.

Methods: A databank of 199 recombinant Carbohydrate-Active enZymes and sulfatases was tested *in vitro* for their action towards *L. digitata* and *U. lactuca* cell wall polysaccharides. The enzymes were incubated with macroalga suspension, either alone or combined. Quantification of released reducing sugars, fluorescence intensity of Calcofluor White stained cell walls, and released nutritional compounds was performed in triplicate.

Results: Alginate lyase (PL7) and ulvan lyase (PL25) were the most efficient in disrupting *L. digitata* and *U. lactuca* cell walls, respectively. The alginate lyase released 7.11 g/L of reducing sugars, 8.59 mmol/100 g dried alga of monosaccharides ($p < 0.001$), and reduced cell wall fluorescence by 39.1% ($p = 0.001$). The ulvan lyase released 4.54 g/L of reducing sugars, 11.4 and 11.2 mmol/100 g of mono- and oligosaccharides, and decreased cell wall fluorescence by 41.7% ($p < 0.010$). No significant release ($p > 0.05$) of total fatty acids, proteins or pigments was observed, except for monounsaturated fatty acids ($p < 0.001$).

Discussion: These results highlight the capacity of single recombinant alginate lyase and ulvan lyase to partially degrade *L. digitata* and *U. lactuca* cell walls. The enzymes could enhance the bioaccessibility of macroalgae bioactive compounds for monogastrics, with further application in feed industry.

Support/interest disclosure: This study was financially supported by Portuguese Foundation for Science and technology (FCT, Lisbon, Portugal) through grants: PTDC/CAL-ZOO/30238/2017 with an associated post-doctoral contract to MMC, LA/P/0059/2020 to AL4AnimalS, UIDB/00276/2020 to CIISA and a PhD studentship to DFC (SFRH/BD/126198/2016).

Keywords: *LAMINARIA DIGITATA*; *ULVA LACTUCA*; CARBOHYDRATE-ACTIVE ENZYMES; CELL WALL DEGRADATION.

Tissue specific variation of aquaporins and cytokines transcriptional profile in piglets under LPS challenge

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Background: Lipopolysaccharide (LPS), an effective stimulator of the immune system, has been widely applied in pigs as an experimental model for human sepsis. Aquaporins (AQPs), a family of small integral membrane proteins responsible for facilitating water fluxes through cell membrane, stand out as promising drug targets for sepsis treatment due to their role in water balance and inflammation.

Methods: In order to investigate the potential effect of a dietary amino acid mixture supplementation on LPS-challenged weaned piglets, twenty-seven 29-day-old males were randomly allocated to one of three experimental treatments for five weeks: control (CTL) treatment (n=10); LPS treatment (n=7), whose piglets were intraperitoneally administered LPS (25 μg/kg body weight); LPS + amino acid cocktail treatment (n=10), whose piglets were intraperitoneally administered LPS and fed a diet supplemented with a mixture of arginine, branched-chain amino acids (leucine, valine, isoleucine), and cystine. Key organs controlling sepsis, as small intestine, liver, kidney and spleen, were collected and processed for AQPs and cytokines transcriptional profile by RT-qPCR.

Results: Minor variations were detected for AQPs and inflammatory markers mRNA levels, upon dependence of LPS or the amino acid cocktail. Using a discriminant analysis tool, we report here for the first time a tissue specific variation of AQPs and cytokines transcriptional profile that clearly apart the small intestine and the kidney from the liver and spleen.

Discussion: Our findings provide a novel insight into the gene expression signature of AQPs and cytokines for each organ in piglets, recognized as a valuable comparative animal model for human metabolic disorders.

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Keywords: LPS; AQUAPORINS; CYTOKINES; AMINO ACID SUPPLEMENTATION; PIGLETS

Unraveling biomarkers of *trans*-10 shift in ruminants: a multi-Omics approach

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Introduction/Background: The *trans*(*t*)10-shift has been recognized as the deviation of the normal ruminal biohydrogenation (BH) pathways of the dietary polyunsaturated fatty acids (FA) in the rumen, with the formation of the deleterious *t*10-18:1 replacing the beneficial *t*11-18:1 as the main BH intermediates. The *t*10-shift has been associated with low-forage high-starch diets. Nevertheless, there is a high variability among animals feeding the same diet regarding the *t*10-shift expression. Moreover, the mechanisms that drive the *t*10-shift are not well known. This work aimed to use several levels of information, lipidomics, metabolomics, and proteomics of ruminal contents, to search for biomarkers associated with *t*10-shift.

Materials & methods: Rumen contents from sixteen male lambs distributed by two diets with distinct forage:concentrate ratios (20:80 and 80:20) were collected after 42 days on ad libitum feed. FA from ruminal contents were prepared and analyzed by gas-chromatography, metaproteomics analysis was conducted from ruminal bacterial pellets using LC-MS/MS based label-free quantitation, and metabolomics was conducted from ruminal fluid supernatant using IMS-TOF technology. Multivariate analysis and correlations were conducted using MetaboanlystR' package.

Results: Several FA, proteins and metabolites were highly correlated with the proportion of *t*10-18:1 in the ruminal contents, the 17:0, 14:0, a-15:0, and *t*15-18:1 were the FA most positively correlated, while *t*11-18:1, *t*11,*c*13-CLA and *c*9,*t*11-CLA were negatively correlated. The metabolites, thiamine aldehyde, mevinolinic acid, 2-Hydroxy-2H-benzo[h]chromene-2-carboxylate, or biotin were positively correlated with *t*10-18:1, as well as proteins associated with gluconeogenesis, stress response or amino acid metabolism.

Discussion: This work provides for the first time, a multi-level integrated view on t10-shift phenomena in sheep.

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Keywords: BIOHYDROGENATION; FATTY ACIDS; TRANS-10 SHIFT; RUMEN; OMICS

ORAL COMMUNICATIONS

SESSION 3
ANIMAL HEALTH

Chairpersons: Isabel Pereira da Fonseca & José Correia Costa

O 017

What lives in the fur of shelter animals? A mycological survey

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In the microbiological world, the relationship between pets (dogs/cats) and their owners contributes to fungal species sharing. Some of these species can be potential pathogens. Some pathogenic fungi in animal (dogs and cats) skin are potentially transmitted to humans, being responsible for superficial mycozoonoses.

The aim of this work was to study fungal diversity in fur samples from dogs and cats from shelters in Portugal Samples were collected using the Mackenzie method, and mycological routine techniques were applied to achieve fungi identification.

In this study, 156 fur samples were analyzed from animals living in the shelter of Terra Quente, Mirandela, Portugal. Regarding animal species, 78.2% were dogs (n=122) and (21.8%) were cats (n=34). Regarding breed, the vast majority, 93.6% (n=146) were not breed specific, 1.9% (n=3) were from the Portuguese breed Transmontano Mastiff, 0.6% (n=1) were Dogue de Bordeaux, 1.3% (n=2) were Portuguese Pointer, 1.3% (n=2) was Portuguese Warren Hound, and 1.3% (n=2) were Siamese cats. Concerning the presence of skin lesions, only 5.1% (n=8) of the animals had lesions, while the majority had no lesions at all (94.9%; n=148). The following medically important fungal genera/species were isolated: *Alternaria* spp.; *Aspergillus* spp.; *Aspergillus fumigatus*; *Aspergillus niger*, *Beauveria* spp., *Chaetomium* spp, *Cladosporium* spp.; *Cladophialophora* spp., *Curvularia* spp., *Epicoccum*; *Fusarium* spp., *Microsporum audouinii*, *Mucor* spp.; *Penicillium* spp.; *Rhizomucor* spp., *Scopulariopsis* spp., *Sporothrix* spp., *Verticillium* spp..Based on the One Health concept, as these animals can serve as reservoirs for fungi that can affect humans, especially immunocompromised individuals and bring harmful consequences to them.

Support: This research was supported by CECAV - project UIDP/CVT/00772/2020 and AL4AnimalS - LA/P/0059/2020

Keywords: CAT; DOGS*;* FUNGI; ONE HEALTH; SHELTER

Significance of uterine cytology macrophage counts on endometritis recovery in postpartum dairy cows

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Introduction: Postpartum clinical endometritis, even following appropriate therapy, frequently evolves to a persistent status named subclinical endometritis (SCE) that causes subfertility and repeat-breeding. Diagnosis of SCE require invasive uterine cytology and/or biopsy. Whereas the uterine cytology populations of polymorphonuclear neutrophils (PMN) are well characterized, the presence and significance of macrophages remain elusive.

Materials & methods: This study related the uterine cytology PMN and macrophage populations in postpartum dairy cows with different endometrial health status. Based on uterine cytology, cows were allocated to groups Healthy (n=16; PMN<18% at 21 days postpartum (dpp) and PMN <5% at 45dpp), Recovered (n=10; PMN≥18% at 21dpp and PMN<5% at 45dpp) and Persistent Endometritis (n=5; PMN≥18% at 21dpp and PMN≥5% at 45dpp).

Results: At 21dpp, Recovered cows had a higher percentage of macrophages than Healthy and Persistent Endometritis cows (7.3% vs. 2.8% vs. 1.3%, respectively; p< 0.01). Among Recovered cows, the percentage of macrophages was higher at 21 than at 45dpp (7.3% vs 1.73%; p< 0.001), the latter stage being coincident with apparent endometrial recovery from inflammation.

Discussion: These results evidence that spontaneous recovery from endometritis is associated with an endometrial infiltration of macrophages, and Persistent Endometritis cows' failure to recover from endometritis is associated with an absence of macrophage endometrial infiltration. Low macrophage infiltration in Healthy cows may be associated with absence of inflammatory stimuli. In conclusion, macrophage counts in uterine cytology results as early as 21dpp may allow differentiation between cows that recover and cows that fail to recover from the endometrial inflammation, thus requiring additional therapy and/or follow-up.

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Keywords: ENDOMETRITIS; DAIRY COW; CYTOLOGY; PMN; MACROPHAGE

Structural characterization of African Swine Fever Virus nucleoprotein p10 interaction with DNA

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Background: African swine fever virus (ASFV) is the etiological agent of a highly contagious, hemorrhagic infectious swine disease, with a tremendous sanitary and economic impact on a global scale. Currently, there are no vaccines or treatments available. The p10 protein, a structural nucleoprotein encoded by ASFV, has been previously described as capable of binding double-stranded DNA (dsDNA), which may have implications for viral replication. However, the molecular mechanism that governs this interaction is still unknown, mostly due to the lack of a structural model for this protein.

Materials & methods: In this work, we have generated an *ab initio* model of the p10 protein and performed extensive structural characterization using molecular dynamics simulations to identify the motifs and residues regulating DNA recognition.

Results: The helix-turn-helix motif identified at the C-terminal region of the protein was shown to be crucial to the dsDNA binding efficiency. Like other DNA-binding proteins, two distinct serine and lysine-rich regions found in the two helices were identified as key players in the binding to DNA, whose importance was later validated using experimental binding assays.

Discussion: The multidisciplinary approach used in this work highlighted the importance of electrostatics in the interactions established between p10 and DNA. The detailed structural characterization of p10 performed in this work, opens the door to further studies on the role of these amino acid residues in p10 function during ASFV replication and assembly.

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Keywords: AFRICAN SWINE FEVER VIRUS; P10 PROTEIN; MOLECULAR DYNAMICS; DNA BINDING FUNCTION; K78R

Iberian hare extinction: the foundation of a Breeding Centre in Portugal

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Introduction: The Iberian hare (*Lepus granatensis*) is an endemic and key species, that has declined by more than 50% in the last four years after the emergence of a natural recombinant myxoma virus (ha-MYXV) and the putative reactivation of Leporid gammaherpesvirus 5 (LeHV-5) in ha-MYXV infected hares which together will lead to the reclassification of its conservation status to Vulnerable by the IUCN this year.

The impact of these viral pathogens prompts us, within the scope of Project +Coelho2, to carry out vaccination trials in hare specimens proving the lack of efficacy of commercial vaccines in protecting hares. In the context of an animal health and conservation emergency, the aim of this work was to reproduce seropositive animals in captivity and to create the Iberian hare breeding centre in Portugal in 2020 and wild rabbit breeding was programmed later.

Material & Methods: Physical and technical facilities (about 2 ha) were created including breeding, feeding and physical training parks as well as methods for disinfection, deworming, capture of animals with minimal stress, and specific probe was built for the Iberian hare for semen collection.

Results: Currently, the centre has 4 epidemiological units with 60 hares and 2 epidemiological units with 40 wild rabbits (Endangered, IUCN). During this year, repopulations were started (43 hares and 67 rabbits) in Évora with 2.7% of mortality.The electroejaculation method has allowed the collection of sperm in about 5-10 minutes, of a concentration of $1.06 \times 10^9 \pm 7.98 \times 10^8$ spz/mL.

Discussion: In the next years the annual restocking will include 200 hares and 150 rabbits. The artificial insemination will be started and it is expected that improve the species' prolificacy, increasing the number of animals released into the wild.

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Keywords: IBERIAN HARE, EUROPEAN RABBIT, CONSERVATION MEDICINE, REPRODUCTION

Circulating cell free miRNA profiles of postpartum dairy cows with subclinical endometritis

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Introduction: Subclinical endometritis (SCE) is an unresolved inflammation of the endometrium of postpartum dairy cows, seriously affecting subsequent fertility, for which current diagnosis relies on invasive uterine cytology and/or biopsy sampling. Due to the known role of miRNAs in disease development, this study evaluated the cell-free circulating miRNA profiles of SCE cows and their value as blood-based diagnostic biomarkers.

Material & Methods: Healthy (H, n = 6) and SCE (n = 11) cows characterized by endometrial cytology and biopsy were blood sampled at 21 and 44 days postpartum. Following extraction of cell-free plasma miRNAs and RNA-seq analysis, differential abundance analysis of miRNAs was performed with the DESeq2 R package (adjusted p-value of 0.05).

Results: Results identified 34 miRNAs with differential abundance between H and SCE cows, from which 22 were more abundant, and 12 were less abundant in SCE cows than in H cows, without the influence of postpartum stage. From the 22 more abundant miRNAs, miR-425-3p and miR-30b-5p had the lowest adjusted p-values and the highest log2 fold change (FC), and from the 12 less abundant miRNAs, miR-10b exhibited the highest normalized read counts and the lowest adjusted p-value and log2 FC.

Discussion: In total, the 34 miRNAs are predicted to interact with 10104 transcripts, including those known to be involved in endometrial control of the inflammatory response, tissue remodeling, immune tolerance and pregnancy establishment. In conclusion, despite being apparently confined to the endometrium, SCE is associated with a distinct circulating miRNA profile, which may render it a source of non-invasive diagnostic biomarkers.

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Keywords: DAIRY COW, SUBCLINICAL ENDOMETRITIS, MIRNA, POSTPARTUM

Carbapenemase-producing Enterobacterales clinical strains causing infections in cats and dogs

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Introduction: Carbapenemase-producing Enterobacterales strains (CPEs) are increasingly being reported in Companion Animals (CAs), despite their use being prohibited in veterinary medicine. The aim of this retrospective study (January-December 2020) was to detect CPEs from clinical strains of companion animals.

Methods: Nine hundred and seventy-seven Enterobacterales clinical isolates were included in the study. Susceptibility data was obtained by the disc diffusion method and/or MICs determination. Phenotypic and genotypic confirmatory tests were also performed to detect beta-lactamase-producing Enterobacterales, including carbapenemases. Positive isolates were characterized by WGS. Assemblies were then used to screen for antimicrobial resistance genes (ResFinder 4.1) and mobile genetic elements (Mobile Element Finder v1.0.3). MLST was performed.

Results: Four ESBL- producing *Klebsiella pneumoniae* isolates were positive for carbapanemase genes: i) OXA-181-producing *K. pneumoniae* ST273, only resistant to ertapenem (MIC > 1 mg/L), ii) two KPC-3-producing *K. pneumoniae* ST147, and iii) one KPC-3-producing *K. pneumoniae* ST392. All isolates belonged to clonal group 147 and were resistant to carbapenems but susceptible to ceftazidime-avibactam. The *bla*$_{\text{KPC-3}}$ gene was located on transposon Tn4401*d* on IncFIA-type plasmid for *K. pneumoniae* ST147 isolates and on a IncN-type plasmid for *K. pneumoniae* ST392. Lastly, one *Escherichia coli* ST127 isolate (uropathogenic lineage) was found to only harbour *bla*$_{\text{OXA-48}}$, being susceptible to 3rd generation cephalosporins and carbapenems.

Conclusion: Carbapenemase detection methods need to be implemented during routine microbiology in Veterinary medicine as to ascertain the impact carbapenem resistance has on Animal and Public Health. More data is urgently needed for the design of preventive measures.

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Keywords: KPC-3-producing *K. pneumoniae*, OXA-181-producing *K. pneumoniae*, OXA-48-producing *E. coli*, CLONAL GROUP 147, CARBAPENEM RESISTANCE

Highest Priority Critically Important Antimicrobial Resistance Gene, *mcr-1*, Shared Within Households, Lisbon, Portugal, 2018-2020

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Background: The increasing reports of plasmid-mediated colistin resistance worldwide raises concern towards the treatment of MDR Gram-negative bacteria nosocomial infections. Our aim was to investigate the possible sharing of colistin-resistant *Escherichia coli* recovered from faecal samples of companion animals (CAs) and their household members in Portugal.

Methods: Between February 2018 and February 2020, faecal samples from healthy and sick dogs and cats (*n*=102) and their owners (*n=125*) were plated onto SuperPolymyxin medium. Species identification was performed by PCR. Susceptibility tests were performed by broth microdilution (SensititreTM FRCOL, Thermo Fisher Scientific and for MicroScan® Neg MIC Panel Type 44, Siemens). All isolates were screened for the presence of colistin resistance genes (*mcr-1* to *mcr-9*) by PCR and sequenced generating libraries of WGS paired-end with 150 bp. Assembly genomes were performed and genetic relatedness between animal and human strains estimated.

Results: Colistin-resistant *E. coli* strains harbouring the *mcr-1* gene, were recovered from faecal samples of CA (7.8%, *n*=8/102) and humans (3.2%, *n*=4/125). In two households from dogs diagnosed with skin and soft tissue infection, had resistant strains in both a CA and owner. Relatedness analysis of the assembled genome suggests the occurrence of transmission within the households. All *mcr-1* positive strains were MDR.

Discussion: The identification of similar *mcr-1*-positive strains in CA and humans is of great concern. These results demonstrate that CAs may act as reservoirs of plasmid-mediated colistin resistance, allowing the spreading of these clinical important genes into the community. Reinforcing the importance of an active monitoring and hygiene practices.

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Keywords: *mcr*; PLASMID-MEDIATED COLISTIN RESISTANCE; HUMAN–DOG TRANSMISSION; MULTIDRUG RESISTANCE: COLONIZATION.

○ 024

“One Health” approach to challenge antimicrobial resistance

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According to the World Health Organization, infections with antibiotic resistant bacteria can cause a total of 700,000 deaths annually globally. The use of antibiotics promotes the development of resistance and influences co-selection processes in bacterial communities leading to the spread of antibiotics, resistant bacteria and resistance genes among humans, companion animals, livestock, wildlife and the environment. Considering the promiscuity of gene transfer systems among bacteria, the presence of resistance genes in the environment is considered an ecological problem. Thus, there is an urgent need to understand the dynamics of antimicrobial resistance (AMR), being the One Health approach essential to assess the origin, spread and flow of AMR mechanisms and to define new strategies to combat the problem. Therefore, it is necessary to address the problem on a number of fronts by promoting studies to elucidate AMR mechanisms in resistant bacteria, and to contribute to an emerging view of the extent of the AMR problem in the context of One Health.

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Keywords: ANTIMICROBIAL RESISTANCE, ONE HEALTH, ANTIBIOTIC RESISTANT BACTERIA

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ORAL COMMUNICATIONS

SESSION 4
CLINICAL RESEARCH

Chairperson: Esmeralda Delgado & Luís Lopes da Costa

Encephalitozoon cuniculi infection in pet rabbits – could it be an issue?

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Introduction: The population of pet rabbits is increasing Worldwide, and, in many countries, they have become the third top pet species. Their appealing character, together with their small size and low maintenance costs have all accounted for rabbits' growing popularity. *Encephalitozoon cuniculi* is an ubiquitarian Microsporidian agent, described in rabbits and in many other mammalian species, including humans, to whom it is considered an opportunistic agent. In rabbits the infection can range from asymptomatic to severe eye, kidney, or nervous disease. Therapeutic protocols for pet rabbits are far from ideal and it is not yet possible to eliminate completely the Microsporidium from carrier nor diseased rabbits. The most accepted therapeutic protocol consists of 20 mg/kg fenbendazole, daily, for 28 days.

Material & Methods: A retrospective review was performed out of the 132 rabbits that were presented for consultation in FMV-ULisboa Teaching Hospital from September 2021 until September 2022. 21 animals were tested for *E. cuniculi*. Criteria for testing included: clinical signs, in contact positive rabbits and owner permission for testing. From each rabbit, 1 ml blood was collected from the lateral saphenous vein, and serum was submitted to ELISA techniques for detection of *E. cuniculi* antibodies (IgG and IgM).

Results: 18 out of 21 samples tested positive, and only three clinically suspected animals tested negative for *E. cuniculi*. All in contact animals tested positive.

Discussion: In our Teaching Hospital, due to owners' financial constraints, screening was not always performed in all rabbits, but our limited review accounted for seropositivity of 85,7% (18/21) in clinically suspected rabbits and rabbits in contact with positive animals. One study carried out in Lisbon, in 2014, determined a seroprevalence of 42,7%, in a sample of 44 asymptomatic rabbits (Patrício, 2014). These results suggest that prevalence of *E cuniculi* infection in pet rabbits is likely to be very high, raising the interest in investigating the subject, namely in terms of prevalence, prevention and therapeutics. Considering these data, and the fact that the prevalence of the infection in pet rabbits has been determined between 20 and 70%, this diagnostic should be integrated as part of the routine check-up for pet rabbits in Lisbon's Teaching Hospital.

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Keywords: *ENCEPHALITOZOON CUNICULI*; RABBIT, INFECTION, ELISA, IGM/IGG

Nanoparticulate system carrying epoetin beta as a neuroprotective agent: topical ocular administration in glaucomatous rats

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Purpose: To evaluate the effects of chitosan-hyaluronic acid-epoetinβ (CS/HA-EPOβ) nanoparticles through topical ocular route in glaucoma

Material / methods: Wistar Hannover rats (n=24) were split into treatment (n=12) and control (n=12) groups. All animals underwent complete ophthalmological examination, electroretinography, microsurgery for glaucoma induction through cauterization of three episcleral veins plus topical ocular administration of nanoparticles in the right eye (OD) (1 drop TID during 3 days). The treatment group received nanoparticles loaded with EPOβ while the control group received empty nanocarriers. Electroretinography was repeated on day 3, and before euthanasia on days 7, 14, and 21. Both eyes were enucleated and histological processing (hematoxylin-eosin staining) and immunofluorescence for EPOβ detection were performed.

Results: Animals demonstrated good tolerance to the nanoparticles. After glaucoma induction, intraocular pressure (IOP) values in the OD were 62.6 ± 8.3 mmHg for treated and 63.6 ± 7.9 mmHg for control group. Electroretinography at day 3 showed a significant decrease of retinal electrical activity, confirming glaucoma induction. Later, the treated group showed a faster retinal recovery with an improvement in b-waves amplitude. Hematoxylin-eosin staining revealed thinner retinas in the control group ($p < 0.05$). EPOβ was detected by immunofluorescence in the retina of all treated eyes, and also in the corneal stroma, indicating transcorneal permeation.

Discussion: Topical administration of CS/HA-EPOβ nanoparticles in glaucomatous rats allowed EPOβ's transcorneal permeation and delivery to the inner retina, promoting an earlier recovery of retinal thickness and electrical activity. Topical drug delivery of neuroprotective agents targeting the posterior segment of the eye could become a safe and user-friendly adjuvant treatment for neurodegenerative ocular diseases.

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Keywords: NANOPARTICLES, CHITOSAN-HYALURONIC ACID-EPOIETINβ, TOPICAL ROUTE, GLAUCOMA

Biocompatibility and Tolerance of the InEye® ophthalmic insert in rabbits

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Introduction: Presently, more than 90% of all ophthalmic preparations are delivered as eye drops. Despite being a non-invasive, safe, inexpensive and easy-to-use route of administration, it has several disadvantages. An ocular insert named InEye® was developed to address them. The present study aimed to evaluate biocompatibility and tolerance of the InEye® in rabbits.

Materials & methods: Six male, New Zealand white rabbits, with normal ophthalmological exams, were used. Experiments were conducted in one eye of each rabbit, the contralateral eye serving as control. An interval of at least one week was respected between trials. The insert was placed in the lower conjunctival sac and ophthalmic examinations were performed at 6 h, 24 h and daily until 7 days after placement to check for patency and recognize any signs of discomfort or inflammation. In total 52 inserts were tested, half of them spheres (n=26) and half hemispheres (n=26). The mass and equatorial diameter of the inserts varied according to 11 categories.

Results: There were no ocular sequelae due to the insert's presence. From the total of inserts, 19.2% stayed in the conjunctival sac of rabbits for at least 7 days. Both mass and equatorial diameter variation had implications on its biofunctionality. The inserts with longer residence time were the 15 mg mass (96 hours) and 3 mm equatorial diameter (108 hours). It was not possible to conclude if either shape or pre-hydration had influence on biofunctionality.

Discussion: InEye® presence was well tolerated in this study. Concerning biofunctionality, it may vary according to the anatomical characteristics and behavior of the patient, so further studies are needed with additional shapes and sizes depending on the target species.

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Keywords: INEYE®, RABBITS, BIOCOMPATIBILITY, TOLERANCE

O 028

Decitabine effect on collagen type I and III in TGF-β1 treated mare endometrial fibroblasts

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Introduction: Mare endometrial fibrosis has a deleterious effect on fertility. It is characterized by excessive deposition of collagen type I (COL1), collagen type III (COL3) and α smooth muscle actin (α-SMA), a marker of myofibroblasts. Epigenetic alterations in chromatin structure may change fibroblast phenotype. Transforming growth factor (TGF-β1) induces myofibroblast differentiation and promotes collagen synthesis by epigenetic mechanism in humans. Nevertheless, this has not been referred to in the mare endometrium. We hypothesized that mare endometrial fibroblasts might be under epigenetic regulation. Therefore, the objective was to evaluate the *in vitro* epigenetic modulation in equine endometrial fibroblasts treated with TGF-β1, as well as the use of an epigenetic inhibitor drug 5-aza-2’-deoxycytidine (5-aza-dc or decitabine) to impair fibrogenesis.

Materials and methods: Fibroblasts isolated from mare endometria (n=5) were treated with TGF-β1 (10 ng/mL) for 48h, followed by decitabine (1 μM) for additional 48h. As controls, fibroblasts were incubated alone, or with decitabine or TGF-β1 for 96h. The effect of TGF-β1 and decitabine DNA methylation inhibition on DNA methyltransferases (*DNMT1, DNMT3A* and *DNMT3B*), COL1, COL3 and α-SMA mRNA levels (qPCR) were evaluated in fibroblasts, and collagen (EIA) in conditioned medium.

Results: TGF-β1 increased *DNMT3A* mRNA levels and COL1 and COL3 protein. Decitabine diminished COL1 and COL3 mRNA levels and secretion, but not *α-SMA* transcripts in TGF-β1 challenged endometrial fibroblasts.

Conclusion: These data pinpoint the involvement of epigenetic pathways in fibrogenesis, as observed by evident decitabine inhibition of TGF-β1 profibrotic action in equine endometrial fibroblasts. A putative treatment option for mare endometrial fibrosis with decitabine should be further considered.

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Keywords: ENDOMETRIUM FIBROSIS, MARE, EPIGENETICS, FIBROBLASTS, DECITABINE

O 029

Hip Dysplasia in a New Functional Rabbit Model: Computed Tomography Characterization

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Introduction: Hip dysplasia (HD) is a developmental orthopaedic disease, with clinical relevance mainly in dogs and humans. Current HD models are centered on long immobilization of the rabbit hindlimb, leading to permanent malfunctions. Therefore, new HD rabbit functional models may provide valuable medical inputs in HD studies.

Materials & Methods: Thirteen 6-weeks-old male New Zealand white rabbits were randomly allocated into 3 groups: GI (n=3) - control; GII (n=5) - left hip instability surgery and right hip sham; GIII (n=5) - left hip instability surgery, followed by hindlimb cast immobilization for 3 days. The instability surgery was performed by sectioning the round ligament and the sham by accessing the capsule without sectioning. HD imaging characterization was evaluated in: normal hips (NH, left and right hips-GI); instability surgery hips (ISH, left hips-GII); sham surgery hips (SSH, right hips-GII); instability surgery hips with limb immobilization (ISHI, left hips-GIII), and hips without surgery (HWS, right hips-GIII). In computed tomography (CT), the Lateral Center-Edge Angle (LCEa) and Dorso Subluxation Score (DLS) were evaluated.

Results: The LCEa and DLS mean were respectively: NH-88.6º^{a}, 0.49^{a}; ISH-77.0º^{a}, 0.38^{a}; SSH-84.9º^{a}, 0.46^{a}; ISHI-62.1º^{b}, 0.25^{b}; HWS-79.4º^{a}, 0.41^{a}. In each variable, mean values with different superscripts were statistically different in the One Way-ANOVA LSD Post-hoc test ($P<0.05$).

Discussion: The present model was efficient in promoting HD and maintaining the limb functionality. The CT assessment demonstrated differences in the joints of the studied groups consistent with HD development. This HD rabbit model can be even recommended for HD studies that intend to test therapeutic osteoarthritic responses in vivo.

Support/interest disclosure: The work has been funded by the project TraDACa (POCI-01-0247-FEDER-72229), co-financed by the European Regional Development Fund (ERDF) through COMPETE2020 – the Operational Programme for Competitiveness and Internationalisation (OPCI) and by the projects UIDP/CVT/00772/2020, LA/P/0059/2020, and CEECINST/00127/2018 UTAD from the Portuguese Foundation for Science and Technology (FCT).The authors have no conflicts of interest regarding the content of the abstract.

Keywords: FUNCTIONAL INDUCTION MODEL, HIP DYSPLASIA, COMPUTED TOMOGRAPHY, OSTEOARTHRITIS, RABBIT.

Preliminary data on the owner's insights for an effective weight management programme in dogs and cats

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Introduction: Weight management programmes (WMP) for pets should be managed by veterinary teams (VT). This incorporates all available information on the human-animal bond, pet environment and routines. According to recent studies, the primary cause of WMP failure is owner's noncompliance. The goal of this study was to evaluate owners' impressions of a WMP that resulted in their pets achieving a healthy weight (HW).

Materials & methods: A 10-question survey was posted in Google Forms and emailed to owners whose animals achieved the HW with the help of a WMP at Hospital Escolar Veterinário FMV-ULisboa throughout the previous year. It inquired about the difficulty of implementing the recommendations and the critical success factors for reaching the animal's HW, among other topics.

Results: A total of 9 answers were obtained so far. The average perceived difficulty in achieving IW was set at 6/10 (scale 0-10). The difficulty in convincing other family members that the pet was overweight (67%), altering pets' feeding habits (33%) and restricting the amount of food offered by cohabitants (22%), were cited as reasons for this issue. Moreover 88% of respondents began measuring the exact amount of pet food using a scale, and 44% reported that their pet became more active during/after WMP. The animal's quality of life (89%), the animal's health being harmed (78%), the pet's lifespan (78%), and the VT's support and encouragement (67%) were all critical in achieving the required WMP goals.

Discussion: Backing owner's motivations and concerns throughout a WMP is critical for obtaining effective outcomes and providing superior veterinary care.

Support/interest disclosure: This study was funded by Fundação para a Ciência e a Tecnologia (FCT) under the Project UIDB/00276/2020) and the Project LA/P/0059/2020 - AL4AnimalS).The authors have no conflicts of interest to declare.

Keywords: PET OBESITY;WEIGHT MANAGEMENT PROGRAMMES; VETERINARY CARE, OWNER´S PERCEPTION

Plasma and urinary Normetanephrines – a promising biomarker for future diagnosis of feline pheochromocytoma

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Introduction: Feline pheochromocytoma (PCC) is rare and literature is limited to few case reports. In dogs, the measurement of plasma (PL) and urinary (U) metanephrines and normetanephrines (NMN) are the most sensitive tests for diagnosis of PCC, being NMN more often preferred. In cats, little is known about these biomarkers. This pilot study evaluates the feasibility of PL-NMN and U-NMN measurement in healthy cats using liquid chromatography with tandem mass spectrometry (LC-MS-MS), comparing these results with those obtained from a cat with a final diagnosis of PCC (PheoCat).

Material & methods: A cross-sectional pilot study was conducted, using a group of healthy adult cats recruited from a veterinary teaching hospital. Urine and plasma samples collected from the healthy cats and the PheoCat were stored at -80ºC until measurement of PL-NMN and U-NMN. Urinary creatinine (Creat) was assessed to calculate the U-NMN ratio.

Results: A total of 10 healthy cats were recruited, with a median age of 6 years (IQR=4.5). The PL-NMN and the U-NMN/Creat ratio median values of healthy cats were 7.02nmol/L (IQR=5.2) and 139µg/g (IQR=77), respectively. Results obtained from the PheoCat revealed a PL-NMN of 66.27nmol/L and a U-NMN/Creat ratio of 1262µg/g.

Discussion: The PheoCat had a substantial increase in both parameters when compared to the healthy cats, highlighting the clinical applicability of these findings. This is the first study supporting the feasibility of PL-NMN and U-NMN measurement by LC-MS-MS in healthy cats and will contribute to the biochemical diagnosis of feline PCC in the future.

Support/interest disclosure: This work was supported by FCT - Fundação para a Ciência e Tecnologia IP - grant UIDB/00276/2020 (CIISA) and by LA/P/0059/2020 (AL4AnimalS).

Keywords: FELINE PHEOCHROMOCYTOMA; PLASMA AND URINARY NORMETANEPHRINE; LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY; HEALTHY CATS.

O 032

Comparison of Skin Allergy tests, Intradermal tests and Skin Prick tests in the characterization of Insect Bite Hypersensitivity in a population of Lusitano horses

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Background: Insect Bite Hypersensitivity (IBH) is a worldwide, mainly type I, IgE-mediated, hypersensitivity reaction to *Culicoides spp* salivary allergens. Skin Allergy tests, Intradermal tests (IDTs) and Skin Prick tests (SPTs) results were compared in IBH-diagnosed Lusitano horses from Portuguese stud farms.

