

1st Meeting of the Associate Laboratory for Animal and Veterinary Sciences (AL4Animals)

Faculdade de Medicina Veterinária da Universidade de Lisboa, MAY 6th-7th 2022 | Auditório B

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PROGRAMME

MAY 6th, Friday

08:15 Registration

09:00 Welcome and Opening Session

AL4AnimalsS Overview and Goals of the Meeting

Luís Lopes-da-Costa, Director AL4AnimalsS

Opening Word

Helena Pereira, Presidente da Fundação para a Ciência e Tecnologia

The AL4AnimalsS Partner's Collaboration and Public Policies

Chairman: Luís Lopes-da-Costa

09:15 INSA - Instituto Nacional de Saúde Dr. Ricardo Jorge

Ana Vasconcelos Correia, Director INSA, Porto

09:30 INIAV - Instituto Nacional de Investigação Agrária e Veterinária

Nuno Canada, President INIAV

09:45 DGAV - Direção Geral de Alimentação e Veterinária

Susana Pombo, Director DGAV

10:00 Coffee Break – Poster Session

AL4AnimalsS Thematic Lines

Thematic Line | "Green Animal Production": From Farm to Fork

Chairman: Rui Caldeira

10:30 CIISA, Quality of Animal Products Lab:

Novel Feeding Strategies for the Sustainable Production of Monogastrics and Enhanced Meat Quality

José Prates

10:45 CECAV: Valuation of Animal By-Products in Animal Nutrition

André Almeida

11:00 CIISA, Animal Production Systems Lab:

Insights in Rumen Metabolism and Microbial Ecosystem: From Biohydrogenation to Methanogenesis

Rui Bessa

11:15 CECAV, Animal Production Group:

Body, Carcass Composition and Meat Quality of Animals - Use of Non-Invasive Imaging and Spectroscopic Methods

Severiano Silva

11:30 CECAV, Animal Production Group:

Experimental Facilities for Production Animals

Divanildo Monteiro

11:45 CIISA, Food Safety and Technology Lab:
A Sneak-Peek into the Food Technology and Safety Laboratory
 Maria João Fraqueza

12:00 CECAV, Veterinary Public Health Group:
Zoonosis and Zoonotic Agents in Meat Production – One Health Concept
 Maria Madalena Pinto

12:15 CECAV-Veterinary Public Health Group:
Microbial Safety of Processed Meats with Reduced Preservatives. Sensory Implications
 Luís Patarata

12:30 Lunch – Poster Session

Thematic Line | “Emergent Infectious Diseases and Zoonosis”: A One Health concept
Chairman: Cristina Guedes

14:00 CIISA, Antibiotic Resistance Lab:
Dynamics of Multidrug Resistant Bacteria Transmission in Companion Animal Health
 Maria Constança Pomba

14:15 CECA, Antibiotic Resistance Group:
Antibiotic Resistance from a One Health Perspective
 Manuela Caniça

14:30 CECAV, Microbiology and Antibiotic Resistance Team (MicroArt):
Antibiotic Resistance in the One Health Concept
 Patrícia Poeta

14:45 CECA, Emergent and Zoonotic Diseases Group:
West Nile – One Health Approach to Ensuring Surveillance
 Maria João Alves

15:00 CIISA, Infectious Diseases Lab:
Development of a DIVA Vaccine Against African Swine Fever and Epidemiological Studies on Infectious Diseases of Animals
 Fernando Boinas

15:15 CIISA, Parasitology and Parasitic Diseases Lab:
New Insights on Therapeutic and Prophylactic Strategies on Parasitic Diseases: A One Health approach
 Isabel Fonseca

15:30 CIISA, Tropical Animal Health and Production Lab:
Apicomplexan Parasites and the Challenge for the Development of Effective Vaccines
 Alexandre Leitão

15:45 CECA, Emergent and Zoonotic Diseases Group:
Risk Assessment of Emergent and Zoonotic Protozoa
 JM Correia da Costa

16:00 Coffee Break – Poster Session

Thematic Line | “Comparative and Translational Medicine and Biotechnology: Towards Precision Medicine”
Chairman: António Duarte

16:30 CIISA, Microbiology and Immunology Lab:
New Therapeutic Strategies to Overcome Infectious, Oncologic and Degenerative Animal and Human Diseases in a One Health Context
 Luís Tavares

16:45 CECA, New Diagnostic and Therapeutic Tools Group:
Natural Extracts with Antioxidant and Antimicrobial Capacity from Plants and By-Products of the Food Industry
 Ana Sanches Silva

- 17:00** CECA, Metabolic Diseases in Animals Group:
Development of RNA Therapies and their in vitro and in vivo Experimentation Models
Sandra Alves
- 17:15** CECA, Experimental Surgery and Regenerative Medicine Group:
Novel Therapies for Regenerative Medicine in One Health Perspective
Ana Colette Maurício
- 17:30** CIISA, Pathology Lab:
Biomarkers and Targeted Therapies in Feline Mammary Carcinoma: Past, Present and Future
Jorge Correia
- 17:45** Water Break
- 18:00** CECAV, Veterinary Public Health Group:
Translation and Comparative Research on Animal Spontaneous Disease
Maria dos Anjos Pires
- 18:15** CIISA, Clinical Research Lab:
New Trends in Translational Clinical Research: Animal Models of Disease
Esmeralda Delgado
- 18:30** CIISA, Animal Genetic Resources Lab:
Animal Genetic Diversity and Adaptation to Climate Change
Luís Telo da Gama
- 18:45** CECA, Biotechnology in Animal Reproduction Group:
Biothechnologies in Animal Reproduction: History, Perspectives and Collaborations
António Rocha
- 19:00** CIISA, Reproduction and Development Lab:
Meeting Scientific Unresolved Questions to Address Medical and Zootechnical Challenges
Luís Lopes-da-Costa
- 19:30** Depart for Dinner

- 09:00** Assembling of groups
- 09:15** Group meetings and interplay
- Room 1 | Green Animal Production**
Moderator: Rui Bessa
- Room 2 | Emergent Infectious Diseases and Zoonosis**
Moderator: Alexandre Leitão
- Room 3 | Comparative and Translational Medicine and Biotechnology**
Moderator: Rui Damásio
- 10:30** Coffee Break – Poster Session
- 11:00** Group meetings and interplay (cont.)
- 12:45** Closing Session – Auditório B
- 13:00** Lunch and farewell

POSTER SESSION

P1	CIISA, Animal Behaviour and Welfare Lab: <i>Welfare Assessment in Farm Animals – Ensuring Sustainability and Acceptability of Animal Production</i>	P11	CECAV, Veterinary Public Health Group: <i>Multispecies Approach to Fertility Preservation</i>
P2	CECAV, Animal Production Group: <i>Animal Welfare and Behaviour</i>	P12	CIISA and AL4Animals: <i>Endangered indigenous pigs of Angola: a key for rare mutations for adaptation</i>
P3	CECAV, Animal Production Group: <i>Animal Nutrition - Experimental Unit and Laboratory</i>	P13	CIISA, Clinical Research Lab <i>Cannabidiol clinical applications in oral health</i>
P4	CECAV, Veterinary Public Health Group: <i>Bioterium</i>	P14	CIISA, AL4Animals <i>A novel tolerogenic liposome-based allergen immunotherapy for dogs in the horizon</i>
P5	CECAV, Veterinary Public Health Group: <i>Laboratory of Parasitology, Department of Veterinary Science (ECAV, UTAD)</i>	P15	CIISA, AL4Animals <i>CBD-containing drug delivery systems as a novel therapy in dogs with atopic dermatitis</i>
P6	CECAV, Veterinary Public Health Group: <i>CRAS-HVUTAD: Expertise for Wildlife</i>	P16	CIISA, AL4Animals / NOVA PHRC, CHRC <i>Effectiveness of antimicrobial strategies directed at tackling antimicrobial resistance in animal production: a systematic review and meta-analysis protocol</i>
P7	CECAV, Veterinary Public Health Group: <i>HVUTAD: Education, Science and Clinical Service</i>	P17	CIISA, Clinical Research Lab <i>Therapeutic strategies in oral cancer</i>
P8	CECAV, Veterinary Public Health Group: <i>The Importance of Normal in the Concept of "One Health"</i>	P18	CIISA, AL4Animals, FF <i>A topical bioactive moisturiser for canine atopic dermatitis: the dawn of a new era for primary prevention?</i>
P9	CECAV, Veterinary Public Health Group: <i>Genotoxicity and DNA Repair</i>	P19	CIISA, AL4Animals, FF <i>Lipidomic analysis of Stratum Corneum in Canine Atopic Dermatitis</i>
P10	CECAV, Veterinary Public Health Group: <i>Hip Dysplasia - Diagnosis and Treatment</i>	P20	CIISA, AL4Animals, <i>Delivery of epoetin beta by chitosan-hyaluronic acid nanoparticles through subconjunctival and topical ocular administrations in rats</i>



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POSTERS

Welcome to the first Meeting of the Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)!

Following the signature of the contract programme with FCT and the holding of the first Scientific Council, this Meeting of AL4AnimalS represents the starting point of the Associate Laboratory activity.

First objective of this Meeting is to illustrate the research currently conducted within the three Centers – CIISA, CECA and CECAV, by the different laboratories and groups. Here, the idea is to know each other's research lines and goals, consolidated methodologies, infrastructures available and teams. This is presented by the Lab/Group Leaders during the brainstorm immersion session of Friday, 6th May.

The above session is expected to allow the kick-off in close interactions between the Labs/Groups, regarding the joint submission of projects, supervision of students, publications, organization of seminars/workshops and courses, training and mobility between Labs and share of equipment and infrastructures. Indeed, AL4AnimalS established himself as the platform for the synergic development of research activities of the three Centers exclusively devoted to Animal and Veterinary Sciences. These synergies are expected to start to arise in the interplay sessions of Saturday, 7th May.

A second objective of this Meeting is to bring us our Strategic Partners. We will hear from INSA, INIAV and DGAV, the current agendas for which AL4AnimalS research is welcome. Of mainstream relevance for AL4AnimalS mission, our Strategic Partners will point out the public policies, addressing current and future challenges that need research, scientific and technical support, potentially representing opportunities of collaboration.

I hope that this atmosphere of scientific share and personal interplay establishes the foundation for fruitful synergic realizations and the start of AL4AnimalS mission statement and recognition!

Sincerely,

Luís Lopes-da-Costa
Director AL4AnimalS

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ORAL COMMUNICATIONS

Thematic Line

“Green Animal Production”: From Farm to Fork

Novel feeding strategies for the sustainable production of monogastrics and enhanced meat quality

José A. M. Prates, CIISA - Centre for Interdisciplinary Research in Animal Health, Laboratory of Quality of Animal Products

The research team of Quality of Animal Products (QAP) lab is composed by 1 Full Professor, 2 Auxiliar Professors, 4 Post-Doc Researchers and 4 PhD Students. The QAP lab is currently dedicated to the societal challenge of sustainable production and quality improvement of poultry meat and pork. In fact, the estimated increase in world population (from 7 to near 10 billion; UN, 2020), combined with the expected improvement in income, will double the global demand for animal-derived products by 2050 (FAO, 2007). As poultry meat and pork are the two most consumed meats worldwide, this increased demand will be particularly problematic for monogastric production systems, due to the unsustainability of the current allocations of corn and soybean for food and feed productions (FAO, 2011). In addition, the nutritional value of lipids from these meats is poor due to the low contents of the beneficial n-3 long-chain polyunsaturated fatty acids and lipid-soluble antioxidants.

