CIISA
ACTIVITY REPORT
2013-2017

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A WORD FROM THE COORDINATOR

A Word from the Coordinator

The Centre for Interdisciplinary Research in Animal Health – CIISA - the Research Centre of the Faculty of Veterinary Medicine of the University of Lisbon, is commemorating its 25-year jubilee in 2018. Following a governance and scientific restructuring in 2014, accompanying the evaluation of the Foundation for Science and Technology, CIISA has been consolidating as the top ranking national Animal and Veterinary Sciences research unit.

During the 2013-17 period, CIISA took a leading role in the coordination of national and international research networks and consortiums. This conveyed a highly interdisciplinary nature to CIISA's research, encompassing animal, veterinary and biomedical sciences. This multi- and interdisciplinary nature is reflected on the broad scientific background of the team. CIISA showed a steady increase trend in scientific indicators, and most importantly in the quality and impact of its outputs at the scientific, economic and societal levels. Along with the scientific performance, advanced training, technology transfer and consulting to stakeholders, outreach and dissemination have all been ancillary targets of CIISA's agenda.

The above was the result of the combined efforts of a highly motivated, committed and entrepreneurial team, including CIISA's Coordination, Lab leaders and principal researchers, Post-Docs, PhD and Master students, technicians and administrative staff.

This publication showcases the activities encompassed in the 2013-2017 period.

Luís Lopes da Costa

A SNAPSHOT OF CIISA FACTS AND STATS

MISSION

Mission

The Centre for Interdisciplinary Research in Animal Health – CIISA – initially emerged as a R&D Unit to stimulate, implement and coordinate the research activities at the Faculty of Veterinary Medicine of the University of Lisbon (FMV-ULisboa). This mission evolved to its present status of also consolidating and coordinating the research activities established at multiple institutions, within national and international networks. CIISA develops fundamental and applied research in the areas of animal, veterinary and biomedical sciences. Scientific advance in these mutually linked areas of knowledge generally lacks a common framework that can effectively foster knowledge and innovation. At a national level, CIISA provides an organization platform that leads and integrates research performed at the major Portuguese institutes and research centers acting in the Animal Science and Animal Health fields. For this purpose, in the last 5 years (2013-17), CIISA extended its networks to integrate R&D institutions spread throughout the country, including Universities, Polytechnic Institutes, Research Institutes, National State Labs, pharma, biotech and food producing (animal and human) industries, private enterprises, cooperatives, producer's associations and other non-profit organizations. This allowed the rational use of research infrastructures and resulted in a high level of internationalization, arising from an impressive number of collaborations with foreign labs. Internationalization is a full priority of CIISA's policy, and CIISA has ongoing strong collaborations with top-ranking international R&D Institutions, within EU and outside EU. As a result of all the above, CIISA achieved its status in the international scenario, where CIISA's scientists have lead international consortia, have been invited to integrate networks, present key-note conferences and support consultancy and advice at the highest levels. This highlights the scientific merits of CIISA's scientists with respect to international recognition.