Material & Methods: 30healthy/30 IBH-affected horses were evaluated and submitted to IDTs/SPTs with 14 specific allergens: 13 recombinant proteins from *Culicoides (Cul) spp.* salivary glands (Cul n 1-11, Cul o 1, Cul o 2), *C. nubeculosus (Cul n)*. Twelve IBH–affected horses were tested with 6 more allergens: 4 from *C. nubeculosus* (Cul n 3 Bac, Cul n 3 Bar, Cul n 4 Bac, Cul n 4 Bar), 2 from *C. obsoletus* (Cul o 142, Cul o ext). Allergen concentrations were: 10µg/ml (IDTs) and 100µg/ml (SPTs). Wheal diameters were assessed at 20min (SPTs/IDTs) and 6+48hours (IDTs). For SPTs a cut-off≥0.9cm was determined. Positive IDTs had wheal diameter≥50%histamine wheal.

Results: Statistically significant differences ($p \leq 0.05$) were found for: SPTs'1st allergen panel -Cul n, Cul n 1, 7, 8, 9, Cul o 23+110; 2nd allergen panel -Cul n 3 Bar, Cul n 4 BAc, Cul o Ext (classification matrix≥73%); IDTs' similar allergens, exceptions Cul n 7, Cul o 23, Cul o ext and Cul n 4 Bac, as C/Tratio≥1 (classification matrix≥68%).

Discussion: Overall SPTs 100µg/ml, 20min, 0.9cm, presented better discriminatory diagnostic potential than IDTs. Cul n, Cul n 1, 7, 8, 9, Cul o 23+110 seem a good choice for an SPT IBH diagnostic panel. Although tested on fewer horses, Cul n 3 Bar, Cul n 4 Bac, Cul o Ext may also be relevant, but should be further tested before recommendations are made.

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Keywords: IBH, SPTS, IDTS ALLERGENS, HORSES

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SESSION 1
BIOMEDICAL SCIENCES AND BIOTECHNOLOGY

Siglec10 – CD24 axis as a new therapeutic target for feline mammary carcinoma

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Introduction: The interaction between tumoral cell-surface sialoglycans and immune cell sialic-acid-binding immunoglobulin-like lectin (Siglec) receptors has emerged as a key immune response modulator. Recently, the Siglec-10-CD24 axis was proposed as the major immune checkpoint in human breast cancer, promoting immune evasion by preventing macrophage dependent phagocytosis of tumoral cells. Due to the similarities between feline mammary carcinoma (FMC) and human breast cancer we sought to identify and characterize the Siglec-10-CD24 axis in FMC, with the aim of developing a novel immunotherapeutic approach based on targeted tumour cell desialylation.

Materials & methods: Expression of CD24 and Siglec-10 on FMC was evaluated using immunohistochemistry on samples from 17 feline patients. To develop the ideal enzyme for the targeted desialylation strategy, 74 putative sialidase genes were selected from the Cazy.org database, expressed and purified in a high-throughput platform. The enzymatic activity of the sialidases was evaluated using the 4-methylumbelliferyl-α-N-acetylneuraminic acid.

Results: Immunohistochemistry analysis showed marked Siglec-10 and CD24 staining in tumour associated macrophages and in poorly differentiated neoplastic cells, respectively. All selected recombinant sialidases were soluble and approximately 20 displayed a good yield-activity combination.

Discussion: High expression of Siglec-10 was observed in the macrophages infiltrating the tumour samples. Furthermore, CD24 expression in FMC cells appears to be correlated with poor differentiation. This is highly suggestive of the presence of Siglec-10-CD24 signalling in FMC, likely leading to immune evasion, similarly to what is observed in human breast cancer. Finally, a group of promising novel sialidases was identified, which will be further characterized and have their cell desialylation potential assessed.

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Keywords: SIALOGLYCANS; SIGLEC-10; CD24; CANCER; IMMUNOTHERAPY

Panobinostat-loaded liposomes as a drug delivery system for the treatment of canine lymphoma

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Introduction: Liposomes are among the most promising nanocarriers for clinical applications. Owing to their unique characteristics, liposomes can entrap different drugs and provide site-specific delivery by adding targeting molecules. The folate receptor is often overexpressed in tumor cells and is a suitable target for drug delivery. Thus, folic acid has been added to nanoparticle surfaces to enhance liposomal endocytosis and anti-tumor activity. Therefore, we aimed to develop folate-targeted liposomes encapsulating panobinostat and investigate its antitumor properties against canine non-Hodgkin lymphoma (cNHL), an animal model of NHL.

Material and Methods: Folate receptor expression and acetylation of H3 histone were evaluated in cNHL cell extracts using immunoblotting. A cell viability assay was performed on cNHL. The caspase 3/7 activity levels and percentage of apoptotic cells were determined using the Caspase Glo 3/7 and Guava Nexin assays. Liposomes cellular uptake by cNHL cells was determined by immunofluorescence and flow cytometry. Biodistribution was performed in CD1 mice and in a cNHL xenograft mouse model using radiolabeled liposomes.

Results: Overexpression of the folate receptor in a cNHL cell line was confirmed. All panobinostat-liposomes exhibited dose-dependent inhibitory effects on CLBL-1 cells and the acetylation of H3 histone. Folate-liposomes obtained the lowest EC50. Both panobinostat-liposomes were able to induce caspase 3/7 while promoting a higher percentage of apoptotic cells. Lastly, biodistribution studies demonstrated a higher tumor uptake by folate liposomes.

Discussion: Our work validates this drug delivery system as an effective cancer treatment that enhances the therapeutic potential of panobinostat, thus contributing to the treatment of cNHL.

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Keywords: LIPOSOME; FOLATE; PANOBINOSTAT; NANOMEDICINE; NON-HODGKIN LYMPHOMA

iPSCs-derived Cardiomyocytes and Future Perspectives

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Introduction: Fabry disease (FD), one of the commonest Lysosomal Storage Disorders, is caused by mutations in the alpha-galactosidase A gene (*GLA*) resulting in deficient activity of alpha-galactosidase A (α-Gal A), particularly in the target cells affected by the disease, such as cardiomyocytes. In this work, after obtaining induced Pluripotent Stem Cells (iPSCs) derived from a FD patient, we intend to correct the patient's mutation through gene editing. We also anticipate obtaining a reliable disease model to further study the disease pathophysiology in iPSC-derived cardiomyocytes (iPSCs-CMs).

Methods and Results: We first reprogrammed FD patient cells into iPSCs using the non-integrative episomal vectors (Epi5). The obtained iPSCs were differentiated using PSC Cardiomyocyte Differentiation Kit (Gibco®). We obtained beating cardiomyocytes; the results were confirmed using antibodies for cardiac markers. In order to correct the missense mutation present in this particular cell line (p.W287X) we will use Prime Editing (PE) with a PEA1-GFP plasmid vector (ongoing strategy for PE experiments).

Discussion: To validate our iPSC-CMs as a good disease model, we have to fully characterize the cell line (gene expression and calcium handling), and further compare with the initial FD fibroblast. For gene editing, the use of PE has the advantage to mitigate the need of double strand break (DSB) repair machinery, which is notoriously error prone. Therefore, off-target effects are almost undetectable in comparison to CRISPR-Cas9 DSB-dependent repair system.

Support: Foundation for Science and Technology (FCT): PTDC/BIM-MEC/4762/2014 and SFRH/BD/118009/2016.

Keywords: INDUCED PLURIPOTENT STEM CELLS; CARDIOMYOCYTES; LYSOSOMAL STORAGE DISORDERS; FABRY DISEASE; GENE EDITING.

Induced pluripotent stem cells (iPSCs) and NGS as the basis for a Tay Sachs disease variant B1 cellular model

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Tay Sachs disease variant B1 (TSD B1; OMIM 272800) is a neurodegenerative lysosomal storage disease (LSD) which, although rare, it is the most frequent form of TSD in Portugal. The availability of disease-relevant cell types derived from induced pluripotent stem cells (iPSCs) provides a model for studying the pathogenic mechanisms and, eventually, test therapeutic approaches for TSD B1 patients.

The main objectives of this project are: establish iPSCs from patient fibroblasts, generate a neuronal TSD B1 specific cellular model and implement the genetic profiling by Next Generation Sequencing (NGS) to examine potential changes in the manipulated cells.

First, iPSCs from a control fibroblast cell line and from TSD B1 fibroblasts were obtained by using a non-integrative approach with episomal vectors, the control was further differentiated into neural progenitor cells (NPCs). Those results as well as the NGS results from the donor cells are presented in this work.

By using a customized NGS panel, we obtained results of the cells in a “naïve” state to later compare with TSD B1 iPSCs, TSD B1 NPCs and NPCs obtained from control iPSCs.

The iPSC reprogramming was accomplished and differentiation into NPCs was also achieved.

Perseverance is crucial in this type of highly meticulous work where minor things, such as lack of liquid nitrogen or a mycoplasma infection, may force a start over.

Acknowledgements: We thank the collaboration of INSA’s DGH and the support from FCT PTDC/BIM-MEC/4762/2014 in personal grants and consumables.

Keywords: TAY SACHS DISEASE VARIANT B1; CELL MODEL; iPSCs

DNA damage and repair response in peripheral blood lymphocytes from patients with breast and colorectal cancer: Clinical response to oncologic treatments

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Introduction: Genomic instability play a key role in carcinogenesis, particularly in the conversion of a normal cell into a premalignant cell when DNA damage induced from endogenous and exogenous agents is not repaired. Dysregulation in these repair mechanisms may contribute to cancer development and their increase may lead to cell resistance to DNA damage-based therapy. In this study, the effect of anti-neoplastic drugs and radiation used in treatment of breast and colorectal cancer was evaluated in patients' lymphocytes.

Material & Methods: Blood samples were obtained from 80 cancer patients, undergoing chemotherapy and/or radiotherapy. DNA damage levels and DNA repair capacity were evaluated by alkaline comet assay before and after a few cycles of treatment.

Results: DNA strand breaks levels of cancer patients were higher when compared to healthy individuals. After treatment administration, damage levels increased significantly, and viability of lymphocytes decreased compared to their pre-treatment values. Conversely, the variations observed in colorectal cancer biomarkers levels were not significant, and there was even a significant increase of Cancer Antigen 15.3 levels in breast cancer patients after some cycles of treatment, suggesting that tumour would not yet be in remission. Additionally, preliminary repair results from colorectal patients showed that cancer patients presented a suppression in repair response compared to healthy individuals and, after treatment administration, there was a significant increase in nucleotide excision repair capacity.

Discussion: Results suggest that a repair efficacy adaptation of lymphocytes to anti-neoplastic agents occurred. These findings could impact in diagnosis and prognosis, contributing to cancer´s prevention and cure.

Support: This work was supported by the projects UIDB/CVT/00772/2020, UIDB/04033/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

Keywords: DNA DAMAGE; REPAIR RESPONSE; COMET ASSAY; BREAST CANCER; COLORECTAL CANCER

Whole-genome sequencing reveals unique regions of selection for metabolism and feed efficiency in endangered native pigs of Angola

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Introduction: Angola has undergone dramatic social events, leading to the disappearance of native pig populations that nowadays are further threatened by the recent introduction of European exotic breeds.

Materials & Methods: In this study we performed the genetic characterization of endangered native pigs of Angola sampled at *Bailundo district*, using whole-genome sequencing (N=4).

Results: After read quality filtering, variant calling resulted in the identification of ~8 million SNPs which were compared with the variants identified in 78 genomes from European and Asian domestic pigs and wild boars, currently in the public domain. Population structure analyses showed that *Bailundo* pigs are closely related to European pig breeds and very distant from Asian pig breeds. Pairwise F_{ST} ranged from 0.14 to 0.26. *Bailundo* pigs displayed lower levels of genetic differentiation with European breeds. Finally, we identified candidate genomic regions under strong effect of selection, using a complementary approach based on three different methods. These analyses allowed the identification of selection footprints emerging in different time periods. Candidate genes under selection in *Bailundo* pigs were mostly involved in biological processes related with feed efficiency and metabolism. The identification of *CDKAL1* gene (which is related with insulin and cholesterol metabolism) as a target of selection was consistent using methods that detect ancient and as well as recent selection events.

Discussion: This study represents the first assessment of the genetic relationship between a native pig population from Angola and pigs from around the world, showing that these pigs harbour unique genetic attributes that should be further explored and preserved.

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Keywords: BAILUNDO PIGS; WHOLE-GENOME SEQUENCING; SIGNATURES OF SELECTION; GENOMICS; ADAPTATION.

Retinal iron overload is associated with BRB breakdown and leakage

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Introduction: Retinal iron overload affects blood-retinal barrier (BRB) integrity and accelerates retinal cell loss by enhancing oxidative stress. Alterations and loss of function of the BRB play a crucial role in the development of retinal diseases and vision loss, such as diabetic retinopathy. This study aimed to assess BRB integrity in a mouse model of retinal iron overload.

Materials & methods: Four- to six-month-old TIM2 heterozygous (TIM2$^{+/-}$) mice and wildtype littermates were used (n=6). In order to evaluate BRB integrity, *in vivo* scanning laser ophthalmoscopy (SLO) fluorescein angiography was performed. Mice were injected intravenously with fluorescein isothiocyanate conjugated-bovine serum albumin (FITC-BSA, 100 mg/kg) to study retinal vascular permeability. Albumin extravasation was evaluated *in vivo* by means of SLO as well as confocal laser microscopy. Transmission electron microscopy (TEM) was also performed.

Results: SLO fluorescein angiography revealed focal points of vascular leakage in TIM2$^{+/-}$ mice. Serum albumin has been used as a tracer to assess BRB function and vascular integrity. After injection of FITC-BSA, diffuse leakage of FITC-BSA in TIM2$^{+/-}$ retinas was observed by means of SLO, confirming BRB breakdown. Accordingly, confocal laser microscopy analyses of whole-mount retinas and paraffin embedded retinal sections from TIM2$^{+/-}$ mice injected intravenously with FITC-BSA showed diffuse extravasation of FITC-BSA around blood vessels. TEM analysis confirmed also BRB breakdown by the formation of perivascular spaces filled with plasma-like material compatible with oedema in TIM2$^{+/-}$ retinas.

Discussion: The presence of vascular leakage was an indicative of BRB breakdown, confirming the increased vascular permeability probably due to iron overload-induced damage.

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Keywords: VASCULAR PERMEABILITY; BRB BREAKDOWN; IRON OVERLOAD; RETINOPATHY

Structural and biochemical characterization of two novel β-glucanases belonging to glycoside hydrolase family 157

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Introduction: β-glucans are a class of polysaccharides whose hydrolysis is catalysed by β-glucanases. Due to the complex relationship between the molecular structure and functional profile of β-glucans, there is a continuous need for highly specific β-glucanases that can fully reveal β-glucans' potential applications. Here we report the first biochemical and structural characterization of two β-glucanases from the recently founded glycoside hydrolase family 157 (GH157) and investigate their molecular basis for substrate recognition and hydrolysis.

Materials & methods: Genes encoding the GH157s from the human gut bacteria *Bacteroides cellulosilyticus* and psychrophilic bacteria *Labilibaculum antarcticum* were cloned, expressed and structurally characterized by X-ray crystallography. Their specificity and activity were analyzed with reducing sugar assays, enzyme kinetics and product analysis by HPAEC-PAD and LC-MS.

Results: Specificity screening revealed that both enzymes are active on β-1,3-glucans and mixed-linkage glucans. Temperature catalytic dependency reflected the psychrophilic nature of *La*GH157 (4-32.5°C) while *Bc*GH157 was active in a broader range of temperatures (12-55ºC). HPAEC-PAD and LC-MS on hydrolysis products revealed that both enzymes display an endo mode of action, capable of cleaving β1-3 and β1-4-linked glucoses, when preceded by a β1-3 linkage. The structure of *La*GH157 revealed a classic TIM-barrel fold and suggested a retaining mechanism of hydrolysis, with two glutamates serving as the catalytic residues, confirmed by site-directed mutagenesis.

Discussion: We have performed the first structural and biochemical characterization of GH157 members, which were found to be retaining endo-β-1,3(4)-glucanases. This provides insight into β-glucan deconstruction in the human gut and identifies novel catalysts for the production of value-added products through β-glucan depolymerization.

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Keywords: β-GLUCANS; β-GLUCANASES; CAZymes; GH157; CARBOHYDRATES

Mutations in the β-strand DNA-binding region of African Swine Fever virus protein A104R disrupt function and lead to a loss of immune recognition

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Background: African swine fever (ASF) is a severe, highly contagious, and economically important transboundary viral disease. Significant advances in live attenuated vaccine (LAV) development were made over the last decade however issues with residual virulence remain and none of the experimental candidates allow differentiation of infected from vaccinated animals (DIVA). It is therefore necessary to identify novel strategies to improve safety, by genetic modification for further attenuation and generation of DIVA markers.

Materials and Methods: Sequence analysis and docking studies were used to identify residues involved in protein-DNA interactions; mutants were expressed in E. coli and binding kinetics analysed by EMSA. Immunoinformatics tools were used to predict linear epitopes and the generated peptide libraries screened by ELISA with sera from domestic pigs.

Results: Effects of mutations at residues K64, K66 and R69 of the β-strand DNA binding region (BDR) were found to be additive in reducing DNA binding activity. Three regions were identified as immunodominant for IgM (V21-K45, α2helix; V61-A85, β2-4sheets, and T76-N104, β4-5sheets, α3helix) and two for IgG (I56-I80, β2-α3helix; and K66-A90 β2-5sheets), Mutations at residues K57 to V61 and A95 to K97 in these regions were found to reduce immune recognition and had an additive effect in reducing DNA binding activity.

Discussion: Results obtained demonstrate that rational mutation of the BDR reduces DNA binding activity and immune recognition, resulting in functional disruption that can potentially be used to increase the safety profile of currently experimental vaccines as well as to provide them with DIVA capabilities.

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Keywords: ASFV; pA104R; VACCINE; DIVA; MUTAGENESIS

Effect of a mitochondriotropic antioxidant based on caffeic acid (AntiOxCIN4) on spermatozoa capacitation and in vitro fertilization

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Introduction: Germ cells are particularly vulnerable to the oxidative stress, which results from an imbalance between the reactive oxygen species (ROS) and antioxidants levels, limiting their fertilizing potential. Mitochondria are major sources of ROS being relevant targets for therapeutic intervention. Therefore, we hypothesized that mitochondria target antioxidant based on caffeic acid could increase the resistance of bovine gametes to oxidative stress improving fertility outcomes.

Materials & methods: Our goal was to study the effect of a novel and natural mitochondrial-directed antioxidant, on bovine spermatozoa (spz) capacitation and fertilization process. This antioxidant AntiOxCIN4 was synthesized from the caffeic acid and added to sperm capacitation medium (CAP), during the swim-up process, and to the fertilization medium (FERT) during the co-incubation of matured oocytes and capacitated spermatozoa, in concentrations of 0 (control), 0.1, and 1 µM (5 sessions). After the swim-up, sperm motility (CASA analysis), concentration, vitality (eosin-nigrosin) and mitochondrial membrane potential (JC1) were evaluated. Embryo development was also assessed.

Results A positive effect of 1µM AntiOxCIN4 on CASA parameters (higher total and progressive motility, number of rapid spz, beat cross frequency (BCF), and lower static spz compared to control and 0.1 µM dose, $p \leq 0.01$) and, consequently, on the sperm quality was identified. Both doses more than doubled the number of good quality embryos.

Discussion A beneficial effect of AntiOxCIN4 (1 µM) on sperm capacitation process was identified demanding further studies.

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Keywords: MITOCHONDRIA-TARGETED ANTIOXIDANTS; SPERMATOZOA; OOCYTES; FERTILIZATION; ASSISTED REPRODUCTIVE TECHNOLOGIES

The coccidian Toxoplasma gondii's MOB1: an unexpected story

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Introduction: Monopolar spindle One Binder1 (MOB1) proteins participate in key events such as cytokinesis, centrosome biology, tissue homeostasis, morphogenesis, and stem cell maintenance and differentiation, thus acting both at the cell and tissue level. The presence of conserved MOB1 proteins in apicomplexans, namely in cyst-forming coccidia, suggest these may provide advantages in these parasites' complex life cycles leading us to study its function in *Toxoplasma gondii*.

Materials & methods: In tachyzoites, we quantified the *mob1* expression and obtained transgenic lines with ligand-controlled destabilization domain gain-of-function and loss-of-function. We also used overexpression and endogenously C-terminal tagged lines to study the MOB1 subcellular localization.

Results: *Mob1* transcript levels decrease abruptly following host cell invasion while *mob1* overexpression lines show a decline in replication efficiency *in vitro*. Contrary to most organisms studied to date, *mob1* is not essential in *T. gondii* with knockout lines showing an increase in replication efficiency and marked decrease of bradyzoite cyst formation. We observed this phenotype with both CRISPR/Cas9 and homologous recombination knockout systems. In endogenously tagged tachyzoites, MOB1-HA is localized between the newly individualized daughter nuclei at the end of mitosis in a dot-like pattern. The MOB1 interactome identified through proximity-biotinylation includes conserved and novel components, coherent with the detected lack of conservation of some key amino acid residues in coccidians.

Discussion: The effect of *mob1* knockout is surprising as most *mob1* genes studied are essential for cell survival by ensuring accurate cytokinesis. Our results point to an intricate evolutionary history and a multifaceted function of *mob1* in *T. gondii.*

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Keywords:*Toxoplasma gondii*; TACHYZOITE; BRADYZOITE CYST; *Mob1*; REPLICATION

Development of potent and broadly neutralizing single-domain antibodies for SARS-CoV-2 treatment and prevention

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Introduction: SARS-CoV-2 and the resulting disease, COVID-19, are one of the greatest public health concerns worldwide. The evolution of the virus and the emergence of new variants has compromised the efficacy of approved vaccines and highlighted the need for new therapeutic approaches. To enter into target cells, SARS-CoV-2 uses the receptor-binding domain (RBD), which specifically binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2). Our goal is to develop a rabbit derived bispecific single-domain anti-RBD antibody that can block the interaction between the RBD and ACE2 and consequently neutralize the SARS-CoV-2 infection.

Materials and methods: One New Zealand White rabbit was immunized and boosted with SARS-CoV-2 RBD domain from the delta variant. The rabbit immune response was monitored by ELISA by screening the bleeding collected before, during and after immunization. After the final boost, spleen and bone marrow were harvested for cDNA synthesis and the antibody library was constructed by polymerase chain reaction (PCR).

Results: Screening of serum samples collected at different time points demonstrated a high antibody titer and specific response against the RBD domain. From the cDNA synthetized, the families of rabbit antibodies were efficiently amplified by PCR and used to construct the sdAb library.

Discussion: The rabbit immunization allowed the production of a high titer of antibodies against the RBD domain and the construction of a specific antibody library. The antibody library is being screened by Phage Display to select potent and broadly neutralizing antibodies against SARS-CoV-2.

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Keywords: SARS-CoV-2; COVID-19; NEUTRALIZING ANTIBODIES; PHAGE DISPLAY

Pluripotent cells and zebrafish: two complementary platforms for modelling Lysosomal Storage Disorders

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Complementary models of the same disease are fundamental tools in biomedical research to validate pathophysiological mechanisms or therapies.
Being a R&D group actively involved in developing novel therapies for Lysosomal Storage Disorders, we face a constant need for more effective models than those commonly available both *in vitro* and *in vivo*.
Therefore, we are implementing three types of patient-specific multipotent/pluripotent cells for *in vitro* disease modelling: dental pulp (DPSCs), urine-derived (USCs) and induced pluripotent (iPSCs) stem cells. The first two result from non-invasive procedures and can be easily collected. DPSCs are multipotent neuronal precursors that reside in dental pulp. As we already demonstrated, DPSCs self-maintain through several passages and can give rise to a variety of cells including neurons (see the abstract by Carvalho S). USCs, on the other hand, have characteristics of mesenchymal cells, which can differentiate into chondrocytes, adipocytes, osteocytes and skeletal myogenic cells. We are optimizing a protocol to establish USCs and its further characterization. Finally, iPSCs are reprogrammed from fibroblasts and, while more expensive, can potentially be indefinitely maintained and give rise to any cell type (see the abstracts by Duarte A and Ribeiro D).
In vivo, we are generating zebrafish knock-outs for disease-causing orthologue genes by CRISPR/Cas. Zebrafish have high genetic homology with humans and fecundity rate, while being amenable to genetic manipulation, and relatively low-cost.
Once well-established, these independent but complementary platforms have the potential to model different diseases, providing further insights on their pathophysiology and into the safety and efficacy of new therapies.

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Keywords: DISEASE MODELS; PLURIPOTENT CELLS; MULTIPOTENT CELLS; ZEBRAFISH; CRISPR/Cas

Carbohydrate active enzymes as a potent biofilm degradation tool for the treatment of Pseudomonas aeruginosa and Staphylococcus aureus infections

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Introduction: Biofilm-associated infections are a major public health concern. Conventional treatment is hampered by the low penetration of antimicrobials in biofilms. Biofilms are bacterial communities surrounded by an extracellular matrix, which has exopolysaccharides as one of its major components. As such, enzymatic polysaccharide degradation could pose an alternative approach to improve biofilm susceptibility to antimicrobials. Here, we constructed a library of highly specific carbohydrate active enzymes (CAZymes) with potential to promote biofilm degradation by digesting the main exopolysaccharides produced by *Staphylococcus aureus* (PNAG) and *Pseudomonas aeruginosa* (Pel, Psl and alginate).

Materials & methods: A library of 135 CAZymes was established through a bioinformatic analysis. Genes encoding the enzyme library were chemically synthesized, cloned, expressed and purified in a high-throughput platform. Enzyme activity was tested using chromogenic substrates and biofilms biomass assessment.

Results: All proteins were soluble and 30 had high production yields. A preliminary analysis of the enzymes' efficacy allowed selecting 4 enzymes capable of efficiently depolymerize PNAG, Psl, Pel and alginate, respectively, promoting bacterial biofilm degradation. Three enzymes were also found to be able to effectively inhibit PNAG, Pel or Psl-based biofilm formation. One alginate lyase in particular, was capable of promoting a biofilm biomass reduction of around 70% after 6.5h of incubation with 24h old biofilms of a mucoid *P. aeruginosa* strain.

Discussion: This study allowed the identification of novel enzymes with the potential for *P. aeruginosa* and *S. aureus* biofilm degradation. Overall, we expect that these enzymes, in combination with antimicrobials, can constitute a promising approach for the treatment biofilm associated infections.

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Keywords: BIOFILMS; ENZYMES; EXOPOLISSACHARIDES; *S. AUREUS*; *P. AERUGINOSA*

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Evaluation of antibacterial activity of rifabutin and vancomycin against planktonic and biofilm forms of staphylococcal clinical isolates

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Introduction: *Staphylococcus aureus* is an important opportunistic pathogen in both human and animal medicine, strongly associated to hospital-acquired infections. The major reasons of concern in dealing with these type of infections are the ability of *S. aureus* to form biofilm structures and the emergence of multidrug-resistance strains. A potential treatment alternative is the repurposing of antibiotics. In this work, the antibacterial activity of rifabutin, a broad-spectrum antibiotic, was evaluated and compared with the gold standard antibiotic vancomycin, against a set of *S. aureus* clinical isolates, in both planktonic and biofilm forms.

Materials & methods: 116 clinical isolates recovered from invasive staphylococcal disease were characterized in terms of antibiotic susceptibility and growth profiles. A susceptibility screening was performed for rifabutin and vancomycin for the reference *S. aureus* strain (ATCC®25923™) and all clinical isolates, in planktonic form. Based on these results a subset of 34 clinical isolates were selected, characterized in terms of biofilm assembly and the minimum biofilm inhibitory concentration of 50% of the two antibiotics was determined.

Results: Rifabutin displayed a minimum inhibitory concentration of 15 to 1000-fold lower than vancomycin. All selected strains were able to assembly biofilm. Rifabutin also demonstrated a high anti-biofilm activity even against MRSA strains.

Discussion: Rifabutin demonstrated to be a potent antibiotic against staphylococcal clinical isolates with different resistance patterns in both planktonic and biofilm forms. The antibacterial evaluation of this antibiotic incorporated in a drug delivery system, constitutes further studies to assess the benefit of specifically targeting and releasing the incorporated antibiotic at the infected sites.

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Keywords: *S. AUREUS* INFECTION; CLINICAL ISOLATES; BIOFILMS; RIFABUTIN; VANCOMYCIN

A specific phage lysin, Lysk, as a natural targeting protein for Staphylococcus aureus

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Introduction: *Staphylococcus aureus* is one of the most infectious bacterial pathogens responsible for hospital-acquired infections worldwide. The ability of *S. aureus* to form biofilms associated with the emergence of multidrug-resistant strains poses a huge challenge to infection eradication. This is hampered by to the low penetration capacity of antibiotics along the biofilm structure at effective therapeutic concentrations. Therefore, there is an urgent need to develop new *S. aureus*-targeted strategies to achieve effective antimicrobial treatment. To address this, we explored the potential use of LysK as a targeting approach for *S. aureus* biofilm-associated infections.

Materials & methods: LysK expression and purification were optimized by cloning assays using a pET21a-derived LysK expression vector. LysK binding studies were optimized for the reference *S. aureus* strain (ATCC®25923™) at different time points (15, 30, 60, 90 min, 3h and 24h), followed by WB analysis. To evaluate lysin-binding activity and specificity, a binding assay was performed in non-staphylococcal clinical isolates.

Results: LysK was successfully cloned and expressed in a pET21a expression vector. Binding assays showed that LysK presented high binding activity just after 15 min of incubation with the reference strain. A marked signal was observed only in the case of LysK incubated with staphylococci species in comparison with non-staphylococci species.

Discussion: LysK was validated as a potential vector for targeting *S. aureus* with high specificity. This protein can be coupled to drug delivery systems incorporating antimicrobials to develop highly potent nanobiotics.

Support: This work was supported by FCT – Fundação para a Ciênica e Tecnologia IP, grants UIDB/00276/2020, DL57/2016/CP1438/CT0002 and by Centre for Interdisciplinary Research in Animal Health (CIISA), grant UIBD/INOV20Set04. M.F. is funded by PhD grant BD/2018 from Universidade de Lisboa (UL).

Keywords: *S. AUREUS* INFECTION; BIOFILMS; LYSIN; LYSK; BACTERIAL TARGETING

Effect of the application of organic acids and chitosan on viscera inoculated with Listeria

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Introduction: The consumption of viscera is increasing, representing an important source of nutrients. Visceras are highly perishable, and decontamination can ensure product quality and safety. This study aimed to evaluate the effectiveness of lactic acid and acetic acid at 1% and 2% and chitosan at 0.2% and 0.5% on bovine liver samples inoculated with *Listeria* over storage time.

Material & Methods: The samples were inoculated with a mix of *Listeria monocytogenes* ATCC 4b and *Listeria* sp. ambiental in a concentration of 1.42 x 10^5 CFU/g, then decontaminated with the solutions. The evaluation was performed in five moments (0h, 12h, 24h, 48h, and 72h) whit the samples were stored at 7°C. Performing *Listeria* counts, pH and color evaluations.

Results: No significant differences in counts were observed. However, in the treated samples lower counts of Listeria could be observed compared to the control. Nevertheless, it should be noted that the treatment with 2% acetic acid had the highest reduction in counts and the 0.5% chitosan had the lowest counts at 0, 24 and 48h of storage. The parameters L, a* and b* were not affected by the application of the solutions. As for the pH, with the application of 1% lactic acid, there were very significant differences, with a reduction from 6.26 at 0h to 5.45 at 72h.

Discussion: The results showed that the use of organic acids and chitosan can be used as an additional step to control microbial growth.

Keywords: VISCERA, PORK MEAT, DECONTAMINATION, ORGANIC ACID, CHITOSAN

Effects of Notch and Wnt blockades at the early embryonic stage on subsequent placental development

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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 trophectoderm and inner cell mass populations and affect fetal weight. This study evaluated if placental constituents are also affected by early Notch and/or Wnt blockades.

Material & Methods: 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 pregnancy placentas were weighted (n=177) and placental components (choroid plate–CP, labyrinth–L, basal layer–BL, decidua basalis–DB) quantified by stereology (n=24).
Results: DAPT placentas were heavier than control placentas (0.1684g vs. 0.1644g, p=0.03), and had a larger CP compared to control (6.5% vs. 9.1%, p=0.027) and double blockade (5.7%; p=0.005) placentas. Compared to control placentas, DKK1 tended to decrease DB (15.1% vs. 11.0%, p=0.085), whereas double blockade tended to increase it (19.8%, p=0.051).

Discussion: Notch and Wnt signaling blockade at the first cellular differentiation affected placental development. Notch signaling limits choroid plate proliferation, while Wnt may be involved in regulation of decidua basalis formation. These findings offer a new insight into early placental regulation, namely in exclusively fetal or maternal components (CP and DB, respectively). Further studies analyzing specific placental cell populations are necessary to fully characterize the observed phenotypes.

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Keywords: PLACENTA; NOTCH; WNT; DECIDUA; CHORIONIC PLATE; EMBRYO.

Designing a multi-epitope vaccine against Campylobacter fetus subsp. venerealis using a reverse vaccinology approach

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Introduction: Bovine Genital Campylobacteriosis (BGC) is a worldwide distributed venereal disease of cattle caused by *Campylobacter fetus* subsp. *venerealis* (*Cfv*). Vaccination against *Cfv* is a critical step for the control and prophylaxis of BGC at the herd level. However, the efficacy of bacterin vaccines remains less optimal and controversial. This study aimed to identify novel vaccine candidates using a reverse vaccinology approach to design a multi-epitope vaccine against *Cfv*.

Materials & methods: The whole proteome of *Cfv* strain NCTC 10354 was retrieved from GenBank database, and proteins analysed for its subcellular location, virulence potential, antigenicity, non-allergenicity, non-similarity to host proteome, physicochemical properties, and the number of transmembrane helices. The most promising vaccine candidates were further analysed for B and T cell epitope prediction, and the most antigenic epitopes were selected for vaccine design.

Results: From the 66 out of 1849 *Cfv* proteins initially selected (23 with outer membrane location and 43 with extracellular location), 21 were predicted to be virulence factors and selected for further analysis. Nine proteins were identified as antigenic and non-allergenic, with suitable physicochemical properties and none or one transmembrane helices. From these, a flagellar protein – FliK – and an outer membrane protein – OmpA – were selected for B and T cell epitope prediction. The most promising conserved epitopes, antigenic and non-allergenic, were used in the design of a vaccine, which is being evaluated *in silico.*

Discussion: In conclusion, this study identified novel vaccine targets and proposed a multi-epitope vaccine for further experimental validation, which ultimately may contribute for the control and prophylaxis of BGC.

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Keywords: BOVINE GENITAL CAMPYLOBACTERIOSIS; *Campylobacter fetus* subsp. *venerealis*; REVERSE VACCINOLOGY; MULTI-EPITOPE VACCINE.