In line with this, the main thematic line of QAP lab is the valorisation of sustainable feed resources, predominantly from marine origin (microalgae and seaweeds) but also terrestrial by-products, to improve monogastric production and its meat quality. This thematic line is currently funding with the three following projects: SW4F (PTDC/CAL-ZOO/30238/2017, 231 m€, Sep 2018 - Jun 2022), NF4T (PTDC/SAU-NUT/2017/30455, 232 m€, Sep 2018 - Sep 2022) and AlgaDigest (P2020 17/SI/2019/70114, 960 m€, Jun 2021 - Jun 2023). Seventeen papers on this topic were published in the last year (2021-22) by the QAP lab.

For a brief contextualization, the use of algae as feed ingredients, mainly microalgae (e.g. *Spirulina* and *Chlorella*) but also seaweeds (e.g. *Laminaria* and *Ulva*), is very promising as an alternative to corn and soybean, thus mitigating the current competition among food-feed-biofuel industries. However, algae have low nutrients digestibility due to the recalcitrant cell walls and to the resistance of protein complexes to the action of endogenous peptidases. Therefore, algae are largely indigestible by monogastric animals and, therefore, our scientific challenge is to improve algal nutrient accessibility and utilization by animals through the use of rational mixes of enzymes.

The research plans of this thematic line have typically the following steps: production in *E. coli*, by high throughput protocols, and selection of the recombinant enzymes from marine origin most efficient for the *in vitro* fine-tuning degradation of algae recalcitrant structures; cost-effective large-scale production (in fungi) of the selected enzymes for the degradation of algae target structures; growth and digestibility trials in poultry/swine fed with algae and supplemented with exogenous enzymes; assessment of the effect of feed enzymes on growth performance, health status, carcass traits and meat quality of broilers/swine fed algae-containing diets; elucidation of the mechanisms involved: impact on feed digestibility, gut microbiota, serum metabolites and integrated transcriptomic-proteomic-metabolomic analysis of gut, muscle and liver; influence on eating quality and lipid nutritive value of meat.

The equipment available at CIISA enables the in-house execution of meat chemistry (including HPLC-DAD/FD/ECD and GC-FID/MD techniques), meat quality (including a meat trained sensory panel), biochemistry and molecular biology methodologies. Our national and international

collaborations provide the animal house facilities and experimental abattoirs required for poultry and swine (piglets and pigs) trials, as well as assistance in haematological and multi-omics analyses.

Supported by FCT PTDC/CAL-ZOO/2017/30238, PTDC/SAU-NUT/2017/30455, UIDB/00276/2020 and LA/P/0059/2020-AL4Animals, and P2020 17/SI/2019/70114 grants.

Valuation of animal by-products in animal nutrition

André Almeida, ETSA and CECA

The transformation of animal by-products into animal feeds, protein meals or processed animal proteins and animal fats, is a very old practice known as rendering. In the year 2000, due to the Bovine Spongiform Encephalopathy (BSE) crisis, the European Commission implemented a strong Legislation regarding the categorization and uses of animal by-products and derived products not intended for human consumption. 3 categories of animal by-products were defined regarding the risks to public and animal health, the way these by-products are processed, placed into the market and how they circulated in the European Union. The so called “Animal by-products Regulation” nº 1069/2009 and others were and still are in place.

Rendering is a very sustainable activity which applies both a nutritional and economic circular model in up-cycling value of the animal by-products. The use of sustainable ingredients and products is more mandatory than ever, and the rendering activity is a clear positive contribute to the Circular Economy and sustainability of the animal protein as an ingredient. Following the sanitary rules in the control of Encephalopathies and other diseases, Europe has been recovering proteins and fats from animal by-products and introducing these ingredients into animal feeds in a unique way in the world.

These animal by-products need to be processed in an authorized unit according to standardized and defined processing methods and the final derived products, protein meals and animal fats, may enter or not into the animal feed chain, depending on the category they derived from. These products may also be used to produce fertilizers or energy production.

In the last 20 more years, Europe has clearly set very high standards of animal by-products traceability and processing into high value products. Complying with a very strong European Legislation, companies operating in Europe also developed strong technological skills which improved the value of the final products. Under certain conditions, the correct separation of animal parts and species segregation, together with the use of robust technologies, allow the obtention of high value products, useful in the pharmaceutical and nutraceutical market. The application of

hydrolyzation technologies has recently allowed also a very high value products in the animal feed, particularly in the petfood and aquaculture field. In this last case, the use of high value hydrolyzed proteins is particularly interesting in the feed of young stages of several fish, the larvae, in which the nutritional profile, the digestibility and bioavailability of the proteins are crucial for the fish growth.

Due to a long and impeccable track record of traceability and feed safety, rendering and feed companies operating in Europe are assisting to an adaptation in the Regulation where the BSE-derived feed ban is less restricting. Recently, a very significative and important measure was taken: the use of porcine processed animal protein is now allowed in the poultry feed. Meals containing ruminant proteins are still banned in the animal feed, but its exportation outside Europe is allowed. Always committed with animal safety, the animal derived proteins and fats are gaining more value in the animal nutrition.

Insights in rumen metabolism and microbial ecosystem: From biohydrogenation to methanogenesis

Rui J. B. Bessa, CIISA, Animal Science and Food Safety Group, Animal Production System Lab

Research goals and ongoing projects: The CIISA's Animal Production System (APS) lab is generally involved in increase animal/herd productivity and optimize feeding to improve animal product quality. Although we could study most farm animal species and equines, our major research target have been the ruminants. In fact, most APS lab research has been on the study on rumen metabolism and its impact on productivity and product quality. The main rumen metabolic trait under study by APS lab has been the biohydrogenation (BIOH) of dietary unsaturated. In particular, we are interested in understanding the biology behind the alterations of BIOH pathways occurring on intensively finished animals (i.e. trans-10 shift) that negatively affect the nutritional quality of products. This basic knowledge is indispensable to develop practical production strategies for preventing the occurrence of the trans-10 shift and produce healthier meat and milk. These line of research have been funding for several projects in the last decade and there are still ongoing (PDR2020-101-031179 LegForBov, PTDC/CAL-ZOO/29654/2017 RUMOMICS, PTDC/CAL-ZOO/4515/2021 Gene2Rumen - New insights into the variability of ruminal lipid metabolism in lambs). More recently we have been initialing the studies on the strategies to mitigate methane production of beef cattle production systems (Project LISBOA-01-0247-FEDER-047050 GreenBeef) and in, particularly on the use of the macroalgae *Asparagopsis taxiformis* as antimethanogenic feed additive.

Consolidated methodologies: The lab team has a long experience in conduction ruminant nutrition experimentation including rumen in vitro methodologies and in vivo feeding trials with lambs and cattle, as well as carcass and meat

quality evaluation. One of the most distinctive traits is the deep expertise on detailed fatty acid (FA) analysis and other lipid metabolism analytical techniques, allowing us to study fatty acid metabolic pathways and identification of unusual FA.

Methodologies to study in vitro and in vivo rumen methanogenesis have been implemented more recently. In last month's we just start to implement LC-MS lipidomic and metabolomics based techniques.

Infrastructure and facilities (equipment): Facilities to conduct lambs and cattle feeding experiments as well as the experimental slaughterhouse are located at Fonte Boa (EZN-INIAV). The equipment for in vitro studies are available both at FMV (i.e. Gas Endeavour®) and EZN-INIAV (Ankon RF) facilities. Gas chromatography equipments for lipid and methane analysis are available both at EZN-INIAV (2 GC FID) and FMV (2 GC-FID and 1 GC-MS). EZN-INIAV has a classic animal nutrition lab able to conduct most of common feed and meat analysis. EZN-INIAV acquired recently the Greenfeed® instrument for measuring in vivo methane production in cattle. Recently the CIISA-FMV acquired a high-resolution LC-MS instrument.

Team: The core of APS lab team comprises senior researchers from both FMV and EZN-INIAV that have been involved in a long-term collaboration that started for more than two decades at EZN. Younger researchers, including MSc and PHD students are being recruited and involved in the APS lab research activities.

Body, Carcass Composition and Meat Quality of Animals - Use of Non-Invasive Imaging and Spectroscopic Methods

Severiano Silva, CECAV, Animal Production Group

The evaluation of body composition, carcass and meat composition and quality is an inescapable need in different animal and veterinary science fields. Examples are animal nutrition, breeding programs and a value-based payment and marketing system in the meat production chain. These aspects play an essential role in animal production in the present and more significantly in the future, contributing to being more resilient, adaptable and generating safe and healthy better food. Over the years, the assessment of body composition, carcass and meat composition and quality has been researched to develop fast, non-destructive and non-invasive techniques. Thus, non-invasive imaging and spectroscopic methods are under the scope of the CECAV research field and also aligned with Green Animal Production of the Lab4Animals thematic area. Under this research field, CECAV has developed work in vivo, in the carcass or meat, with imaging techniques such as real-time ultrasound, video image analysis, computer vision and computed tomography, as well as spectroscopic techniques such as near-infrared spectroscopy and hyperspectral imaging. These techniques have been applied to different farm species such as cattle, swine, sheep, goats, chickens, rabbits and fish. Many of these

works are carried out in collaboration with national and international partners.

Team: Cristina Guedes, Divanildo Monteiro, Jorge Azevedo, José Manuel, José Silva, Mariana Almeida, Mário Ginja, Miguel Quaresma, Severiano R Silva, Victor Pinheiro, Virginia Santos

Experimental Facilities for Production Animals

Divanildo Monteiro, CECAV, Animal Production Group

A Sneak-Peek into the Food Technology and Safety Laboratory

M.J. Fraqueza, CIISA, Animal Science and Food Safety Group, Food Technology and Safety Lab

The CIISA's Food Tech & Safe Lab is involved with emergent technologies improving food quality and proactively managing the health risks associated with biological and chemical hazards arising from the food chain in order to control zoonotic agents (namely *Campylobacter*, *Salmonella* and *Listeria monocytogenes*) and food chemical hazards (e.g. Polycyclic Aromatic Hydrocarbons, nitrosamines, biocides residues and contaminants). The food chain is one of the main critical routes of transmission of antimicrobial resistance from animal to human bacterial populations. Our team's main interest is to understand the strategies necessary to manage and implement preventive measures to protect consumer health in a one health approach. The possible sources and transmission routes of antibiotic resistance of pathogens associated with the pork and the poultry chain and its relationship with human infections is a primary interest of our lab. The assessment of foodborne risks and the burden of foodborne diseases are also the main research goals, which are necessary for the subsequent plan and implementation of preventive measures to reduce disease risk. One central research line of the Food Tech & Safe Lab is related to the development of healthy and safe food since there is a demand by consumers for safe, low-salt and low-fat meat products without chemical additives. The meat industry and all traditional sausage producers with rural insertion and low technological differentiation may be threatened due to concerns associated with the consumption of these products. So, it is essential to help them to achieve the goal of healthy products. The Food Tech & Safe Lab have been working in partnership with several industries developing and innovating meat products. So the use of new ingredients is important namely those with bioactive compounds with a beneficial impact on human health. These lines of research have been funded for several ongoing projects (PTDC/CVT-CVT/29510/2017, Farm2Fork-Identification of key steps on Antibiotic resistance dissemination through the food chain from farm-to-fork; PDR2020-101-031254 Campyfree: Strategies for *Campylobacter* control on poultry meat and meat products;

PDR2020-1.0.1-FEDER-031359 Safemeatprod: Strategies for meat products quality and safety with the reduction of potential carcinogenic hazards PTDC/OCE-ETA/1785/2020 Authentic CheesOMICs: from big data, through laboratory, towards the future of protected designation of origin). More recently, we have been initialling the studies on various strategies to mitigate Polycyclic Aromatic Hydrocarbons (PAHS) in smoked foods (LISBOA-01-0247-FEDER-068048 CleanSmoke-Industrial smoking products and processes). The Food Tech & Safe Lab team is experienced in food microbiology (OMIC technologies), chemistry, and optimisation of food processes. Our expertise is related to safety preventive measures and food quality and safety management systems. Methods such as biogenic amines and bases on chromatography and HPLC are implemented. In microbiology, all analytical ISO methods are in place, and various molecular biology methods such as the classic polymerase chain reaction (PCR), Multiplex PCR, pulse-field gel electrophoresis (PFGE) among other OMIC methods are utilised. The Food Tech & Safe Lab is equipped with food microbiology and food physicochemical lab facilities and a “Technological workshop”, a differentiated pilot-scale premise equipped to allow the reproduction of industrial food processes and offer the possibility of new food and processes development. The Food Tech & Safe lab team comprises young and senior researchers and technicians. Younger researchers, including MSc and PhD students, are recruited and involved in the Food Tech & Safe lab research activities.