Origin and mechanisms involved in the acquisition of NOTCH2, DLL4 and JAGGED1 by bull spermatozoa

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The investigation of male factor subfertility and infertility routinely uses semen quality parameters. However, their fertility predictive value remains sub-optimal as they fail to predict the fertilizing ability of spermatozoa (SPZ). In this context, sperm proteins known as key regulators of SPZ competence were proposed as male fertility markers. Previous team work identified NOTCH signalling proteins (NOTCH2, DLL4, JAGGED1) in bull ejaculated SPZ, which are relocated during acrosome reaction (AR), supporting a role in sperm competence. This study aimed to identify the molecular mechanisms involved in the acquisition of NOTCH2, DLL4 and JAGGED1 by bull SPZ. The presence of NOTCH proteins was evaluated in the testis and along the epididymis segments by immunofluorescence and in epididymosomes (extracellular vesicles secreted by epididymis cells) by Western blot. Epididymosomes were purified from caput, corpus and cauda epididymis, and their presence confirmed by exosomal protein markers (HSP70, TGS101; Western Blot) and transmission electron microscopy. Results first identified DLL4 in testicular SPZ, mainly localized in the acrosomal cap region and tail, whereas NOTCH2 and JAGGED1 were identified in the epididymis SPZ head and tail of the caput and corpus segments, respectively. Additionally, NOTCH2 and JAGGED1 were found in epididymosomes from the three epididymis segments, indicating that these proteins are transferred to SPZ *via* epididymosomes during epididymal maturation. In conclusion, this study identified the origin of SPZ NOTCH proteins which are related with AR and potentially sperm competence.

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Keywords: BULL SPERMATOZOA; NOTCH PROTEINS; EPIDIDYMOSOMES;

Antigenotoxicity of Natural Ingredients: an in vivo study in Drosophila melanogaster

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Introduction: Toxic substance exposure is much more problematic today than it would have been in the past. The environmental repercussions include DNA damage, and this genome instability leads to diseases such as cancer, degenerative diseases, infertility, diseases associated with aging, among many other issues. A healthy lifestyle can reduce these issues, including consuming substances that protect the genome by several mechanisms reducing DNA damage. Genotoxicological studies are fundamental for knowing the hazards to genome and health, and antigenotoxicological studies are the answer to minimize genome instability. Thus, the use of natural ingredients has become increasingly important. Natural ingredients such as Almonds (*Prunus dulcis*), Elderberry (*Sambucus nigra*), Olives (*Olea europaea*), and Grapes (*Vitis vinifera*) have been shown to possess a variety of biological activities and to hold therapeutic promise. They are the most common in the Trás-os-Montes region of Portugal. This region has the most organic farmers and has climatic, topographic, and pedological differences that contribute to agriculture diversity. This study aimed to demonstrate*, in vivo*, the genotoxicological effects of Elderberry berry and flower, olive leaf, olive pulp, almond, almond shell, and grape pulp in *Drosophila melanogaster*.

Materials and Methods: For each natural ingredient quantity tested (Elderberry: 5 g; Elderberry flower: 10 g; Olive: 10 g; Olive leaf: 1 g; Grape pulp: 10 g; Almond: 10 g; Almond shell: 1 g) Comet assay was performed based on the described method.

Results: The streptonigrin-challenged group exhibited an overall increased DNA damage in all ingredients assessed. Flies fed with only the instant medium and challenged with streptonigrin presented with the highest levels of DNA damage, while flies fed with Elderberry showed the lowest levels in both unchallenged and streptonigrin-challenged groups.

Discussion: Flies fed with Elderberry showed the lowest levels in both unchallenged and streptonigrin-challenged groups.

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Keywords: ANTIGENOTOXICITY; *Drosophila melanogaster*; GENOTOXICITY; NATURAL INGREDIENTS, TRÁS-OS-MONTES

Combined TLR2/TLR4 activation equip non-mucosal dendritic cells to prime Th1 cells with gut tropism

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Introduction: The capacity to programme properly polarized T cell responses at the port-of-entry of pathogens may improve the efficacy of vaccines. Targeting dendritic cells (DCs) in vaccination is a promising strategy to achieve this goal, since DCs play a major role at determining the fate of T cell differentiation (e.g. towards Th1, Th2, Th17) and in instructing the localization of lymphocytes to different tissues. Although non-mucosal DCs are inefficient to induce tropism of T cells into mucosal surfaces, their activation through TLR2 has been shown to promote the homing of primed T cells to the gut mucosa. This capacity relies on the induction of ALDH enzymes on activated DCs that become able to metabolize vitamin A into all-trans retinoic acid, which imprints the expression of mucosal-homing molecules (α4β7 and CCR9) in the primed T cells. Clarifying how this TLR2 property is modulated in the presence of other innate stimuli may guide the rational incorporation of combined PRR ligands in vaccines formulations to better control the type and location of adaptive immune mechanisms.

Materials & methods: Here, we evaluated whether the TLR2 property in inducing mucosal-homing molecules is maintained when TLR2 stimulation is associated with TLR4 agonists, which, alone, do not induce ALDH activity on DCs.

Results: We found that the simultaneous activation of non-mucosal DCs through TLR2 and TLR4 maintains such capacity while reinforces the polarization of primed CD4 T cells towards Th1.

Discussion: These results suggest that the inclusion of TLR2/TLR4 agonists in non-mucosal vaccines could promote the gut localization of Th1 cells.

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Keywords: TOLL-LIKE RECEPTOR 2; TOLL-LIKE RECEPTOR 4; DENDRITIC CELLS; MUCOSA; VACCINES

P 023

Tumoral Expression of the Immune Checkpoint TIM-3 in Feline Mammary Carcinoma

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Background: Feline mammary carcinoma (FMC) is a high prevalence disease which often presents an aggressive behavior. Recent findings in human breast cancer showed that blocking T-cell Immunoglobulin and Mucin-domain containing molecule-3 (TIM-3) has remarkable anti-tumor effects, suggesting that TIM-3-targeted therapies are very promising. Although FMC is a valuable cancer model, no studies on TIM-3 receptor have been developed in this species.

Materials and Methods: In this study, the TIM-3 expression was evaluated by immunohistochemistry in total (*t*), stromal (*s*) and intratumoral (*i*) tumor-infiltrating lymphocytes (TILs) and in cancer cells, of 52 feline mammary carcinomas.

Results: sTILs-TIM3+ are more frequent than iTILs-TIM-3+, which are more abundant in luminal A/B subtypes. An association between *s*TILs-TIM-3+ and TIM-3+ cancer cells and higher tumor malignancy grade was found ($p=0.044$ and $p=0.018$, respectively), whereas higher *i*TILs-TIM-3+ densities were mostly associated with a benign tumoral behavior. Additionally, the solid histological subtype showed a positive association with the percentage of *s*TILs-TIM-3+ ($p=0.020$), with higher densities of cancer cells overexpressing TIM-3+ being associated with positive lymph node status ($p=0.025$) and with triple negative basal-like ($p=0.038$) and luminal B carcinomas ($p=0.010$) subtypes. Finally, higher *t*TILs-TIM-3+ densities were predominantly associated with more favorable clinicopathological features, namely the percentage of *t*TILs-TIM-3+ was negatively associated with tumor metastasis ($p=0.010$) and tumor recurrence ($p=0.013$).

Discussion: Altogether, in line with previous findings in human breast cancer reports, our results suggest that the expression of TIM-3+ among TILs subpopulations and cancer cells may differently influence the clinical outcome of cats with mammary carcinoma.

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Keywords: FELINE MAMMARY CARCINOMA; TIM-3; TILs; IMMUNOTHERAPY

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POSTERS

SESSION 2
ANIMAL AND FOOD SCIENCE

The influence of rumen ciliates´ community composition on methane emissions of lambs

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Ciliate protozoa are the main ruminal hydrogen producers, that is used by the methanogenic archaea to produce methane (CH_4). Ruminants contribute relevantly to the total of anthropogenic emissions of this potent greenhouse gas. To mitigate the ruminants´ CH_4 emissions many strategies as ciliates´ elimination from rumen, are proposed. However, there is still a strong lack of knowledge on the relationships between the ciliates´ community and rumen methanogenesis. We evaluated the relationships between the rumen ciliates community composition of lambs and CH_4 produced. Data derived from 54 animals raised for meat production and fed with a conventional, high-cereals and low-forage, finishing diet. Rumen fluid was collected at slaughter and ciliates analyzed by optical microscopy. Gas and CH_4 productions were evaluated *in vitro*, in glass bottles with gas detectors (Ankom System), incubating for 48 h individual rumen fluid samples with the diet provided to the donor. The ciliates´ total abundance ($\log_{10}$cells/ml rumen fluid), the *Isotricha*, *Dasytricha*, *Entodinium*, *Epidinium* and *Ophryoscolex* genera relative abundances (% total ciliates) and the CH_4 proportion (% total gas) were subjected to a regression analysis with incubation as random effect. CH_4 proportion increased linearly with the total abundance of ciliates in rumen fluid ($P<0.0001$), but different relationships were identified for ciliates´ genera. CH_4 proportion was negatively and quadratically related with *Entodinium* proportion ($P=0.0231$ and $P=0.006$ for linear and quadratic coefficients, respectively) and negatively and linearly related with *Isotricha* ($P= 0.002$) and *Epidinium* ($P=0.035$) proportions. The results suggest that ruminal CH_4 emissions seems to depend on the rumen ciliates´ community composition.

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Keywords: RUMEN CILIATE PROTOZOA; RUMINANTS´ METHANE EMISSIONS; ENTERIC METHANOGENESIS; LAMBS; INTENSIVE PRODUCTION SYSTEM

Personal hygiene requirements assessment – a cross-sectional study of butchers and fishmongers

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Introduction: Food handlers (FH) represent an important source of cross-contamination to foods. This work aimed to assess specific personal hygiene requirements compliance in butchers and fishmongers of Almada municipality in 2021.

Material & Methods: A cross-sectional study was performed including 140 FH that were interviewed for data collection purposes and retail establishments that were audited considering specific hygiene requisites based on European food hygiene regulations, including procedures, practices and premises assessment.

Results: Men were overrepresented (72%) and while 58% of the butchers were over 45 years of age, 70% of the fishmongers were <45 years. Despite low educational levels, FH with high school or university degree were also present. Most FH (95%) had also attended specific training courses on food hygiene and safety.
Regarding hand hygiene procedures and practices, a sufficient level of compliance was found with non-conformities related to the available handwashing premises and procedures, and to glove wearing practices. Although routine hand swabs for microbiological monitoring were performed in 82% of the establishments, several FH disclosed not to understand the real purpose of this monitoring, nor its relationship with hand washing and cross-contamination.

Discussion: These findings suggest the need for regular and continuous training in food hygiene and safety as an improvement tool, contributing to an increase in food safety procedures and practices awareness among FH.

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Keywords: FOOD ESTABLISHMENTS; FOOD HANDLERS; PERSONAL HYGIENE; HAND WASHING.

Metaproteomics of the ovine ruminal microflora affected by the forage-concentrate ratio

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Introduction: Ruminal microbiome is strongly influenced by dietary forage-concentrate ratio. Advances in metaproteomics can elucidate the role of active microbial populations involved in essential rumen metabolic functions according to the forage-concentrate ratio.

Material & Methods: Sixteen male lambs were distributed to two treatments (2 basal diets fed *ad libitum*: 80% concentrate and 20% forage and vice versa) for 42 days. Upon slaughter, rumen content was collected for metaproteomics analysis, conducted using LC-MS/MS based label-free quantitation. Purified peptides were identified and quantified using FragPipe v.17 and LFQ-MBR workflow against a metaproteomics database containing known mass spectrometry contaminants, and reviewed sequences from uniprot combined with the reference proteomes of glycine max, sheep and hungate1000 fasta database. Quantitative protein profiles were analyzed using prolfqua R-package.

Results: From 3723 identified microbial proteins, 398 bacterial differentially expressed proteins were found in the ruminal content of concentrate fed lambs when compared to forage fed lambs. From those, 301 proteins were identified and classified into one of thirty-two categories according to their biological function. Most of the proteins belonged to protein translation (19%), followed by structural role (10%), carbohydrate metabolism (8%), stress response (8%) and protein folding (8%). In total, 108 bacterial species were identified, being *Prevotella sp. AGR2160* (6%), *Ruminobacter sp. RM87* (5%), *Selenomonas bovis DSM 23594* (4%), *Prevotella bryantii C21a* (4%) and *Prevotella sp. KH1P2* (4%) the most abundant.

Discussion: *Prevotella* has been associated to concentrate-rich diets and degradation and utilization of plant noncellulosic polysaccharides. Also, *Ruminobacter* and *Selenomonas* are starch-degrading bacteria, therefore their higher abundance in concentrate-rich diets is expected.

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Keywords: METAPROTEOMICS; LAMB; RUMEN MICROFLORA; CONCENTRATE

Production season influence in the fatty acid composition of lamb fat from southern Portugal

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Introduction: Diets affect the fatty acid (FA) composition of ruminant products. Forage/pasture diets are associated to higher amounts of vaccenic (t11-18:1) and rumenic acid (c9,t11-18:2) in fat, both with beneficial health effects. Conversely, cereal-rich concentrates stimulate a shift in biohydrogenation pathway with higher t10-18:1 quantity, trans-FA associated with detrimental health effects. This study aim was to characterize the FA composition of fat from lambs produced in Alentejo region in two production seasons (autumn/winter (A/W) vs spring (Sp)).

Materials & methods: In A/W, season characterized by absence of pasture, were collected 142 samples of lamb kidney knob channel fat (KKCF), and in Sp season, with higher pasture availability, 130 KKCF samples. The FA methyl esters were analysed by GC-FID.

Results: Production season influenced the fat content of saturated FA (A/W: 53.0 and Sp: 60.5 g/100g total FA (TFA)) and monounsaturated FA (A/W: 40.0 and Sp: 32.3 g/100g TFA). The t10-18:1 was the main trans-FA in 60% of samples from A/W, ranging from 0.15 to 11.1 g/100g TFA. In these samples the t11-18:1 varied between 0.22 and 7.3 g/100g TFA. Conversely, in Sp the t11-18:1 was the main trans-FA in 80% of samples, ranging from 0.60 to 7.3 g/100g TFA, while t10-18:1 varied from 0.12 to 9.0 g/100g TFA. Higher c9,t11-18:2 contents was found in Sp (0.15-2.03 g/100g TFA) than in A/W (0.06-1.46 g/100g TFA).

Discussion: Lamb fat from Sp had higher levels of healthy trans-FA. In both seasons, the trans-FA contents showed great variations, and even in Sp, high levels of t10-18:1 were found in some samples.

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Keywords: FATTY ACIDS; LAMBS; RUMINAL BIOHYDROGENATION; ALENTEJO; NUTRITIONAL VALUE.

Does chromium propionate affect growth and fatty acid composition of lamb tissues?

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Introduction: Chromium (Cr) has been reported to improve sensibility to insulin. As insulin participates in the regulation of lipogenesis, thus it is expected that Cr increases the intramuscular fat (IMF) and meat fatty acid (FA), particularly on forage-fed ruminants which have low insulinemia and meat fat content.

Materials & methods: We tested that hypothesis using 32 lambs randomly distributed to four treatments, resulting from the 2×2 factorial arrangement of basal diet (BD: HC: high-concentrate; HF: high-forage) and chromium propionate (CrC3) supplementation (CrC3: 0 or 800 ppb) main effects. After 42 days lambs were slaughtered, blood was collected for insulin and glucose determination and after 72h the longissimus muscle was collected for IMF and FA analysis.

Results and discussion: Supplementation with CrC3 had no effects on the performance of lambs, but as expected, lambs fed HC presented higher average daily gain and slaughter body weight than those fed HF. The insulinemia was highest in lambs fed HC than HF diets and was not affected by CrC3. However, CrC3 increased glycemia in the lambs fed HF but not in those fed the HC diet (DB*CrC3, $P < 0.05$). Lambs fed HC present higher ($P < 0.03$) IMF than those fed HF (4.91 vs. 4.09 g/100 g of meat) but CrC3 had no effects on IMF. The effects on meat FA were mainly due to BD and not to CrC3 supplementation although the 16:0 increased with Cr supplementation.

Conclusion: The CrC3 supplementation did not increase the growth, or IMF content in lambs and exerted only minor effects on meat FA composition.

Support: Financial support was provided within the scope of the projects PTDC/CAL-ZOO/29654/2017, UIDB/ 00276/2020 (CIISA) and LA/P/0059/2020 (AL4Animals) and Kemin is great acknowledge for providing the chromium propionate.

Keywords: LAMBS, FAT ACID, MEAT, CHROMIUM.

Influences of Fatty Acid Moiety and Lipid Class in Ruminal Lipolysis and Biohydrogenation

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Introduction: Lipid metabolism in the rumen involves the lipolysis of dietary triacylglycerols (TAG), phospholipids (PL) and glycolipids (GL), releasing free-fatty acids (FA), which are metabolized by ruminal bacteria forming *trans* and saturated FA during the ruminal biohydrogenation (RBH). The aim of this study (Study 1) was to evaluate the effect of the FA in the lipolysis of different TAG (TAG-16:0/16:0/16:0, TAG-18:0/18:0/18:0, TAG-18:1/18:1/18:1, TAG-18:2/18:2/18:2 e TAG-18:3/18:3/18:3). We also aimed (study 2) to evaluate the effects of different lipid classes (TAG, PL, and GL) in releasing 18:3n-3 and its subsequent RBH.

Materials & methods: For that, two anaerobic in vitro batch incubations were performed using rumen inoculum with 3 mg of each lipid for 0, 0.5, 2, 4 and 6 hours (study 1) or extended to 12 and 24h (study 2).

Results: The disappearance of all TAG increased with increasing incubation time excepting for TAG-18:3. There were no great differences in the FA composition of free-FA fraction during the 6h of incubation with the saturated-TAG, whereas the unsaturated-TAG were largely biohydrogenated. Also, the lipolysis was faster for TGL-18:3 compared with the other TAGs. In study 2, we observed that the 18:3n-3 released from TGL was faster at initial times than PL and GL but no differences were observed at 24h (reaching ~94%). After 24h of incubation, the 18:3n-3 from TAG was almost completely converted into 18:0, while the RBH of PL and GL was less complete, accumulating more intermediates of 18:3n-3.

Discussion: In conclusion, the dietary lipid class and its composition affect the FA generated in the rumen.

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Keywords: LIPIDS, FATTY ACIDS, LIPOLYSIS, BIOHYDROGENATION, RUMEN

Impact of Laminaria digitata as feed ingredient supplemented with exogenous enzymes on plasma metabolites and liver metabolism of broilers

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Background: *Laminaria digitata* has emerged as potential feed ingredient to partial replace conventional feedstuffs in poultry nutrition. Nonetheless, the recalcitrant cell wall of macroalga can undesirably affect feed digestion and absorption efficiency by trapping valuable nutritional compounds. The objective of this study was to assess if dietary supplementation with Carbohydrate-Active enZymes (CAZymes) would improve nutrients availability from *L. digitata*, incorporated at 15% in broiler's diet and how this strategy might impact on hepatic lipid metabolism and systemic metabolic profile.

Materials & Methods: Twenty-one Ross 308 22-d-old birds were randomly allocated into 4 groups and fed a corn-soybean basal diet (Control) plus 15% *L. digitata* (LA) supplemented or not with 0.005% Rovabio Excel AP (LAR) or 0.01% alginate lyase (LAE) during 14 days.

Results: The dietary incorporation of *L. digitata* had a negative effect on broilers productive parameters by reducing final body weight. In contrast, *L. digitata* decreased total cholesterol ($P = 0.037$) and LDL-cholesterol ($P = 0.004$). However, total lipids and TAG ($P < 0.001$) were increased by CAZymes relative to control and LA diets. In liver, n-3 and n-6 fatty acids, β-carotene, copper and iron were enhanced with *L. digitata*, individually or combined with CAZymes.

Discussion: Overall, *L. digitata* incorporated at high level in broilers' diet compromised birds' growth performance but decreases systemic lipemia, suggesting a hypocholesterolaemic action, and improves hepatic composition. Moreover, the effect of *L. digitata* suppresses the effect of both enzymes. In forthcoming studies, lower level of *L. digitata* inclusion and new exogenous CAZymes should be evaluated.

Support disclosure: Financial support by CIISA (UIDB/00276/2020), AL4AnimalS (LA/P/0059/2020) and LEAF (UIDB/04129/2020) grants. It was also supported by Fundação para a Ciência e Tecnologia (FCT, Lisbon, Portugal) through PTDC/CAL-ZOO/30238/2017 grant associated post-doc contract to M.M.C., SFRH/BD/126198/2016 for PhD fellowship to D.F.C., SFRH/BPD/116816/2016 post-doc fellowship to J.M.P. and DL57/2016/CP1438/CT0007 contract to P.A.L.

Keywords: *Laminaria digitata*; CAZymes; PLASMA METABOLITES; LIVER COMPOSITION; BROILER.

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Holistic approach to evaluate the metabolic impact of dietary Ulva lactuca and ulvan lyase supplementation in the diet of weaned piglets

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Background: *Ulva lactuca*, a green seaweed, is a putative alternative to conventional crops, that can help piglets cope with *post*-weaning stress. However, its cell wall is rich in ulvan, a recalcitrant polysaccharide. The objective of this work was to evaluate the effects of dietary *U. lactuca* and ulvan lyase supplementation on piglet performance, feed digestibility, serum biochemical profile and hepatic proteome.

Materials and Methods: Forty piglets (8.56 ±0.85 kg, Large White × Duroc) were randomly divided across four experimental groups: control, UL (7% *U. lactuca* replacing control), ULR (UL+0.005% Rovabio Excel AP) and ULU (UL+0.01% ulvan lyase). Each piglet was housed individually in a metabolic cage with free access to water and fed with 50 g feed/kg live weight/day. At the end of two weeks, piglets were slaughtered, and serum and liver were sampled.

Results: Diets had no effect on growth performance parameters (P>0.05). There was a significant effect for decreased dry matter (P=0.0297) and organic matter (P=0.0491) digestibility in ULU compared to control. Ulvan lyase promoted increased urea (P=0.0003) and total lipids (P<0.0001) in the serum. Seaweed alone increased serum IgG (P=0.0361) by 45% compared to control. Ulvan lyase inclusion decreased serum cortisol by more than 50% (P=0.0012) compared to control and UL. The latter had an increase in the abundance of hepatic ENO1, a protein involved in carbohydrate degradation pathway, whereas ULU supplementation increased fatty acid synthesis (CYP2E1).

Discussion: This study indicates that the piglet benefits from *U. lactuca* and ulvan lyase supplementation by increasing anti-inflammatory activity and decreasing stress-related biomarkers.

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Keywords: *Ulva lactuca*; CAZymes; PIGLET; SERUM; LIVER

Effects of inclusion of the aerial part and condensed tannin extract from Cistus ladanifer L. in lamb diets on muscle antioxidant status and lipid stability

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Introduction and Aim: *Cistus ladanifer* L. (CL) is an abundant perennial shrub in Mediterranean countries, containing high levels of bioactive compounds with recognized antioxidant activity, including vitamins and phenolic compounds such as condensed tannins (CT). The aim of this study was to evaluate the effect of utilization of CL, as source of CT, in lamb diets on muscle antioxidant capacity, phenolic and vitamin E content and oxidative stability. The presence of CT monomers in the muscle was researched. Antioxidant capacity and CT, total phenols and vitamin E contents was evaluated in diets.

Material & Methods: Six diets were formulated considering three CT levels (0, 1.25 and 2.5% CT) and two ways of CT supply (CL aerial part vs CL CT extract). Basal diet was composed by dehydrated lucerne supplemented with soybean oil (60 g/kg). Thirty-six lambs were individually housed, with six lambs per diet. The trial lasted for 35 days.

Results and Discussion: Increasing levels of CL aerial part and CT extract in diets increased the antioxidant activity and total phenols and CT contents of diets, while the 〈-tocopherol content was not affected. Muscle antioxidant activity and total phenols and 〈-tocopherol contents were not affected by dietary treatments. No CT monomers were found in muscle. Meat lipid oxidation increased over storage time from 0.09 to 0.40 mg MDA/kg at day 0 and 7 of storage, respectively, and was not affected by the dietary treatments. The higher phenolic compounds content in diets with CL aerial part and extract did not result in higher antioxidant capacity of lamb muscle.

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Keywords: CISTUS LADANIFER; CONDENSED TANNINS; LAMB MEAT; ANTIOXIDANT ACTIVITY; LIPID OXIDATION

Asparagopsis taxiformis vegetable oil extracts: a stable alternative processing method to freeze-dried

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Introduction: The red macroalgae *Asparagopsis taxiformis* contains bromoform ($CHBr_3$), a highly volatile potent rumen methanogenesis inhibitor. To prevent the volatilization of $CHBr_3$ the algae need to be freeze-dried. Practical and cost-effective alternatives to freeze-drying must be developed to allow the use of *A. taxiformis* in ruminant feeding. This work aimed to compare the bromoform concentration of *A. taxiformis* subjected to different types of post-harvest initial processing.

Materials & methods: *A. taxiformis* was harvested and kept at 4ºC for 20 min, and then either frozen and freeze-dried (FD) or immersed in 3 different oils (sunflower, linseed, or coconut). Oil-plus-algae mixtures were filtered after 15 d, and the filtrated oil enriched in $CHBr_3$ (ATOil) was stored at room temperature and protected from light. The $CHBr_3$ concentration was determined by GC-MS.

Results: The $CHBr_3$ concentrations were $1.3^a \pm 0.06$, $0.94^b \pm 0.06$ and $0.82^b \pm 0.06$ mg/mL, respectively for the ATOils prepared with sunflower, coconut and linseed oils. The efficacy of extraction was quite high with sunflower oil immersion method as the $CHBr_3$ concentration expressed as mg/g DM of algae was comparable ($P > 0.05$) to that of freeze-dried biomass (14.7 mg/g DM algae in sunflower ATOil vs. 11.5 mg/g DM algae in FD biomass). These $CHBr_3$ measurements were obtained after a 16 months storage period showing the very high stability of the ATOils.

Discussion: The ATOil processing method provides a cheaper and more stable alternative to freeze-drying that can support the practical use of *Asparagopsis* as a feed additive to mitigate methane production in ruminants.

Support/interest disclosure: Project GreenBeef (LISBOA-01-0247-FEDER-047050), FCT Project UIDB/00276/2020 (CIISA) financed by national funds through FCT - Fundação para a Ciência e a Tecnologia, I. P. and LA/P/0059/2020 (AL4AnimalS).

Keywords: METHANE, RUMINANTS, RED ALGAE, BROMOFORM, ANTI-METHANOGENIC.

Methane emissions of beef cows grazing natural or biodiverse sown pastures

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Introduction: Beef production is often pinpointed as one of the main causes for emissions of greenhouse gases (GHG) contributing to climate change. In Portugal, the official roadmap towards carbon neutrality by 2050 proposes a sharp 20-30% decrease in beef cattle stock. Despite that drastic proposal, there are no direct measurements of digestive CH_4 emissions by cattle in Portuguese production systems. The Greenbeef project aims to fill that gap and establish the baseline GHG emissions of grazing suckler cows by monitoring their digestive CH_4 emissions using a GreenFeed® apparatus.

Materials & Methods: Ten cows were kept in natural pastures other ten on biodiverse sown pastures. The GreenFeed unit was placed in with each group during two periods of 15 d for monitoring the emissions of CH_4. The pasture intake was estimated using the n-alkane method using a concentrate feed marked with dotriacontane (C_{32}) and beeswax and administered to the animals one to five times a day for 11 consecutive days. On the last 4 days of each period, pasture and faecal samples were collected to be analysed for n-alkanes.

Results & Discussion: The data already obtained are yet preliminary. Some cows refused to use the GreenFeed unit. Still, the average value for digestive CH_4 emissions is 118 g/d for cows (average of 9 cows) grazing in the natural pasture and 192 g/d for cows (average of 4 cows) grazing the biodiverse sown pasture. Although pasture intake estimates are not yet available, we anticipate that pasture intake differences mainly explain the difference in CH_4 emission.

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Keywords: GREENHOUSE GASES; METHANE, PASTURE; GREENFEED; SUCKLER COWS

Fully feathered and naked neck slow growth broilers fed with Spirulina (Arthrospira platensis) dietary inclusion: Productive performances under high ambient temperatures

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Background: One of the biggest challenges in poultry production is heat stress (HS). HS significantly influences production, behavioral and psychological traits in poultry. Microalgae, as Spirulina, provide a sustainable source of nutrients for poultry.
The objective of this study was to evaluate the impact of HS on productive performances and carcass yield of two slow-growing strains, Naked Neck (NN) and Fully Feathered (FF) fed with Spirulina.

Materials & methods: Twenty-day-old male chicks of each strain (NN and FF) were individually housed at 30 °C with *ad libitum* access to water and feed. Each group received either a Control diet (C) or a diet with 15% Spirulina (SP). The animals were randomly assigned in 4 groups: CNN; CFF; SPNN and SPFF. Animals were weighed and feed intake (FI) monitored weekly to determination Feed Conversion Ratio (FCR) and Average daily gain (ADG). After 84 days of experiment, broilers were slaughtered.

Results: FCR were not significantly affected ($p > 0.05$). The final live weight was 2741, 2526, 2536 and 2141g for CNN, CFF, SPNN and SPFF groups, respectively. SPNN animals had the highest ADG and FI, 53.3 and 1012 while CFF had the lowest, 39.53 and 774 g, respectively. The incorporation of SP negatively influenced the carcass yield ($p< 0.05$) in both strains however, no significative differences were found in the breast muscle yield.

Discussion: HS affects the growth performance of the animals, but was mitigated by using NN animals, even on SP diets. Future information provided by digestibility coefficients and meat quality will complement these results.

Support/interest disclosure: Funded by Fundação para a Ciência e Tecnologia (FCT) under the Collaboration Protocol for Financing the Pluriannual Plan for Research Fellowships for PhD Students, signed between FCT and the R&D Unit -LEAF (FCT UID/AGR/04129) based at Instituto Superior de Agronomia of Universidade de Lisboa.

Keywords: HEAT STRESS, NAKED NECK, FULL FEATHERS, SPIRULINA, POULTRY

Whole genome sequencing of Landim pigs from Mozambique suggests selection for immune response and a close relationship with native pigs from Angola

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Introduction: Landim pigs, a population of native pigs from Mozambique, suffered a significant decrease in population size over the last decades. The remaining populations are threatened by the recent introduction of European exotic breeds. This population has never been characterized at the genome level. In this study, we aimed to provide a comprehensive genetic characterization of Landim pigs using whole-genome sequencing (WGS).

Materials & Methods: We obtained genomes of Landim pigs (N=6), that were further compared with the genomes of local pigs of Angola (N=4) and with genomes from European and Asian domestic pigs and wild boars, currently in the public domain (N=74). Identification of polymorphisms and population structure analysis were performed.

Results: Results showed that Angolan and Landim pigs are closely related, and highly related to European breeds. In Landim pigs, genes harbouring missense SNPs are associated with immune system response. Our analysis suggests that Landim pigs display a large duplication in chromosome 4 reported only in Chinese domestic pigs, overlapping the *TBX19* gene, a transcription factor involved in the regulation of developmental processes, that can activate *POMC* gene expression and repress thyroid-stimulating hormone beta promoters. Analysis for the detection of regions under selection is currently ongoing.

Discussion: This study represents the first assessment of the genetic relationship between native pig populations from Mozambique and pigs from around the world using WGS, showing that these pigs might have been selected towards disease resilience and metabolic adaptation.

Support/interest disclosure: Authors have no conflict of interest to declare. Authors acknowledge the support of Centre for Interdisciplinary Research in Animal Health - CIISA (Project UIDB/00276/2020, funded by FCT) and of Associate Laboratory for Animal and Veterinary Science (LA/P/0059/2020 - AL4AnimalS). AJA was funded by FCT 2021.02058.CEECIND.

Keywords: LANDIM PIGS; WHOLE-GENOME SEQUENCING; SIGNATURES OF SELECTION; GENOMICS; CNV.

Screening of new Tunisian lactic acid bacteria with antimicrobial properties to be used in biopreservation

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Background: Lactic acid bacteria (LAB) are a ubiquitous group found in various niches, which are used to preserve and/or transform products, due to increasement of shelf life, enhancement of organoleptic properties, digestibility of proteins/carbohydrates and bioavailability of vitamins/minerals. The objective of this study was to isolate new Tunisian LAB and evaluate their biological features, such as antimicrobial and enzymatic activities.

Materials/Methods: LAB were obtained from various Tunisian fermented products, purified and identified using Gram, growth at various temperatures and capacity to produce organic acids. Subsequently, we screened for antimicrobial activity (agar diffusion or overlaid methods). *Listeria monocytogenes*, *Escherichia coli* were used as indicators for antibacterial activity. *Penicillium expansum*, *Aspergilus niger*, *Fusarium culmorum*, and *Botrytis cinerea* were used as indicators for the antifungal activity. The semi-characterization of the antibacterial substance was done by treatment at 100°C/15 min and/or neutralisation of the supernatant pH.

Results: Six *L. plantarum* showed the highest inhibition against all indicators, especially against *Listeria* and *E. coli* (diameter ~11 mm). The radia of inhibition halos was the same when we performed the antibacterial activity with LAB culture and with the crude cell free supernatant -CFS-; however, the antibacterial activity CFS was lost after treatment with NaOH (pH neutralization), suggesting the nature of the antibacterial substance, since lactic bacteria are known to produce organic acids as principal metabolite.

Discussion: Preliminary results confirmed the nature of the inhibitory substance to be organic acid, further investigations are needed to confirm the putative use of the isolated LAB for the biopreservation of fresh fruits and vegetables.

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Keywords: LACTIC ACID BACTERIA, TUNISIAN FERMENTED PRODUCTS, BIOPRESERVATION, ANTIMICROBIAL ACTIVITY

The mineral profile of cow's milk and plant-based milk alternatives (PBMA)

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The consumption of plant-based milk alternatives (PBMA) as a substitute of cow's milk (CM) is a matter of concern due to the variability verified in their nutritional composition. To avoid situations of deficiency/toxicity to humans, the equilibrium in the mineral profile is essential. The aim of this study was to characterize the mineral profile of CM and PBMA. Forty samples of pasteurized CM and 60 samples of PBMA (soya, oat, rice, almond, coconut, and hazelnut) were analysed by ICP-OES for the determination of their mineral contents. The results showed that the contents of calcium and iron were not significantly different ($P>0.05$) between beverages, averaging 1.28 g/kg and 0.001 g/kg. The potassium, phosphorus, and sulphur contents in CM and soya PBMA displayed no significant differences in between ($P>0.05$; averaging 1.60, 1.04, and 0.32 g/kg) but were higher ($P<0.05$) than the contents of others PBMA. Soya PBMA had the highest ($P<0.05$) contents of magnesium, cooper, and manganese (0.203, 0.001, and 0.001 g/kg), while coconut PBMA presented the highest sodium content (0.493 g/kg). CM, soya and oat PBMA showed the lowest ($P<0.05$) values of calcium/phosphorus and sodium/potassium ratios, averaging 1.3 and 0.37. Some PBMA showed a mineral profile resembling CM, which reveals mineral supplementation during processing, that does not guarantee equal nutritional quality. Furthermore, some PBMA showed values on sodium/potassium ratio quite above the guidelines, which is of concern. In conclusion, some PBMA have a mineral profile close to CM, however more studies are needed to ensure nutritional similarity.