Zoonosis and Zoonotic Agents in Meat Production – One Health Concept

Maria Madalena Pinto, CECAV, Veterinary Public Health Group

Microbial safety of processed meats with reduced preservatives. Sensory implications.

Luis Patarata, CECAV – Animal and veterinary Research Center, Integrated Meat Quality

The team’s research aims to assess factors that may affect sensory and chemical characteristics of meat and meat products and evaluate feasible and cost-effective strategies (e.g. packing, natural additives) to control spoilage and pathogenic microflora.

Framed in the project “SafeMeatProd (PDR2020-1.0.1-FEADER-031359), coordinated by CIISA-FMV we studied the

effect of eliminating nitrite from the processing of cured meat products on the survival of *Clostridium sporogenes* (used as a surrogate for *Cl. Botulinum*) *Listeria monocytogenes* and *Salmonella* spp. The use of phenolic compounds, from wine or other origins, with potential antimicrobial and antioxidant activity to improve the safety and the shelf life of meat and processed meats are the goal of the ongoing AgriFood (NORTE-01-0145-FEDER-000041) coordinated the CQ-UTAD and the CleanLabel (COMPETE) coordinated by CITAB-UTAD. The formation of Zn-protoporphyrin in cured meat products without nitrite is an ongoing work made with the strict collaboration action with the Chemistry Center. The problem of colour stabilisation in cooked, processed meats is being studied in both of these projects, namely by using red ingredients without nitrate. In the project OneCancer (Norte 20202) we intend mainly to consolidate knowledge on the safety of traditionally processed meats without nitrite, and evaluate the risk associated with traditional cooking (particularly barbecuing) on the deposition of harmful polycyclic aromatic hydrocarbons in meat. We also preview to evaluate the formation of other harmful compounds, like acrylamide during culinary processing. The consumption practices and behaviour of the consumers towards these products will be evaluated. From a slightly different perspective, non-meat ingredients in the so-called hydride products are previewed in the project Funghi4Health (NORTE-01-0247-FEDER-070171) coordinated in UTAD by CITAB, where the use of mushrooms will be tested to include in meat products.

There are other projects with involvement of the elements of the group, namely related to microbial assessment of meat, meat products and other foods, and evaluation of quality parameters of meat – instrumental texture, colour, chemical parameters associated with meat quality, and sensory analysis (for details, please check <https://cecav.utad.pt/>).

The methodologies used are based on classical microbiology and general chemistry laboratory, sensory analysis. The analytical approaches based on chromatographic methods are made with external collaborations. The pilot plant for (certain) meat products allow us to made pilot experiments with processed meats and packaging. The LTeQSA (Laboratório de Tecnologia, Qualidade e Segurança Alimentar) includes six career professor/researchers (Alexandra Esteves, Cristina Saraiva, José António Silva, Luis Patarata, Madalena Vieira-Pinto, Maria da Conceição Fontes) two laboratory technical assistants (Ana Leite e elena Santos) and a variable number of PhD fellows.

Thematic Line

“Emergent Infectious Diseases and Zoonosis”: A One Health concept

Dynamics of multidrug resistant bacteria transmission in companion animal health

Maria Constança Matias Ferreira Pomba, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal CIISA - Centre for Interdisciplinary Research in Animal Health, Antibiotic Resistance Lab

- Research goals and ongoing projects

An increase and spread of hospital infections caused by multidrug-resistant (MDR) bacteria has been observed which represents a global threat to patient safety and healthcare systems. Furthermore, as the present Covid-19 pandemic moves across the world, it covers a second slow-motion pandemic with it: the rise of antimicrobial use and antimicrobial resistance. Additionally, the current weak pipeline for antibiotic agents, in particularly against Gram-negative bacteria, may worsen the resistance problem. Animal health is also an important setting as such resistances have been found on animals. Lastly, the common environment is also a favored site for transmission from humans. Our Lab has during the last years produced a solid body of evidence on the role of animals as reservoirs of multidrug-resistant bacteria. Furthermore, our molecular epidemiology work as profiled the main antibiotic resistance microbiological hazards for companion animal health. Recently, we have described KPC-3 -, OXA-181 - and OXA-48 –producing Enterobacterales found on clinical isolates from companion animals. The ongoing research lines are also a natural evolution of the previously conducted research work (PET- Risk consortium JPIAMR 0002/2016). Particularly, the study on the role of companion animals as a direct setting for transmission of MDR bacteria to humans and their common environment. Recently, we have identified *E. coli* lineages harboring *mcr-1* in dogs and humans in the community and an unexpectedly high-frequency of *mcr-1* in commensal *E. coli* strains from dogs. To the best of our knowledge, this is the first worldwide report of fecal colonization and sharing of *E. coli* ST744 lineages harboring the same plasmid-mediated *mcr-1* gene, between humans and dogs (Eurosurveillance, 2022 in press). The ongoing research at the Antibiotic Resistance Lab on transmission of MDR bacteria on both the level of bacterial species and mobile genetic elements will provide a sound foundation for tailoring specific measures to prevent the development of an integrative approach that will enable rapid tracing of antibiotic resistance transmission in One-Health settings. This will ensure the establishment of interventional measures for mitigation of MDR bacteria transmission and shield public-health.

- Consolidated methodologies Antimicrobial Susceptibility Testing, Antimicrobial Molecular Mechanisms Characterization, NGS sequencing (short and long read), 16S metagenomics

- Infrastructure and facilities (equipment) Standard Microbiology Facility Biohazard II Molecular Biology Equipment (Conventional and real-time thermocyclers, Electroporator, DNA and Protein electrophoresis) Genomics (ISeq 100, McMinion)

Team Constança Ferreira Pomba, PhD Cátia Marques, PhD Adriana Belas, PhD Juliana Menezes, PhD student

Antibiotic resistance from a One Health Perspective

Manuela Caniça, National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRLAMR/HAI), National Institute of Health Dr. Ricardo Jorge (NIH), Lisbon, Portugal; Laboratório Associado para a Ciência Animal e Veterinária (AL4AnimalS), University of Porto, Porto, Portugal*

**Integrated member of CECA/ICETA; Collaborating member of CIISA; Leader of Antibiotic Resistance Group at CECA*

Different reservoirs play a crucial role in the spread of antibiotic resistance. In fact, human, food from animal and vegetable origin, food-, companion-, wild-, and zoo-animals, as well as soil, air and aquatic environment are hotspots for antibiotic resistance. The extent of the relationship between the occurrences of antibiotic resistant bacteria and pathogens in those reservoirs should be better clarified. How resistant bacteria in the environment can offer barriers and/or opportunities for antimicrobial drug discovery is also a relevant issue nowadays. Biofilm formation, namely in food chain, as well as non-expressing bacteria that stay susceptible to the antibiotics although carrying potentially transmissible resistance genes, and proteins providing resistance to two structurally and functionally different classes of antibiotics by acquisition of a single gene, are all interesting dimensions in the microbial adaptability, implicating in the performance of antibiotics in several reservoirs. The role of vertical and horizontal gene transfer (HGT) of antibiotic resistance needs to be deepened; this HGT either directly through contact or indirectly through the different niches (people, animals and environment) is assuming serious proportions. The responsibility of harmonization of phenotypic and genotypic methods and the implementation of guidelines is of up most interest, namely to measure the fitness cost of antibiotic resistance and to predict and/or decipher the real impact of this threat. How coordinated must be the human, veterinary and environment sectors to prevent the spread of antibiotic resistance is also an important topic (such as the antibiotic consumption in all sectors).

These scientific/strategic topics or related, in the field of Antibiotic resistance from a One Health Perspective, are proposed for discussion within the scope of the 1st AL4AnimalS meeting with a view to their collaborative development.

Antibiotic resistance in the one health concept

Patricia Poeta

MicroART – Microbiology and Antibiotic Resistance Team, develops research work focused on the use of omics tools, particularly genomics and proteomics applied to antibiotic resistance, at the molecular genetics and biotechnology level of various microbial species. The group performed studies in nosocomial Multidrug-resistant (MDR) organisms including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci and certain Gram-negative bacilli like *Pseudomonas aeruginosa* and Enterobacteriaceae like *Klebsiella pneumoniae*, *Salmonella* sp. and *E. coli* that cause severe and lethal human infections especially in critically ill patients. Research is focused in order to improve knowledge on prevalence, definition of contamination sources, risk to public health and strategies to improve One Health approach.

Emergent Infectious Diseases and Zoonosis: West Nile – One Health Approach to Ensuring Surveillance

Maria João Alves, INSA - National Institute of Health Dr. Ricardo Jorge, CECA - Centre for the Study in Animal Science

The One Health approach encourages the collaboration of many specialists and promotes interdisciplinary communication, namely between laboratories, physicians, veterinarians and can guarantee the effectiveness of surveillance and the prevention of many cases.

West Nile (WN) fever is a zoonotic viral disease currently considered a public health challenge due to its re-emergence in Europe. WN virus maintains itself in nature in enzootic cycles that involve mosquitoes as vectors and some bird species as reservoirs. Humans and horses are accidental hosts and transmission is mainly through mosquito bites, transmission by blood transfusion and organ transplantation has already been documented and represents concerns to the health services mainly in non-endemic countries.

Since the first discovery of West Nile virus in Portugal in the 60' in mosquito, birds, equines and humans, there was four human cases, all in the south, very well documented, and outbreaks of equine WN neurological disorders are reported almost every year.

Since 2008 we have been performing extensive mosquito surveillance under REVIVE (Rede de Vigilância de Vetores) and the geographical distribution of the WN vectors is well known as the probable effects of the climatic changes effects.

Bringing together case surveillance in humans, horses and vector population studies on the same work platform represents a benefit and defines the One Health concept.

Development of a DIVA vaccine against African swine fever and Epidemiological studies on Infectious diseases of animals

Fernando Boinas, AL4Animals - CIISA - Centre for Interdisciplinary Research in Animal Health, Infectious Diseases Laboratory

The Infectious Diseases Lab is organized in two different units:

- The African Swine Fever (ASF) Unit (ASFU) conducts studies related to the host response to relevant pathogens, namely ASF virus, aiming the modulation of host immunological responses towards development of vaccines, diagnostic and therapeutic tools;
- The Epidemiology Unit (EPIU) develops studies on the epidemiological evaluation of infectious diseases, zoonosis and emergent infectious diseases affecting livestock species, companion animals and wildlife, risk analysis, as well characterization of clinical presentations and predictors of outcomes of dog and cat infectious diseases.

The research goals and ongoing projects are: ASFU: Under the framework of the Horizon 2020 European Project VACDIVA are being performed studies aiming at the development of effective DIVA vaccines against ASF; EPIU: Several projects have been implemented with financial support from EU-COST, DGAV, the pharmaceutical industry and CIISA, namely on farm biosecurity assessment, biocides evaluation, wildlife tuberculosis and companion animals infectious diseases.