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Keywords: COW'S MILK; PLANT-BASED MILK ALTERNATIVES; MINERAL PROFILE.

Cull beef cow from extensive systems (Southern Portugal): high n-3 PUFA

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Introduction: mature or cull cows are animals that have ended their productive life. The total amount of beef from cull beef cows is variable and, under normal conditions, the proportion of meat obtained is between 20 to 25% in Portugal. The goal of this research work was to characterize carcass, meat quality traits, proximate composition and lipid profile of cull beef cows reared in extensive systems across various seasons.

Material & methods: mature beef cows (n=65), mean age of 14.12 years, and culled from commercial farms were slaughtered in the Spring, Summer, Autumn and Winter, after a finishing period of about 4 weeks. The meat quality traits were analyzed (7 d *postmortem*) in *Longissimus, Semimembranosus, Psoas major* and *Semitendinosus* muscles.

Results: the highest carcass yield was obtained in Winter ($P<0.05$), but the intramuscular fat (IMF) means were similar in the different seasons. The meat samples showed high average shear force (between 5.41 to 6.86kgF), which may be considered as tough (according to the scale used), except for the *Longissimus thoracis* which showed an intermediate average (5.13kgF). However, *Psoas major* obtained the highest overall acceptance-scores. Meat from animals kept in extensive under natural conditions of Southern Portugal, present high n-3 PUFA.

Discussion: In Autumn and Summer, portions of 100 g/day of meats with 4.03% and 4.25% of IMF with 1.12 and 2.20% of n-3 PUFA in total fatty acids, can provide between 72.96 and 143.31mg of n-3 PUFA or 31.99 and 61.21mg/d of EPA plus DHA, which corresponds to 12.47% and 24.48% of the requirements recommended by EFSA authorities (250mg/d of EPA plus DHA).

Support/interest disclosure:
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Keywords: MATURE COWS; MEAT QUALITY; SLAUGHTER SEASON; MONTADO AGROFORESTRY SYSTEM

Does reticulo-rumen morphology explain lambs´ susceptibility to altered rumen biohydrogenation pathways?

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Introduction: Reticulo-rumen are gastric compartments of ruminants that harbour a complex microbiota that digest fiber efficiently but also isomerizes and hydrogenates extensively the dietary unsaturated fatty acids through biohydrogenation metabolic pathways (RBH). Generally, RBH yields t11-18:1 as the main intermediate, but when ruminants are fed high-starch diets tend to develop sub-acute ruminal acidosis and to present altered RBH (i.e. t10-shift) where the t10-18:1 isomer becomes main intermediate with potential deleterious effects on meat nutritional value. Lambs susceptibility to express the t10-shift widely varies, but individual variability remains unexplained. The project Gene2rumen proposes to sample up to 700 intensively finished lambs and use rumen morphological data in a GWAS study.

Materials & methods: Lamb´s reticulo-rumen are being collected from a slaughterhouse for morphometric data, including maximal length and width, and rumen volume, registered. After dissection, the colour of rumen mucosa was determined by visual determination and by using a Minolta Chroma Meter for lightness, redness and yellowness in 5 different areas.

Results: Preliminary data (19 animals) indicates that 68% of rumen mucosa were dark-brownish, while 32% were yellower. In accordance, a significant difference in yellow/blue coordinate values was confirmed for the same samples using a chromameter (13.5±2.4 *vs* 11.7±3.0; p=0.006), but no differences were found for its lightness and redness. Averages for maximal rumen length and width were 28.5cm and 27.43cm, respectively, and the average estimated rumen volume was 6.75L. In summary, a morphological and colorimetric assay was successfully developed and two distinct chroma phenotype were established as far as rumen mucosa yellowness variation.

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Keywords: RUMEN, MORPHOLOGICAL PHENOTYPE, COLORIMETRY, MORPHOMETRY, SHEEP

Are consumers ready for eggs and meat of Portuguese local poultry breeds?

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Consumers' attitude towards local poultry breeds is of utmost importance for maintaining traditional production, and promoting environmental, social and economic sustainability of local farms. The objective of this preliminary study was to assess Portuguese consumers' egg and meat consumption preferences and willingness to pay for local products.

A questionnaire was applied to consumers (N=585) using online survey tools including consumer characterization, preferences on egg and meat consumption (shell and yolk color, meat color and production system) and willingness to pay for autochthonous-breed products. Data was analyzed using descriptive frequency and Chi-square tests.

Most interviewed consumers would be willing to consume local-breed eggs (94.0%) and meat (93.0%). Very few consumers appreciate white-colored eggs (1.4%) or yellow yolk (12.5%) ($p<0.001$). Most consumers have no preference regarding egg shell color (55%) and prefer light and dark orange (59%) colored yolk ($p<0.001$). Consumers declare to consume preferentially eggs of chicken bred in free-range production systems (50.2%). Most consumers prefer dark-colored meat (59.8%) of poultry bred in free-range production systems (47.4%). Consumers would be willing to consume eggs and meat of autochthonous poultry breeds (94.0 and 93.0% respectively) ($p<0.001$). Portuguese consumers would be willing to pay up to 25% more (51.9%) for local than for industrial hybrid eggs and up to twice as much for meat of local breeds (64.7%)($p<0.001$).

Portuguese consumers are interested and recognize the added value of local breed productions although some consumption preferences seem to be in disagreement with intrinsic characteristics (e.g. egg yolk color) of local products.

Keywords: PORTUGUESE LOCAL BREED; POULTRY; CONSUMER PREFERENCES; EGG; MEAT

BROILERNET: an international network to bring science and the broiler production sector together

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There is a continuous need to improve the animal production sector, namely broiler production, to meet the various demands arising globally. BROILERNET is a European Commission funded project in which the FMV-UL is a partner, aiming to assist the broiler sector in overcoming the foreseen challenges. BROILERNET is based on creating twelve national-level innovation networks, including in Portugal, and three EU-level networks of broiler farmers, advisors, supply chain integrator companies, farmers' organizations, researchers and veterinarians to address the upcoming tribulations faced by broiler farmers. The project design focuses on three main fields of ongoing concern: environmental sustainability, animal welfare, and animal health management. Through a multi-actor approach, the most urgent needs of broiler farmers will be identified, and the network will collect and evaluate good practices that can meet these needs. BROILERNET will also engage with existing and new broiler-focused EIP-AGRI Operational Groups (OGs) and enhance their impact. The selected best practices will be spread through various communication and dissemination activities to reach the vast majority of broiler farmers in the countries of the participating consortium partners, but also to all broiler farmers across Europe. A multi-target communication strategy will be implemented at the national and international level, with diverse multi-language flyers, videos and national roadshows as the major channels within the activities dedicated to communicating and disseminating the information.

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Keywords: BROILERNET; INTERNATIONAL NETWORK; BROILER PRODUCTION; GOOD PRACTICES; COMMUNICATION AND DISSEMINATION.

Effect of ram breed, season and cryopreservation on spermatozoa motility and morphologic traits of 10 Portuguese local breeds

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Introduction: Semen quality is essential for successfully cryopreserve spermatozoa (SPZ) to storage in germplasm bank for posterior use, and artificial insemination. The present study aimed to characterize seasonal ram semen variations of 10 Portuguese local breeds, hypothesizing that the breed and season had lower effects on the main spermatozoa traits.

Materials & methods: A total of 1471 semen collections from 85 rams between 2004 and 2020 (EZN) were obtained and evaluated in fresh, and re-evaluated after freezing/thawing. A multivariable mixed linear model for repeated measures was used to test the effect of breed, season, and semen processing on nine semen/SPZ traits.

Results: The volume per ejaculate, SPZ concentration and total number of SPZ per ejaculate were affected by breed ($P <0.001$) but not by season ($P >0.05$). As expected, the semen cryopreservation process was the most significant ($P <0.001$) factor of variation. Also, breed and interactions between breed and season, or semen processing, have modulated the response of SPZ viability, abnormal morphology, head and intermediate piece defects. The season effect only affected the intermediate piece defects due to the highest percentage observed between February and April period in the fresh semen of some breeds.

Discussion: Breed and semen processing had significant effects on all SPZ traits (except for tail). Semen processing was the most significant effect. The seasonal effect on ejaculates and SPZ traits is marginal in our latitude, because only intermediate piece defect was affected. Therefore, rams from local breeds can be semen donors for cryopreservation or mating all year round at our latitude.

Support/interest disclosure: Projects FCT POCTI/CVT/57148/2004, FCT PTDC/CVT/098607/2008, UIDP/CVT/00772/2020 (CECAV), LA/P/0059/2020 (AL4AnimalS), and ALT20-03-0246-FEDER-000021.

Keywords: SEMEN CRYOPERSERVATION; SHEEP; LOCAL BREEDS, SEASONALITY, GERMPLASM BANK

Influence of 15% Ulva lactuca alone or combined with CAZymes on broilers meat quality and nutritional value

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Background: Having in mind the benefits of *Ulva lactuca* as feed ingredient, several studies reported its impact on the meat quality of poultry. Nonetheless, high inclusion levels of this seaweed may impair nutrient digestibility as a result of indigestible cell wall polysaccharides. Thus, the use of Carbohydrate-Active enZymes (CAZymes) emerges as a putative solution to degrade *U. lactuca* cell wall. The aim of this study was to test if feeding 15% of *U. lactuca* to broilers, with or without carbohydrases, can improve meat quality.

Methods: One hundred and twenty 22-day-old broilers were randomly allocated to 4 diets during 14 days: (1) maize and soy-based diet (control); (2) control with 15% of *U. lactuca* (UL); (3) UL diet supplemented with 0.005% of a commercial carbohydrase mixture (ULC); (4) UL diet supplemented with 0.01% of a recombinant ulvan lyase (ULE).

Results: Meat lightness and redness scores, off-flavours, and total carotenoids increased ($p < 0.001$), while yellow values, tenderness, juiciness, overall meat acceptability, α- and β-tocopherol, and total lipids decreased ($p < 0.001$) in broilers fed *U. lactuca*. The n-3 polyunsaturated fatty acids (PUFA) increased ($p < 0.050$), and the n-6/n-3 PUFA ratio decreased ($p < 0.001$) in broilers fed *U. lactuca* with the recombinant ulvan lyase. The incorporation of 15% of *U. lactuca* into broilers' diets increased meat nutritional value through the incorporation of health-promoting antioxidant carotenoids and n-3 PUFA, although negatively reducing meat acceptability.

Discussion: In view of these results, the incorporation of lower levels (<15% feed) of *U. lactuca* into broilers' diets could represent a promising strategy to counteract the negative effects of *U. lactuca* on meat sensory perception by the consumers.

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Keywords: *Ulva lactuca*; CAZymes; MEAT QUALITY; SENSORIAL ANALYSIS; BROILERS.

Biodiverse haylages in beef fattening diets – Effect on lipid oxidation of cooked meat

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Introduction: Portugal has bulls and heifers fattened with diets based on concentrates rich in cereals and oilseed derivatives, raw materials strongly dependent from the external market. Nowadays is possible to produce high-quality haylages, even in unfavorable Spring climate conditions. In the project "LegForBov – Alternative feeds in beef production", haylages were used to replace part of conventional raw material, reducing the external dependence of beef production systems.

Materials & Methods: In 3 experiments, diets of bulls and heifers (64 animals) had different levels of haylage (50, 54, 60 and, 67.5% DM) produced from a biodiverse mixture (Speedmix – Fertiprado, Portugal). The diets with 54 and 67.5% DM haylage contained sunflower seed (10% DM). The effect of haylage on meat lipid oxidation was compared with a conventional concentrate-based diet. Lipid oxidation (TBARS) was evaluated in muscle samples stored under vacuum (2°C) from 3 to 14 days after slaughter and maintained at 4°C during 0 and 3 days after cooking.

Results and discussion Meat lipid oxidation was lower in haylage diets ($P<0.001$; 0.12 mg malonaldehyde (MDA)/kg muscle) than in concentrate-based diet (0.18 mg MDA/kg muscle) on cooking day. Lipid oxidation was not affected by the diet on cooked meat preserved for 3 days, averaging 3.09 mg MDA/kg muscle. Higher contents of antioxidant compounds, such as phenolic compounds and vitamins, in haylage diets than in concentrate diet, may have contributed to the meat lipid protection. However, in more severe oxidative conditions (cooking and storage) the antioxidant effect of haylage was not enough to limit meat lipid oxidation.

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Keywords: MEAT LIPID OXIDATION; HAYLAGE DIETS; BEEF PRODUCTION.

Use of almond hull in lamb diets – effect on growth performance and meat quality

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Introduction and objective: Almond hull (AH) is an abundant by-product in the Mediterranean region, namely in southern Portugal. The objective of this work was to evaluate the effect of partial replacement of cereals in the diet by increasing levels of AH on the growth performance and meat quality of lambs.

Material & methods: Twenty-four ram lambs were individually housed and assigned to 3 diets (8 lambs/diet) offered *ad libitum*. All diets included 40% dehydrated lucerne, 6% soybean oil, soybean and sunflower meal, and cereals, which were stepwise replaced by AH, reaching 0% dry matter (DM) (Control), 9% DM (AH9) and 18% DM (AH18) of AH in diets. The trial lasted 6 weeks after 7 days of adaptation. Average daily gain (ADG) and feed intake were evaluated. In meat was determined the colour, cooking loss, shear force, and sensory attributes.

Results and discussion: The partial replacement of cereal in the diets by the AH up to levels of 18% DM did not affect the ADG (P=0.152, 349 g/d) and the dry matter intake (P=0.468, 1783 g/d). However, 18% of AH in diets increased the feed conversion ratio (P=0.004, 6.54 (AH18) vs 5.31 (Control/AH18)). No effects were observed in meat colour parameters and cooking loss. Increasing levels of AH in diets reduced the meat shear force (P=0.017, 5.83, 5.55 and 5.39kg in Control, AH9 and AH18, respectively). Regarding to meat sensory attributes, only flavour acceptability was lower in AH9 diet (P=0.013; 6.70 (AH9) vs 6.97 (Control/AH18)). AH can be incorporated in lamb diets without compromising the growth performance or meat quality.

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Keywords: ALMOND HULL; LAMB DIETS; RUMINANT NUTRITION; GROWTH PERFORMANCE; MEAT QUALITY

Resistance of Salmonella enterica subsp. enterica isolated from slaughtered pigs to a chlorine-base biocide

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Introduction: In the European Union foodborne non-typhoidal salmonellosis is frequently associated with pork, thus the implementation of effective cleaning and disinfection programmes is paramount. However, decreased susceptibility to biocides can result in *Salmonella* persistence in the abattoir environment.

Materials & Methods: Forty-five *S. enterica* isolates from slaughtered pigs were studied regarding the presence of efflux pump-associated genes (*acrA*, *acrB*, and *tolC*) by PCR. The susceptibility to different concentrations of Mida FOAM 193 (MF193 – a chlorine-based biocide formulation) with three levels of organic matter (absent, low, and high OM) was tested in 12 isolates.

Results: PCR amplification confirmed that the *acrA/acrB/tolC* genotype was present in all isolates (100%). The Minimum Bactericidal Concentration (MBC) of MF193 in all isolates was 20x lower than the manufacturer's recommended application concentration (10%), corresponding to an average of 0.5%, in the absence and with low levels of OM. Resistance to MF193 occurred at concentrations <2.500% with high OM levels. In the present study conditions, the Minimum Inhibitory Concentration (MIC) was only possible to determine with high levels of OM, with a mean value of 1.542%. The 12 isolates screened for susceptibility had the *acrA/acrB/tolC* genotype, which is constitutively expressed.

Discussion: Resistance to MF193 was only observed in high OM levels and low biocide concentration conditions, which could result from the combination of efflux pump function and OM barrier effect.Our results stress the relevance of the application of biocides according to the in-use recommended concentrations to effectively eliminate *Salmonella* from the slaughterhouse environment, as incorrect dilutions can promote its persistence.

Support/Interest disclosure: This work was supported by CIISA (UIDB/00276/2020), AL4AnimalS (LA/P/0059/2020) and Project UIDB/00276/2020 (Funded by FCT).

Keywords: *Salmonella enterica* subsp. *enterica*; CHLORINE-BASED BIOCIDE; MINIMUM INHIBITORY CONCENTRATION; MINIMUM BACTERICIDAL CONCENTRATION; RESISTANCE GENES.

Hygiene and Health Safety of Edible Viscera Obtained at a Slaughterhouse

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Introduction: Viscera are highly perishable, mainly due to deteriorating microbial growth. A decontamination step can be considered to reduce microbial contamination and thus increase product safety and quality. This study aimed to evaluate the effectiveness of three solutions, lactic acid and acetic acid at 1% and 2% and chitosan at 0.2% and 0.5% on bovine liver samples inoculated with *Salmonella* over storage time.

Material & Methods: The samples were inoculated with a mix of *Salmonella* Typhimurium ATCC 14028 and *Salmonella* Derby at a concentration of 1,60 x 10^8 CFU/g, and then decontaminated with the solutions. The work was performed in five times (0h, 12h, 24h, 48h, and 72h) and the samples were stored at 7°C. At the tested times *Salmonella* counts were made and pH and color were evaluated.

Results: No significant differences in the counts were observed. The average range of counts obtained was between 6.37 and 6.92 log CFU/g. However, lower *Salmonella* counts could be seen in the treated samples compared to the control. In particular at 12 hours, for 2% lactic acid and at 24 hours for 0.2% chitosan. L, a* and b* parameters were not affected by the solutions application. Regarding pH, there was a decrease, with significant differences. For the 1% lactic acid, where highly significant differences were seen, there was a reduction from 6.26 at time 0h, to 5.45 at time 72h.

Discussion: The results showed that the use of organic acids and chitosan can be used as an additional step to control microbial growth.

Funding: This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

Keywords: VISCERA, PORK MEAT, DECONTAMINATION, ORGANIC ACID, CHITOSAN

Genome-Wide analysis of genetic markers associated with carcass weight in Portuguese Preta cattle

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Introduction: Preta is a Portuguese local cattle breed raised in the Ribatejo and Alentejo regions. A genetic management program has been in place for nearly 20 years, essentially based on the collection of phenotypic and pedigree data, with the aim of selection for production traits and controlling the levels of inbreeding.

Materials & Methods: Genotyping using the *GGP Bovine 100K SNP array* was performed for 317 animals with the aim of identifying markers associated with carcass weight. Data on carcass weight per day of age were first analysed with an Animal Model, using phenotypic records from 4194 animals, collected since 2002, and the full relationship matrix (n= 48,728). The estimated breeding values were deregressed for genome-wide association analyses.

Results: After quality filtering, 83,689 SNPs remained. We have identified markers located in BTA6 and in BTA26 which displayed significant association with carcass weight gain. These overlapped *LCORL* and *RNLS* genes, respectively.

Discussion: The identified markers also overlap previously described QTLs for body height and carcass weight, which suggests that these novel variants are relevant for the future improvement of this trait in Preta cattle breed.

Support disclosure: Financial support by CIISA (UIDB/00276/2020) and AL4AnimalS (LA/P/0059/2020)

Keywords: GWAS; LOCAL BREEDS; SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS); CARCASS WEIGHT

Feasibility of the Welfare Quality protocol in a Mertolengo cattle farm in Évora

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Introduction: The importance of animal welfare has had an exponential growth, not only due to consumer demand, but also by increasing animal production profitability and sustainability. Animal welfare certification depends largely from the application of assessment protocols, one of them being the Welfare Quality protocol (WQ).

The WQ Protocol is based on four Principles – Good Nutrition, Good Housing; Good Health and Proper Behaviour – which are fulfilled by the assessment of indicators associated to 12 Criteria.

Materials and Methods: This study aims to test the feasibility of this protocol in the conditions of many Portuguese fattening farms. The study farm is located near Évora and keeps Mertolenga cattle. The welfare indicators were assessed and the constrains were registered. A critical analysis was also applied to the worst scored indicators. The negative points and the potential for improvement were interpreted.

Results: The farm was classified as Good. However, there were two Principles scored only as Sufficient - Good Feeding and Proper Behaviour. The Criteria classified as Sufficient were: Absence of prolonged thirst, Absence of diseases and Expression of other behaviours.

Discussion: Some of these more negatively scored indicators can be easily corrected, such as the prolonged thirst criterion which can be solved by adding more troughs or ensure water cleanliness. However, one of the lowest ranked criteria was Human-Animal Relationship which is more difficult to change as it implies keepers' education and ensuring more positive interactions with humans. In conclusion, feasibility was demonstrated and the protocol proved to be useful in showing the points to be improved.

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Keywords: BOVINE; BEEF; ANIMAL WELFARE; PROTOCOL

Antibiotic-resistant coagulase-negative staphylococci in the pork-meat chain

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Introduction: Coagulase-negative staphylococci (CNS) can be a potential reservoir of antibiotic-resistance genes in the food chain. This work aimed to identify and characterize the antimicrobial profile of CNS isolated in a pork-meat chain.

Material & Methods: A Portuguese pork-meat chain was sampled on two different working days. Swine's anus and ears (n=20) were sampled, as the slaughter and room deboning workers' hands, the environment surfaces, and the cut pieces that originated on the swine sampled. In addition, sliced pork from the shoulder was distributed to consumer families (n=20), with the meat and consumer hands being sampled (before and after washing and after meat preparation).

Results: A total of 414 CNS isolates were clustered according to their fingerprinting profile and identified by PCR. The antibiotic sensibility was tested (n=140) using the disc diffusion method (EUCAST). The majority of CNS in this study were recovered from swine and families. With the most common species being *S. carnosus* (27%), *S. equorum* (25%) and *S. saprophyticus* (19%). Antibiotic resistance was found in 91% of isolates, mainly *S. equorum* (presenting 68% of multiresistance). It was possible to detect a higher number of isolates resistant to clindamycin associated mainly with swine and slaughter workers but not with families.

Discussion: The CNS isolates from human sources had a higher presence of resistance to gentamicin mainly *S. saprophyticus* and *S. carnosus*. The antibiotic resistance profiles of the CNS isolates from swine were not like those found in the families. However, CNS are still a reservoir of resistance.

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Keywords: COAGULASE-NEGATIVE STAPHYLOCOCCI; ANTIBIOTIC RESISTANCE; PORK MEAT-CHAIN

Effects of enzymatic and non-enzymatic pre-treatments on Arthrospira platensis proteins

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Background: *Arthrospira platensis* has emerged as a novel protein source, but its thick recalcitrant cell wall makes this microalga difficult to digest especially by monogastric animals. Therefore, it is important to disrupt the cell wall using pre-treatments and improve protein accessibility. The objective of this work was to evaluate the effect of bead milling, extrusion, freeze-drying, heating, microwave and sonication in combination with trypsin on *A. platensis* protein solubility.

Methods: After *in vitro* incubation and collection of supernatant and pellet, Bradford method (ProtBrad) and 14% SDS-PAGE electrophoresis gel (ProtGel) were applied to quantify protein content and solubility. The amount and proportion of the two most prominent protein fractions in the gel (F1, 18.5-26 kDa, and F2, 40-48 kDa) was also assessed. Peptide formation was evaluated by *o*-Phthaldialdehyde (OPA) assay.

Results: Combination of pre-treatments and trypsin showed a significant decrease ($P<0.001$) in ProtBrad in supernatant fraction, exception for sonication, and in ProtGel with extrusion and microwave. Concerning the protein quantification in the gel, only extrusion and microwave caused a significant decrease ($P<0.001$). None of pre-treatments showed significant differences in peptide formation. In pellet fraction, ProtBrad increased ($P<0.001$) with sonication but decreased with extrusion, and ProtGel decreased ($P<0.001$) with heating, extrusion and sonication. Fraction 2 significantly decreased ($P<0.001$) with heating, extrusion and microwave but fraction 1 and other proteins ($P<0.001$) just decreased with extrusion and sonication, respectively, comparing with control.

Discussion: Results indicate the presence of strong protein aggregation caused by extrusion conditions, possibly due to a profound change on protein structure.

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Keywords: *A. platensis;* EXTRUSION; PRE-TREATMENTS; PROTEIN; SOLUBILITY.

Green nitrite options for cured meat sausage: Thymus citriodorus and Salvia elegans

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Introduction: Consumers are now demanding for meat products without synthetic nitrate and nitrite. However, it is necessary to ensure their desirable characteristics. This work aims to evaluate *Thymus citriodorus* and *Salvia elegans* as natural replacers of nitrate in cured meat sausage (CMS), combined with the addition of a *S. equorum* starter.

Material & Methods: Three batches of six CMS formulations were produced: C1- Control without nitrate nor starter; C2- Control with starter without nitrate; F1- 150 mg KNO3/kg; F2- 150 mg KNO3/kg with starter; F3- Sage10.6% infusion; F4- Sage10.6% infusion with starter; F5- Thyme10.6% infusion; F6- Thyme10.6% infusion with the starter. Analysis was performed on days 0 and 60 (final product and end of shelf-life). Microbial analysis was performed according to ISO Standards: Lactic Acid Bacteria (LAB), Coagulase Negative Staphylococci (CNS), and *Enterobacteriaceae*. Water activity ($A_{w)}$ *and pH were evaluated.* The colour using L*a*b* colour space was measured with a Konica Minolta CR-400/410 (Konica Minolta, Japan) illuminant D65.

Results: *CNS* counts in the product conditions with the starter were approximately 7 log cfu/g for both days 0 and 60. *Enterobacteriaceae* counts were inferior to 4 log cfu/g, indicating that the product was satisfactory. Regarding CMS color, sausages F2 (a*=13.16) and F6 (a*=13.19), inoculated with *S. equorum* presented significantly higher a* values ($p<0.05$). The effects described were maintained until the end of storage.

Discussion: CMS formulated with thyme and inoculated with *S. equorum* seems to be a good strategy for the replacement of nitrate, since it presented a reddish colour similar to products with synthetic nitrate.

Support/interest disclosure
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Keywords: MEAT PRODUCTS; ALTERNATIVES; CLEAN LABEL; PLANT EXTRACTS; STARTER.

Are rumen mucosa histological traits related with susceptibility to rumen acidosis and altered biohydrogenation in lambs?

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Ruminants host a complex anaerobic microbial ecosystem in the rumen that digests fiber efficiently but also isomerizes and hydrogenates extensively the dietary unsaturated fatty acids through biohydrogenation metabolic pathways (RBH). Generally, RBH yields t11-18:1 as the main intermediate, but when ruminants are fed high-starch diets tend to develop sub-acute ruminal acidosis (SARA) and present altered RBH (i.e. t10-shift) where the t10-18:1 isomer becomes the main intermediate with potentially deleterious effects on meat nutritional value. Lambs vary widely regarding to susceptibility to express the t10-shift but that individual variability remains unexplained. The project Gene2rumen proposes to conduct an intensively finishing lamb sampling where the rumen phenotypes will be used in a GWAS study and here we present the first preliminary results regarding the histological traits of rumen mucosa.

Lamb´s ventral sac rumen mucosa samples have been collected at slaughterhouse, fixed by immersion in 10% buffered formalin, embedded in paraffin, sectioned with an automatic microtome at a thickness of 5 mm, and stained with hematoxylin-eosin and visualized under a light microscope. Five papillae were randomly chosen per slide and four images were captured per papillae for a total of three measurements per papillae and a total of 24 measurements per animal and measurements were by ImageJ software. The preliminary data (30 animals) indicates that the papillae length, width and thickness averaged 6.5 mm, 1.16 mm and 3.6 mm, respectively. The stratum corneum thickness averaged 15 μm, whereas the non-keratinized epithelium and connective tissue width was 36 μm and 44 μm, respectively.

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Keywords: RUMEN HISTOLOGY, HIGH-CONCENTRATION DIET, SARA, RUMEN BIOHYDROGENATION

Risk factors and consequences of bimodal milk flow and overmilking in dairy cows

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Introduction: Bimodal milk flow and overmilking affect the efficiency of the milk removal process and teat health. This observational study aimed to investigate the prevalence of bimodal milk flow and overmilking, determine individual and farm variables associated with these occurrences, and determine the association of overmilking and bimodal milk flow with milk yield and teat changes.

Materials & methods: Twenty-one farms were visited during the study, wherein the milking routine was timed, the teat condition was assessed, and dynamic evaluation of milking vacuum was performed.

Results: A total of 606 vacuum graphic records were obtained. There was an average of 41.7% bimodality per farm. Median overmilking time was 59 seconds. An association was found at cow level between occurrence of bimodal milk flow and days in milk, total stimulation time, parity, and preparation lag time. Increased stimulation time and number of passes during preparation were associated with decrease in herd bimodality. Parity, reattaching the milking unit and milking in manual mode were associated with increased overmilking time of individual cow. The presence of a clogged air bleed hole and the reduction of cluster removal milk-flow threshold were associated with an increase in the herd's median overmilking time. An association was found between occurrence of bimodal milk-flow and decreased milk yield. A mean of 78.4% of cows per farm had short-term teat changes and 33.6% of cows displayed at least one teat with hyperkeratosis.

Discussion: These results reinforce the importance of the milkers' actions and the functioning of the milking parlour for prevention of bimodality and overmilking.

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Keywords: BIMODAL MILK FLOW; OVERMILKING; MILKING EFFICIENCY; TEAT HEALTH

Milk flavouring incorporation in beef calf feed in an intensive fattening system

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Introduction: Calves weaning time is a period of diet change and adaptation, with adverse effects on both production and wellbeing, mainly due to stress. Milk flavouring added to concentrate feed can lead to stress reduction, as this aroma resembles the smell of breast milk. This study's main goal was to analyse milk feed additive effect on beef calves during their first 45 days post-weaning, recording feed conversion ratio (FCR), live weight gain, daily consumption (DC), and faecal cortisol (FC) levels.

Material & methods: 120 calves (6 months old) were randomly divided into two groups; Control group (CG) fed normal concentrate and a group fed milk flavoured feed (MFF). Each experimental group was distributed in 4 different parks, with 15 animals respectively, in a final total of 8 parks. During the experimental period, clinical signs, consumption levels and life weights were recorded. FC concentrations were also determined to compare stress levels.

Results: Results showed that feed consumption was higher in the CG, except for the first 10 days, in which the MFF group presented better results. FCR and DG were superior in CG, with higher respiratory disease incidence, contrary to the study group, which showed more digestive signs. FC concentration was lower in CG, presenting a lower stress level.

Discussion: This study with milk flavoured feed is pioneer in Portugal, and although zootechnical indexes were higher in the CG at the end of the experimental period, the first 10 days were crucial for weaned calves, which presented a higher feed consumption. However, the hypothesis that milk flavoured feed would enhance zootechnical indexes and reduce stress was contradicted in this work.

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Keywords: CALVES; WEANING; MILK FLAVOURING; CORTISOL LEVELS; ZOOTECHNICAL INDEXES

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ProteOMICs in Azeitão and Nisa PDO cheeses

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Introduction: Protected Designation of Origin -PDO- cheeses are manufactured throughout Portugal. Specific organoleptic features of each product being associated with artisanal practices, particular geographical areas, raw materials and autochthonous microbiota. Among the variety of Portuguese PDO-cheeses, Azeitão and Nisa were selected for the present study. Both are produced using sheep's raw milk, salt and *Cynara cardunculus*, but harbour rather distinct characteristics. Azeitão is cured with a semi-soft texture, while Nisa it is also cured, but presents a semi-hard paste. Material & Methods: The present research focused on the proteomic analysis of the aforementioned cheeses, aiming to identify differences between proteome profiles, regarding region and/or producer, namely eco-specific biomarkers.

Results: Label-free proteomic analysis, and comparison with public available databases (National Center for Biotechnology Information and Uniprot), revealed the presence of 296 *Ovis aries* proteins, and global analysis showed the presence of several *Ovis aries* proteins in both regions under study. Subsequently, the results were further explored using region-derived heatmaps, which showed no differences between Azeitão PDO producers, but identified differential proteins amongst the two Nisa cheesemaking factories. These results were further confirmed by Principal Component Analysis.

Discussion: Overall, although both similarities and differences could be observed between the PDO cheeses under investigation, further insights may be revealed by exploring the microbial proteins associated with each sample. Considering the importance of the fermentative autochthonous microbiota for the development of specific organoleptic features, it is foreseen that microbial proteome profiles may allow the additional identification of differential molecules.

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Keywords: PROTEOMICS, TRADITIONAL CHEESE, ECO-SPECIFIC BIOMARKER.

Silages of agro-industrial by-products in lamb diets – Effect on carcass, meat quality and methane emissions

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Introduction: The use of agro-industrial by-products in animal feed is an opportunity to reduce imports, food waste and promote a clean and circular economy, turning worthless raw materials into high-quality, low-cost animal feeds that does not compete with human food. This study aimed to produce silages with by-products of carrot, sweet potato, potato, and tomato and integrate them in lamb diets, replacing 50% of concentrate. The effects on growth performance, meat quality and methane production were evaluated.

Material and Methods: Three silages were prepared using 35% tomato pomace, 20% wheat bran, 15% of lucerne hay and 30% potato (Psil) or 30% sweet potato (SPsil) or 30% carrot (Csil). Thirty-two lambs were individually housed and divided into four groups (8 animals/group) in the following diet: Control - 85% concentrate and 15% hay; P - 50% concentrate and 50% Psil in DM; SP - 50% concentrate and 50% SPsil in DM; C - 50% concentrate and 50% Csil in DM. The trial lasted 6 weeks after 7 days of adaptation. Methane production was evaluated *in vitro* by the Ankom system, using as inoculum rumen content samples collected individually from 6 lambs on each diet.

Results & Discussion: The diet had no effect on growth performance, carcass quality traits, and meat pH, IMF, colour parameters (L*, a*, b*), shear force and sensorial attributes. The diets had no impact on total methane. By-product silages can be a good option to replace concentrated feed in lamb diets, allowing a reduction of 0.29€ in feed cost per kg of live weight.

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Keywords: BY-PRODUCT SILAGES; LAMB DIETS; GROWTH; MEAT; METHANE

Multi-OMIC insights towards the characterization of Portuguese traditional PDO cheeses

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Introduction: Cheese is a worldwide-appreciated delicacy, with special emphasis on raw milk traditional cheeses harboring the Protected Designation of Origin (PDO) label. These foods are associated with unique organoleptic features, commonly attributed to the autochthonous fermentative microbiota present in the raw materials, or introduced during manufacturing. The complex dynamics and interactions between microbes and growth substrates (milk proteins and fatty acids) highly influence the quality and safety of the final product. Thus, cheese properties are expected to diverge between type of cheese, region and producers, due to differences in the manufacturing technologies, slight variations in the chemical/microbial composition of the milk and microbial populations from the cheese-making environment.

Material and Methods: Hence, for a comprehensive characterization of PDO-cheeses, our interdisciplinary research team applied a multi-OMIC approach, comprising culturOMICs, metagenOMICs, proteOMICs, LipidOMIcs and volatilOMICs. Briefly, samples from Azeitão and Nisa were collected between 2016-22 (including all producers and replicate samples) and further characterized by multi-OMICs.