The consolidated methodologies are: ASFU: the characterization of the biological role of several non-assigned ASFV genes involved in viral replication and transcription and identification of ASFV-host cell interactions at molecular level; EPIU hospital-based surveillance strategies for companion animal infectious diseases, epidemiological studies of the wild fauna-livestock-environment interface for *Mycobacterium tuberculosis*-complex, and ecological studies on vector-borne diseases.

The infrastructures and facilities are: ASFU: A Biosecurity Laboratory of Level 3 (equipped namely with an horizontal autoclave, flow chambers, an ELISA system, centrifuges including a Cytospin, microscopes, CO2 incubators and ultra-freezers), a Cell Culture Laboratory and a Molecular Biology Laboratory. EPIU: The Biological Isolation and Containment Unit of the Veterinary Teaching Hospital (facilities for small and large animals) and a computer room at the Epidemiology Unit.

Presently the Infectious Diseases Lab gathers 13 researchers: ASFU: 4 internal researchers with PhD (Fernando Boinas, Carlos Martins, Fernando Ferreira, Claudia Istrate) and 1 external researcher (Ferdinando Freitas); 3 PhD students (Andreia Cruz; Catarina Urbano, Nuno Jordão); EPIU: 3 internal researchers with PhD (Fernando Boinas, Virgílio Almeida e Isabel Neto); 1 researcher with a MSc (Telmo Nunes); 1 PhD student 1 (Sara Madeira).

New insights on therapeutic and prophylactic strategies on parasitic diseases: a One Health approach

Isabel Pereira da Fonseca, CIISA – Centre for Interdisciplinary Research in Animal Health, Animal Health and Veterinary Medicine, Laboratory of Parasitology and Parasitic Diseases (LPDP), Faculty of Veterinary Medicine, University of Lisbon; Associate Laboratory for Animal and Veterinary Sciences (AL4Animals)

Research goals and ongoing projects: LPDP research interests include a diversity of parasites of Small/Exotic Pets, Livestock and Wildlife, some of them with zoonotic impact. Currently our goals are: (i) to investigate the potential role of exosomes to modulate the host immune response against trypanosomatids; (ii) to evaluate canine dendritic cell activity after exposure to different leishmaniosis prophylaxis strategies; (iii) to determine prevalence and diversity of gastrointestinal parasites in free-ranging Iberian wolf and other wild carnivores; (iv) biological control using predatory fungi as natural antagonists of gastrointestinal parasites in birds; (v) characterization of *Cryptosporidium* spp. and *Giardia intestinalis* genotypes aiming the creation of a surveillance online platform in Portugal.

Concerning the ongoing projects it is worth highlighting: EXOTRYPANO (IHMT/FMV) FCT - PTDC/CVT-CVT/28908/2017 (<http://exotrypano.fmv.ulisboa.pt>); Gastrointestinal parasites in the Iberian Wolf and other wild carnivores; DogIPM (IHMT/FMV) FCT, PTDC/CVT-CVT/0228/2020 (<https://dogipm.fmv.ulisboa.pt>); Gastrointestinal Parasites of Domestic Birds and their Biological Control with Predatory Fungi (FCT 2020.09037.BD); Resistant and tolerant autochthonous cattle breeds to *Theileria annulata* infection; Avian malaria in Strigiformes birds; Detection of vector borne diseases agents in ticks collected in mainland Portugal (MSD/FMV); Studies on *Culicoides* genus regarding wing interference patterns, sounds, insecticides and endosymbionts.

Consolidated methodologies: Direct morphological identification of several parasites and vectors such as *Culicoides* spp. and validation of mini-FLOTAC technique for exotic birds have been achieved; Serological (IFAT, ELISA, IM) and molecular (PCR and qPCR) techniques are currently used for diagnostics and research; Canine blood cultures for monocytes and dendritic cells differentiation; Predatory fungi cultures and molecular identification. Infrastructure and facilities: 5 rooms (routine diagnosis, entomology, tick breeding lab, fluorescence microscopy/PCR, helminthology); microscopes and stereomicroscopes, thermocycler, ultrasonicator, refrigerators, freezers, hotte.

Team: The LPDP' team is composed by six PhD, four PhD students and one laboratory technician.

Apicomplexan parasites and the challenge for the development of effective vaccines

Alexandre Leitão, Faculdade de Medicina Veterinária, Universidade de Lisboa CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, TAHP – Tropical Animal Health and Production Lab

Our laboratory has been focused on developing research and promoting scientific and technical capability on animal health and animal production issues with relevance in tropical environments, with a particular emphasis on Portuguese speaking countries. At present, our main research subjects are: 1) ticks and tick transmitted pathogens, 2) the Apicomplexa parasites *Toxoplasma gondii* and *Besnoitia besnoiti* and 3) the modulation of the host immune response as a basis for the future development of novel veterinary vaccines.

1) In animal production systems worldwide, ticks represent major threats for their direct blood spoliation and their vectorial capacity to transmit pathogens that include bacteria, viruses and protozoa. Presently, we focus on ticks and tick-borne pathogens distribution and molecular epidemiology, in Tropical and subtropical regions, where we have established partnerships on the ground (Angola, Guiné-Bissau, S. Tomé, Mozambique and Macaronesia islands).

2) Apicomplexa is a large group of protozoan parasites and *T. gondii* is simultaneously a well characterized Apicomplexa model and a serious one-health problem. Using reverse genetics tools such as random integration, homologous recombination and CRISPR/Cas9 based approaches, we have established a set of transgenic *T. gondii* strains to characterize the function of genes that participate in key aspects of the parasite's life cycle. MOB1 (Mps one binder 1) that regulates *T. gondii* replication, influencing tachyzoite to bradyzoite conversion. An analysis of the TgMOB1 interactome using a combination of bioinformatics and proximity biotinylation methods indicates that this conserved protein may act in *T. gondii* through both conserved and *T. gondii* specific pathways. TBCB (Tubulin cofactor b) is an essential protein in *T. gondii* that plays an important role in parasite invasion and replication. Fluorescence and high-resolution microscopy positioned TBCB as a polarity marker, being present at the anterior region of the parasite, under the conoid, and in close association with the polar ring and subpellicular microtubules, fundamental components of the Apicomplexa invasion machinery. Host cell invasion has also been studied in *B. besnoiti*, particularly the role of protein disulfide isomerase, using monoclonal antibodies and specific inhibitors. Moreover, the tissue tropism and large size of *B. besnoiti* cysts offers an excellent model to better understand the group of cyst forming coccidia that we aim to further explore.

3) When available, vaccines are the best approach to the control of infectious diseases. However, for several infectious diseases the development of safe and effective vaccines remains a challenge. We assume that disclosing

the mechanisms that link the activation of innate immunity and the differentiation of effector and memory lymphocytes is the best strategy towards programming properly polarized T cell responses at the point-of-entry of pathogens and, therefore, towards a rational development of effective immunogenic formulations. In the last years, we established an *E. coli* expression system to produce antigens in fusion with a Toll like receptor 2 (TLR2) ligand – the Opr1 lipoprotein – and showed that chimeric antigens induced protective responses against melanoma and *Neospora caninum* vertical transmission, in murine models. At present, our main goal is to determine how the association of TLR2 activation with other innate stimuli can be used to tailor long-lasting adaptive immune responses, with different properties, at the relevant tissues. For the challenging experiments, we are working on mouse models of tick-feeding, to challenge the skin, and of *T. gondii* pregnant mouse infection to challenge the gut mucosa and vertical transmission. Our ultimate goal is to translate our findings into immunogenic formulations with application in veterinary species.

Team: Alexandre Leitão, Sofia Nolasco, Afonso Basto, Inês Delgado, Sara Zúquete, Eduardo Marcelino (20%), Helga Waap (20%), Sílvia Correia (20%)

Emergent and Zoonotic Diseases Group: ideas ongoing for major collaboration inside AL4Animals.

José Manuel Correia da Costa, Scientific Director of Centre for the Study in Animal Science (CECA/ICETA), University of Porto, Porto, Portugal.

The One Health Concept is generally defined as a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment. The synergism achieved will advance health care for the 21st century and beyond by accelerating biomedical research discoveries, enhancing public health efficacy, expeditiously expanding the scientific knowledge base, and improving medical education and clinical care. When properly implemented, it will help protect and save untold millions of lives in our present and future generations. Accepting these principles and consequently, we propose some scientific topics for collaboration within the AL4Animals scientific network:

- 1) Parasites and carcinogenesis – how to understand them
- 2) Animal Fascioliasis, a big veterinary concern in Europe: a better proposal for parasiticide drugs and a new diagnostic tool (intradermal reaction).
- 3) *Toxoplasma gondii*, new diagnostic tool and host-parasite molecular mechanisms. Identification of a specific biomarker for protozoa oocysts and the concept design of a laboratory procedure for protozoa monitoring. In addition, how Protozoa drive Pathogenesis and Virulence; and Host resistance? Finally, Protozoa and Chronic Diseases, namely Neuropsychiatric Diseases. What lies beneath?

Thematic Line

“Comparative and Translational Medicine and Biotechnology: Towards Precision Medicine”

New Therapeutic Strategies to Overcome Infectious, Oncologic and Degenerative Animal and Human Diseases in a One Health Context

Luís Tavares - Microbiology and Immunology Lab, CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa

Our Lab aims at studying bacterial, viral and oncogenic diseases, affecting both animals and humans, to develop novel therapeutic, diagnostic and control strategies for a sustainable health. These include antimicrobial peptides, biotechnologically based immunotherapy strategies, phage therapy and biocides. We also focus on microbial surveillance, in FMV's isolation and biocontainment unit where companion animals' and potential zoonotic infectious agents are monitored, controlled and studied.

The main contributions to health quality of animals and humans, under the wide concept of “One Health”, occur within three core research lines studying:

1- Innovative antimicrobial approaches to assess and prevent the emergence of antibiotic resistance. Our team has started working on Diabetic Foot Infections (DFI) focusing on bacterial isolates from patients with Diabetes Mellitus hospitalized in Lisbon Medical Centers. The DFI samples were analyzed, allowing gathering a collection of bacterial isolates. Then, we developed innovative antimicrobial strategies to tackle DFI, with special focus on antimicrobial peptides (AMP) and confirmed the antimicrobial activity of the AMP nisin against *Staphylococcus aureus* isolates from our DFI collection. We also studied the potential of a guar gum biogel to be used as a delivery system for the topical application of this AMP onto infected foot ulcers. Our team also evaluated the potential of this new antimicrobial approach for the prevention of periodontal disease (PD) in dogs, one of the most prevalent diseases in these animals, demonstrating that AMP also constitute one of the most promising innovative approaches to control the spread of antimicrobial-resistant bacterial strains in veterinary medicine. An increasing number of molecules will be evaluated in clinical trials in the near future.

2- Development of novel immunotherapy strategies to overcome infectious, oncologic and neurodegenerative disorders. We apply rational design and molecular evolution approaches, such as phage display selections, functional screenings and next generation sequencing analysis, to develop future generations of therapeutic antibodies. Based upon this integrated approach, we aim to develop a new generation of “smart bomb antibodies” against challenging targets and unmet diseases. For this purpose,

our lab is mainly working with antibody fragments (Fab and scFv) and single-domain antibodies (sdAbs) as therapeutic scaffolds in the areas of infectious diseases, cancer and neurodegenerative disorders.

3- Biological Isolation and Containment Units (BICU): Surveillance of companion animal' infectious diseases and Prevention of Nosocomial Infections using Biocides. The BICU has a passive surveillance database, including samples from over 1500 companion animals hospitalized since 2013. This data collection contributes for different studies allowing characterization and standardization of cases to identify most frequent and relevant ID patterns, and the definition of parameters to include in the system, classification of syndromes and diseases according to literature and evidence based medicine concerning ID prevention and treatment. Also, as nosocomial infections are a rising problem worldwide, the study of different biocide strategies and efficient disinfection protocols are investigated in our Lab and in the BICU.