Results: CulturOMICs and metagenOMICs confirmed the lactic acid bacteria group as the most abundant, *Lactococcus* genus being the predominant, whilst no putative foodborne pathogens were detected. Slight variations were observed between origin (region and manufacturer) and production year. The uniqueness of each sample was further confirmed by proteOMICs, LipidOMIcs and volatilOMICs. For each OMIC technique the selection of eco-specific biomarkers, associated with origin and/or production year, is ongoing.

Discussion: Overall, the integration of distinct OMIC technologies offered in-depth insights on the complex microbial ecology of cheese fermentation, providing wide-range information on cheese flavor development, overall safety and quality.

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Keywords: TRADITIONAL CHEESE; OMIC TECNOLOGIES; BIOMARKERS; FOOD SAFETY; FOOD QUALITY

Meat and fish consumption in EU and Portugal between 2000-2020: What are we bringing to our tables?

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Introduction: In the early 2000s a study was conducted on the analysis of EU consumers` changes in meat consumption habits throughout the1990s. Now, 20 years later, and after various socio-economic circumstances, the growth of alternative food movements, the negative beef image among some consumers, and the growing concern, sometimes misdirected, with animal welfare and environmental sustainability, it became relevant to analyse the 2010 and compare it with the results previously obtained, to understand the impact of these socioeconomic changes on meat and fish consumption habits in EU and in Portugal.

Materials & methods: Consumption data for EU and Portuguese consumers was collected from FAO database and analysed by calculating percentage changes and average annual growth rates.

Results: The analysis of the results shows a drop in meat consumption between 2010 and 2012, years highly demanding in financial terms due to the 2008 global crisis. It is also possible to see that the consumption of cheaper meats, namely poultry, was favoured over the consumption of more expensive meats, such as beef. In the case of fish, it decreased between 2010-2013, though for the period 2010-2019 it increased by 0,8 %. Between 2000 and 2020, fish consumption in the EU has increased, poultry and pig meat considerably increased, and beef meat consumption decreased. Notice that a different trend took place in Portugal, though with lower changes, as beef consumption increased (0,05 %) and pig meat decreased (-0,03 %).

Discussion The trend we witness is in accordance with previous studies. Poultry and pig meat are becoming the major meats consumed in the EU and in Portugal, with steady increases. We confirm that beef is undergoing a different trend. Economic factors justify these trends but non-economic factors such as demand for more sustainable products and healthy diets also have a word.

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Keywords: MEAT; FISH; CONSUMPTION; CONSUMER TRENDS

Correlations of age or live weight of growing rabbits with the development of internal organs and parts of the carcass

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Introduction: In growing rabbits, the development of different organs and body regions follows a pattern that is very dependent on biological factors, age and live weight. In this work, we studied correlations between age or live weight at slaughter with the weight and length of digestive organs and weight of carcass parts.

Materials & methods: Six male rabbits were slaughtered at 63, 70, 77 and 99 days of age (live weight between 1892 and 4445g). The small intestine, cecum and colon were measured and weighed and the stomach was weighed (organs with contents). After refrigeration, the kidneys, liver and the set of lungs, thymus and heart were also weighed. The reference carcass was prepared and divided into anterior, middle and posterior parts. Correlations between live weight or age and each of the above referred parameters were determined.

Results: All correlations were significant ($P<0.05$) with the exception of those between small intestine length and dressing out percentage with weight and age, which also had lower $R2$ values. The length of the tubular digestive viscera showed higher correlation values with age with a maximum value of $R2=0.752$ between age and colon length. The weight of digestive organs was better estimated by the live weight ($R2=0.851$ for the weight of the digestive tract and $R2=0.840$ for liver weight). Regarding carcass characteristics, the best correlation was achieved for live weight and chilled carcass weight ($R2=0.988$) and weight of the anterior part ($R2=0.984$).

Discussion: It is important in the future to incorporate more animals into the model, particularly females and of intermediate slaughter ages.

Support: The participation of Victor Pinheiro and Divanildo Monteiro was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT); Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS) LA/P/0059/2020 (funded by FCT) and UTAD FOOD ALLIANZ: Infraestrutura de Investigação em Nutrição e Alimentação. A ligação com a Ciência Animal. - (NORTE-01-0145-FEDER-072687).

Keywords: *RABBITS*, INTERNAL ORGANS, CORRELATION, AGE, LIVE WEIGHT

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POSTERS

SESSION 3
ANIMAL HEALTH

ESBL-positive Enterobacteriaceae isolated from faecal samples of dogs from Santiago and Boa Vista Islands, Cape Verde

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Introduction: Antimicrobial resistance is a major Public Health threat that is spreading fast all over the world. In low- and middle-income countries, like Cape Verde, bio-surveillance programs are lacking, particularly in the veterinary setting, contributing for resistance development. Additionally, the country suffers from poor sanitation facilities and inefficient waste treatment, facilitating the dissemination of resistant microorganisms among humans, animals, and the environment. This study aimed to analyse the presence of bacteria producing Extended Spectrum β-Lactamases (ESBL) among faecal samples of dogs from Santiago and Boa Vista Islands, and to assess resistance profiles of the isolates obtained and their relevance for Public Health.

Material & Methods: After collection of 100 faecal samples using a sterile swab (VWR™) and transportation at 4ºC to the Laboratory of Bacteriology, Faculty of Veterinary Medicine, University of Lisbon, screening and confirmation of ESBL-positive bacteria were performed using ChromID®ESBL agar (bioMérieux) and Modified Double-Disk Sinergy Test, respectively, after which ESBL-positive isolates were subjected to antimicrobial susceptibility testing towards 11 antibiotics according to CLSI guidelines.

Results: It was possible to collect ESBL-producer isolates from 29 faecal samples, in a total of 48 isolates. *Escherichia coli* was the most prevalent bacterial species isolated (n = 29; 60.42%) and half of the isolates (n = 24; 50%) were classified as multidrug resistant, being frequently not susceptible to β-lactams, fluoroquinolones, and tetracyclines.
Discussion: These findings suggest that dogs in Cape Verde may act as reservoirs of antimicrobial resistant bacteria, representing a Public Health concern especially considering the overpopulation of stray dogs in the country.

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Keywords: DOGS, CAPE VERDE, ANTIMICROBIAL RESISTANCE, ESBL, PUBLIC HEALTH

Development of Immunoliposomes with Dual-Targeting Against Canine B-Cell Lymphoma

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Introduction: Non-Hodgkin lymphoma (NHL) is a common cause of cancer related deaths worldwide. Even though treatment options have improved, refractory/relapsed disease remains a clinical challenge, urging the development of novel treatment strategies. Canine lymphoma (CL) shares many similarities with human NHL, making dogs with spontaneous lymphoma promising preclinical models for new therapies. In this context, we assessed liposomes dual-targeted with folate and a single domain antibody (sdAb) C5 highly specific for CL, as a way to overcome the tumour resistance mechanisms and deliver a potent cytotoxic drug (panobinostat) for a targeted treatment of CL.

Materials & Methods: Immunoliposomes were developed by conjugation of the sdAb C5 to the liposome surface, followed by the evaluation of all the liposomes (PEG, PEG-FA, PEG-C5, PEG-FA-C5) targeting properties through flow cytometry and fluorescence microscopy. Subsequently, the cytotoxic activity of the liposome formulations and their ability to induce histones H3 acetylation were evaluated *in vitro*.

Results: Results showed that targeted liposomes have a higher internalization compared with non-targeted liposomes, both *in vitro* and *in vivo.* Despite dual targeting not presenting a higher internalization compared with single sdAb targeted liposomes in the *in vitro* assays, preliminary results show that IC$_{50}$ for dual-targeting panobinostat liposomes was significatively lower than for the Lip-PEG-C5 formulation. Moreover, all liposome formulations increased H3 histone acetylation.

Discussion: This study demonstrated the targeting and activity of dual-targeted Panobinostat liposomes against CL, confirming this strategy's promise.

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Keywords: CANINE LYMPHOMA; NON-HODGKIN LYMPHOMA; IMMUNOLIPOSOMES; FOLATE; PANOBINOSTAT

Canine Parvovirus 2: subtyping matters?

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Introduction: Canine parvovirus (CPV2) genetic diversity within the *vp2* gene allows the identification of three viral variants, CPV2a, CPV2b, CPV2c with specific antigenic profiles. Due to the availability of commercial vaccines with different CPV2 variants, veterinarians commonly request CPV2 subtyping to identify the subtype associated with clinical disease.
Routine molecular diagnosis of CPV2 at the National Reference Laboratory is performed by quantitative real time PCR (qPCR) (developed by Decaro et al., 2005) which allows the detection of Canine and Feline parvoviruses, followed by molecular subtyping of the *vp2* gene. This allows unravelling the virus subtype.

Materials and methods: In this study we explored the qPCR developed by Decaro et al (2006), using specific TaqMan⟶ probes for each CPV2 variant. This qPCR allows not only the detection of the subtype/subtypes present in the biological sample, but also its quantification. A set of stool samples (n=22), collected from dogs (n=17) and wolves (n=5) between 2018 and 2022 (2018 n=4; 2019 n=12; 2020 n=3; 2022 n=3), was processed. Viral nucleic acids were extracted from biological samples and a molecular survey was conducted.

Results: We detected co-infection by the three subtypes in 19 samples (CPV2a, b, c; 86.4%) and two subtypes in 3 samples, (CPV2a, b; 13.6%). Also, viral loads revealed the predominant subtype.

Discussion: Non-synonymous mutations may imply different pathogenicity patterns in CPV2 infections.
The insight within CPV2 genetic diversity using a qPCR approach allowed us to rapidly identify the predominant subtype in co-infections within the same animal but also to re-evaluate the genetic and clinical importance of subtyping.

Support/interest disclosure: This study was supported by Centre for Interdisciplinary Research in Animal Health - CIISA (Project UIDB/00276/2020, funded by FCT) and by the Associate Laboratory for Animal and Veterinary Science (LA/P/0059/2020 - AL4AnimalS) and by the National Institute of Agrarian and Veterinarian Research, Public Institute.

Keywords: CPV2; GENETIC SUBTYPING; qPCR

Effects of Biochar supplementation on the microbiota of Recycled Manure Solids

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Introduction: The use of Recycled Manure Solids (RMS) as cow bedding has been increasing globally as an environmental-friendly strategy for manure reuse. However, RMS may disseminate pathogenic and resistant bacteria to farm staff, animals, and later to soil and water, representing a potential health hazard. Biochar, an inorganic char coproduct obtained from thermochemical processing of biomass, is considered a promising RMS amendment to eliminate relevant bacterial species.

Material & Methods: To evaluate the effect of biochar addition on the elimination of Enterobacteriaceae and Enterococcaceae in RMS, an incubation experiment was designed using 5 groups: negative (RMS only) and positive (RMS+H_2SO_4) controls; RMS+2.5%Biochar; RMS+5%Biochar; RMS+10%Biochar. Thirty-days incubation experiments were performed in two periods, April-May and June-July. Samples were collected on days 0, 5, 15, 30, and inoculated on MacConkey and Slanetz-Bartley agar for Enterobacteriaceae and Enterococcaceae quantification, respectively.

Results: During the first season, Enterobacteriaceae quantification increased over time in all groups. The group supplemented with 5%biochar presented the best results, showing Enterobacteriaceae quantification bellow the negative control throughout the experiment, revealing a 41% decrease on day 30. Regarding Enterococcaceae elimination, biochar supplementation was more effective, since by day 30 growth was only observed in the negative control plates. Second season results followed the same pattern regarding Enterococcaceae. For Enterobacteriaceae, RMS treatment with 10%biochar promoted a 84% decrease in bacterial quantification overtime, being the most promising concentration for controlling these bacteria during this season.

Discussion: Results support our hypothesis of the beneficial effect of biochar supplementation on RMS microbiota, prompting further studies focusing on biochar antimicrobial potential.

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Keywords: RECYCLED MANURE SOLIDS (RMS), BIOCHAR, ENTEROBACTERIACEAE, ENTEROCOCCACEAE, ANTIMICROBIAL RESISTANCE.

Evaluation of the repellent action of thyme (Thymus vulgaris) and eucalyptus (Eucalyptus globulus) essential oils in wild rabbit's parasitic ixodids

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Introduction: The Rabbit Haemorrhagic Disease Virus (RHDV) is the main responsible for the huge decline of the European rabbit, *Oryctolagus cuniculus* population, on the Iberian Peninsula. Ixodid ticks are vectors of this virus and therefore there is an urgent need to create sustainable forms of control. The aim of the present pilot study was to evaluate the repellent action of the *Eucalyptus globulus* and *Thymus vulgaris* essential oils, both at 2.5% and 5%, used *in vitro* bioassays against nymphs and adults *Hyalomma marginatum*, *Dermacentor marginatus* and *Rhipicephalus sanguineus*.

Material & Methods: Tick specimens were captured in field using flag method and when necessary, reared under laboratory conditions. This study comprised 6 trials with 14 *D. marginatus*, 12 *R. sanguineus* and 54 *H. marginatum*. Aiming to test the capacity of ticks to engorge, an acrylic box with a Hemotek™ system, filed with rabbit blood and rabbit hairs and CO2 as stimuli. Essentials oils were placed at a filter paper disk around the blood reservoir.

Results: The data obtained showed that none of the essential oils were able to completely repel the ixodids as the arthropods were able to cross the filter paper disks containing essential oils. Nonetheless, it seems that some repellent activity was observed, being slightly more evident with the *T. vulgaris* essential oil. The essential oils tested also showed some acaricidal activity.

Discussion: Although some evidence of repellency was observed, further studies are needed to find sustainable alternatives to repel arthropods and be suitable to rabbits in the wild.

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Keywords: IXODIDS; ESSENTIAL OIL; THYME, EUCALYPTUS; EUROPEAN RABBIT

Staphylococci antimicrobial resistance patterns from companion animals with skin and soft tissue infections in portugal 2020

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Background: The spread of antimicrobial resistance has become a challenge to veterinary practice, due to the limited options of therapeutic.
Staphylococci are a commensal organism of the animals' skin, but also an opportunistic pathogen responsible for the majority of bacterial skin and soft tissue infections (SSTIs)**.**
Moreover, the increasing reports of Methicillin-resistant *Staphylococci* strains (MRS) isolated from companion animals, with MRSP and MRSA being the most detected within the Coagulase-positive *Staphylococci* group (CoPS).

The aim of this retrospective study (Jan-Dec 2020) was to investigate the prevalence of MRS isolated from SSTIs in dogs and cats, in Portugal and evaluate the efficacy of the phenotypic methods for MRS detection.

Materials & Methods: Samples submitted for culture were analyzed according to microbiological standards. All samples were plated on Brilliance™ MRSA 2 agar. Susceptibility testing by disc diffusion was carried out for all positive samples and results were interpreted according to EUCAST and CLSI guidelines.
MecA gene presence and Staphylococci species were confirmed by PCR.

Results: A total of 275 Staphylococci were isolated from 288 SSTIs samples.
Eighty-eight MRS were isolated, of which, 86,3% (n=76/88) harboured the *mecA* gene. MRSP was the most prevalent specie within CoPS group, detected in 34,2% (n=26/76) of isolates. A total of 11 MRSA were detected in dogs and cats [5.3% (4/76) and 9.2% (7/76), respectively]. The MRS false-positives corresponded to 10% (9/88).

Discussion/Conclusions: The rising number of MRS is a concern and emphasizes the need to control the spread of antibiotic resistance.

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Keywords: MRS; MRSP; MRSA*; mecA*.

Molecular characterisation of Leishmania spp. in Cats from Portugal

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Leishmaniosis is a widespread vector-borne disease caused by protozoa belonging to the *Leishmania* genus. The hypothetical involvement of cats in the maintenance and dissemination of human and animal leishmaniosis has become increasingly evident. However, the genetic diversity of *Leishmania* spp. detected in cats has been poorly explored. This study aimed to assess the genetic diversity and phylogenetic relationships of *Leishmania* parasites identified in cats from Portugal by the analysis of multiple genetic markers (fragments of *cytB*, g6pdh, hsp70, and ITS-rDNA genes). A total of 25 cat buffy coat samples where the presence of *Leishmania* SSU-rDNA was revealed by PCR were included in this study. Phylogenetic analyses showed that most of *Leishmania* parasites molecularly detected in cats did not display singular genetic features, sharing the same genetic type with *L. infantum* strains isolated from dogs, humans and phlebotomine sand flies. Unexpectedly, DNA of *L. major* was identified in a cat from the Lisbon region and DNA of *L. major*/*L. donovani* sensu lato hybrid in a cat from the Algarve region, representing the first evidence for the presence of *Leishmania* hybrid parasites in these felids. The results reported here not only reinforce the idea that cats can play a role in the epidemiology of zoonotic leishmaniosis but also indicate the circulation of *L. major* and *L. major*/*L. donovani* s.l. hybrid parasites in Southern Europe. Combined analysis of multiple genetic markers seems to be essential for *Leishmania* typing, whenever approaches based on complete genome sequencing cannot be carried out.

Support: Fundação para a Ciência e a Tecnologia, I.P. (SFRH/BD/116516/2016; GHTM-UID/Multi/04413/2020)

Keywords: CATS; *LEISHMANIA*; PORTUGAL; MOLECULAR CHARACTERISATION

Isolation and virulence characterization of Enterococcus from surfaces of a Biological Isolation and Containment Unit from a Veterinary Teaching Hospital

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Introduction: One of the most common bacteria associated with hospital-acquired infections (HAIs) are enterococci, due to their intrinsic and extrinsic resistance to a variety of antimicrobials, biofilm-forming capacity and genomic flexibility. This means it is important to control the presence of these bacteria in different hospital surfaces and characterize them in terms of virulence.

Material & Methods: Environmental samples were collected from different surfaces of the BICU (n=200), inoculated in Slanetz-Bartley medium and colonies with macroscopic characteristics compatible with enterococci were presumptively identified through Gram staining and catalase and esculin reaction. Isolates were evaluated phenotypically for the production of virulence factors using Columbia agar + 5% sheep blood (cytolysin), gelatin agar (gelatinase), DNase agar (DNAse), skim milk agar (proteinase) and tryptic soy agar supplemented with egg yolk (lecithinase). Biofilm production was evaluated in Red Congo agar.

Results: From all samples collected, 8% were positive for enterococci (n=16/200). Infusion pumps were the area with the highest number of positive samples (21%), followed by cages (11%). In Columbia blood agar, the majority of isolates (n=39/73) presented β-haemolysis and 29 presented α-haemolysis. All isolates were DNase and lecithinase negative, 18% were proteinase positive and 3% were gelatinase producers. In Red Congo agar, 96% of all isolates were biofilm producers.

Discussion: The strict disinfection methods used in the BICU are a simple explanation for the low number of positive samples. Concerning virulence factors, the abundance of haemolysin and biofilm producers raises concerns not just about potential HAIs but also about environment permanence (biofilm production).

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Keywords: *ENTEROCOCCUS*; HOSPITAL ACQUIRED INFECTIONS; VIRULENCE; BIOLOGICAL ISOLATION AND CONTAINMENT UNIT

Case study: management of the infection by the nematode Foleyella sp. in a Panther Chameleon (Furcifer pardalis)

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Introduction: Exotic pet reptiles are often carriers of underlying parasitic diseases. Parasites are a major component of biodiversity, but remain largely uncharacterized in wildlife. Adults of the superfamily Filarioidea can live in blood vessels, body cavities, connective tissues and subcutaneously, in reptilian definitive hosts. Filarial parasites affecting chameleon species belong to the genus *Foleyella*, namely *Foleyella furcata* and *F. candezei*, which are commonly found in subcutaneous tissues, and *F. brevicauda* which is normally found in muscles. The current case study aimed to optimize a treatment procedure for a Panther Chameleon specimen (*Furcifer pardalis*) infected with the nematode *Foleyella* sp.

Material & Methods: Initial procedures compraised routine fecal smears, total CBC and biochemistry. Blood smears were screened initially at 400x total magnification to search for extracellular haemoparasites, such as microfilariae, and at 1000x for intracellular parasites. The treatment protocol included calcium, injected vitamins, Fenbendazole and Sylimarin, following the guidelines of Carpenter Exotic Formulary. Blood smears were performed at days 1, 7, 30, 60 and 90, as well as at 6 and 12 months, after treatment.

Results: An improvement regarding clinical data such as appetite and body weight was recorded. From day 1 to 60, improvements on calcium, AST levels and red blood cell count were observed.

Discussion: These results confirm that chameleons are frequently exposed to parasitism and suggest that parasitological tests should be performed when owners purchase reptiles from foreign countries. Imported chameleons may be reservoirs of *Foleyella* species and the risk to human health should be considered. Also, Fenbendazole is effective and supportive care provides improvement of the animal's general condition.

Support: CIISA/FMV Project UIDB/00276/2020 and LA/P/0059/2020 – AL4AnimalS (funded by FCT); João Lozano owns a PhD research fellowship 2020.09037.BD (funded by FCT).

Keywords: CHAMELEONS; *FURCIFER PARDALIS;* PARASITOLOGY; HEMATOLOGY; *FOLEYELLA* SP

Prevalence of zoonotic gastointestinal helminths in dogs and cats in the Lisbon Metropolitan

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Introduction: Pets may carry zoonotic parasites that can be transmitted to humans through direct contact with infected animals or exposure to contaminated soil, food and water. The study aimed to assess the prevalence of zoonotic gastrointestinal (GI) parasites in dogs and cats in the Lisbon Metropolitan area (LMA).

Materials and Methods: A total of 146 fecal samples were collected from homed (n=38), sheltered (n=88) and sheep farm dogs (n=20) and from homed (n=8) and sheltered (n=35) cats in the LMA and examined for helminth eggs using the Mini-Flotac® device. Samples from farms were additionally screened for *Echinococcus granulosus* eggs by PCR targeting the small subunit of the ribosomal RNA (Trachsel et al. 2007).

Results: The overall prevalence of endoparasites in canine and feline samples was 23.3% and 30.2%, respectively. The prevalence of helminths was highest in farm dogs (70%), followed by owned (10.5%%) and sheltered (6.5%) dogs. 15.5% of dogs were positive for Ancylostomatidae and 14.6% for *Toxocara canis*. 18.6% of cats were positive for Ancylostomatidae and 7% for *Toxocara cati*. None of the farm samples was positive for *E. granulosus*. Egg burdens of Ancylostomatidae and *Toxocara* ranged between 0-4005 and 0-5225 eggs per gram, respectively.

Conclusion: The study showed a significant prevalence of zoonotic parasites in different dog and cat populations in the LMA. The high prevalence in farm dogs, translates into an increased contamination of the farm's environment with parasite eggs. People living on farms may therefore constitute a risk group for human toxocariasis and zoonotic ancylostomiasis.

Seroprevalence of Fasciola hepatica in Cattle in the Madeira Island, Portugal

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Introduction: In temperate climates, bovine fasciolosis is caused by *Fasciola hepatica*, a digenetic trematode found in the liver parenchyma and bile ducts. Economic losses due to fasciolosis are associated to reduced meat and milk production and condemnation of livers. Though it is found worldwide, little is known about the epidemiology of this parasite in the autonomous region of Madeira, Portugal. The study aimed to estimate the prevalence and distribution of *F. hepatica* in the Madeira Island.

Material & Methods: A total of 342 sera from cattle in 40 farms distributed in 8 municipalities were randomly selected and tested by a commercial ELISA test (IDEXX Fasciolosis Verification Test). Spatial clustering of seropositive farms was analyzed with the free SaTScan™ software version 9.7 using the Bernoulli probability model for high rates.

Results: Considering published test specificity and sensitivity data, true prevalence of *F. hepatica* was estimated at 7.7% (95% CI: 5.3 - 11). Positive animals came from 7 of the municipalities (87.5%). Seropositivity was significantly associated with municipality, but neither with sex, age or production type. Spatial statistics revealed one significant cluster of infection with a radius of 13.8 km. The proportion of positive cases within the cluster area was 39.4% and the relative risk of an individual animal being compared to animals outside the cluster was 9.4.

Discussion: The study provided new data on the prevalence and geographic distribution of *F. hepatica* in the Madeira Island. Spatial analysis results highlight the need for targeted intervention in specific risk areas.

Support/interest disclosure: 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).

Keywords: *Fasciola hepatica*; SEROPREVALENCE; CATTLE; MADEIRA ISLAND; PORTUGAL

Evaluation of normal aerobic oral flora of ball pythons (Python regius)

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Introduction: Ball pythons are one of the most common species commercially bred and kept as exotic pets. Pet reptiles can serve as reservoirs of a variety of pathogenic bacteria and are a potential source of multidrug-resistant bacteria transmission, which poses a serious concern for public health safety. Reptile bacterial diseases can be caused by primary pathogens but are frequently the result of immunocompromising conditions that allow overgrowing of commensal bacteria. Thus, to correctly interpret the significance of bacterial isolates from ill animals, there is the need to first determine the normal flora, that is relatively unknown in ball pythons.

Materials & methods: In this study, twenty healthy ball pythons were selected. Using aseptic technique, oral swabs were collected and preserved in Amies medium. Samples were supplemented with Brain-heart infusion broth, incubated at 37ºC for 24h and then streaked onto selective and chromogenic media. Plates were incubated at 37ºC for 24-72h followed by Gram staining and bacteria identification using a VITEK® 2 system.

Results:A total of 57 colonies were isolated, resulting in the identification of 12 species, five Gram-positive (*Enterococcus faecium*, *E. gallinarum*, *Staphylococcus xylosus*, *S. sciuri*, and *Micrococcus luteus*) and seven Gram-negative (*Aeromonas hydrophila*, *Salmonella* spp., *Morganella morganii*, *Acinetobacter* spp., *Enterobacter* spp., *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*).

Discussion: This study provides a better knowledge of ball pythons' normal oral flora and identifies potentially pathogenic bacteria. Considering the growing popularity of ball pythons, these findings caution the need to better characterize the normal flora and further determine the presence of drug-resistant bacteria in this species.

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Keywords: ORAL FLORA; AEROBIC BACTERIA; *PYTHON REGIUS.*

Can an antimicrobial biogel influence the virulence profile of canine oral enterococci?

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Background: Periodontal disease (PD) is a prevalent oral inflammatory disease in dogs, with previous studies showing that a nisin-biogel may be useful for PD control. Enterococci are ubiquitous bacteria with high genome plasticity, frequently used as bacterial models in antimicrobial studies. We aimed to evaluate the influence of nisin-biogel topic application on the virulence profile of oral enterococci from dogs.

Materials/Methods: Twenty dogs were selected and randomly allocated to one of two groups: treatment group (TG), with a nisin-biogel (200 µg/mL) dental application every 48 h, or control group (CG), with no treatment, for 90 days. Dental plaque swabs were obtained from each animal at day 0 and 90. Enterococci were isolated using conventional bacteriological techniques. PCR based methods allowed identification and typing. The phenotypic virulence profile was assessed using selective media.

Results: A total of 70 enterococci were identified as *E. faecalis* (42.9%), *E. faecium* (20%), *E. hirae* (5.7%), and *Enterococcus* spp. (27.1%). Virulence profiling revealed that 77.1% of the isolates were able to produce lipase, 30% gelatinase, 27.1% lecithinase, 25.7% protease, 60% haemolysins, and 95.7% biofilm. None of the isolates was able to produce DNase. No differences (p-value>0.05) were observed in the virulence index between timepoints or TG and CG.

Discussion: Enterococci with high virulence potential may be present in the oral cavity of dogs. After the *in vivo* application of a nisin-biogel, no differences were observed in the virulence signature of isolates from the TG or CG, reinforcing its potential to be used in PD control.

Support/interest disclosure: This work was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Project UIDB/00276/2020 (Funded by FCT); and by the Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020 - AL4AnimalS).

Keywords: ENTEROCOCCI; NISIN-BIOGEL; VIRULENCE PROFILE.

Bagaza Virus in Red-legged Partridges in Portugal

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Introduction: Bagaza virus (BAGV) is a zoonotic ssRNA(+) virus associated to human encephalitis, first identified in 1966 in female *Culex* mosquitoes in Central African Republic. In 2010, the virus was isolated from partridges and pheasants in Spain. Susceptible red-legged partridge (*Alectoris rufa*), the wood pigeon (*Columba palumbus*) and the common pheasant (*Phasianus colchicus*) develop weight loss, apathy and neurological signs. Mortality rates are estimated in 23-30% in *A. rufa*, the most sensitive species. Partridges underwent a reduction of 86% in southwest Spain, in 2010.

Material & Methods: In October 2021, two carcasses of partridges, found dead in Serpa, Portugal, were submitted for necropsy and diagnostic analysis in the National Reference Laboratory (INIAV, I.P.). Nucleic acids were extracted from several organs. Among other virological analysis, a RT-qPCR (Buitrago et al, 2012) was used to test for BAGV, directed to NS5. BAGV full genome was sequenced and submitted to GenBank.

Results: The cadavers were positive to BAGV RNA with compatible lesions (lymphoid depletion in the spleen and severe congestion, diffuse mononuclear inflammatory infiltration, and focal necrosis in several organs). All the sampled tissues were positive: brain (Cq 25.29±5.33), heart (Cq 26.06±8.00), kidney (Cq 29.33±6.83), and small intestine (Cq 38.04±1.87). BAGV/PT/2021 is closely related (99.6 % of identity) to BAGV detected in Spain.

Discussion: BAGV was detected for the first time in Portugal in 2021. Phylogeny indicates a common ancestor with the Spanish isolate 2010. Given the location of Portugal along the Atlantic-East migratory flyway and the current conservation state of the red partridge, it is paramount to maintain a sanitary surveillance on BAGV.

Support: This study was carried out as part of the mission of the National Reference Laboratory (INIAV, I.P.). This research was also funded by Fundação para a Ciência e Tecnologia (FCT), Project UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS, and by the Interdisciplinary Research Center on Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon (Portugal)."

Keywords: RED-LEGGED PARTRIDGE, *ALECTORIS RUFA*, NATURE CONSERVATION, WILDLIFE

Comparison of serological techniques to detect Toxoplasma gondii antibodies in serum of naturally exposed lambs

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Background: Several serological tests are used for the diagnosis of *Toxoplasma gondii* infection, each with different performance characteristics. There is a lack of standardized protocols for the use of the Direct Agglutination Test (DAT) and Immunofluorescence Antibody Test (IFAT) in livestock species and only a few comparative studies to assess the sensitivity and specificity of current commercial Enzyme-linked Immunosorbent Assay (ELISA) kits have been published so far.

Materials and methods: 330 merino lambs raised in the Alentejo region were tested for the presence of antibodies to *T. gondii* by a commercial DAT (Toxo-screen DA, bioMérieux), an in-house IFAT and the ID Screen Toxoplasmosis Indirect Multispecies ELISA (IDvet). The level of agreement between the three serological tests was calculated using Cohen's Kappa.

Results: The percentage of *T. gondii* positive lambs was 7.0% (95% CI:4.5-10.3%) by the DAT, 7.9% (95% CI: 5.2-11.3%) by the IFAT and 0.9% (95% CI: 0.2-2.6%) by the ELISA. Only one sample tested positive by all 3 techniques. The DAT and IFAT showed a poor agreement (k= -0.036; $p = 0.515$); the DAT and ELISA a slight significant agreement (k= 0.140; $p < 0.01$) while the IFAT and ELISA demonstrated a slight but not significant agreement (k=0.054; p=0.100).

Discussion: The poor agreement between serological techniques in this study highlights the importance of confirming results with a more specific test, like the Western blot. Also, more effort should be put on the standardization of protocols, especially the definition of optimal cut-off points for serological tests commonly used in the diagnosis of *T. gondii* infection in livestock.

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Keywords: *Toxoplasma gondii;* SHEEP; DAT; IFAT; ELISA

Molecular detection of canine herpesvirus in the reproductive tract of the bitch in different stages of the estrous cycle

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Introduction: Canine herpesvirus type I (CHV-1) is widely spread among dogs, with a distribution, in Europe, of 40% to 90%, mainly found in breeding kennels. In adult animals, the infection ranges from asymptomatic to mild illness with genital, respiratory and ocular clinical signs. It is also considered to be a major cause of infertility, reproductive disorders and neonatal mortality. In a large part of the adult canine population, infection remains latent and can be reactivated during stressful events. However, the correlation between the shedding of the virus and estrous cycle phase is still unknown.

Materials & methods: The aim of this work was to detect CHV-1 in the different phases of the estrous cycle and correlate it with the reproductive health of bitches. Between September 2021 and April 2022, vaginal samples were taken from 60 bitches of various ages and breeds, presented to the reproduction consultation at Anicura Atlântico Veterinary Reproduction Centre. For each animal, the phase of the estrous cycle was determined, a vaginal swab was performed and the owners filled in a questionnaire about the bitches' health and reproductive history. The vaginal swabs were submitted to DNA extraction and real-time PCR to detect the presence of CHV-1DNA.

Results: CHV-1 DNA was detected in 2 of the 60 samples (3,3%). Both were adult animals in estrus, belonging to breeding kennels with history of infertility.

Discussion: Although the frequency of infection was low, the association of CHV-1 infection in animals with reproductive disorders should be assessed. The follicular phase seems to be associated with virus reactivation, which may impair ulterior fertility. Further studies are needed to understand CHV-1 activation throughout the estrous cycle.

Disclosure: This work was supported by Lusófona University (Bolsas Estágio MV 21-22) and was approved by the Ethics Committee.

Keywords: CANINE HERPESVIRUS; INFERTILITY; PCR.

Profiling Patients Admitted To A Veterinary Teaching Hospital Isolation Unit For Early Detection Of Infectious Cases

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Introduction: Routine analysis of clinical data from patients with infectious diseases (ID) generates information that helps to determine the ID patient profile supporting early suspicion of ID.
Our aims were to identify host and environmental determinants to help canine and feline infectious patient detection, triage, early segregation, and treatment.

Materials/Methods: Data from 534 dogs and 788 cats admitted due to ID suspicion were retrieved from the Veterinary Teaching Hospital information system over a 7-year period (2013-2020). A descriptive analysis was performed assessing age, lifestyle, sex, neuter status, breed, vaccine status, concomitant disorders, and presence of cohabitant pets in confirmed ID patients.
Results: 263 dogs (49.3%) had a definitive ID diagnosis,being mainly aged <2 years-old (59.3%), outdoor lifestyle (65.4%), males (57.4%), intact (89.7%) and breed dogs (57.4%). Only 14.8% had a complete vaccination program, most had no concomitant disorders (56.3%) and were single pets (53.2%). The most frequent ID diagnosed were parvovirosis (n=130) and leptospirosis (n=57).
405 cats (51.4%) had a definitive ID diagnosis,being mainly aged ≥2 and <10 years-old (49.9%), living indoors (47.9%), males (56.8%), neutered (55.8%) and mixed breed (91.4%). Only 19.3% had a complete vaccination program, most had concomitant disorders (67.2%) and a large proportion lived with other pets (65.9%). The most diagnosed ID were feline retroviruses (n=214), upper respiratory tract disease (n=88) and panleukopenia (n=64).