Natural Extracts with Antioxidant and Antimicrobial Capacity from Plants and By-Products of the Food Industry

Ana Sanches Silva, CECA- Center for Study in Animal Science (New diagnostic and therapeutic tools)

Food additives have a key role in the preservation of food quality and safety. However, some synthetic food additives have been associated with adverse health effects. In this line, in the last years, considerable research studies were devoted to the analysis of natural extracts from plants, such as rosemary, oregano and thyme extracts, and food by-products, namely fruits' by-products, in order to find alternatives to synthetic additives.

In this presentation the main methods applied to the characterization of the antioxidant and antimicrobial capacity of these extracts will be addressed. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay is one of the most frequently used methods to determine the antioxidant capacity of natural extracts, besides the evaluation of individuals antioxidants, such as phenolic compounds and carotenoids. In what concerns to antimicrobial capacity, disc diffusion test is one of the preferred methods.

The factors affecting the variability of the natural extracts such as the plant species, part of the plant used, edaphoclimatic

conditions and extraction methodology, will be discussed as well as the need of standardization of these extracts to guarantee their effectiveness, quality and safety.

On the other hand, the dark side of natural extracts, i.e., the possibility of contamination of the natural extracts by contaminants such as toxins (both plant toxins and mycotoxins), pesticides or heavy metals will also be focused. The preparation of extracts from plants can concentrate active antioxidants or antimicrobials but, if these plants and by-products are contaminated, they can ultimately originate contaminated extracts.

In this communication, the use of natural extracts in releasing active packaging intended for food purposes will also be addressed. Examples of releasing active systems developed using aromatic plants extracts and industrial fruits' by-products will be presented.

Acknowledgments:

ValICET (Valorise foods and Improve Competitiveness through Emerging Technologies applied to food by-products within the circular economy framework) project is supported by the PRIMA program (Partnership for research and innovation in the Mediterranean area, Section 2 Funded Projects 2020, Agri-Food Value Chain). A. Sanches Silva is grateful to the Foundation for Science and Technology (FCT) for funding the ValICET project in Portugal (PRIMA/0001/2020). The work was also supported by UIDB/00211/2020 with funding from FCT/MCTES.

Development of RNA Therapies and their in vitro and in vivo Experimentation Models

Sandra Alves, Unidade de Investigação e Desenvolvimento - Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge/Centro de Estudos de Ciência Animal, CECA-ICETA, Universidade do Porto/Laboratório Associado para a Ciência Animal e Veterinária (AL4AnimalS)

A better understanding of metabolic diseases is the major challenge of the CECA Research Group "Inherited metabolic diseases in human and animals: mechanisms and therapies". Our research focus is on the field of inherited metabolic diseases (IMD), and encompasses the biochemical and molecular basis of those disorders, but also includes the development of innovative disease-or mutation-specific RNA therapies as well as the indispensable generation of models for their testing. Currently, we are running projects related to the application of RNA therapies (using antisense oligonucleotides, siRNAs and modified U1snRNAs) to IMDs, but also projects related to the generation of disease cellular and animal models. We will present some of the results already obtained as well as some lines of research that we aim to develop.

Novel therapies for regenerative medicine in one health perspective

Ana Colette Maurício, CECA-ICETA, Centro de Estudos de Ciência Animal, Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto. Experimental Surgery and Regenerative Medicine Group.

Recent advances in Tissue Engineering have promoted the development of scaffolds that may be implanted in combination with cell-based therapies. Mesenchymal stem cells (MSCs) obtained from the umbilical cord tissue are a promising alternative due to: i) high number of cells obtained from ethically acceptable sources, ii) low expression of HLA-ABC antigens and absence of HLA-DR expression, iii) procurement ease and iv) high numbers of quality samples cryopreserved in biobanks worldwide. Recently, an increasing clinical interest on dental pulp stem cells (DPSCs) and from synovial membrane (SM-MSCs) have also been noticed given the large number of samples being cryopreserved in biobanks for Human and Veterinary patients. The main research goal is to evaluate the therapeutic effect on tissue regeneration of several resorbable structures/medical devices mostly produced by electrospinning/ 3D bioprinting combined with cell-based therapies. Therefore, the ongoing projects aim to develop combined therapy products (CTP), according to the best practices delineated by European Medicines Agency (EMA), fully characterized with respect to both, cell safety and therapeutic potency, involving karyotyping, epigenetic profiling, cytocompatibility, gene expression by RT-PCR, multi-lineage differentiation capacity, immunocytochemistry, flow cytometry, secretome and metabolic profiling, as well as optimization of isolation, and expansion protocols under GMP conditions. The products are always in vivo tested, using an One Health approach, in rat model, for initial validation of the scaffold; in the ovine model, for the study of critical defects and finally, in pilot clinical trial with dogs or equine patients using real clinical cases, and, in some cases, in human clinical trials (like Bonelike®, dextran-based tube-guides, and DPSCs which are 3 international patents from UPorto). Outcomes of the regenerated tissues are assessed by histomorphometry and stereology. Functional recovery concerning especially bone and neuromuscular regeneration has been assessed serially using video recording of the gait for biomechanical analysis. The research team involved is widely experienced in the proposed methodologies, which is able to provide precious insight on the ethical and regulatory requirements of cell-based therapies for clinical applications and has more than 20 years of experience in veterinary surgery and biomechanical functional evaluation. The involved projects present a multidisciplinary effort where Stem Cell Biology, Physics, Engineering, Medicine and Veterinary Medicine, and Bio-entrepreneurship come together that through Experimental Surgery have a crucial role in the development of biomaterials and cell-based therapies, allowing a close share of knowledge between biomaterials design, development of cellular systems, and surgeons' needs, with an extensive experience in advanced therapy medicinal product (ATMP) development in the bio-industry point of

view, with a clear vision of what is required regulatory wise to achieve a translational final production. With this final goal in view, a detailed functional and morphological analysis of the regeneration process is carried on for proof of concept, involving a trained team in microsurgery techniques for implantation of the biomaterial systems and MSCs based-therapies. All activities have in mind the future applicability of the results and the development of the combined medical device/ATMP will be subject to, and confined, to the stipulated ATMP directives, namely to the guideline on the risk-based approach according to Directive 2001/83/EC applied to Advanced Therapy Medicinal Products (EMA/CAT/CPWP/686637/2011), which directs product development towards a preliminary risk assessment to the patient and to the patient requirements. The team includes several members ICBAS-UP staff with Felasa C accreditation, and access to facilities dedicated to Experimental Surgery and Regenerative Medicine approved by ORBEA and DGAV. Several collaborations mainly with IPLeia/CDRsp and FEUP allow access to innovative biomaterials. The functional and biomechanical evaluation has been carried out by UTAD, which is an international reference in biomechanical analysis concerning pre-clinical animal models and computerized sciences. Morphological investigation has been developed in collaboration with Pathologists from ICBAS/i3S, UTAD, and University of Turin, internationally recognized in this field. The projects also highly benefit from the large experience brought about by CECA related to ATMP development and access to GMP rooms (from protocols between UPorto and biotechnology and pharmaceutical companies from the North of Portugal).

Biomarkers and targeted therapies in feline mammary carcinoma: past, present and future

Jorge Manuel Jesus Correia, CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon; Associate Laboratory for Animal and Veterinary Sciences (AL4Animals); Pathology Lab.

The Pathology Lab research team is composed of: 2 Full Profs. (Maria Peleteiro; Jesús París), 4 Associate Profs. (Mário Pinho; Ana Romay; Marc Beltrán; Victor García), 10 Assistant Profs. (Jorge Correia; José Silva; Fernando Afonso; Maria Jorge; Hugo Pissarra, Rute Noiva, Maria Soares, Andreia Valença, Joana Catita, Carlos Pinto), 3 PhD Students (Ana Nascimento; Andreia Gameiro; Fábio Santos) and 1 Laboratory Technician (Sandra Carvalho), amounting to 16 Professors, 3 PhD Students and 1 Laboratory Technician for a total of 20 researchers. A main oncology research line addresses feline mammary carcinomas. This thematic line stems from an extensive case samples available on our Pathological Anatomy Lab (LAP, FMV-ULisboa), as well as being an ideal model for comparative studies to the women mammary carcinoma. These studies began with the establishment of a tumor's molecular classification, using immunohistochemical methods resorting to markers for cytokeratin, estrogen and progesterone receptors and Her-2

molecules. Currently, new biomarker studies address their role on the inflammatory response both at the intratumoral and stromal levels, laying the foundations for new prognosis indicators, and assessment of neoplastic cell culture sensibility to novel drugs as possible therapeutic target molecules (Tyrosine kinase inhibitors, histone deacetylase inhibitors and microtubule inhibitors). Other important tumor biology molecules and biomarkers were evaluated, using various methodologies optimized for the feline species, applied to the tumoral tissue (immunohistochemistry) or blood, namely Ki67, Her-2, CD3 (3, 4, 8, 20, 56, 68 and 163), VISTA, Leptin, TIM3, CXCR4/CXCL12, PD-1/PD-L1, CTLA-4, LAG-3, IL-6, TNF- α , SDF-1, microRNAs, VEGF-A, TOP2 α , VEGFR-1 and VEGFR-2. The LAP Lab has: a small and large animal necropsy facility and laboratories for histopathology, cytology and immunohistochemistry analysis. A second research line studies the vascular modifications in human retinopathies. Recently the focus has been on TIM2 retinal iron uptake and its implication for the diabetes macular edema. Transmission electron microscopy, scanning electron microscopy and confocal laser microscopy methodologies were involved, as well as immunohistochemistry and retinal laser scanning ophthalmoscopic retinal angiography techniques. Another research line focus on wild leporids (wild-rabbit and Iberian-hare), endangered and key species from the Mediterranean ecosystems. Study objectives include: i) minimization of the impact of Viral hemorrhagic disease virus, via feedstuff incorporation of an edible virus-like-particle (VLP) vaccine; ii) the study of an emergent wild-rabbit and Iberian-hare recombinant myxomatosis virus, named ha MYXV; iii) and the identification of the first Iberian-hare herpesvirus, denominated Leporid gammaherpesvirus 5 (LeHV-5). Wild-rabbit related main results include: i) world first reported cases of wild and domestic rabbit recombinant myxomatosis virus (ha-MYXV); ii) detection of a recombinant RHDV2 GI4P.GI2 virus in Eurasian badger (*Meles meles*) which constitutes a Lagomorpha to Carnivora order species barrier jump; iii) development of the first quadruplex real-time PCR (qPCR) for the detection, quantification and differentiation of the different Myxoma virus strains from wild-rabbit and Iberian-hares; iv) development and production of an Baculovirus-Insect cell Expression system for the production of optimized VLPs used in feedstuffs for wild-rabbit immunity induction, currently under assays; v) Isolation and sequencing of the complete genome of the first recombinant Myxoma virus strains; and vi) optimization of an immunohistochemistry technique using a pool of three monoclonal antibodies against Viral hemorrhagic disease virus. Iberian-hare related main results were: i) the detection of the first reports of myxomatosis in the Iberian-hare, after 70 years of wild-rabbit only dissemination; ii) the first report of the *Lepus* herpesvirus genus (LeHV-5) with cutaneous and reproductive involvement; and iii) development of the first real-time PCR (qPCR) method for the detection and quantification of LeHV-5. Another research line involves bivalve's health monitoring, by studying Ostreid herpesvirus 1 μ var (OsHV-1 μ var) in Portuguese oysters (*Crassostrea angulata*), Pacific oysters (*Crassostrea gigas*) and associated bacteriosis in Portugal, aiming to collect

and analyze national samples, establishing a nosological disease characterization and, subsequently, the implementation of control and eradication procedures and biosecurity and animal health and welfare best practices and codes of conduct protocols. The Pathology Lab team has collaborations with the following institutions: IMM-FM-ULisboa; CAG-UTAD; CBATEG-UAB-Espanha; INIAV-I.P.; IPMA-I.P. and DGAV.

Translation and comparative research on animal spontaneous disease

Maria dos Anjos Pires

This research group (CollabGroup for Animal Spontaneous Diseases) aims to contribute to the knowledge on spontaneous degenerative, oncological and inflammatory animal's diseases. By studying these diseases, the group aims at unveiling molecular pathways that may be useful to devise new diagnostic or therapeutic approaches, improve animal health and that could also be translated to equivalent human diseases. To succeed this goal, the group will also evaluate physiologic and immune parameters. The deeper knowledge of such parameters in animals will foster the translation to human medicine, favouring the welfare and bridging to the One Health concept since animals and humans share the same environment. Sporadically, experimental models of disease are also performed.

This group brings together a multidisciplinary team, combining different skills and integrating all the valences of the Veterinary Teaching Hospital (HVUTAD), as well as the Laboratory of Laboratory of Genotoxicity and DNA Repair and the Laboratory of Histology and Anatomical Pathology (LHAP) of UTAD, with these labs contributing transversally to different investigations. All the animal species are tackled in our investigation. Albeit a strong focus is posed on small domestic animals, farm, equines, exotic/wild, and experimental animals (laboratory animals) are also covered within the group research. The group uses the facilities and equipment belonging to the main structures mentioned above (as it can be inferred from the different posters): several services and labs at the Veterinary Teaching Hospital (clinical, surgery, imaging, clinical pathology), the Wildlife Rehabilitation Centre (CRAS, the only ICNF network hub integrated into a Portuguese university facility), Genetic Cytotoxicity lab, and LHAP, which further includes the necropsy room. Through these state-of-the-art structures, advanced diagnostic tools and techniques are easily accessed by the group team. Among them, the most used consolidated methodologies include diagnostic imaging, clinical pathology and endocrinology determinations, histopathology, "Comet Assay" for DNA fragmentation, PCR, immunohistochemistry, electron microscopy (SEM and TEM), stereology, and ELISA. Several granted projects are ongoing (FCT, COMPETE 2020, FEDER) such as "Chronic wasting disease risk assessment in Portugal", "ICas_Bísaro - Immunocastration protocols for "Bísaro" pigs", "OneHcancer - One health approach in animal

cancer", "Dys4Vet - Dysplasia software for veterinary professionals" and "TraDACA - Diagnosis and preventive treatment of hip dysplasia in dogs", among others.

Our results are routinely published in high impact international peer-review journals, books and book chapters, including the international reference book of the Classification of Mammary Tumors of Domestic Animals. Some patents are already registered and others are in progress.

Translational clinical research: animal models of disease

Esmeralda Delgado^{1,2}

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CIISA – Centre for Interdisciplinary Research in Animal Health, Clinical Research Lab Research performed at the Clinical Research Lab (CRL) aims to improve scientific knowledge in diseases that affect animals and to develop new prevention strategies, discover diagnostic methods, understand pathological mechanisms and unravel new therapeutic approaches. Clinical studies using naturally occurring or experimental animal models of disease fulfill the basis of translational medicine and contribute for advances in human medical knowledge in a One Medicine – One Health perspective.

The CRL congregates several research areas that work under the umbrella of the Teaching Hospital of FMV-ULisboa, which aims to provide the highest standard of veterinary medical care, generating a pool of clinical cases in which research studies on multiple scientific areas can be carried out, allowing for a multidisciplinary approach. The CRL uses all the facilities and equipments of the Teaching Hospital.

The CRL comprises several research areas with different responsible investigators: Clinical Ophthalmology (Esmeralda Delgado), Allergy and Immunology (Ana Mafalda Lourenço), Farm animals (Miguel S. Lima), Pharmacology and Toxicology (Berta S. Braz & Anabela Moreira), Equine Internal Medicine (Paula Tilley), Equine Surgery and Biomechanics (Luis Lamas), Estomatology and Oral Surgery (Lisa Mestrinho) and Small Animal Internal Medicine (Rodolfo Leal). The CRL has ongoing work in the fields of ocular drug delivery and neuroprotection, allergic conjunctivitis, canine atopic dermatitis and immunotherapy, animal intoxication and poisoning, equine respiratory diseases and hypersensitivities, equine surgery and biomechanics, oral inflammatory diseases and oncology, new vaccines, gastrointestinal disease, endocrinology and leishmaniosis. The clinical research team comprises 30 researchers, 18 doctorate and 12 non doctorate, their main expertise being their clinical experience in different veterinary specialties. This team has several national and international collaborations.

Animal Genetic Diversity and Adaptation to Climate Change

Luis Telo da Gama, Animal Genetic Resources Lab

Since they were domesticated, animals have adapted to a wide diversity of environmental conditions, including climatic constraints, territorial restrictions, social pressure, etc. Currently, the challenge represented by climate change is of outmost importance and a major concern for the future of livestock production worldwide. Therefore, selection of breeds and breeding systems with a focus on their adaptation and resilience to face the expected changes in temperature and precipitation, among other constraints. Research in this area is therefore crucial, enabling the identification of genes and genetic markers associated with resilience, which may allow the continued adaptation to the occurring environmental changes. To achieve this goal, Creole livestock breeds represent a unique resource, as they cover a very wide representation of environmental constraints in very different latitudes. Livestock species were brought to the Americas from the Iberian Peninsula since the late 15th century, starting with the second trip of Columbus. These animals subsequently expanded throughout the Americas, adapting to a wide range of environmental conditions and giving origin to the populations currently known as Creole, which may have received influences also from other origins. From the narrow genetic base represented in the populations initially established, the expansion throughout the Americas resulted in the wide genetic variability currently existing in Creole breeds. This diversity largely reflects the adaptation of Creoles to local environments which are extremely diverse, ranging from tropical climates to high snowy mountains, from deserts to lush pastures. Creole breeds represent, therefore, a unique resource to investigate genes and genetic mechanisms associated with adaptation to climatic extremes, both through detection of genetic signatures or landscape genetics approaches. These investigations are currently underway, and are of crucial importance to address the need for improved resilience and adaptation in a future scenario of climate change.

CECA's group on Biotechnologies in Animal Reproduction: History, Perspectives and Collaborations

Antonio Rocha, CECA-ICETA, Centro de Estudos de Ciência Animal, Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto.

This group was formed based on ICBAS' staff working on a variety of theriogenology subjects, with particular emphasis on domestic ruminants, dogs, and wild lagomorphs. Later on, the Center of Animal Reproduction of Vairão (CRAV) was formed, and in the last years, work emphasis has shifted to equine reproduction. CRAV was also the basis to the establishment of a European Board of Veterinary Specialization (EBVS) approved center, to train European Veterinary Specialists on Animal Reproduction. This group's work has always been characterized by positive feedback between applied research,

extension work/transfer of technology, as well as services to the public in the area of reproductive biotechnologies, including cryopreservation of genetic material to stock gene banks of endangered national breeds. Some of the highlights of our research activity, often as collaborators with other institutions, were: the characterization of the reproductive biology of the wild rabbit and hare in Portugal; studies on in-vitro maturation of dog oocytes; methods to improve the cryopreservation of canine semen; the first diagnosis of bovine neosporosis in the country and the study of its epidemiology; evaluation of seasonal influence on ewe's superovulation response and embryo cryopreservation; assessment of the prevalence of bacterial agents of venereal-transmitted diseases in horses and its potential impact on fertility and embryo quality; efficacy of silica colloids to increase the fertility of stallions and decrease bacterial content of the ejaculates using antibiotic-free extenders, and insights into the limitations of in vitro semen capacitation and fertilization in the horse. Relevant transfer of technology and continuous education training focused on oocyte aspiration in mares and cows, embryo transfer in horses, embryo cryopreservation in horses, and diagnosis and treatment of pathologies in stallions. Presently, active research programs are on the microbiome of the mare's genitalia and its relation with uterine health and pathology; the use of more efficient methods to diagnose endometritis in the mare, and vitrification of stallion semen. In addition, we collaborate on a study on semen characteristics of Lusitano stallions (University of Évora), one on sperm proteins as targets for contraception and fertility biomarkers (University of Aveiro), and a study on the influence of epigenetics of the spermatozoa on embryo metabolism (University of Porto). Facilities of this group include a fully equipped (with the exception of equipment for ICSI and cloning) equine theriogenology lab, an IVF laboratory, paddocks, boxes, and examination stocks for horses. The core of this group has always been very small. Presently, new elements have been admitted, and a future research program with a larger scope is under discussion. Possibly, one of the contributions of our group with higher potential for this new associate laboratory is the tight, informal network of international collaborators developed over the years, with colleagues from Europe, South, and North America, Australia, and Middle-East, mainly on equine theriogenology and bovine reproductive physiology.

Meeting scientific unresolved questions to address medical and zootechnical challenges

Luís Lopes-da-Costa, CIISA – Centre for Interdisciplinary Research in Animal Health – Reproduction & Development Laboratory

The R&D Lab of CIISA congregates teams located at FMV and INIAV (EZN – Santarém). A main research area addresses the inflammatory status of the uterus. Endometritis is a principal cause of cow infertility and economic inefficiency of dairy herds. Early diagnosis of it is elusive and there is no effective treatment. The Lab leads a pioneer search for specialized

Mediators (SPM) and adipokines as diagnostic biomarkers and therapeutic targets of cow endometritis and mastitis. Also, in Bovine Genital Campylobacteriosis, a venereal disease of worldwide concern, causing endometritis and infertility, the microbiologic diagnosis has a low sensitivity, current molecular assays have low specificity and there is no effective vaccine. The Lab developed a novel molecular diagnostic tool under field validation and is scrutinizing genetic targets for the development of a vaccine. In the mare, where postpartum or post-insemination endometritis is a relevant cause of infertility, the Lab has focused on the mechanisms of endometrial fibrosis establishment, ascribed to neutrophil extracellular traps (NETs) proteins, inflammatory mediators and epigenetics. A second main research area considers the embryo maternal interactions leading to pregnancy establishment, as well as developments in Assisted Reproductive Technologies (ART). Using the mouse and bovine models, the Lab received international recognition in the evaluation of Notch signaling in gametogenesis, embryo development and cancer biology. Results evidenced that Notch regulates the pace of gametogenesis, embryo kinetics and differentiation, and tumor development. Male factor accounts for 30% of infertility cases in domestic animal species and Humans. The team currently decipher the Notch

proteins interactomes involved in sperm capacitation, to uncover mechanisms behind sperm fertilizing capacity and assemble a male fertility biomarker algorithm. A third more recent research area addresses the identification of genetic, molecular and cellular adaptive mechanisms of native breeds conveying resistance to heat stress effects in reproductive function, in the present climatic change scenario. The Lab has expertise on molecular biology and cell techniques (RNAseq, qPCR, WB, IHC, zymography, tissue explant and cell culture, in vitro NETs formation), cell imaging and isolation (fluorescence and confocal microscopy, Laser Capture Microdissection) and all ART. The R&D Lab host DGAV licensed Germplasm Banks with cryopreserved sperm, oocytes, embryos and cells, units for ART, an Endocrinology Lab and an IPAQ (Portuguese Quality Agency) accredited lab (LabRepA) for the diagnosis of reproductive infectious diseases, also providing ART and clinical services to community.