Discussion: Dogs with ID tend to be young, non-vaccinated, intact, pure breed with outdoor lifestyle. Cats with ID were mainly characterized by absence of vaccination, cohabitation with other pets and presence of concomitant disorders.

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Keywords: INFECTIOUS DISEASES; TRIAGE; DOGS; CATS

Characterization of the oral microbiota in Albino Reticulated Python (Python reticulatus): antibiotic resistance

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Introduction: The oral microbiota presents a diversity of microorganisms which protect the oral cavity and the systemic balance of the animal. Changes in this microbiota allow the entry of pathogenic organisms and infections.Snakes are new companion animals and important attractions in zoos. Snakebites are a major public health problem, due to infections caused mainly by multidrug-resistant bacteria. Thus, the characterization of the oral microbiota is essential to carry out monitoring studies on antimicrobial resistance, aiming to promote greater antibiotherapy effectiveness, considering a One Health approach.

Materials & methods: This study reports a male reticulated albino python (*Python reticulatus*) of undefined age which, during routine examinations, presented stomatitis. An oral swab revealed bacterial pathogens associated with this finding. In addition, blood was collected from the tail vein to haematological analysis.

Results: The results of the bacteriological analysis revealed the presence of *Staphylococcus sciuri* with resistance to ceftazidime, piperacillin and intermediate phenotype to ceftrioxone. The isolate also showed sensitivity to aminosidine and enrofloxacin.The infection was treated with enrofloxacin (5-10 mg/Kg, q48h) over a 15-day period, intramuscularly and then orally. Afterwards, a new collection was performed and no growth was observed, confirming the elimination of the bacteria.

Discussion: The complete blood count was considered normal and an increase in uric acid concentration was detected, probably associated with kidney disease, gout or infection. The blood smear revealed no morphological alterations or forms compatible with haemoparasites, and the heterophils showed no signs of toxicity. To complement the diagnosis, the animal was submitted to a blood analysis which was found normal.

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Keywords: ORAL CAVITY; *Python reticulatus*; *Staphylococcus sciuri*; ANTIBIOTIC RESISTANCE

Monitoring the antibiotic resistance profile in bacteria isolated from Ambystoma mexicanum: case report

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Introduction: Axolotls (*Ambystoma mexicanum*, class Caudata, family Ambystomatidae) are pedomorphic salamanders, maintaining certain larval characteristics in adult. They have three pairs of external gills and a high regenerative capacity.The present study reports an axolotl belonging to a zoological park, with undefined age and sex and presenting whitish dots disseminated on the skin.

Materials & methods: A swab maintained in Cary Blair medium was performed for bacterial agent research and determination of susceptibility profile. Bacteriological analysis using the Vitek®-2 compact revealed the presence of two distinct isolates, namely *Pseudomonas aeruginosa* (gram-negative bacteria) and *Streptococcus* spp. Bacterial agents of the genera *Pseudomonas* and *Streptococcus* are very often associated with skin lesions. For antimicrobial sensitivity testing, eight antibiotics belonging to various classes were tested: ß-lactams, aminoglycosides, quinolones, tetracyclines, polymyxins and fusidic acid.

Results: Resistance to fusidic acid and cephalexin and sensitivity to neomycin were observed in both bacteria. For *Pseudomonas aeruginosa*, sensitivity was observed to kanamycin and polymyxin B, which presented an intermediate phenotype for the gram-positive isolate. The presence of multidrug-resistant bacteria (resistance to more than three classes) was also noteworthy.The animal was given neomycin, as both isolates were sensitive to this aminoglycoside. A new collection was not carried out as the animal died.

Discussion: This clinical report reinforces the importance of the knowledge of microbial agents, namely in amphibians in captivity, and the monitoring of antibiotic resistance for a better efficacy in the treatments implemented. Promoting and improving surveillance of antibiotic use is fundamental in assessing the spread of multi-resistant bacteria from different ecological niches.

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Keywords: *AMBYSTOMA MEXICANUM;* AMPHIBIAN; MULTIRESISTANCE; *PSEUDOMONAS AERUGINOSA*; STREPTOCOCCUS SPP.

Susceptibility of pets to SARS-CoV-2 Infection: Serological survey for SARS-CoV-2 antibodies in cats and dogs from households with confirmed cases of COVID-19

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Introduction: SARS-CoV-2 and the resulting disease, COVID-19, are one of the greatest public health concerns worldwide. To enter target cells, SARS-CoV-2 uses the receptor-binding domain (RBD) of the spike glycoprotein that specifically binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2). Pets, owing to their close contact with humans, have been the subject of numerous studies. Here, we performed a serological survey for SARS-CoV-2 antibodies in cats and dogs from households with confirmed cases of SARS-CoV-2 infection.

Materials and methods: A biobank was constructed using the serum samples from 78 cats and 75 dogs. These samples were tested against SARS-CoV-2 RBD proteins from alpha, delta and omicron variants by ELISA. The positive samples were subjected to a surrogate virus neutralization test (sVNT).

Results: Among the samples tested, 26/78 (33.3%) cat samples and 20/75 (26.7%) dog samples were positive in the ELISA assay. In the sVNT none of the dog samples tested positive, whereas 18/26 (69.2%) of the cat samples tested positive and four of them showed neutralizing antibodies against alpha, delta and omicron variants.

Discussion: Compared to a previous study from our group in which the exposure status of the animals to the virus was unknown, in households with a confirmed diagnosis of COVID-19, the seroprevalence of antibodies against the virus was higher. Our study attests the susceptibility of cats to SARS-CoV-2 infection and the development of potent antibodies in these animals. Cats naturally infected with SARS-CoV-2 may be a promising source for the development of antibodies against the virus.

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Keywords: SARS-CoV-2; COVID-19; NEUTRALIZING ANTIBODIES; CATS

Physical, chemical, and microbiological assessments of drinking water of small layer farms in Mozambique

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Introduction: Water quality is critical for poultry farming. This study assessed the physical, chemical and microbiological quality of drinking water in small layer farms in Southern Mozambique and identified potential risk factors for total coliform (TC) and *E. coli* (EC) contamination of drinking water.

Materials & Methods: In 20 farms, 57 samples were collected and examined for pH, nitrate content (NC), nitrite level (NL) and total hardness contents (TH). Furthermore, TC and EC growth were assessed at 37ºC.

Results: 100% of the drinking water was of acceptable quality in terms of pH (6.5-8.5), NC (50 mg/L), and NL (3 mg/L). TH exceeded the recommended standard in 37.5% of borehole water samples and 91.7% of tap water samples, respectively. TC and EC were found in 40 and 15% of water samples. Tap water samples had the greatest contamination, TC and EC levels of 41.7 and 16.7%, respectively.

Discussion: Although not statistically significant, sampling from the beginning of the nipple line (p=0.101, OR=7.357 [0.678-79.886]) and not cleaning the equipment regularly (p=0.098, OR= 3.966 [0.766-20,280]) were factors affecting the TC growth. Sampling from the tank water source (p=0.001, OR=0.005 [0.000-0.121]), borehole water source (OR = 13,585), and not cleaning the equipment consistently (p=0.073, OR=9.682, 95% CI [0.810-115.68]) were factors affecting EC growth. It is concluded that TH and microbiological quality of the drinking water of the study region are inadequate. Regular water quality assessments should be incorporated into Mozambican layer farm management to limit the potential for health concerns, and farmers should thoroughly clean and disinfect the rearing equipment.

Financial support: The study was supported by InterMed Mozambique, Lda.

Keywords: WATER QUALITY; LAYERS; COLIFORMS; RISK FACTORS; MOZAMBIQUE.

Vector-Borne Diseases agents detected in travelling dogs and cats from Sub-Saharan Africa

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Introduction: Pets travelling with owners may contribute to spread vector-borne-diseases (VBD) agents, with impact on animal and human health. The lack of information about these agents in sub-Saharan Africa pets lead us to determine the current risk of exposure in dogs and cats.

Material & Methods: Serum samples of 112 dogs and 69 cats from Mozambique, Angola and Cape Verde (36, 67 and 9 dogs; 35, 31 and 3 cats, respectively), aged 5 months to 13 years for dogs and until 15 years for cats, were screened by IFAT for *Ehrlichia canis, Rickettsia conorii, Anaplasma phagocytophilum* and *Leishmania infantum* antibodies.

Results: In this study, it was demonstrated seroprevalence for all VBD agents, with the dogs being the species with the highest number infected (≥1 agent), about 84.85% (56/66). The agent with the lowest seroprevalence was *Leishmania infantum*, corresponding to 10.71% (12/112) of dogs and 1.45% (1/69) of cats. Antibodies against *Ehrlichia canis* were detected in most dogs, about 62.12% (41/66), whereas in cats it was only observed in 7.5% (3/40). In contrast, *Anaplasma phagocytophilum* obtained the highest prevalence in cats, about 45% (18/40), while in dogs it was demonstrated in only 15.15% (10/66). The second most prevalent infection was by *Rickettsia conorii*, corresponding to 27.27% (18/66) of dogs and 10% (4/40) of cats.

Discussion: This study confirms the exposure of travelling dogs and cats from Mozambique, Angola and Cape Verde to different VBD agents, some of them zoonotic, emphasizing the importance of applying preventive strategies to mitigate their spread.

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Keywords: VBD AGENTS; DOG; CAT; IFAT; SUB-SAHARAN AFRICA

Environmental contamination with Staphylococci and OXA-23-producing Acinetobacter spp. in Portuguese small animal veterinary practices

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Introduction: Little is known about the prevalence and transmission of multidrug resistant (MDR) bacteria in Veterinary Medicine, particularly in small animal veterinary practices (SAVPs).The aim of this study is to determine prevalence of multidrug resistant bacteria in SAVPs.

Methods: Seven SAVPs were studied. Environmental samples from critical surfaces were collected. Nasal swabs were voluntarily obtained from workers. All swabs were plated on specific media selective for resistant bacteria: ESBL- and carbapenemase-producing Enterobacterales; Methicillin-Resistant *Staphylococcus* (MRS) and MDR Acinetobacter. Gram negative isolates were screened by PCR for the presence of major families of beta-lactamases and carbapenemase genes. Staphylococci isolates were screened by PCR for the presence of *mecA* gene.

Results: At least one resistant isolate was found in 18.9% (n=28/148) of surfaces analysed: i) 50% (n=14/28) were positive for MDR *Acinetobacter* spp.; ii) 7.1% (n=2/27) were positive for MRSP; iii) 14.3% of surfaces (n=4/28) were positive for MRSE. In SAVP4, 18.2% of surfaces analysed (n=4/22) tested positive for OXA-23-producing *Acinetobacter* spp. In SAVP6, 13% of surfaces (n=3/23) tested positive for the presence *Staphylococcus* spp. carrying *qac*A/B genes. Forty-five percent of nasal isolates carried the *mecA* gene, of which 21% (n=7/34) were MRSA.

Conclusion: This study highlights the need for implementation of infection, control and prevention guidelines in Veterinary Medicine to prevent the dissemination of multidrug resistant bacteria onto the community.

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Keywords: INFECTION AND PREVENTION CONTROL GUIDELINES (IPC); METHICILLIN-RESISTANT STAPHYLOCOCCI; CARBAPENEM RESISTANCE *ACINETOBACTER* SPP.

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Antimicrobial resistance and virulence profiles of yeasts from zoo animals

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Background: Intestinal microbiota role in animal health is well established. Several studies have characterized the bacterial species of zoo animals GI tract; however, little is known about the fecal yeasts carried by these animals and their potential role as vehicles of resistant and virulent species.

Materials & methods: Ninety-three fecal samples from animals of different species belonging to a zoological collection were collected and analyzed using conventional mycological techniques (incubation in Sabouraud Dextrose agar, 27ºC, 48h). Yeast isolates were identified according to their macro and microscopic morphologies and using the biochemical gallery API®20CAUX. Isolates susceptibility to six antifungals was evaluated using the disk diffusion method and the multiple antimicrobial resistance index (MAR index) assessed. The phenotypic virulence profile was evaluated by testing isolates' ability to produce enzymes associated with pathogenicity (haemolysins, lipase, DNAse, protease, gelatinase, lecithinase) and biofilms using specific media, and their virulence index (V.index) determined.

Results: A total of 45 yeast isolates were collected from 42 samples and identified as *Candida* (22/45), *Rhodotorula* (13/45), *Cryptococcus* (6/45), *Saccharomyces* (2/45), *Geotrichum* (1/45) and *Trichosporon* (1/45). Isolates' antifungal susceptibility varied from 16% for posaconazole to 82% for voriconazole, with a mean MAR index of 0.57. Most isolates (33/45) expressed at least two virulence factors, frequently DNase and lipase, simultaneously with biofilm-forming ability. The mean V.index was 0.34.

Discussion: This study brings relevant information not only concerning the occurrence of resistance and virulence among yeasts of zoo animals' intestinal microbiota, but also to the framework of conservation of wild animals under captivity.

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Keywords: YEASTS; ANTIMICROBIAL RESISTANCE; VIRULENCE; ZOO ANIMALS

Presence of Phyllobothrium delphini (Cestoda: Phyllobothriidae) in a striped dolphin (Stenella coeruleoalba) stranded in the Tagus estuary (Alcochete, Portugal)

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Introduction: The range of striped dolphins (*Stenella coeruleoalba*) extends across the warm-temperate to tropical waters of the world and this cetacean is the most frequently occurring dolphin in the Mediterranean Sea. Tetraphyllidean merocercoids have been commonly reported from most cetacean species and some pinnipeds world-wide, including *S. coeruleoalba*. Two types of merocercoids have been widely recognized: *Phyllobothrium delphini* (Bosc, 1802), encysted in the subcutaneous blubber, usually in the abdominal area, and *Monorygma grimaldii* (Moniez, 1889), encysted mainly in the peritoneum of the abdominal cavity.
The authors refer to a recent parasitological finding regarding this host and these parasites in Portugal.

Material & Methods: On 4th April 2019, a female *S. coeruleoalba* specimen was found stranded in Alcochete and was necropsied at the Pathological Anatomy Service of FMV-ULisboa. Several cystic structures were collected from the subcutaneous blubber of the inguinal area, preserved in 70% ethanol, and analyzed under a stereoscope.

Results: The observed parasitic cysts had a length between 4-8 mm and were identified as intermediate stages of *P. delphini*.

Discussion: *P. delphini* is considered one of the most common parasites of cetaceans. Despite being common, the life cycle of this parasite is not clear, and little is known about the biology of its larval forms. Published reports of its presence in *S. coeruleoalba* are available from England, Wales, United States, Spain, Italy, and Costa Rica until nowadays. To the best of our knowledge this is the first published reference of *P. delphini* in a striped dolphin from Portugal.

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Keywords: HELMINTHS, PARASITES, PORTUGAL, *STENELLA COERULEOALBA, PHYLLOBOTHRIUM DELPHINI.*

Helminths found in wild thrushes (Turdus spp.) from Portugal

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Introduction: Thrushes (Passeriformes: Turdidae) are small to medium-sized songbirds and one of the most popular groups of game birds in Europe. Several helminths have already been detected in *Turdus* spp. Since these birds are predominantly migratory and feed mainly on invertebrates (insects, earthworms, terrestrial crustaceans and snails), they can act as pathogen reservoirs to other hosts.

Material & Methods: Four *Turdus merula* and one *Turdus philomelos* were taken to the laboratory for parasitological screening. Parasites collected from the coelomic cavity, liver, gallbladder and intestine were preserved in 70% ethanol until visualization and were: i) observed with stereomicroscopy or; ii) prepared in slides with Hoyer's Medium and observed with a compound microscope. Helminths were identified using dichotomous keys based on their morphology.

Results: Several helminths were identified in *Turdus merula*, including nematodes (e.g. *Porrocaecum* spp.), cestodes (e.g. *Passerilepis* spp.), trematodes (e.g. *Brachylecithum filum*) and acanthocephalans (e.g. *Plagiorhynchus cylindraceus*) and a trematode was identified in the coelomic cavity of *Turdus philomelos* (*Morishitium* spp.).

Discussion: Most of the parasites found in this study have intermediary hosts (e.g. molluscs and crustaceans), which are included in thrushes' diet. To our knowledge, the helminths found in this study are being reported for the first time in our country. Since works regarding parasites in thrushes from Portugal are scarce, this study adds valuable information on the helminthofauna of *Turdus* spp. from our country.

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Keywords: HELMINTHS, PARASITES, PORTUGAL, *TURDUS* SPP.

Implementation of Mini-FLOTAC in the routine assessment of gastrointestinal parasitic infections in exotic birds from an ornithological park of North-western Spain

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Introduction: Mini-FLOTAC (MF) is considered worldwide as a more sensitive alternative to the traditional McMaster method (McM) for the diagnosis of gastrointestinal (GI) parasitic infections in different animal species. Yet, the implementation of this technique in exotic animals is still scarce. This research aimed to implement MF in the routine diagnosis of GI parasitic infections in several exotic bird species.

Material & Methods: Between 18 May – 19 July 2022, a total of 48 fecal samples were collected in an ornithological park located at Lugo (Spain) and from the following bird species: Greater Rhea, Curassow, Pheasants, Monk Parakeet, Flamingos, Capercaillie, Black Swan, Ruddy Shelduck, Cockatiel, Parrots, Motmot, Cockatoo, Eagle-owl, Superb Parrot and Eastern Rosella. Samples were processed using the MF protocol optimized for exotic species, aiming to identify avian GI parasites and calculate their prevalence. Also, 17 fecal samples from different pheasant species were processed using MF and McM for comparing the prevalence of parasitic forms.

Results: MF implementation allowed to identify: coccidia (33%), ascarids (44%), *Capillaria* sp. (44%), *Strongyloides* sp. (6%) and *Syngamus trachea* (6%) in pheasants; *Strongyloides* sp. in curassows (67%) and flamingos (33%); coccidia (67%) and *Capillaria* sp. (100%) in capercaillie; *Capillaria* sp. in parakeets (33%); *Capillaria* sp. (100%) and ascarids (100%) in motmot. Also, MF detected a higher prevalence of coccidia (35%), *Capillaria* sp. (41%) and ascarids (41%) in pheasants, in comparison with McM (12%, 24% and 35%, respectively). Discussion: This research provided more scientific support regarding the benefits of using MF in routine diagnosis of GI parasitic infections in zoological collections.

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Keywords: EXOTIC BIRDS; AVIAN PARASITOLOGY; DIAGNOSIS; MINI-FLOTAC; GALICIA – SPAIN.

Analysing the effect of gelatines enriched with predatory fungi in the avian gut microbiota: preliminary results

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Introduction: The biocontrol of animal gastrointestinal parasites using predatory fungi is globally considered an accurate and sustainable approach for farms and zoos. However, there is no information regarding the effect of these fungi in animals' gut microbiota. This research aimed to analyse the avian gut microbiota composition and diversity, following feeding an ostrich with gelatines enriched with predatory fungi spores.

Materials & Methods: The experiment was performed in an exotic animal collection located in Abrantes, Portugal, between 15-20 March 2021. Two fungal formulations were used: 5 sticks of *Duddingtonia flagrans* (CECT20823), and 5 gelatines containing *D. flagrans* and *Mucor circinelloides* (CECT20824) (10^6 spores per formulation). Only 1 ostrich specimen was available for this study, which received 1 stick and 1 gelatine per day, P.O., during 5 consecutive days. A total of 4 fresh fecal samples were collected before (n=2) and after (n=2) the assay. Total genomic DNA was extracted from all samples and used for long-read 16S sequencing.

Results: The ostrich's fecal microbiota was composed mainly by Firmicutes (81% and 56% of relative abundance, before and after fungal administration, respectively). Also, Proteobacteria and Bacteroidetes phyla were highly represented, with both increasing their frequency after treatment (5% to 23% and 5% to 14%, respectively). No differences were identified for potentially pathogenic bacteria such as *Escherichia coli*, *Salmonella enterica*, *Campylobacter jejuni*, *Pasteurella multocida* and *Clostridium* spp. after fungal treatment, and the OTU diversity also remained similar during the assay (Shannon index, p=0.42).

Discussion: This research suggests that feeding predatory fungi to birds does not alter their gut bacterial equilibrium.

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Keywords: BIOCONTROL; PREDATORY FUNGI; BIRDS; INTESTINAL MICROBIOTA; PORTUGAL.

ESBL/AmpC-Producing Enterobacterales Colonization And Sharing By Co-habiting Companion Animals and Humans In The Community, Portugal And United Kingdom

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Background: The role of companion animals (CAs) as potential reservoirs of antimicrobial resistant bacteria represents a growing concern worldwide. The aim of this study was to evaluate the possible sharing of ESBL/AmpC and Carbapenemase-producing Enterobacterales between healthy CAs and their household members.

Methods: Between 2018 and 2020, healthy dogs and cats (n=110) and their co-habiting humans (n=119) from 41 households from Portugal and 42 households from the United Kingdom (UK). Informed consent was obtained. Fecal samples were inoculated on MacConkey agar plates supplemented with 1.5 µg/mL cefotaxime and 1.0 µg/mL meropenem. Beta-lactam genes were screened by PCR and sequenced. Species identification was performed by PCR. Susceptibility tests were performed by microdilution with MicroScan® Neg MIC Panel Type 44 (Siemens, US). Isolates clonality was assessed by rep-PCR. WGS producing pair-end libraries with 150 bp., was performed for CAs/owner pairs with the same rep-PCR pattern. Genomes assembly was conducted and genetic relatedness estimated.

Results: No carbapenem-resistant Enterobacterales were found. ESBL/AmpC-producing Enterobacterales were detected in CA (10.9%, *n*=12/110) and humans (13.4%, *n*=16/119) from both countries. Identical rep-PCR pattern was observed in CAs/owner pair *Escherichia coli* strains from 2 Portuguese households and one UK household. Phylogenetic analysis of core-genome assemblies confirmed sharing of *E. coli* pandemic lineages within the two Portuguese households.

Discussion: The identification of ESBL/AmpC from healthy companion animals is of great concern and should not be neglected. These results are crucial to demonstrate the importance of CA/human unit in the dissemination of clinically important genes in the community setting.

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Keywords: ESBL-SHARING; HEALTHY ANIMALS; GUT COLONIZATION; HEALTHY HUMANS;

Characterization of Klebsiella spp. virulence from different small animal infection sites

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Introduction: In recent years, *K. pneumoniae* strains with both multidrug-resistance and unusually invasive, hypervirulent phenotypes have emerged in the human healthcare setting, raising the question whether such concerning strains are already present in the small animal setting. Our aim was to characterize the antimicrobial resistance and virulence of *Klebsiella* spp. from different small animal infection sites.

Material & methods: Sixty-two *Klebsiella* spp. isolates obtained during 2020 from clinical samples from small animals were categorized according to infection site and antimicrobial susceptibility testing followed the EUCAST guidelines. Beta-lactamase genotyping was further performed by PCR and sequencing. Virulence characterization included the string test and microplate crystal violet biofilm quantitation, as well as PCR screening of 15 virulence encoding genes.

Results: Half of the isolates were obtained from urinary tract infections (UTI), and sixty-one were multidrug-resistant. Four carbapenemase producers were identified, and forty-five isolates carried the $bla_{\text{CTX-M-15}}$ gene. Two *Klebsiella pneumoniae* isolates were positive on the string test but *rmpA* negative. The virulence genes *fimH-1* and *entB* were present in more than 80% of the isolates, followed by *mrkD* (79.0%), *kpn, ycfM, kfu* were present in more than 30%, Yersiniabactin High-Pathogenicity Island was present in 22.6% and *allS* was absent. No apparent relationship between infection site and biofilm production was found.

Discussion: *Klebsiella* spp. from different types of small animal infections can harbour virulence determinants associated with increased invasiveness and be multidrug-resistant, constituting an emerging problem. The two *strains* showing a hypervirulent-suggestive phenotype (positive string test) require further studies, highlighting the need for continuous surveillance of this serious phenotype.

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Keywords: CARBAPENEMASE PRODUCER; *KLEBSIELLA* SPP.; SMALL ANIMAL INFECTION; HYPERVIRULENCE; MULTIDRUG RESISTANCE

Presence of a KPC-3-producing Klebsiella pneumoniae in a dog with chronic rhinitis

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Introduction: A 10-year-old female dog presented with upper respiratory signs which continued after an empirical round of doxycycline. Biopsy of the nasal mucosa revealed chronic purulent inflammation, and nasal exudate was submitted as a sample for microbiological aerobic culture, from which a MRSA and an ESBL-producing *Klebsiella pneumoniae* resistant to carbapenems were isolated. Our aim was to characterize the *K. pneumoniae* isolate's antimicrobial resistance and virulence through whole-genome sequencing (WGS).

Materials & methods: WGS (Illumina NovaSeq) produced pair-end libraries with 150 bp. *De novo* genome assembly was used to screen for antimicrobial resistance, virulence genes and mobile genetic elements using ResFinder 4.1 and Mobile Element Finder v1.0.3. MLST was performed.

Results: *K. pneumoniae* ST392 was identified, carrying a KPC-3 carbapenemase. It was resistant to carbapenems (MIC >8 mg/L) but susceptible to ceftazidime-avibactam by disc diffusion. The $\mathit{bla}_{\text{KPC-3}}$ gene was located on transposon Tn4401*d* on a ~50 kb IncN-type plasmid. Virulence genes *traT* and *iutA* were identified. *traT* was located on a IncFII(K)-type plasmid. The absence of other relevant virulence factors classifies this strain as belonging to the classical non-hypervirulent *K. pneumoniae* pathotype. Both the *K. pneumoniae* and the MRSA were susceptible to trimethoprim/sulfamethoxazole.

Discussion: A 1-month course of cotrimoxazole was performed and the dog made a full recovery, with no further relapses, highlighting the importance of 1st line antibiotics as possible choices to treat multidrug resistant infections without using human-reserved antimicrobials. This is particularly important in the treatment of biofilm-producing bacteria that are resistant to critically important antimicrobials such as carbapenems.

Disclosures: This study was financed through CIISA and Fundação para a Ciência e Tecnologia (FCT) Project UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS; LF holds a FCT PhD Grant UI/BD/153070/2022; JMS holds a FCT PhD Grant 2020.06540.BD; JM holds a FCT PhD grant 2020.07562.BD.

Keywords: CARBAPENEMASE PRODUCER; *KLEBSIELLA PNEUMONIAE*; SMALL ANIMAL INFECTION; WHOLE GENOME SEQUENCING; ANTIMICROBIAL STEWARDSHIP

Gastrointestinal helminths in Rattus spp.: two new species identified in São Miguel island, Portugal, and a phenomenon of ecological release.

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Introduction: Synanthropic rats have dispersed worldwide with the contribution of maritime traffic. As such, due to their role as vectors and reservoirs of numerous pathogens, including helminths, rats became important dispersers of these agents. In this study, the prevalence of gastrointestinal helminths was evaluated in *Rattus norvegicus* and *Rattus rattus* inhabiting the vicinities of the seaport of a continental capital city (Lisbon) and an insular city (Ponta Delgada, São Miguel island, Azores).

Material & Methods: The gastrointestinal tract and the liver of 192 rats (72 from Lisbon, 120 from Ponta Delgada) were dissected and macroscopically analysed.

Results: In total, 11 species of helminths were identified based on their morphological characteristics under a compound optical microscope. In Lisbon, *Syphacia* spp. was found co-parasitizing the large intestine of rats with *Heterakis spumosa*, with prevalence of 27,8% (20/72) and 29,2% (21/72), respectively. In Ponta Delgada, albeit having been previously identified in S. Miguel island, *Syphacia* spp. was not detected in our dataset and the prevalence of *Heterakis spumosa* was 45,8% (56/120), much higher when compared to Lisbon.

Discussion: All 10 species found in Lisbon had already been identified in continental Portugal. Two new species were recorded for S. Miguel island (*Moniliformis moniliformis* and *Aspiculuris* spp.). The absence of *Syphacia* spp. in Ponta Delgada and the higher prevalence of *Heterakis spumosa*, is consistent with a scenario of ecological release, characterised by the increase in prevalence of one species in the absence of other.

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Keywords: *Rattus* spp.; HELMINTHS; AZORES; ECOLOGICAL RELEASE; SEAPORTS

Haematological profiles in Alentejano and commercial (LWxLR) pigs

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Introduction: The levels of blood elements in pigs vary according to sex, breed, growth rate, diet, stage of gestation, management practices or season. Blood elements may be used to investigate adaptation of pig breeds to different environments and these studies are still lacking in many local pig breeds including Alentejano, a well-adapted breed to the acorn fields of Southern Portugal.

Material & Methods: Alentejano sows born in May 2021 and F1 sows (Landrace x Large White) born in July 2021 were raised in extensive system for a study aiming to investigate the effect of epigenetic regulation on litter size. Sows were not of the same age, but at similar physiological development since breeds have different growth rates and different age at sexual maturity. Sows were submitted to estrous synchronization in May 2022. Peripheral blood was also collected in EDTA tubes and hemograms were performed using a CELL-DYN 3700 System Analyzer which performs simultaneous impedance measurements of various blood elements.

Results: Hemograms displayed values within normal range for the species. When compared with Alentejano, the F1 sows had higher number of erythrocytes and platelets ($P \leq 0.05$) and lower levels of MCV, MCH and of MCHC ($P \leq 0.05$).

Discussion: These differences may be attributed to physiological differences between breeds, but can also be due to the adaptation of F1 sows to the extensive system. Future perspectives should include the comparison of F1 sows raised in intensive system in order to discriminate between the effect of management practices and breed.

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Keywords: LOCAL BREEDS; ALENTEJANO; HEMOGRAM; BLOOD

Susceptibility of pets to SARS-CoV-2 Infection: Serological survey for SARS-CoV-2 antibodies in a cat population of Lisbon, Portugal

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Introduction: SARS-CoV-2 and the resulting disease, COVID-19, are one of the greatest public health concerns worldwide. To enter target cells, SARS-CoV-2 uses the receptor-binding domain (RBD) of the spike glycoprotein that specifically binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2). Cats, due to their high susceptibility to infection and close contact with humans, have been the subject of numerous studies. In this study, we performed a serological survey for SARS-CoV-2 antibodies in a cat population in the Lisbon region.

Materials and methods: A biobank of serum samples was constructed from cats admitted to the Veterinary School Hospital (HEV) of the FMV-ULisboa. These samples were tested against SARS-CoV-2 spike and RBD proteins from alpha, delta and omicron variants by ELISA. The positive samples were subjected to a Surrogate Virus Neutralization Test (sVNT). Serum samples with high neutralizing activity were tested using a pseudotyped virus infection assay.

Results: Of the 761 cat serum samples collected 118 (15.5%) tested positive in the ELISA assay. These 118 samples were tested in the sVNT and 22 (18.6%) presented neutralizing antibodies, with four serum samples neutralizing the alpha, delta and omicron variants. The serum samples with the highest titer of neutralizing antibodies showed a greater ability to inhibit infection in a SARS-CoV-2 pseudotyped assay.

Discussion: Our study attests the susceptibility of cats to SARS-CoV-2 infection and the development of potent antibodies in these animals. Cats naturally infected with SARS-CoV-2 may be a promising source for the development of antibodies against the virus.

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Keywords: SARS-COV-2; RBD; CATS; NEUTRALIZING ANTIBODIES

Tick-borne diseases in asymptomatic cattle from São Miguel Island, Azores – A clinical perspective

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Introduction/Background: The Azores archipelago is one of the regions with the highest cattle production, in Portugal. Outbreaks of tick-borne (TB) diseases have been reported by local field veterinarians and different tick species are described in São Miguel. At this study, we decided to investigate the presence of some of these agents in asymptomatic cattle, in S. Miguel.

Materials & methods: In October 2019, 10 blood samples from each of 10 farms were randomly selected. Species belonging to the Babesia/Theileria and Anaplasma/ Ehrlichia genus were tested by PCR screening.

Results: There were 45 positive samples later confirmed as species belonging to the Theileria orientalis group (45/100, 45%), in 9 farms (9/10, 90%) and it was also possible to identify Anaplasma bovis, in 2 animals, at two farms (2/10, 20%).

Discussion: As far as we were able to verify, this is the first reference to the presence of these agents in S. Miguel. However, although asymptomatic, the repercussions associated with these infections are probably being underestimated or confused with other health problems. Since ixodids were not found in cows, other factors may be contributing to the high percentage of positive samples found, such as poor management practices. This is clearly an interesting subject for further investigation, as a better understanding of the life cycle of these parasites will contribute to their control.

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Keywords: TICK-BORNE PATHOGENS; AZORES; DAIRY CATTLE; IXODIDAE

Bee or not to Bee? Present and future of Portugal's honeybee colonies

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Introduction: Pollination is crucial to maintain the balance in the ecosystems and food chains. The honeybee (*Apis mellifera*) is one of the most important pollinators, improving the food's quantity and quality. Unfortunately, the number of honeybee colonies has been declining, urging to ensure these insects' health. With this project, we aimed to understand the health status of the Portuguese honeybees.

Materials & Methods: 550 and 273 samples of adult bees collected in 2021 and January to June of 2022, respectively, sent to the Bee Health Laboratory, coming from continental Portugal and Azores islands, were analyzed using macroscopic and microscopic techniques to diagnose *Varroa destructor*, *Aethina tumida, Nosema* spp., *Tropilaelaps* spp., *Acarapis woodi*, *Senotainia tricuspis* and *Braula coeca.*

Results: In 2021 and 2022, the most prevalent pathogen in the continent was the mite *V. destructor* 31.64% (174/550) and 26.74% (73/273), respectively, followed by *Nosema* spp. 26.18% (144/550) and 24.18% (66/273). *Senotainia tricuspis* in association with *Varroa destructor* is the most common mixed infection. *Aethina tumida* and *Tropilaelaps* spp. were not detected. In 2021, the Azores islands were the only region simultaneously free of *Varroa destructor* and positive to *Acarapis woodi* and *Braula coeca*.

Discussion: *V. destructor'*s the main threat to *Apis mellifera* in Portugal. Nonetheless, the small sample size from Azores and Madeira islands interfered with this sanitary survey. Due to the adverse global scenario for beekeeping, we stress the importance of continuing to collect and analyze national bee samples to better monitor the health of Portuguese colonies.

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Keywords: *APIS MELLIFERA*; POLLINATION; PATHOGENS; PORTUGAL; *VARROA*

Comparison of different laboratory techniques for detection of Cryptosporidium spp. and Giardia duodenalis in lambs

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Introduction: Cryptosporidium spp. and Giardia duodenalis are protozoa that cause diarrhoea in humans and animals. These parasites are considered important enteric pathogens associated with neonatal diarrhoea in lambs.

The objective of this study was to compare the sensitivity and specificity of Ziehl-Neelsen (ZN) staining and direct immunofluorescence (IFD), with and without faecal concentration (FC) method, for the detection of Giardia duodenalis cysts and Cryptosporidium spp. oocysts in lambs.