Team – Luís Lopes-da-Costa, Graça Ferreira-Dias, António Duarte, Luísa Mateus, Rosa Lino Neto Pereira, João Pedro Barbas, Elisabete Silva, João Nestor Chagas e Silva, Ana Catarina Torres, Marta Silva, Gonçalo Pereira, Patrícia Diniz, Luís Capela, Catarina Anastácio, Maria Rosa Rebordão, Joana Alpoim-Moreira, Ana Sofia Amaral, Mariana Batista, Daniel Murta

POSTERS

P1

The Animal Behaviour and Welfare Lab (ABWL) research lines

George Stilwell, Ilda Rosa, Alexandre Azevedo, Manuel Magalhães Sant'Ana, Animal Behaviour and Welfare Lab, Centre for Interdisciplinary Research in Animal Health – CIISA

The Animal Behaviour and Welfare Lab (ABWL) from CIISA has been following four main areas of work: Farm animal welfare assessment. Since its involvement in the European project AWIN – Animal Welfare Indicators, in which the first welfare assessment protocol for dairy goats was developed, ABWL has been studying the validity, repeatability and feasibility of welfare indicators to be incorporated in protocols to be used in different production systems (e.g. outdoor feedlots, suckler herds, fattening lambs) for which there is no current systematic and science-based approach. At this moment three lines of studies are on course – validation of a protocol for extensively kept autochthonous breeds, especially in mountain environments; comparison of the welfare in suckler herds in Portugal and Mozambique using the same protocol; comparison of the welfare in organic and conventional beef herds. Associated to this line of research, ABWL has been

involved in the training of auditors in the use of the AWIN and Welfare Quality protocols, especially in collaboration with the Azorean Government that intends to certify the welfare of its dairy and beef farms. Good practices that ensure the welfare of farm animals. Pain management is a traditional topic in ABWL research. Several small projects that involve 6th year FMV students, are looking at ways to reduce pain and improve healing after dehorning or castration. Also, ABWL is a partner in two European projects, BOVINE and WELFARUMINAT (and similarly in the recently finished ANICARE project), in which good farming practices that ensure health and welfare of farmed ruminants, are validated and disseminated. In these projects ABWL has established very close and fruitful cooperation with partners from several EU countries and also Turkey. Evidence based veterinary medicine education and Ethics in the veterinary profession. The EVIEDVET project, hosted by ABWL in partnership with UTAD, and CIIE and I3S from UP aims to, firstly, comprehensively map and critically evaluate how evidence-based veterinary medicine is currently being taught to veterinary students and practitioners in Portugal, and compare the Portuguese landscape with examples from selected European veterinary schools. Behaviour of farm and companion animals. A PhD project will be looking at the relationship between canine epilepsy and

anxiety and the possibility to control canine epilepsy through managing canine anxiety. Another expected line of investigation on behaviour of companion animals (in partnership with TKF- Research Group in Anthrozoology) will study the psychological and physiological benefits of human-animal bond in Animal-Assisted Services and Interventions. In the case of farm animals, a FCT funded project is looking at the possibility of using behaviour and temperament tests in the selection of Mertolenga-breed sire bulls, while a Masters' project is studying the effect of positive treatment to under 15 days-old dairy calves. In summary, ABWL strive to ensure the acceptability and sustainability of livestock farming by improving animal welfare; to up-hold scientific evidence and ethical basis in veterinary education and profession; and to understand better the behaviour of companion animals and how these can influence and be influenced by their owners. Finally, it should be added that ABWL is hosting a residency in Animal Welfare Science, Ethics and Law.

P2

Animal Welfare and Behaviour

CECAV, Animal Production Group

P3

Animal Nutrition - Experimental Unit and Laboratory

CECAV, Animal Production Group

P4

Bioterium

CECAV, Veterinary Public Health Group

P5

Laboratory of Parasitology, Department of Veterinary Science (ECAV, UTAD)

CECAV, Veterinary Public Health Group

P6

CRAS-HVUTAD: Expertise for Wildlife

CECAV, Veterinary Public Health Group

P7

Education, Science and Clinical Service

CECAV, Veterinary Public Health Group: HVUTAD

P8

The Importance of Normal in the Concept of "One Health"

CECAV, Veterinary Public Health Group

P9

Genotoxicity and DNA Repair

CECAV, Veterinary Public Health Group

P10

Hip Dysplasia - Diagnosis and Treatment

CECAV, Veterinary Public Health Group

P11

Multispecies Approach to Fertility Preservation

CECAV, Veterinary Public Health Group

P12

Endangered indigenous pigs of Angola: a key for rare mutations for adaptation

Andreia J. Amaral, CIISA and AL4Animals

Angola includes one of the regions that has been a long refugee for pigs derived from ancient domestication derived from Near Eastern pigs introduced by land through Egypt. Angola has been through dramatic social events that have led to a drastic reduction of the population of indigenous pigs, which is currently endangered. These pigs are well adapted to poor diets and harsh environments. We used whole-genome-sequencing to explore the genetic diversity of indigenous pigs from Angola and to study their relationships with worldwide breeds. Results show that these pigs harbour thousands of unique mutations and high levels of heterozygosity. We have identified unique mutations related adaptation events causing differences in metabolism and tissue remodelling.

P13

Cannabidiol clinical applications in oral health

Lisa Mestrinho, Clinical Research Lab

Cannabidiol (CBD) is the most well-known non-psychoactive phytocannabinoid with recognized therapeutic benefits, particularly for the treatment of chronic pain and inflammatory diseases. This research line aims to evaluate CBD's pharmacokinetics, safety, and efficacy in pets with oral disease. This research team performed a pragmatic clinical trial (phase IV) using a CBD commercial formulation. Methods included clinical trial protocols and mass spectrometry. A pool of clinical cases

routinely attended at the teaching Hospital of FMV-ULisboa (dentistry and oromaxillofacial surgery service), where CIISA's Clinical Research Lab holds part of its work gathers unique circumstances where this (and other future planned trials) take place. The ultra-high performance liquid chromatography (UHPLC) equipment coupled to a triple quadrupole mass spectrometer (MS/MS), Waters Acquity™ (Waters®, Ireland) is used for the quantitation of CBD in both formulation and serum of studies individuals. This part is assured by the external collaboration with the faculty of pharmacy at ULisboa. This research line was supported by CIISA's internal funding (INOV) and the pharmaceutical industry. Outputs so far include 3 poster presentations in national and international conferences, one Integrated Master thesis, one invited lecture at a national meeting and one paper submitted in a peer review journal.

Research team: Lisa Mestrinho¹, Joana Chambel Coelho Andreia Bento Silva², Maria Rosário Bronze², Noélia Maria Duarte²*

**MsC Student 1 Clinical Research Lab, Centro de Investigação Interdisciplinar em Sanidade Animal 2 Grupo Química de Produtos Naturais, Instituto de Investigação do Medicamento*

P14

A novel tolerogenic liposome-based allergen immunotherapy for dogs in the horizon

Ana Mafalda Lourenço, CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon; AL4Animals – Associate Laboratory for Animal and Veterinary Sciences; Allergy and Immunology Unit.

The main research goal of this pioneering study lies on the pharmaceutical development and clinical administration of highly efficient allergen immunotherapy formulations through both non-invasive Epicutaneous and Oro-mucosal routes. These formulations will be potentiated for efficacy and safety using adjuvant-like nanoparticle-based manipulations that enhance allergens' potency and tolerogenicity. This approach aims to address some of the major current challenges for creating the ideal vaccine, namely (1) the development of a delivery system designed to overcome the skin/oral mucosa barrier, (2) the use of adjuvants endowed with de-novo Th2 priming suppressive properties, (3) in vivo targeting of tissue-resident dendritic cells to enhance vaccine potency, and (4) the avoidance of allergen cross-linking to pre-existing IgE on mast cells. The specific objectives outlined for Epicutaneous Immunotherapy consist in setting up a more pet-friendly administration and evaluating this route in a longer-term pioneer protocol. Noteworthy, our six-month, proof-of-concept, pilot study on Epicutaneous immunotherapy has previously shown promising preliminary results. Therefore, an Epicutaneous Immunotherapy Delivery System is one of the consolidated methodologies of this team. For oro-mucosal immunotherapy, the specific scientific goals concern studying the ideal formulation application site within the oral mucosa, creating a mucoadhesive formulation

enhanced for greater adhesion and longer contact time with the oral mucosa, and reducing the protocol administration frequency to foster pet owner's compliance with the proposed treatment. Ultimately, this project aims to maximize the allergen immunotherapy potential as the only current aetiological therapeutic tool for Canine Atopic Dermatitis, a quality-of-life disruptive and highly prevalent disease worldwide. The theoretical knowledge will translate into practical value once applied in real-life experience, being expected to clinically achieve promising, non-invasive, patient- and owner- friendly, effective, and safe immunotherapy protocols. This is an exciting strategy, aligned with the complementary background and expertise of the team and institution thematic areas. Team: Ana Mafalda Lourenço^{1,2}, Joana Marto³, Solange Gil^{1,2}, Marta Pinto^{1,2}, Rodolfo Oliveira Leal^{1,2}, Lídia Gonçalves³, Sandra Simões³, Frederico Silva^{1,2}, Berta São Braz^{1,2}, Esmeralda Delgado^{1,2}, Hugo Pereira^{1,2}, Helena Ribeiro³, Laura Ramió-Lluch⁴, Vanessa Schmidt⁵, Mário Morais-Almeida⁶. 1 CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon 2 AL4Animals – Associate Laboratory for Animal and Veterinary Sciences 3 Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon 4 LETI Pharma S.L.U., Barcelona, Spain 5 School of Veterinary Science, The University of Liverpool, Liverpool, United Kingdom 6 Allergy Centre, CUF Descobertas Hospital, Lisbon, Portugal

P15

CBD-containing drug delivery systems as a novel therapy in dogs with atopic dermatitis

Ana Mafalda Lourenço, CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon; AL4Animals – Associate Laboratory for Animal and Veterinary Sciences. Allergy and Immunology Unit.

Cannabidiol (CBD) is the second most abundant phytocannabinoid within the Cannabis sativa plant. It has promising anti-inflammatory, immunomodulatory, and antipruritic properties but without the psychoactive effects of delta-9-tetrahydrocannabinol (THC). Therefore, it may help treat dermatologic conditions, such as atopic dermatitis (AD). Moreover, recently a pilot study found preliminary evidence that supplementation with a CBD-containing hemp oil without THC effectively controlled pruritic behaviour in dogs with canine atopic dermatitis (CAD) (Mogi et al., 2022). CAD affects 15-20% of the dogs' population, and one of its major clinical signs is pruritus which can negatively impact the quality of life of both dogs and owners. The primary objective of this project is to evaluate the efficacy, tolerability, and safety of two newly developed CBD formulas, a CBD-infused oil for oral administration and a CBD cream for topical application. Hence, we will assess whether there is a statistically significant difference between Canine Atopic Dermatitis Extent and Severity Index (CADESI)-04, Pruritus

Visual Analog Scale (PVAS), Owner Global Assessment of Treatment Efficacy (OGATE) and time until relapse between the CBD groups and the respective vehicle group. Additional objectives are: 1) Evaluate whether there are statistically significant differences between CBD and respective vehicle groups regarding objective skin barrier function measurements – transepidermal water loss (TEWL) and skin's pH and hydration. 2) Assess the pharmacokinetics of the CBD-infused oil formulation. 3) Evaluate CBD serum concentrations after a 42-day daily CBD administration and after every 48 hours administration regimen. Team: Ana Mafalda Lourenço^{1,2}, Joana Marques Marto³, Vanessa Schimdt⁴, Ana Bizarro Camões^{1,2}, Hugo Pereira^{1,2}, Beatriz Pinto Fernandes^{1,2}, Marta Pinto^{1,2} 1CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon 2AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences 3Research Institute for Medicines (iMed. ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal 4School of Veterinary Science, The University of Liverpool, Liverpool, United Kingdom References Mogi, C., Yoshida, M., Kawano, K., Fukuyama, T., & Arai, T. (2022). Effects of cannabidiol without delta-9-tetrahydrocannabinol on canine atopic dermatitis: A retrospective assessment of 8 cases. *The Canadian veterinary journal = La revue veterinaire canadienne*, 63(4), 423–426.