Materials and Methods: Thirty-seven faecal samples were collected from newly arrived lambs under 60 days old on a feedlot farm. For detection of Cryptosporidium spp. oocysts and Giardia duodenalis cysts, ZN staining and IFD test (Crypto/Giardia CEL, Cellabs) were used with and without previous concentration using an adaptation of Ritchie's Method.

Results: Twenty samples were positive for Cryptosporidium spp.. Positive samples for ZN with and without FC were 4 and 16; for IFD with and without FC were 5 and 10, respectively. ZN detected more positive animals (n=18) for Cryptosporidium spp. than IFD (n=10).

Giardia duodenalis was detected on 20 samples, although only with IFD. For IFD with and without FC, 19 and 16 samples were considered positive, respectively. Overall, FC increased the number of samples and quantity of Giardia duodenalis cysts detected.

Discussion: Ziehl-Neelsen staining doesn't allow the identification of Giardia duodenalis cysts. Nevertheless, it remains an excellent technique for identifying Cryptosporidium spp. oocysts. The concentration method with IFD increases detection of Giardia duodenalis cysts, especially in lower burden infections. However, the concentration method before ZN staining is not suitable for identifying Cryptosporidium spp. oocysts, since a significant number are lost during this procedure.

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Keywords:ZIEHL-NEELSEN, DIRECT-IMMUNOFLUORESCENCE, CRYPTOSPORIDIUM SPP.; GIARDIA DUODENALIS; LAMB

Project CryGiarPT: Unravelling animal cryptosporidiosis and giardiosis in Portugal

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Background: Cryptosporidium spp. and Giardia duodenalis are protozoan parasites responsible for intestinal infection of humans and animals causing high morbidity and death in immunocompromised hosts. The main transmission route is faecal-oral, mainly through the ingestion of contaminated water, food or contact with infected animals. Together, they represent a serious problem in animal and public health since some genotypes and assemblages are zoonotic. This project intends to clarify the epidemiology of Cryptosporidium spp. and Giardia duodenalis in Portugal.

Material & Methods: The detection and characterization of Cryptosporidium spp. and Giardia duodenalis will be done in fecal samples from domestic and wild animals using conventional methods (Ziehl-Neelsen and Trichrome staining and direct immunofluorescence assay) and molecular techniques (PCR, molecular cloning and sequencing). The obtained data will later be used to create an online Geographic Information System platform.

Results: We expect to estimate the occurrence of both parasites in domestic and wild animals and to assess different Cryptosporidium spp. genotypes and Giardia duodenalis assemblages which will be compared with worldwide genotypes in outbreaks.

Discussion: Identifying the presence of both parasites in Portugal can help to create effective prevention measures, while genotyping Cryptosporidium spp. and Giardia spp. can allow earlier detection and track the sources of infection for animals and humans.
The creation of an online Geographic Information System platform with all the data collected, will be used in the future by Medical and Veterinarian professionals as a tool to evaluate the risk of outbreaks and to identify areas where control measure must be applied.

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Keywords:CRYPTOSPORIDIUM SPP.; GIARDIA DUODENALIS; ZOONOSIS; GENOTYPING

In vitro evaluation of bacteriophage host range against Pseudomonas aeruginosa otitis isolates from dogs

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Introduction: Canine otitis externa caused by *Pseudomonas aeruginosa* is a concerning issue in Veterinary Medicine, due to *P. aeruginosa* high biofilm-forming ability and multidrug resistance profile. Considering that, it is difficult to select antimicrobials capable of eradicating *P. aeruginosa* infections, being mandatory to find innovative antimicrobial strategies, such as bacteriophage therapy. Lytic bacteriophages are viruses able to infect bacteria, with high specificity to their target hosts, that can cause bacterial cell lysis without integrating into the host DNA, making them an excellent choice for therapeutic application, in both human and veterinary medicine. The main goal of this work was to evaluate the inhibitory potential of two selected bacteriophages against a collection of *P. aeruginosa* isolates obtained from dogs with otitis externa.

Material & Methods: A total of 49 isolates, obtained from dogs with otitis externa and two bacteriophages specific for *Pseudomonas aeruginosa* isolates, JG005 (DSM 19872) and JG024 (DSM 22045) were used.
To assess the bacteriophage inhibitory potential, a bacteriophage spot-test procedure was applied.

Results: Regarding bacteriophage JG005, 38.8% (n=19) of the isolates were susceptible to this bacteriophage, including biofilm-producing isolates (42.1%). Regarding bacteriophage JG024, 61.2% (n=30) of the isolates were susceptible, with 26.7% being biofilm producers. It was also possible to observe that 21.1% of the biofilm producer isolates were susceptible to both bacteriophages.

Discussion: Considering the difficulty in treating otitis externa, our results reinforce the potential of bacteriophages, specifically JG005 and JG024, to treat these complicated infections. Results are encouraging for future research examining the eradication potential of these two bacteriophages against established biofilms.

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Keywords: *PSEUDOMONAS AERUGINOSA*; BACTERIOPHAGE; OTITIS EXTERNA; DOG

Differential expression of genes in Theileria annulata infected and non-infected Limousin cattle

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Introduction: *Theileria annulata* is a protozoan that causes Tropical theileriosis in cattle. Several cattle breeds show different resistance or tolerance to infection with less severe clinical signs. Limousin is a cattle breed from a non-endemic area of theileriosis, which is established in Portugal since the last century, in regions where *T. annulata* is present. The objective of this study is the evaluation of differential expression levels of several genes from PBMC cells from infected and non-infected animals.

Materials and methods: Blood samples were collected from 29 Limousin cattle for DNA extraction and testing for *T. annulata*. Five positive animals and four negative animals were selected for gene expression studies in genes putatively associated to resistance or tolerance to infection (18S rRNA, BoLA, C1R, CD9, CR2, GPR177, ICAM1, PRNP, TLR10). PBMC extraction was performed followed by RNA extraction, cDNA synthesis and Realtime PCR.

Results: 18s rRNA and CR2 genes amplified in all 9 samples. CD9 gene amplification occurred in 7 animals, and PRNP amplification occurred in 6 animals. BoLA gene was amplified in 5 animals and ICAM1 amplified in 4 infected animals. All genes tested were up-regulated in infected compared to non-infected animals.

Discussion: Up-regulation of several genes is similar in susceptible cattle breeds, but in some genes the opposite is seen. Despite the differences in both groups, further studies are required including more replicates and genes associated with the infection and resistance of bovines to *T. annulata*. Also, studies after *T. annulata in vitro* infection can put in evidence possible resistance genes.

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Keywords: *Theileria annulata*; PBMC; BOVINE; RESISTANCE; TOLERANCE

Molecular survey of Theileria orientalis in Portuguese cattle

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Introduction: Cattle piroplasmosis has been a well-recognised disease among veterinarians, but usually the identified pathogens are *Theileria annulata*, *Babesia bigemina* and *Babesia bovis*. However, other pathogens such as *Theileria orientalis* may also be present. Despite being previously considered non-pathogenic, clinical cases have been identified in different countries. The objective of this study was the identification of *T. orientalis* infection in cattle from different regions in Portugal.

Materials and methods: Blood samples were collected from 264 bovines from 27 farms of 5 NUTSII from mainland Portugal. Samples were tested for *T. orientalis* DNA with a PCR used for amplification of Major Piroplasm Surface Protein (MPSP) and a PCR for the amplification of ITS1-5.8-ITS2 from the rRNA region. Results: A total of 47 blood samples out of 264 were positive for *T. orientalis*, with an overall prevalence of 17.8%.PCR results indicated presence of parasites in 6 farms, with a higher prevalence of infection in Alentejo (21.7 %).

Discussion: This study confirmed that *T. orientalis* infection is widespread in Portugal but further studies for genotyping should be done since different genotypes with different pathogenicity have been described. Since the livestock sector plays a critical role in the national economy in Portugal, reduction of risk of tick-borne diseases such as theileriosis is crucial for the good practice of pasturing cattle in this country. Despite being considered non- or less pathogenic than the other species such as *T. annulata*, this tick-borne protozoan should be considered for differential diagnosis in cattle with signs of tick-borne disease.

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Keywords: *Theileria orientalis*; PCR; CATTLE; EPIDEMIOLOGY; PIROPLASMOSIS.

Preliminary study on the effect of bay and eucalyptus leaf's in feed on weaning pigs growth performances

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Introduction: Dietary supplementation with natural substances may improve gut health and growth performances. Bay and eucalyptus leaves are known for their antioxidant and antimicrobial activity, thus having the potential to further improve gut health.

Material and methods: This study aimed to evaluate the effects of dietary supplementation with dried bay leaves (*Laurus nobilis*) and eucalyptus (*Eucalyptus globulus*) on piglets' growth. The animals (n=72) were divided into 24 pens (3 per pen) and randomly divided into 3 treatments: the control diet (CT), the same diet with 0.20% of dried bay leaves (FL), and 0.20% of dried eucalyptus leaves (FE). The trial lasted 29 days (Initial weight 13.5 kg LW) until the pigs were 70 days old (Final weight 34.6 kg LW). Individual live weight (LW), daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR) were weekly monitored.

Results: There were no significant differences in growth performances between the studied groups ($P > 0.05$). Although, considering only the first two weeks we observed a tendency ($P<0.08$) to lower FCR in the FE group (1.40 in CT; 1.33 in FE; 1.42 in FL).

Discussion: Further studies are necessary to assess the effects of bay and eucalyptus leaves on gut health.

Support: The participation of Victor Pinheiro and Divanildo Monteiro was supported by the projects UIDB/CVT/00772/2020, by).and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT); Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS) LA/P/0059/2020 (funded by FCT;UTAD FOOD ALLIANZ: Infraestrutura de Investigação em Nutrição e Alimentação. A ligação com a Ciência Animal. - (NORTE-01-0145-FEDER-072687)

Keywords: *LAURUS NOBILIS*, *EUCALYPTUS GLOBULUS,* WEANING PIGS, GROWTH PERFORMANCES

Effect of Urtica urens in broiler feed on the development of the carcass characteristics, blood composition and caecal Volatile Fatty Acid

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Introduction: *Urtica urens* can be an additive in animal feed due to its antibacterial, antioxidant, and anti-inflammatory properties. Some studies suggest that it has a positive impact on the immune system, development of the digestive tract, voluntary feed intake, quality of meat and eggs and it is also a rich source of vitamins.

Materials & methods: In this work, we aimed to evaluate the effect of *Urtica urens* in broiler feed on the development of carcass characteristics, blood composition, and caecal VFA. For this purpose, 90-day-old chicks, were reared in 30 small pens (10 pens each treatment) and randomly divided into 3 treatments; control (CT), control diet with *Urtica urens* at concentrations of 1% (UU1) and 2% (UU2). On day 36 of the trial, 10 chickens of each treatment were slaughtered to obtain the different measures.
Results: Carcass yield, breast pH, and skin color were measured and we didn't find any differences between the treatments. Regarding blood analysis, TAG Triacylglycerols, Glucose, Total protein, Albumin, Globulin, Total bilirubin, AST- aspartate transaminase, ALT - alanine transaminase+, ALP - alkaline phosphatase, GGT – glutamyltransferase and Uric acid are not different. Also, caecal VFA, Acetic, Propionic, and Butyric did not differ.

Discussion: These results suggest no effect of *Urtica urens on broiler* carcass characteristics, blood composition, and caecal VFA.

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Keywords: *URTICA URENS*, POULTRY, FEED, BLOOD, CARCASS, VFA

Inhibitory effect of Nisin Z against persisters cells of a Pseudomonas aeruginosa Diabetic Foot Infection isolate

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Diabetic foot ulcers constitute one of the major and severe complications of Diabetes *mellitus*, often associated with difficult-to-treat polymicrobial infections. *Pseudomonas aeruginosa* is a Gram-negative bacterium frequently associated with Diabetic Foot Infections (DFI) and, due to its increasing resistance to conventional antimicrobials and ability to form biofilms including persister cells, is a major cause of infection chronicity, prompting the development of therapeutic alternatives, such as antimicrobial peptides (AMP).

This study aimed to evaluate the inhibitory effect of the AMP Nisin Z on *Pseudomonas aeruginosa* persisters. To induce persister cells by a *P. aeruginosa* DFI isolate, both in planktonic and biofilm forms, the isolate was exposed to environmental pressures by two different methods, using ciprofloxacin and carbonyl cyanide m-chlorophenylhydrazone (CCCP). Then, persister status was confirmed by evaluating their susceptibility to ciprofloxacin after recovery. Finally, the effectiveness of Nisin Z was tested against these persister cells by exposing the cultures to various concentrations of this AMP.

Results showed the ability of both methods to induce persister cells, which after the recuperation exhibited an ciprofloxacin susceptible phenotype. Furthermore, assays performed with Nisin Z showed promising results against *Pseudomonas aeruginosa*, as this AMP presented a high inhibitory ability against *P. aeruginosa* persister cells when present in planktonic cultures. However, this nisin variant was unable to eradicate these cells when they were present in established biofilms.

In conclusion, an early treatment with Nisin Z applied to *Pseudomonas aeruginosa* DFI may be promising and constitutes a potential complementary therapy for the treatment of these complicated infections.

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Keywords: *Pseudomonas aeruginosa*; DIABETIC FOOT INFECTION; PERSISTER CELLS; NISIN Z

First report of Aeromonas spp. isolation from Oreochromis angolensis

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Background: *Aeromonas* spp. are gram-negative, motile, facultative anaerobic bacteria, frequently associated with fish infections. These can occur in an acute form, characterized by hemorrhagic septicemia, or as a chronic syndrome, with the formation of deep dermal ulcers.

Materials/methods: Two *Oreochromis angolensis* individuals from Aquário Vasco da Gama were submitted to necropsy. Samples from skin ulcers, gills, liver and heart were inoculated in Columbia Agar+5% Sheep Blood, MacConkey and Brain Heart Infusion agar, incubated at 37°C for 24 hours, allowing to obtain two isolates with different macroscopic morphologies, identified using API 20NE. Isolates' susceptibility profile was determined using 10 antimicrobials, according to CLSI, and they were screened for virulence factors production (biolfim, haemolysins, lipase, DNAse, protease, gelatinase) using specific media. Their virulence index (V.Index) value was determined.

Results: Both individuals had visible skin ulcers throughout the body and a heterogeneous liver texture. No other lesions were observed. *Aeromonas hydrophyla* was isolated from one individual's gills, and *Aeromonas sobria* was obtained from all samples. Isolates were susceptible to the majority of the antimicrobials tested. *A. hydrophyla* presented intermediate resistance to cefalexin, and a V.Index of 50%. *A. sobria* presented intermediate resistance to tetracycline, and a V.Index of 66.7%.

Conclusions: Despite being frequently associated with diseases in other fish species, to our knowledge this is the first report of *Aeromonas* isolation from *Oreochromis angolensis*. Although isolates' susceptibility profile is not worrisome, they present a high V.Index, revealing their pathogenic potential and the need to survey the presence of *Aeromonas* spp. in this species.

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Keywords: AEROMONAS HYDROPHYLA; AEROMONAS SOBRIA; ANTIMICROBIAL RESISTANCE; CEFALEXIN; TETRACYCLINE

Characterization of the effect of the administration of a food supplement on the intestinal microbiome of dogs

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Introduction: Faecal Microbiota Transplantation (FMT) is an experimental and promising method of modulating the gut microbiome. This work aimed to describe the effect of the administration of oral capsules containing freeze-dried faeces to dogs with diarrhoea for 2 months and to evaluate their long-term influence on the gut microbiome.

Material & Methods: The study design included 5 dogs from a kennel, from which 3 exhibited diarrhoea and the rest were included as controls, being developed during 4 months, including the monthly faecal collection, physical examination, and determination of faecal consistency with the Bristol scale. Capsules were characterized by quantifying the total number of viable bacteria present, and then, together with the faecal samples, their bacterial composition was determined by 16S rRNA gene sequencing.

Results: No side effects were reported throughout the study. The animals' faecal consistency improved, and, after the end of the FMT, Bristol scale values were maintained in 2 of the 3 animals. The capsules contained a satisfactory number of viable bacteria and had a high concentration of beneficial bacteria as revealed by 16S rRNA gene sequencing. During FMT, the animals' microbiome gradually changed to a composition associated with a balanced microbiota. After FMT, the opposite variation to that observed during the therapy was detected, but the influence of the capsules remained evident.

Discussion: Results allow to conclude that the capsules appear to have a positive effect on the modulation of the gut microbiota of animals with diarrhoea. However, further studies with a larger number of animals are needed to confirm these preliminary observations.
Support: This research was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Project UIDB/00276/2020 (Funded by FCT); and by the Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020 - AL4AnimalS).

Keywords: FAECAL MICROBIOTA TRANSPLANTATION; INTESTINAL MICROBIOME; DOGS; DIARRHOEA; FAECAL CAPSULES.

Case study: characterization of gastrointestinal parasites in small companion mammals

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Introduction: Exotic pets are increasing their presence in the daily life of the Portuguese population and at veterinary care centers, being highly prone to gastrointestinal (GI) parasitic infections.

Materials & methods: From November 2021 - April 2022, fecal samples were collected from 86 small pet mammals received for consultation at "Exoclinic": 59 rabbits (69%), 25 guinea pigs (29%) and 2 chinchillas (2%), aged between 2 months and 14 years. Samples were checked for the presence of gastrointestinal (GI) parasites by using the following quantitative and qualitative techniques: Mini-FLOTAC, McMaster, Willis flotation, natural sedimentation and modified Ziehl-Neelsen staining, as well as the adhesive tape test for the investigation of *Passalurus ambiguous*. After each positive result, the animals were subjected to treatment and a new sample was collected to compare the shedding levels obtained by Mini-FLOTAC and McMaster methods.

Results: Parasites were identified in 13 animals (15%), including 11 rabbits and 2 guinea pigs. For chinchillas, no GI parasites were detected. Of the positive animals, 92% were infected with coccidia and 8% with the nematode Paraspidodera uncinata.

Discussion: The average oocyst shedding was higher with Mini-FLOTAC in comparison with the McMaster method, although these techniques did not differ significantly. Coccidia were the most common GI parasite in young animals, and therefore it is highly recommended to carry out an early coprological before antiparasitic drug treatment. This study represents the first report regarding the implementation of Mini-FLOTAC in small companion mammals, which allowed the characterization of their GI parasitic fauna.

Support/interest disclosure: CIISA/FMV Project UIDB/00276/2020 and LA/P/0059/2020 – AL4AnimalsS (funded by FCT); João Lozano holds a PhD research fellowship 2020.09037.BD (funded by FCT).

Key Words: SMALL PET MAMMALS; GASTROINTESTINAL PARASITES; MINI-FLOTAC; COCCIDIA; PORTUGAL

CIISA CONGRESS 2022

INNOVATION IN ANIMAL, VETERINARY AND BIOMEDICAL RESEARCH

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POSTERS

SESSION 4
CLINICAL RESEARCH

A pilot study to evaluate the efficacy of omeprazol vs. omeprazol and valerian in the treatment of Equine Gastric Ulcer Syndrome (EGUS)

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Background: Stress from training/handling horses often leads to EGUS, resulting in discomfort/low performance. EGUS prevalence in sport horses rises to 80-90% during training/competition. Standard therapy is omeprazol, in many cases with low efficacy due to lack of appropriate control of risk factors.

Material & Methods: In this pilot study, a double blinded (clinicians+owners), randomized, standard treatment placebo controlled trial, we evaluated the efficacy of NT-new treatment (oral Omeprazol[2]+Valerian[3]) in the reduction of EGUS score[1] as compared to ST-standard treatment (oral Omeprazol[2]+Wheat Bran Placebo), over 56 days, in 6 ridden horses referred to the Equine Hospital, Veterinary College, Lisbon University, for gastroscopic diagnosis of EGUS and diagnosed with score>1[1]. Horses were randomly allocated to treatment group, following blocked randomization (blocks of two), to balance number of participants/group. The outcomes evaluated were physical exam, EGUS questionnaire, Grimace Scale, EGUS grading scale, gastric pH and serum cortisol.

Results: NTgroup n=3; STgroup n=3. Most relevant results refer to EGUS severity score, where mean+/-SD in NTgroup was 5+/-0 before treatment(b.t.), decreasing to 1.67+/-0.58 after treatment(a.t.), whereas in STgroup it was 4+/-1 b.t., only decreasing to 3.67+/-0.58 a.t. Gastric pH and serum cortisol did not show significant differences between groups at both time points. Grimace scale showed larger improvement after treatment in the NTgroup.

Discussion: In this pilot study, NTgroup showed a more marked decrease in EGUS severity score a.t., when compared to STgroup, indicating the importance of carrying out further larger studies with Omeprazole+Valerian. Serum cortisol was not a good indicator of stress.

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Keywords: VALERIAN, OMEPRAZOLE, EGUS, EQUINE, GASTRIC ULCER

Hallmarks of ovarian ageing in domestic species

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Introduction: Ovarian ageing is the major contributor to the age-related loss of fertility. Our previous works in mice evidenced the presence of multinucleated giant cells (MGC) in the stroma of aged ovaries. The aims of the present work were to identify and characterize the presence of ovarian MGC in different domestic species.

Material & Methods: Ovarian slices from 4 queens (seasonal poliestric; aged 6 to 156 months), 5 bitches (non-seasonal monoestric; aged 9 to 110 months), 15 goats (7 juvenile and 8 adults) and 16 sheep (8 juvenile and 8 adults), both seasonal poliestric, were used. Hematoxylin-Eosin and sudan black B (SBB) staining were performed to study ovarian morphology and identity ovarian presence of the specific population of MGC, respectively. Additionally, Picrosirius Red staining was used to evaluate ovarian fibrosis.

Results: Except for the queen, preliminary screening of the ovarian morphology points to a notable age-related decrease in primordial follicle pool. With the aid of SBB staining, MGC were identified in the ovaries of all species studied. They were observed in the ovaries of adult bitches, goats and sheeps before the age at which a decrease in fertility is denoted. Interestingly, MGC were scarce in the ovaries of aged queens. Picrosirius Red staining of goat and sheep ovaries showed a heterogeneous collagen deposition with less collagen in the ovarian cortex surrounding primordial follicles.

Discussion: Our findings show common features of ovarian ageing among different species, as the decline in follicle number and the presence of MGC. Differences observed in the queen point to ovulation as having an important role in ovarian aging.

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Keywords: OVARIAN AGEING, SENESCENCE, GIANT MULTINUCLEATED CELLS

The influence of different collection frequency intervals in the sperm quality of Standard and Miniature Bull Terriers

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Introduction: The quality of an ejaculate is a factor that influences greatly the outcome of matings and AI in dogs. In the present study, ejaculates from six adult Standard (SBT) and four Miniature (BTM) Bull Terriers dogs, were collected at two different time intervals, in order to determine whether these intervals could be associated with better semen quality.

Materials & methods: Two successive time series of 5 consecutive semen collections each (TS1, 24h *vs.* TS2, 48h), with one week apart, were performed in the same animals. The first and second semen fractions were collected and evaluated for volume, sperm concentration, total sperm cells per ejaculate, motility, vigour, normal and abnormal morphology, acrosome defects, and the percentage of live sperm. A multivariable mixed linear model for repeated measures was used to test the fixed effects of session (1 to 10), breed (SBT *vs.* BTM), age (≤4 *vs.* >4 old years), body score (3 *vs.* 4 points), and semen quality based on nine sperm traits; and animal as random effect.

Results: BTM produced less (P <0.001) volume (3.2±0.2 *vs.* 4.3±0.2 mL; LSmean ±SEM), total sperm cells (221.8±19.2 *vs.* 348.6±19.2 x10^6) and total defects (25.0±1.1 *vs.* 31.3±0.9%), but more percentage of live sperm (77.0 ± 1.4 vs. 71.7 ± 1.1%) than SBT. The collections affected (P<0.001) all parameters except volume, sperm concentration and mid piece defects. Overall, better values were obtained in TS2. As expected, ≤4 years old dogs (P<0.001) and body score = 4 (P<0.05) positively influenced several parameters.

Discussion: Despite differences between the two breeds, better values were observed in the TS2. The intensive semen collection (daily collection for 5 consecutive days), although feasible, appears to be less effective to complete the spermiogenesis process. A 48h-interval between collection sessions is recommended for AI purpose in these breeds.

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Keywords: DOG, BULL TERRIERS, BREEDING, FREQUENCY SEMEN COLLECTION, SEMEN QUALITY.

Use of ultrasound guided techniques for the diagnosis of air sac disease in parrots

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Introduction: The diagnostic approach to avian respiratory tract disease is classically preformed through history, physical examination, blood work and radiography. In the latest decades, endoscopy has also been increasingly used in avian patients, mostly because their anatomical features (namely the absence of diaphragm and the presence of air sacs) that make avian patients' perfect candidates for coelioscopy. However, the presence of fluid in the air sacs or in the coelomic cavity poses an absolute contraindication for endoscopy. On the other hand, the small size of many avian patients and the presence of air in the air sacs, has limited the use of ultrasonography in these species.

Material & Methods: Two African Grey parrots were presented to the Veterinary Teaching Hospital with lower respiratory tract disease. X-ray revealed increased density at the air sac level. The patients were anaesthetized with isoflurane for ultrasound guided aspiration of air sac contents, through a caudolateral thoracic air sac approach. Samples were sent for cytologic, bacteriologic and fungal examination.

Results: Positive results were obtained for both patients. In parrot 1, a diagnosis of *Aspergillus* infection was made, while in parrot 2, bacteriological culture yielded *Staphylococcus* sp. growth. Both patients were treated according to etiological diagnosis and made a full clinical recovery.

Discussion: The widespread availability of ultrasonography, together with the small invasiveness of ultrasound guided procedures compared with endoscopy, support the interest of this technique for the diagnostic of specific conditions in avian patients. In fact, a midline approach for ultrasound guided liver cytology is described in avian patients. On the other hand, very few (if any) other approaches seem to be published. These cases present an alternative approach for avian air sac disease.The authors' cases support the application of ultrasound guided techniques for the diagnosis of selected avian diseases.

Support/interest disclosure:
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Keywords: ULTRASOUND, PARROT, LOWER RESPIRATORY TRACT, AVIAN

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MDR1 1-delta mutation detection in Barbado da Terceira in Portugal

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Introduction: The 4-bp deletion in the MDR1 gene has been described to affect herding dog breeds. The aim of the present study was to determine the occurrence of MDR1 mutation in herding dog breeds in Portugal namely Barbado da Terceira and Cão Serra de Aires.

Materials & methods: Sample population included dog breeds affected by MDR1 1-delta mutation and dogs from the 2 portuguese herding dog breeds mentioned. In total 105 dogs were sampled. Oral swab samples were collected, and DNA extraction, PCR and Sanger sequencing were performed to detect the presence of the MDR1 4-bp deletion.

Results: The homozygous mutant MDR1 (-/-) genotype was detected in Australian Shepherds (4/32) and in Rough Collies (3/4). The heterozygous MDR1 (-/+) form was detected on Australian Shepherd (19/32), Swiss Shepherd*Border Collie (2/8) and Barbado da Terceira (7/23) breeds. The mutant allele was not found in the remaining genotyped breeds (Bearded Collie, Belgian Shepherd, Border Collie, Cão da Serra de Aires, German Shepherd, Jack Russel, and Labrador Retriever). The MDR1 mutation was detected for the first time in Barbado da Terceira dog breed.

Discussion: The mutation MDR1 1-delta rates in some of the dog breeds in Portugal seems similar to that obtained in other studies but was detected for the first time in Barbado da Terceira. This information is very important for breeders to develop breeding strategies to reduce the incidence of this mutation in predisposed breeds. And to clinicians to avoid adverse reactions after administration of P-gp substrates to animals.

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Keywords: MDR1 MUTATION; HERDING DOGS; PORTUGAL; PORTUGUESE BREEDS; PHARMACOTHERAPY

Diferential anatomo-pathological diagnosis between methiocarbe and rodenticides intoxication

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Introduction: The lesions most frequently found in the anatomopathological picture of Methiocarb intoxication in domestic animals, dogs and cats, are similar to those that appear in rodenticides intoxication making toxicological testing the key to the final diagnosis. The aim of this study was to better characterize the pathological lesions found in the methiocarbe intoxications detected at the LFT and LAP of FMV.

Materials & methods: The cases of Methiocarb intoxication in domestic and wild animals detected (by thin layer chromatography) in the analyzes toxicological test performed at the LFT, between 2015 and 2022, were reviewed with special focus on the anatomopathological picture found in the necropsies performed by the LAP.
Results: 29 cases of Methiocarb intoxication were confirmed, mostly in dogs (20) and cats (8), and there was also the detection of a positive case in an eagle. Most necropsied cadavers presented severe pulmonary congestion associated with acute pulmonary edema. In five of the cases, extensive intracavitary hemorrhages such as hemoperitoneum and hemothorax and/or pulmonary hemorrhage were also observed.
Discussion: The fact that the anatomopathological picture is non-specific is highlighted, leading to the need to include the investigation of Methiocarb in the differential diagnosis of cases of death of undetermined etiology.

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Keywords: METHIOCARBE, NECROPSY, INTOXICATION, DOGS, CATS

Is endometrosis in Miranda Donkeys influenced by eosinophils and age?

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Introduction: The reproductive system of the endangered Miranda donkeys is still poorly characterized. Endometrial chronic inflammation and subsequent endometrosis is associated with mare infertility, but little is known about similar conditions in jennies. Our study aims at decoding the donkey uterine immunobiology, evaluating the eosinophiles infiltrate and its correlation with age.

Materials and methods: Thirty-one endometrial biopsies from Miranda donkeys were evaluated according to the Kenney-Doig classification. Twenty high-field magnification per slide (5 for each stratum: compactum, spongiosum, luminal and glandular epithelium) were photographed and evaluated with ImageJ® software for cell counting. In addition, eosinophil counts were analyzed by Spearman´s rank correlation coefficient, using IBM SPSS statistics 28.0 software.

Results: Kenney-Doig´s category III showed an association with age (r=0.424, p=0.016). The correlation between eosinophil infiltrate was negative for Kenney-Doig´s categories category III, and category IIA (r=-0.106, r=-0.055). A positive correlation was seen in category IIb (r=0,139), nevertheless any category was significant (p>0.05).

Conclusions: This study sets the ground for further studies in the pathogenesis of donkeys´ infertility. Even though age can lead to several lesions in Miranda jennies, eosinophils in the endometrium seem unrelated with the endometrosis grade. Eosinophils play a significant role in endometrial immunity and homeostasis, although their specific function and mechanism of action are not yet fully understood. In mammals, the gynecologically controlled microenvironment and maintenance of adequate uterine health are promoted by immune cells that enable successful reproduction. Therefore, future studies are foreseen to unveil the role of eosinophils in endometrial homeostasis in the jenny.

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Keywords: DONKEYS; ENDOMETRIOSIS; ENDOMETRIUM; EOSINOPHILS; KENNEY-DOIG'S

Contribution to the evaluation of the benefits of pergolide in the treatment of equine pituitary pars intermedia dysfunction (PPID/Cushing's syndrome): reduction of helminth fecal egg counts (hFEC)

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Introduction: Equine life expectancy is increasing and PPID is the most common endocrine disorder of older equids. The standard treatment is pergolide, but PPID is often underdiagnosed as owners attribute many of its clinical manifestations to old age alone. Due to immunosuppression, these horses may have higher hFEC and need a more aggressive parasite control program.

Material & Methods: The efficacy of oral pergolide in the reduction of hFEC and time to egg reappearance was evaluated, as compared to no treatment, over 6 months. The characterization of the sample population included four steps: clinical examination; owner questionnaire; plasmatic adrenocorticotropic hormone (ACTH) concentration (inclusion criteria ≥29μg/ml); and hFEC. A total of 15 horses were evaluated. The treatment group included 10 animals treated with pergolide; the control group, with 5 animals, received no medication. Initially, all horses were dewormed in order to properly compare subsequent hFEC.

Results: Horses from the treatment group managed to maintain hFEC<200eggs/gram. Although their weight kept almost unchanged, body condition and cresty neck scores decreased to normal values. Horses from control group weren't able to keep hFEC below acceptable levels and body condition and cresty neck scores kept unaltered.

Discussion: The efficacy of pergolide and its benefits in the quality of life of horses with PPID were reinforced, increasing owner awareness. Improvement of body condition and cresty neck scores in the treatment group can be justified by fat redistribution due to pergolide. Horses under treatment did not increase hFEC above the accepted value, possibly for being less immunosuppressed.

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KeyWords: PPID; PERGOLIDE; FECAL EGG COUNT; HORSE; ACTH

Retrograde flushing of the zygomatic duct with acetylcysteine

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Introduction: Acetylcysteine has mucolitic properties and also acts as an antioxidant agent. Furthermore, it has also been used for retrograde flushing of the zygomatic duct in an attempt to dissolve eventual sialoliths and, in conjunction with the use of antibiotics and anti-inflamatories, to solve salivary sialoceles. This therapeutical approach is relevant as it may avoid the need for invasive surgical intervention, while preserving the integrity of the salivary tissue.

Materials & Methods: Medical records were retrieved (2000-2022), and collected data included history, clinical signs, diagnosis, treatment, and outcome. Four clinical cases in which a retrograde flushing of the zygomatic duct with acetylcysteine was carried out were identified.

Results: Our sample included 4 dogs (3 males, 1 female), with mean age of 7 ± 3.3 years (range 3 to 12 years) of the breeds Basset Hound, Boxer, Labrador Retriever and Beagle. Presenting complaints included exophtalmos (4/4), difficulty on opening the mouth (2/4) and ocular discharge (1/4). All the cases corresponded to zygomatic sialoceles and the treatment included antibiotic and anti-inflamatory therapy besides the instilation of N-acetylcysteine in the zygomatic duct. All the animals improved with this treatment. In 3/4 cases it completely solved the clinical signs within a week. The recurrent animal, scheduled for a new round of acetylcysteine flushing, passed away due to concomitant diseases.

Discussion: This technique allowed avoidance of more invasive surgical procedures, was well tolerated by the animals and there were no reports of associated complications. It is recommended as a co-adjuvant approach for zygomatic sialoceles.

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Keywords: ZYGOMATIC SIALOCELE; ACETYLCYSTEINE; MUCOLITIC; TREATMENT.

SNAIL/SLUG expression in feline mammary lesions

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Introduction: Feline mammary carcinomas are associated to high biological aggressiveness, early invasion and metastasis. The development of metastases depends on the ability of clones to migrate and invade the adjacent stroma, which is facilitated by the Epithelial-Mesenchymal Transition (EMT) characterized by the change from an epithelial to mesenchymal phenotype. SNAIL and SLUG transcription factors regulate the EMT and several studies associates it to high malignancy and low survival rates in woman breast cancer and canine mammary tumours.

Materials & methods: SNAIL and SLUG was evaluated in 43 spontaneous mammary nodules from the archive of HAPL-UTAD archive, using immunohistochemistry.