P16

Effectiveness of antimicrobial strategies directed at tackling antimicrobial resistance in animal production: a systematic review and meta-analysis protocol

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Antimicrobial resistance (AMR) refers to the ability of any microorganism to evade or tolerate antimicrobials. It is recognised as a worldwide public health threat due to the declining the efficacy of the available antimicrobials that are needed for the treatment of infections, particularly for human medicine. As the spread of AMR is not slowing down, different strategies and interventions have been implemented in all the affected sectors. In the context of animal production, several attempts have been applied over the years toward selecting and restricting antimicrobial agents used in veterinary medicine. The main objective of this systematic review is to estimate the effectiveness of a certain set of interventions that include guidelines on prescription/use of veterinary antimicrobial agents, monitoring of veterinary antimicrobial prescriptions and/or usage, yellow card initiative and policies applied towards selection and restriction of

veterinary antimicrobial agents. PubMed™, SCOPUS™, The Cochrane Library™ and Web of Science™ databases will be used to search for articles published since 2000 in English, Portuguese, Spanish, French and Italian. Grey literature will be searched from World Health Organization, European Centre for Disease Prevention and Control, Centres for Disease Control and Prevention, European Medicines Agency. Additionally, we will search in OpenGrey™, OAIster™ and RCAAP™ databases for grey literature. Data synthesis will be performed separately for studies assessing broiler and pig populations but also for non-pharmacological and pharmacological interventions. The overall magnitude of any association will be expressed as odds ratio, relative risk or prevalence ratio with 95% confidence intervals. Nominal data will be expressed as frequencies and percentages. Standard pairwise meta-analysis using a random effects model for low risk of bias data or fixed effects model for homogeneous effects across studies will be performed. A narrative synthesis will be performed to summarize data for outcomes that are only reported in one article or if calculation of a summary measure by means of meta-analysis is not applicable. Expected results include a relative ranking of the competing interventions according to AMR prevalence that may include multi drug resistance and resistance to last resort antimicrobials, but also impacts of interventions on animal populations health and on the profile of AMR in bacteria.

P17

Therapeutic strategies in oral cancer

Lisa Mestrinho, Clinical Research Lab, Centro de Investigação Interdisciplinar em Sanidade Animal

Cancer markers and neoantigens can be used for many different clinical applications. They can be used as diagnostic and prognostic indicators, as well as therapeutical targets. This research line aims to contribute with diagnostic and prognostic information in oral cancer markers (melanoma and squamous cell carcinoma) and develop treatment strategies in these tumors for dogs and cats. This research has established important translational bridges between these tumors in pets and similar ones in humans since they can be important spontaneous models of disease. There are three ongoing projects, two projects in canine and feline OSCC evaluating prognostic markers and therapeutic targets in a previously established biobank and one project in the development of a nanovaccine targeting two neoantigens for melanoma - melan A and gp100. Consolidated methods include two computational pipelines, one for automatic immunohistochemistry cell count and one for the selection of antigenic peptides. From the collaborative effort with the Pathology lab from CIISA, there are two established biobanks with complete clinical information where is possible to pursue with more studies in tumor markers. From the collaboration with the BioNanoCiências Group from iMed, one of the

projects uses a validated nanoplatform which can co-deliver other neoantigens, additionally there are established in vitro immunogenicity tests. Infrastructure and facilities include routine access to biological material and patients with potential to enroll in clinical trials. All clinical information associated with a biobank is the platform that supports all the projects and internal and external collaborations. This data occurs under the umbrella of the veterinary teaching hospital at FMV, ULisboa where the Clinical Research labs work. Dental radiography, computed tomography, surgical facilities, etc. provide valuable clinical information that is the base for the laboratory work. Internal collaborations with the Pathology lab able to assure immunohistochemistry protocols for several markers. Nanoplatform development methods are assured by external collaborations.

Team: Lisa Mestrinho¹, Marta Azevedo Silva^{1}, Hugo Pissarra², Helena Isabel Fialho Florindo Roque Ferreira²,*

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P18

A topical bioactive moisturiser for canine atopic dermatitis: the dawn of a new era for primary prevention?

Ana Mafalda Lourenço: CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon; AL4Animals – Associate Laboratory for Animal and Veterinary Sciences. Allergy and Immunology Unit.

Canine atopic dermatitis (cAD) is a highly prevalent (20-30%) and frustrating disease whose incurable and relentless nature impacts affected dogs and their families' quality of life (QoL). Despite cAD primary prevention being a more logical and cost-effective way to manage its burden, compared to focusing research entirely on its treatment, which only aids already suffering patients after a long chain of irrevocable pathological events, it has been a neglected research topic until recently. This project aims to design and develop the first bioactive emollient for a primary prevention protocol in predisposed cAD breeds puppies and evaluate its clinical efficacy, safety, and impact on the skin barrier function using lipidomic techniques. We will apply our novel product in cAD predisposed breeds' puppies without signs of cAD until they reach 1 year of age in the first randomised, controlled, longitudinal, and long-duration study focused on cAD primary prevention, addressing this untapped issue in veterinary dermatology. If successful, the existence of an effective cAD primary prevention strategy would be truly invaluable, not only to spare families of the economic burden of its treatment but, even more importantly, to safeguard the QoL of all involved. Prototypes' formulation design rationale will stem from the most novel knowledge of AD's multifactorial

aetiology to optimise potential success, improving on: 1) cutaneous barrier impairment; 2) microbiome imbalance; and 3) immunity dysregulation. Stratum corneum (SC) will be non-invasively collected and its lipidome will be analysed through HPLC-MS, a methodology already in validation process by our CIISA labs. The main objective will be to assess whether there is a statistically significant difference between cAD prevalence in the intervention and control groups. Additional objectives are: 1) Evaluate whether this intervention produces a statistically significant difference between groups on objective measures of skin barrier function (TEWL, SC's pH, hydration and SC lipidomic analysis); 2) Ascertain any statistically significant differences between groups on the age of onset, clinical severity (CADESI-04 and IGA-2D), pruritus (PVAS10), quality of life (QoL) and medication score (MS) of the patients who develop cAD; 3) Characterise skin barrier integrity and function in cAD predisposed breeds' puppies; 4) Investigate the presence of objective skin barrier function biomarkers which could have predictive power regarding the risk of developing cAD or the severity of the diseases' lesions, pruritus, impact on QoL and management.

Team: Ana Mafalda Lourenço^{1,2}, Joana Marques Marto³, Susana Alves^{1,2}, Vanessa Schimdt⁴, Beatriz Fernandes^{1,2}, Ana Bizarro Camões^{1,2}, Hugo Pereira^{1,2}, Marta Pinto^{1,2}, Rui Bessa^{1,2}, Helena Ribeiro³, Pedro Pinto³,

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P19

Lipidomic analysis of Stratum Corneum in Canine Atopic Dermatitis

Ana Mafalda Lourenço, CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon; AL4Animals – Associate Laboratory for Animal and Veterinary Sciences. Allergy and Immunology Unit

Canine atopic dermatitis (AD) is one of the most common and distressing chronic, inflammatory, and allergic skin diseases in dogs, presenting extremely complex pathogenesis. A crucial gap that has hindered AD management strategies is the absence of treatment modalities tailored to the well-defined AD phenotypes under investigation. It is widely accepted that integrative approaches linking multiple omics with clinical and epidemiological data are necessary to develop better treatment and prediction models. Dermatological research greatly benefits from the galloping advances in lipidomics (8), for it is a tool to assess and predict the progression of inflammatory skin diseases (12) and to discover lipidic biomarkers regarding risk prediction, diagnosis, and evaluation of responsiveness to treatment (9). This project will use lipidomics analysis to profile epidermal stratum corneum's (SC) lipids

on both healthy and atopic dogs to identify qualitative and quantitative differences between healthy and atopic dogs' SC lipids. This pilot study can be the foundation of a more extensive investigation to better portray and define what a standard or abnormal SC lipids' profile is and, consequently, to help evaluate effects of topical treatments and nutritional supplementation, reveal disease biomarkers, and identify potential therapeutic targets. It can even lead to identifying more defined AD phenotypes and better treatment and prediction models, bringing veterinary dermatology one step closer to evidence-based, precision medicine. Stratum corneum (SC) will be non-invasively collected and its lipidome analysed through High-performance liquid chromatography mass spectrometer (HPLC-MS), a methodology already in validation process by our CIISA labs. Our main goal is to characterise both healthy and atopic adult dogs SC's lipidome and their differences. Other goals are to identify possible patterns or biomarkers of the disease and to optimise the collection and analysis technique to ensure further research in this thematic line.

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P20

Delivery of epoetin beta by chitosan-hyaluronic acid nanoparticles through subconjunctival and topical ocular administrations in rats

Esmeralda Delgado^{1,2}

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Delivery of epoetin beta by chitosan-hyaluronic acid nanoparticles through subconjunctival and topical ocular administrations in rats CIISA, Clinical Research Lab Research team: Beatriz Silva^{1,2,3}, Lídia M. Gonçalves³, Berta São Braz^{1,2} and Esmeralda Delgado^{1,2} 1 CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, ULisboa 2 AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences 3 iMed.Ulisboa – Research Institute for Medicines, Faculty of Pharmacy, ULisboa Neurodegenerative ocular diseases, such as glaucoma, have a substantial impact in people's daily lives, as they may cause major visual impairment, and ultimately blindness. Ophthalmology research is shifting towards neuroprotection as a complementary approach to glaucoma treatment, and several strategies are under study, including the use of erythropoietin and its recombinant forms. In this line of research, our team has developed nanoparticles with chitosan and hyaluronic

acid (CS/HA) for topical deliver of epoetin beta (EPO β) to the retina, in a sustained profile. Firstly, in vitro and ex vivo testing of the physicochemical stability, cytotoxicity, permeation studies and mucoadhesive properties of the CS/HA-EPO β nanoparticles were performed. EPO β was successfully encapsulated in CS/HA nanoparticles, and showed no in vitro cytotoxicity, being suitable for ocular administration with an enhanced EPO β permeation through ocular membranes. Then, in vivo studies in healthy Wistar Hannover rats were performed using the subconjunctival route of administration, to assess the formulation's biological tolerance and safety, its local and systemic impact, its influence in retinal electrophysiology, and EPO β 's distribution in ocular tissues by immunofluorescence. These nanoparticles allowed for a sustained EPO β retinal delivery using a minor invasive route of administration. At present, we are exploring the hypothesis of topical delivery of EPO β in glaucomatous animals. This ongoing line of research consists of assessing retinal morphological and physiological changes in response to the nanoformulation applied by topical ocular route, using electroretinography, histological evaluation, and apoptosis quantification (caspase-3 activity). Our ultimate goal is to develop a non-invasive and patient-friendly neuroprotective/ neuroregenerative treatment using CS/HA-EPO β nanoparticles targeting the retina in cases of glaucoma and other retinopathies. This project was made possible due to the team's skills in the field of nanoparticles development, animal experimentation, ophthalmology (examination, electroretinography and microsurgery) and histological procedures (paraffin embedding and immunofluorescence). Three papers have already been published in Q1 peer-reviewed journals: (1) review article on nanoparticulate systems for ocular drug delivery; (2) CS/HA-EPO β nanoparticles' characterization and ex vivo study in pig eyes; (3) subconjunctival administration of the CS/HA-EPO β nanoparticles in a rat model. Results have also been communicated in three oral presentations in national and international conferences and two poster presentations in national congresses.



1st Meeting of the Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

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