Results: Immunopositivity to SNAIL and SLUG was high in all non-neoplastic changes and neoplastic lesions, with more than 50% of the epithelial cells immunopositive. Most carcinomas (20/33) showed extensive expression (higher than 80% of the cells), being observed mostly in high-grade malignancy lesions.

Discussion: All lesions showed intense immunopositivity to these markers. In non-neoplastic alterations, the high expression may be associated to the intense migration of cells during the epithelial branching processes that characterize this alteration. In malignant lesions, immune-expression was associated to higher malignancy features. It is necessary new studies to better understand mechanisms involved in EMT of feline mammary tumours and its prognostic and predictive potential.

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Keywords: MAMMARY, FELINE, TUMOURS, EMT, CARCINOMAS

Is canine leishmaniosis a predictor of T-cell lymphoma?

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Introduction: Leishmaniosis is a disease induced by parasitic protozoa of the genus *Leishmania* and its prevalence is considerably high in the Mediterranean basin. Lymphoma is the most common haematopoietic neoplasia in dogs. Parasitic diseases such as leishmaniosis have been implicated in carcinogenesis, namely the development of lymphomas, thus the presence of these two diseases in the same tissue samples should be investigated.

Materials and Methods: Nine samples from dogs suspected of leishmaniosis and lymphoma were obtained from biopsy or necropsy and fixed in 4% buffered formalin, routinely processed, and stained with haematoxylin-eosin. Immunohistochemistry (IHC) was performed to identify T and B cells (anti-CD3 and anti-Pax-5 antibodies) and to confirm *Leishmania* spp. infection (anti-parasite serum).

Results: All these cases were suspected of *Leishmania* spp. infection and were diagnosed with lymphoma, with 5 in 9 cases classified as T-cell lymphoma and other as T-cell rich lymphoma. In 3 of these T or T-cell rich lymphomas the presence of *Leishmania* spp. was confirmed by IHC.

Discussion: This study attempts to establish a relationship between *Leishmania* infection and the development of T-cell lymphoma. The exact mechanism by which this intracellular parasite can promote the development of T-cell lymphoma is still not entirely understood. However, the balance of Th1/Th2 response associated with chronic immunosuppression and the prolonged antigenic stimulation seem to be important factors, leading to chronic inflammation and possible promotion of tumour development.

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Keywords: LEISHMANIOSIS; LYMPHOMA; IMMUNOHISTOCHEMISTRY; DOGS.

Reasons for sudden onset blindness in dogs and cats

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Introduction: This retrospective study aimed to describe the most prevalent causes of sudden onset blindness in dogs and cats.

Material & methods:The studied population included 32 dogs and 10 cats, admitted to the Teaching Hospital Ophthalmology Service between November 2017 and September 2022. Microsoft Office Excel was used to organize data and perform statistical analysis.

Results: The canine population involved 53% males and 47% females, of which 41% were mixed breed and 13% were Yorkshire Terriers. Amongst the remaining breeds were 6% Samoyeds, 3% Portuguese Water Dogs and 3% Chihuahuas. The cat population included 30% males and 70% females, with 90% mixed breeds and 10% Persians. Median age was 8±3.9 years for dogs and 8±4.6 years for cats, at the time of diagnosis. The most common etiologies in dogs were Sudden Acquired Retinal Degeneration Syndrome (31%) and retinal detachment (22%). The remaining diagnoses were optic neuritis (9%), intracranial tumor (9%), bilateral uveitis (6%), meningoencephalitis (6%), a suspected infection with Canine Adenovirus-1 (3%) and non-confirmed neurological problems (13%). In cats, the most frequent cause was bilateral retinal detachment (50%), followed by optic neuritis (10%), bilateral uveitis (10%), retinal hemorrhage (10%), trauma (10%) and intracranial tumor (10%).

Discussion: In both species, older animals were more prone to sudden onset blindness, but dogs showed a wider range of causes, with more than 50% being related to retinal problems. In most cats, sudden blindness was due to bilateral retinal detachment, often associated with arterial hypertension. Interestingly, neurological problems were more frequently seen in dogs when compared to cats.

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Keywords: SUDDEN; BLINDNESS; RETINAL; NEURITIS

Individual variability in the cryopreservation of Serpentina buck's semen

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Introduction: Cryopreservation is known to reduce semen quality, and to heighten individual variation when thawed. This study analyzes individual variation of cryopreserved semen of buck goats.

Materials & methods: Semen from seven Serpentina bucks was collected by artificial vagina and assessed immediately via optical microscope regarding mass motility, progressive motility (PM) and morphology. The samples were diluted with Andromed® to a concentration of 200Mspz/ml and frozen. The samples, 40 straws in total, were thawed to assess post-thaw PM, morphology and vitality through a smear. Each thawed sample was assessed by CASA system which evaluates sperm kinetics and subjected to hypoosmotic and thermoresistance test (TTR) evaluating plasma membrane integrity.

Results: Between fresh and thawed semen, a significant reduction ($p<0.001$) in subjective PM (80.88% vs 48.5%) was detected. Samples were graded by subjective PM at thawing: high freezability (>30% PM) and low freezability samples (≤30% PM). Significant differences were detected in vitality ($p<0.05$) and motility at 120min of the TTR ($p<0.001$) between groups. Evaluation through the CASA system showed that statistically significant differences were detected between groups: MOT ($p<0.001$), MOTP ($p<0.001$), VAP ($p<0.001$), VCL ($p<0.001$), VSL ($p<0.05$), ALH ($p<0.05$), RAPID ($p<0.001$), MED ($p<0.001$) and STATIC ($p<0.001$).

Discussion: This study demonstrated that there was individual variability in cryopreserved qualities of buck semen within this sample, with only 30% of animals reaching sufficient or adequate quality of ejaculates post-thawing. Therefore, we can conclude that animals used in cryopreservation programs must be carefully selected for their freezability parameters.

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Keywords: SERPENTINA BUCKS; CRYOPRESERVATION; SEMEN

Occurrence of canine lymphoma and tumours and the associated risk factors in a lab in Portugal

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Introduction: Lymphoma has been seen as one of the most common neoplasia in dogs. Our main objective was to assess prevalence and risk factors for canine lymphoma and tumours in general from a lab registry in Portugal.

Methods: Positive and negative cases for canine lymphoma and neoplasia registered at the Laboratory of Histology and Anatomical Pathology of UTAD, between 1992 and 2022 were retrieved. The risk of lymphoma or tumours by sex, age and breed was calculated as odds ratios (OR).

Results: Six thousand, three hundred fifty-six (n = 6,356) neoplasia were registered. The prevalence in male dogs (**44.95%)** was lower compared to the females **(56.57%)** and the males had a reduced odd of tumours **(**OR = 0.66; 95% **CI:** 0.62–0.72; $p<0.001$)**.** The higher than 11-year age group (66.72%) had a high prevalence and increased odds **(OR = 5.96; 95%** CI: **5.15–6.90; $p<0.001$).** Mixed breeds and 13 pure/defined breeds had increased odds of tumours when compared to the Saint Bernard**.** Two hundred thirty-four (n = 234) canine lymphoma cases were registered. Males were over represented compared to females with an OR of 1.91 (95% CI: 1.46-2.50; **$p<0.001$**). Dogs aged 3-5 years were at higher risk compared to lower than 2 age group with an OR of 3.69 **(95%** CI: 2.07-6.60**;** $p<0.001$). The Great Dane, Rottweiler and Dobermann dog breeds had increased odds of lymphoma**.**

Conclusions: This study confirms high risk factors previously reported, therefore this information could be advantageous in establishing early detection techniques and strategies in dog neoplasia management.

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Keywords: BREED; CANINE; LYMPHOMA; RISK; TUMOUR**.**

Urinary clusterin as a potential biomarker for the early diagnosis of chronic kidney disease in cats

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Introduction: Chronic kidney disease (CKD) involves a permanent reduction of functioning nephrons; symmetric dimethylarginine (SDMA) is a biomarker able to provide an earlier diagnosis. Urinary clusterin - a glycoprotein whose levels are altered in kidney injury - has been suggested as a potential biomarker for CKD. This study aimed to investigate if urinary clusterin levels differ between healthy cats and cats with CKD.

Material & Methods: 27 cats were allocated to 3 groups: 15 healthy cats under 7 years (Group 1); 5 healthy cats over 7 years (Group 2) and 7 cats diagnosed with CKD, over 7 years (Group 3). Healthy cats had no signs of disease and serum creatinine levels under 1.6 mg/dL. CKD diagnosis was based on persistent azotemia, increased SDMA levels or decreased urinary specific gravity. Urinary clusterin levels were expressed as urinary clusterin:urinary creatinine (uClu:uCrea) ratio, to account for discrepancies in urine density between animals.

Results: Median values for uClu:uCrea ratio were higher in group 3 (18.73±13.43 ng/mg) in comparison to group 1 (11.86±8.63 ng/mg) and 2 (7.68±7.02 ng/mg), although differences between groups did not show statistical significance. No statistically significant difference was found between SDMA and uClu:uCrea ratio (P=0.07) for group 1.

Discussion: Results suggests a difference in the median values for uClu:uCrea ratio between healthy cats and cats with CKD; however, lack of statistical significance between groups and the levels of SDMA and uClu:uCrea ratio does not allow yet to confirm urinary clusterin as a reliable marker for early CKD diagnosis in cats.

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Keywords: URINARY CLUSTERIN; SDMA; CHRONIC KIDNEY DISEASE; CAT

Canine humerus supratrochlear foramen morphological study

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Introduction: Condylar fractures represent approximately 52% of all humeral fractures in dogs and the majority involve the lateral epicondyle. Canine humerus presents the supratrochlear foramen (STF), a perforation between the epicondyles. Some authors disclose STF absence, but none have studied its morphology. We aimed to describe STF prevalence and morphometry in different size dog humerus.

Material & methods: 27 unpaired adult dog humeri were analyzed. Humeri were categorized into small (≤100mm) and medium/large bones (103-201mm), according to maximum longitudinal length. STF presence, shape, and osteometric measurements were determined using a caliper: a)longitudinal and transverse lengths, b)distance from STF margins to each epicondyle.

Results: STF morphometric analysis revealed that 71.4% (10/14) of small and 15.4% (2/13) of medium/large humeri were STF deficient. When present, STF area was lower in small humeri (2.4 ±6.16mm^2) compared to medium/large (20.5 ±16.24mm^2). Oval-shape STF with the long axis transversal were more common (60.0%; 9/15) than round-shaped (40.0%; 6/15). STF distance to the lateral epicondyle (10.5 ±2.57mm) was inferior to the medial epicondyle (15.9 ±4.37mm).

Discussion: The study provides new data on canine STF variation, valuable for orthopedic imaging diagnostics and treatment. Small dog humerus showed decreased size or absent STF more frequently, and STF was commonly oval-shaped and closer to the lateral epicondyle, where humeral condilar fractures are predominant. In humans, STF presence can act as a stress-riser or modify fracture patterns, as it is associated with a narrow medullary canal. Hence, STF variation in dogs could render different humerus vulnerability to distal condylar fractures.

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Keywords: SUPRATROCHLEAR FORAMEN; DOG HUMERUS; MORPHOLOGY; ORTHOPEDICS DIAGNOSTIC; HUMERAL CONDYLAR FRACTURE.

Cattle postpartum endometrial inflammation and ovulation is affected by season (Winter vs Summer) and breed

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Introduction: Heat stress, amplified by ongoing climatic changes, seriously affects cattle fertility. This study evaluated postpartum endometrial inflammation and return to ovulation in native and exotic breeds in Winter (n=78) and Summer (n=71).

Materials & methods: In each herd cows (Alentejana, AL, n=34; Mertolenga, MERT, n=55 and Holstein, HOL, n=60) had a ultrasound exam and uterine cytology at 33-45 days postpartum.

Results: Breed and season significantly affected the percentage of polymorphonuclear neutrophils (PMN) on endometrial cytology. HOL had higher mean PMN counts than AL and MERT in Winter (7.2±11.2 vs 2.3±5.3 vs 2.1±4.2, respectively; $p<0.001$). In Summer HOL and AL had higher PMN counts than MERT (3.0±5.5 vs 0.7±0.7 vs 0.4±0.9, respectively; $p<0.032$). The percentage of cows with a luteal phase indicative of postpartum ovulation was not affected by breed or season, being 74% vs 31% in AL, 54% vs 48% in MERT and 74% vs 62% in HOL, in Winter vs Summer, respectively.

Discussion: In conclusion, endometrial PMN counts were higher in Winter than in Summer, which may reflect a later uterine involution and/or a higher PMN recruitment against pathogens. In the latter case, this may indicate that Summer heat stress may impair uterine defense mechanisms, potentially contributing to the known Summer decrease in fertility.

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Keywords: CATTLE BREEDS, SEASON, ENDOMETRITIS, HEAT STRESS

Hip Dysplasia in a New Functional Rabbit Model: Histologic Assessment

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Introduction: Background: Hip dysplasia (HD) is an hereditary disease associated with joint malformation. Existing HD rabbit models result in permanent functional deformities. Hence, a functional surgical model is alternatively presented.

Materials & Methods: Thirteen 6-weeks-old male New Zealand white rabbits were randomly assigned into 3 groups: GI (n=3) control; GII (n=5) left hip instability surgery and right hip sham; GIII (n=5) instability surgery and a 3-day left hip cast immobilization. The instability surgery was accomplished by sectioning the teres ligament and the sham by accessing the capsule without sectioning. In the histologic assessment of femoral head cartilage, under Hematoxylin and Eosin staining, the cartilage structure (CS) and chondrocyte pathology (CP) were graded in 5 severity categories (cat) A (normal) to E (worst): normal (N, left and right hips-GI); instability surgery (IS, left hips-GII); sham surgery (SS, right hips-GII); instability surgery with limb immobilization (ISLI, left hips-GIII), and hips without surgery (HWS, right hips-GIII).

Results: The CS and CP categories distribution in different type of hips were statistically significant on Chi-square test (P<0.05), standing out: CS cat-A in N, the absence of cat-A in IS and ISLI, and the cat-E in ISLI; the CP cat-A in SS and HWS, the absence of cat-A in IS and ISLI, the cat-D in IS and cat-E in ISLI.

Discussion: The histologic evaluation revealed that the used rabbit model, IS and ISLI, are both efficient in the development of hip osteoarthritis, with higher severity in the ISLI. This functional HD model can be applied to evaluate new HD aspects in vivo.

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Keywords: FUNCTIONAL INDUCTION MODEL; HIP DYSPLASIA,;HISTOLOGY; OSTEOARTHRITIS; RABBIT

Innovative surgery proposal for restoring cat's facial functional and aesthetics after total rhinectomy using a biomaterial nasal prothesis

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Introduction: Nasal squamous cell carcinoma (NSCC) is an oncological condition common in cats. Resolution usually involves a total rhinectomy surgery resulting in a major patient's facial defect known as "phantom face". Long-term effects of the surgery are associated with chronic rhinitis, sneezing, and stenosis of the affected region. The present study was developed in cat cadavers with NSCC, submitted to a total rhinectomy, and reconstructed with a preliminary biomaterial nasal prosthesis. The anatomical scientific illustration was used to document accurately this new surgical approach.

Material & Methods: Cats with an NSCC underwent a total rhinectomy followed by facial rehabilitation surgery using a silicone elastomer nasal prosthesis. Surgery and Anatomy design a prosthesis, and Biomaterial team members selected silicone elastomer as the biomaterial appropriate for this anatomical area and goal. Due to the anatomical particularities of the nasal cavity being a difficult area to understand and work, strategies to guide and anchor the prosthesis in the caudal border of the horizontal aspect of the palatine bone in the choanal region easily and permanently were discussed.

Results: The prosthesis prototype used and its design showed an adequate fixation in the proper area after the surgery procedure, which was the critical step considering this surgical case study at that time. Surgical steps were registered with pictures supported by scientific drawings.

Discussion: The methodology used and the results obtained, allow us to jump to the next stage of the technique and start testing it on live animals.

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Keywords: INNOVATIVE-SURGERY, PROSTHETIC–REHABILITATION, BIOMATERIALS, SCIENTIFIC -ILLUSTRATION

Canine lymphoma: retrospective study of 100 clinical cases

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Canine lymphoma is a generic term referring to a broad group of cancer subtypes, which are differentiated based on their anatomical location, morphological criteria and immunophenotypic characteristics. It is one of the most common neoplasms in dogs, representing, in this species, close to 83% of hematopoietic neoplasms.
Worldwide, it is estimated that one in four dogs die because of cancer. In Portugal, in an animal cancer registry, of the total reported cases in 2019 and 2020, 80% were from dogs.

For this reason, we developed a study with the aim of characterizing 100 cases of canine lymphoma followed by the Oncology Service of the Hospital Escolar Veterinário da Faculdade de Medicina Veterinária de Lisboa, between March 2018 and April 2022.

The present study considered sex, breed, reproductive status, living environment, weight, classifications of canine lymphomas, as well as the methods used for diagnosis as the parameters under focus.
Through this data, we were able to conclude that multicentric lymphoma, B immunophenotype, older dogs (>7 years), sterilized females, mixed-breed dogs, Boxer dogs and dogs weighing 30 kg or more were predominant in the population sample, in agreement with previous studies. On the other hand, contrary to what has been reported in the literature, our data showed a higher prevalence of canine lymphoma in females (54%) and in dogs that live indoors.
This work highlights the importance of continued research on oncological diseases, so that new diagnostic and therapeutic approaches can be developed to provide better lives for pets.

Keywords: DOG; LYMPHOMA; ONCOLOGY; VETERINARY

Safety and efficiency assessment of inoculation in medium third of the tail for feline vaccination

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Introduction: Feline vaccines, although preventing infectious diseases, can trigger local inflammatory reactions and the development of fibrosarcomas (sarcomas associated with injection sites). Radical surgical excision is the major treatment but it is related with slow recovery, disfigurement and loss of anatomical function.
The aim of this study was to evaluate the efficiency and safety of inoculation in medium third of the tail for felines vaccination - an alternative inoculation local - and the serological responses produced.

Materials & methods: Cats from two shelters were divided into two groups (24 animals per group): vaccinated subcutaneously in interscapular region (Group A) and vaccinated subcutaneously in medium third of the tail (Group B). After the primovaccination protocol [vaccine against feline viral rhinotracheitis (herpesvirus), feline calicivirus and feline panleukopenia virus] was completed, a blood sample was taken from each animal to evaluate and compare the humoral response in both groups. Antibodies were measured with *ImmunoComb Feline VacciCheck® Antibody Test Kit*.

Results: The degree of tolerance was, mostly, favorable. On average, Group B had higher antibody titers for all three agents, although no statistically significant differences were found between the two groups for calicivirus (*p-value* = 0.8281) and feline panleukopenia virus (*p-value* = 0.9407). For herpesvirus statistically significant differences were found (*p-value* < 0.05).

Discussion and conclusions: The diversity of results reinforces the need to determine individual humoral response after primovaccination and before each adult scheduled vaccine booster. Importantly, our study shows that proposed inoculation site does not influence the humoral response obtained.

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Keywords: CATS; FIBROSARCOMAS; VACCINATION; TAIL; VACCICHECK

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Oral leishmanial infection in dogs

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Introduction: Canine leishmaniosis is transmissible to vertebrate hosts by phlebotomine sand flies. It is a parasitic disease caused by protozoa of the genus *Leishmania*, endemic in several countries and with high prevalence in the Mediterranean basin. Manifestations of leishmaniosis in the oral cavity have been reported occasionally. We present a series of three case of oral *Leishmania* infections diagnosed as an incidental finding after histopathology.

Materials and Methods: Two female and one male dogs were attended at the Veterinary Teaching Hospital of UTAD with oral clinical signs, on the tongue and in the mandible. The clinical aspects were different in the three cases, being the lingual lesions incidentally founded by the clinician, and the mandibular case associated with periodontitis and oral discomfort. In all cases, a biopsy of the lesions was collected and the material submitted to the Laboratory of Histology and Anatomical Pathology of UTAD.

Results: The histological examination of the tongue and bone lesions showed large macrophages with intracytoplasmic structures compatible with *Leishmania* spp. amastigotes. In the case of the mandible, the bone also present reabsorption. Immunohistochemistry with an anti-*Leishmania* hyperimmune serum confirmed the presence of amastigotes inside macrophages and free in the surrounded tissue in all cases.

Conclusions: Although oral leishmaniosis affecting the tongue has already been reported, some lesions could go unnoticed without careful clinical examination. We unexpectedly identified the parasites in a routine histopathological examination of the tissues, a circumstance which confirms the use histopathology, including immunohistochemistry, as a fundamental tool for the definitive diagnosis of leishmaniosis.

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Keywords:
DOG; LEISHMANIOSIS; ORAL; BONE; IMMUNOHISTOCHEMISTRY

Canine leishmaniosis: exploring the activity of dendritic cells from dogs under different antileishmanial prophylaxis strategies

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Introduction: Canine leishmaniosis (CanL) is an endemic disease in several countries, including Portugal. It is caused by the protozoan *Leishmania infantum*, a zoonotic species, being the dog its main host and an important reservoir of infection to humans. Therefore, it is important to prevent the spread of CanL, on behalf of the One Health perspective. Preventive measures include using repellents and/or insecticides, to hinder infection, and immune-stimulation techniques, such as vaccination or administration of immunomodulators, to induce a protective immune response, important to the control of infection. Dendritic cells (DCs) are antigen-presenting cells responsible for activating T-lymphocytes and have been investigated for their role as mediators between the innate and adaptive immune response. This PhD work aims to differentiate DC populations from peripheral blood mononuclear cells of clinically healthy dogs under different prophylaxis protocols, to explore their ability to present *L. infantum* antigens to T-lymphocytes and redirect the dogs' immune response.

Materials & methods: Microscopical, flow cytometry, and molecular techniques will be used to characterize monocyte-derived DCs (moDCs), by identifying the molecular signature, cytokine profile, cell morphology, and functionality of *in vitro L. infantum* pulsed-moDCs.

Expected results: It is expected that moDCs from dogs previously subjected to immunostimulant protocols will demonstrate higher ability to present *L. infantum* antigens to T-cells, reprogramming their activation towards a protective proinflammatory immune environment in the dog.

Discussion: This study will provide new insights on the role of DCs in defence against *Leishmania* spp. infection, and as potential tools in immune-precision prophylaxis and treatment strategies.

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Keywords: CANINE LEISHMANIOSIS; DENDRITIC CELLS; CYTOKINES; MULTIPARAMETRIC FLOW CYTOMETRY; PROPHYLAXIS.

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Epicutaneous immunotherapy as a new hope for canine atopic dermatitis: a proof-of-concept study

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Background: Allergen immunotherapy is a well-established treatment for canine atopic dermatitis (CAD), but new, non-invasive, safe, effective, and easy-to-use home-administration routes that promote owner's compliance are needed. Epicutaneous immunotherapy (EPIT) has been suggested as a promising alternative treatment for human allergies. This proof-of-concept study primarily evaluated EPIT's feasibility, effectiveness, and safety for CAD.

Methods: Sixteen client-owned dogs with spontaneous, nonseasonal, mite-sensitive CAD were enrolled for a 6-month, once-weekly, 12-hour EPIT. A costume-made 3D-printed device was designed to deliver the allergen-based formulation. Primary efficacy outcomes included the owner's assessed pruritus (PVAS10) and treatment efficacy (OGATE), and veterinarian-assessed skin lesions (2D-IGA). Secondary efficacy outcomes were the quality-of-life (QoL) and serological allergen-specific IgE's concentrations. Effectiveness was defined by the success of the primary efficacy outcomes, according to the ICADA's COSCAD'18 recommendations. EPIT was deemed safe if no severe side-effects occurred.

Results: EPIT effectively improved clinical condition, with a success rate of 73.3% for pruritus, 66.7% for skin lesions, and 93.3% for QoL. A good-to-excellent response to EPIT was rated by 93.3% of owners in OGATE. EPIT significantly improved PVAS10 ($p=0.000015$), 2D-IGA ($p=0.006$) and QoL ($p=0.000014$) scores over six months. A significant difference was evident within one month for PVAS10 ($p=0.003$) and 2D-IGA ($p=0.009$) scores. Seven dogs were partially desensitised to at least one mite and two fully desensitised to all mites. No severe adverse events were recorded.

Conclusions: This pioneer study emphasises EPIT's great potential as a novel, non-invasive, feasible, effective, safe, and well-tolerated CAD treatment, supporting further investigation on this promising therapy.

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Keywords: ALLERGEN IMMUNOTHERAPY; CANINE ATOPIC DERMATITIS; EPICUTANEOUS IMMUNOTHERAPY; TRANSCUTANEOUS DESENSITIZATION; VACCINE.

Establishment of Reference Intervals of Blood Biochemical and Hematological Parameters for Portuguese Merino Sheep

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Introduction: Reference intervals (RIs) are used for interpretation of clinical laboratory data and serve as a major decision-making tool in veterinary medicine. Merino Branca and Merino Preta sheep are autochthonous Portuguese breeds, mainly raised in the Alentejo Region. While blood-based RIs are available for several sheep breeds worldwide, to date none have been established for these Portuguese breeds. This study now presents RIs for certain common hematological and biochemical parameters for these sheep.

Material & Methods: Blood samples were collected between September 2019 and December 2021 from 1007 Merino sheep, aged 4 - 12 months, distributed among 35 farms in the Alentejo Region. RIs were estimated for five biochemical and 11 hematological parameters according to ASVCP guidelines. Statistical range values of RIs (95% confidence intervals) were calculated using non-parametric methods with R Statistical Software.

Results: The RIs, according to the International System of Units, are: Total protein (g/L) (52.94 - 77.13); Albumin (g/L) (25 – 38); ALP (U/L) (52.97 – 362.6); AST (U/L) (76.52 – 199.06); ALT (U/L) (7.6 – 28); Leukocytes ($10^{3}/\mu$L) (3.97 – 14.02); RBCC ($10^{12}/\mu$L) (7.53 – 11.97); Hemoglobin (g/L) (80.87 – 129); Hct (%) (25.05 – 37.30); MCV (Fl) (26.30 – 38.21); MCH (Pg) (9 – 12.53); MCHC (g/L) (291 – 371.23); Neutrophils segmented ($10^{9}/\mu$L) (0.73 – 6.88); Lymphocytes ($10^{9}/\mu$L) (1.88 – 8.18); Monocytes ($10^{9}/\mu$L) (0 – 0.89); Eosinophils ($10^{9}/\mu$L) (0 – 0.90).

Discussion: The newly determined RIs for blood biochemical and hematological parameters were similar or showed only mild differences from previously published data for other breeds. Differences observed may be explained by geographic, ecologic, climatological and dietary factors.

Support: this work was supported by project MERINOparasite PTDC/CVT-CVT-28798/2017

Keywords: BLOOD BIOCHEMICAL. HEMATOLOGICAL. MERINO SHEEP. REFERENCE INTERVAL

Reproducibility of Distraction Index measurement in the Dys4Vet semi-automatic software

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Introduction: Currently, the computer vision and artificial intelligence have transformed the medical imaging diagnosis. Computer-assisted software allows the automation of this processes. The first step of the development of this informatic programs is the human annotation of images to train computer vision models. On the diagnosis of canine hip dysplasia thousands of images are evaluated daily worldwide. Therefore, a computer program developed for this purpose can be very useful in terms of reducing the human resources involved and even improving the objectivity of the results.

Materials & Methods: Fifty distraction views of dogs older than 4 months (100 hips) were randomly selected from the Veterinary Teaching Hospital of University of Trás-os-Montes and Alto Douro. The DICOM viewer and image analysis semi-automatic software Dys4Vet was used for the measurement of hip distraction index (DI) by a researcher with experience (RWE) and two trained researchers (TR1 and TR2).

Results: The mean±SD DI of RWE, TR1 and TR2 was respectively: 0.446±0.167, 0.442±0.183 and 0.441±0.168. The differences in measurement of researchers RWE-TR1 and RWE-TR2 were not statistically significant in paired t-test ($P>0.05$). The inter-researcher Intraclass Correlation Coefficient between RWE-TR1 and RWE-TR2 measurements sessions was in both 0.97 and the minimum lower limit of 95% confidence interval was 0.95.

Discussion: Both trained researchers reproduce RWE DI measurements and can perform DI annotations in Dys4Vet platform with confidence for purposes of computer vision models training. The accuracy of automatic computer measurements is directly associated with the quality and number of annotations used in training.

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Keywords: DISTRACTION INDEX, HIP DYSPLASIA, DOG, Dys4Vet, COMPUTER VISION MODELS.

Osteomyelitis caused by Aspergillus terreus in a dog

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Background: *Aspergillus* species are ubiquitous organisms, but occasionally can be responsible for local or systemic diseases in humans and animals. In dogs, the most frequently reported mycosis associated with *Aspergillus* spp. is upper respiratory infection, and disseminated aspergillosis is uncommon.

Materials/methods: A 5-year-old dog, male, mix breed, was referred to the Veterinary Hospital of FMV-ULisbon for evaluation of lameness of the anterior arm. Samples of bone lesions found on CT were submitted to histopathological evaluation and bacterial and mycological culture. For bacterial culture, Columbia Agar+5% Sheep Blood, MacConkey and Brain Heart Infusion agar were used, incubated at 37°C for 24 hours. For mycological culture, sample was inoculated in Sabouraud Dextrose Agar at 25°C for 5 days. The isolate obtained was screened for virulence factors production (biofilm, haemolysins, lipase, lecithinase, DNAse, protease, gelatinase) using specific media. The Virulence Index (V. Index) value was determined.

Results: Histopathological evaluation revealed a focal chronic severe osteomyelitis of mycotic origin. Cultures showed no bacterial growth but an abundant growth of *Aspergillus terreus*. Isolate was identified through its macroscopic (brownish, white-bordered colonies, with floccose texture) and microscopic morphologies (hyaline hyphae and biseriate phialides). Isolate was able to produce hemolysin, lipase and DNase and presented a Virulence Index of 42.9%.

Conclusions: *A. terreus* is found worldwide in various environmental habitats, being rarely associated with difficult-to-treat disseminated infections in dogs. Virulence factors produced by this isolate may predispose to invasive infections, showing that virulence factors detection can help to differentiate pathogenic from non-pathogenic isolates.

Support/interest disclosure: 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).

Keywords: *Aspergillus terreus*, DISSEMINATED ASPERGILLOSIS, MYCOSIS, OSTEOMYELITIS, DOG.

The impact of diet on canine chronic diarrhoea: from the first approach to refractory cases – the referral perspective

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Introduction: Dietary management is a relevant therapeutic tool for canine chronic diarrhoea (CD). Diet trials are recommended as first-line therapy in CD, with or without concurrent supportive treatment. This study aims to assess the impact of dietary trials on second opinion or referred canine CD cases.

Materials & methods: A retrospective multicentric study was developed involving three European referral centres. Medical records of dogs presented for second opinion or referred with CD (>three weeks) were reviewed and dietary history detailed. Dogs with a suspected or confirmed extra-digestive disease were excluded. Clinical improvement was assessed according to clinical signs.

Results: 142 dogs were included; 57% (81/142) had never received a hydrolysed diet prior to consultation. Of these, 12% (10/81) were already on antibiotics, and 11% (9/81) on immunosuppressants. All 81 dogs started a hydrolysed diet, which allowed a clinical improvement in 70% (57/81) of them. A total of 18.3% of dogs (26/142) were classified as refractory cases, since they had been previously treated with hydrolysed diet, antibiotics/probiotics and immunosuppressants without clinical improvement. Of these, 89% (23/26) experienced another dietary transition after referral; 69% (16/23) as a single therapeutic change, while 31% (7/23) in association with ancillary therapy. A favourable response was identified in 69% (11/16) and 43% (3/7) of these cases, respectively.

Discussion: This study highlights the paucity of CD cases that have an appropriate dietary transition before referral, supporting its relevance in the therapeutic approach of this disease. Moreover, it supports that diet should also be reconsidered in refractory cases.

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Keywords: DOGS; CHRONIC; DIARRHEA; DIET; REFRACTORY

Minimal invasive technique to resolve impactions of the small colon in horses

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Introduction: Small colon obstructions that require surgical intervention have been reported to represent 4.2% of equine colic cases undergoing surgery in the United Kingdom and United Stated of America. The techniques described for resolution of impactions include enema under surgical guidance with a nasogastric tube, enterotomy and resection and anastomosis.

Material and Methods: Horses in which a small colon impaction (fecaloma) was diagnosed under exploratory laparotomy were included in this report. The small colon was exteriorized, gas decompression performed and a sterile disposable spinal and a non-sterile assistant injected sterile saline solution (1 to 3 liters of LRS) into the lumen. During and after fluid installation the surgeon performed slow gentle manual massage of the impacted mass until breakdown was complete.

Results: The group included 4 females (50%) and 4 males (50%) (1 stallion, 2 colts and 1 gelding), with age ranging between 3 months (1 case) and 23 years old (mean 13.5 years). Time to achieve breakdown of the mass varied from 10-22min and total surgery time ranged between 60 and 150 minutes (mean 115 minutes). All horses survived after hospital discharge (100%). Only 2 had colic signs (25%), following recurrent SCI but resolved with medical treatment within 12 hours. Six horses (75%) developed a transient fever in the post-operative period, no other complications occurred.

Discussion and Conclusions: Although this simple technique might be used by many surgeons, we believe this to be the first clinical report to describe it and its outcome.

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Keywords: COLIC; SMALL COLLON; HORSES; MINIMAL INVASIVE

Treatment of chronic septic arthritis with 0.05% chlorhexidine solution: Outcome and Survival

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Introduction: Septic arthritis is a life threatening condition for a horse. Despite gold standard treatments, recurrence is common and can often lead to euthanasia due to the poor prognosis, or economic constraints [1]. Lavage with 0.05% chlorhexidine solution has been precluded as an alternative but outcomes have not been described. [2] [3]

Materials and Methods: Chronic septic arthritis was defined as unresolved cases following one (often multiple) standard procedures. Due to poor prognosis and/or economic constraints, these cases were treated by joint lavage with 0.05% chlorhexidine solution followed by a combination of regional and systemic antibiotics. The joints were lavaged for a minimum of 15 min and depending on the structure affected at least one litre of normal LRS was flushed after this. The short and long term outcomes were reviewed.

Results: Eight adult horses, age 3 to 18 yo met the inclusion criteria. Synovial structures affected included the fetlock (2), tarso-crural (1), and distal interphalangeal plus carpal joints or the navicular bursa (3). All horses received systemic antibiotics and regional perfusions. Five horses were alive at least six months following discharge. Two of these were in full work, two were sound for breeding purposes and one was still ridden but with limitations.

Discussion/Conclusions: Lavage with 0.05% chlorhexidine solution should be considered as a salvage procedure. Although the outcome for full recovery should be considered guarded (2/8), the outcome for survival was considered to be fair (5/8). We believe that this approach can be used as a salvage procedure.

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KEYWORDS: SEPTIC; ARTHRITIS; EQUINE; CLORHEXIDINE; SURGERY

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