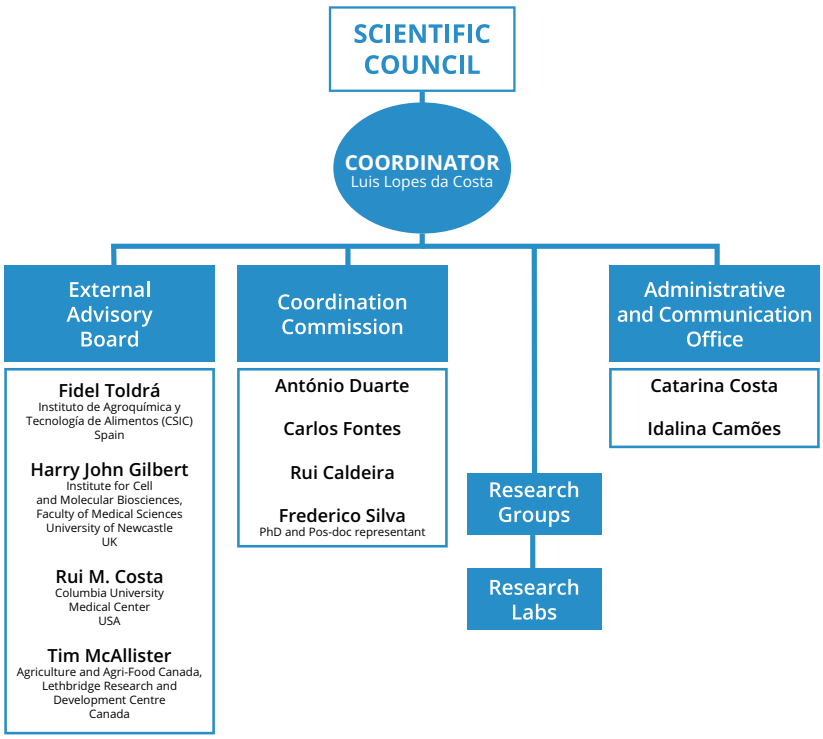
Research at CIISA is based in multidisciplinary approaches to foster innovation in a global international scenario, in articulation with higher education studies and the promotion of scientific careers and entrepreneurism. The highly mature international networks where CIISA is integrated promote a high mobility for students, junior and senior scientists. CIISA's research policies addresses 12 of the 17 specific United Nations Sustainable Development Goals and Targets. Also, CIISA's research is tuned with Europe's increasing concerns with animal health related issues that have considerable impact in the global economy and in public health, and with the general claims for the development of novel environmentally sustainable systems for animal production and agriculture. CIISA publications and its citations can easily be explored in the Clarivate Analytics Web of Science Database by searching the following author identifier: D-2242-2016.

A SNAPSHOT OF CIISA FACTS AND STATS

GOVERNANCE AND ORGANIZATION

Governance and Organization

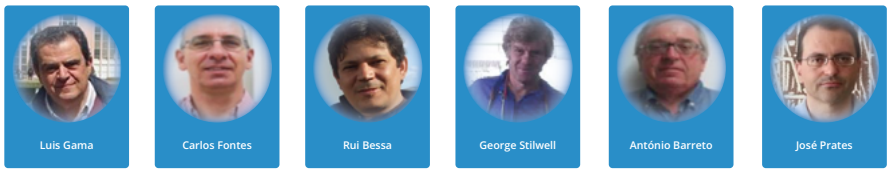
An updated CIISA's Statute was recently approved (2016). CIISA Scientific Council is composed of all PhD entitled members, elects the Coordinator and approves the annual scientific and financial reports, and the annual and future strategic plans. According to CIISA Statute, CIISA Coordinator is elected for a 4 years mandate and nominates the members of the Coordination Commission, which work in close association with CIISA Coordinator to execute the strategic research plan. The Coordination Commission members (presently 5) represent all the major research lines in development within the Centre and include one representative of the PhD students and Post-Docs. The research policies are designed by CIISA's Coordination in accordance with scientific, economic and societal challenges, and including inputs from the External Advisory Board (EAB), which comprises 4 world-renowned scientists in CIISA's research expertise areas. An administrative and communication office deals with all necessary informatics, accounting and divulgation procedures. CIISA's scientific policies are synergistically developed with those of its Management Institution, the Faculty of Veterinary Medicine of the University of Lisboa (FMV-ULisboa). This is achieved by a close relationship between the management bodies of both institutions, namely by integrating the President of the Scientific Council of FMV in CIISA's Coordination Commission. In addition, 3 CIISA members are elected to integrate the Scientific Council of FMV that has a total of 15 members.



CIISA's scientific organization considers two research groups, each of them with several laboratories. These two research groups develop four main thematic lines. There is a high level of collaboration between laboratories, both within and between research groups, which is associated to extensive collaborations with other national and international institutions and laboratories.

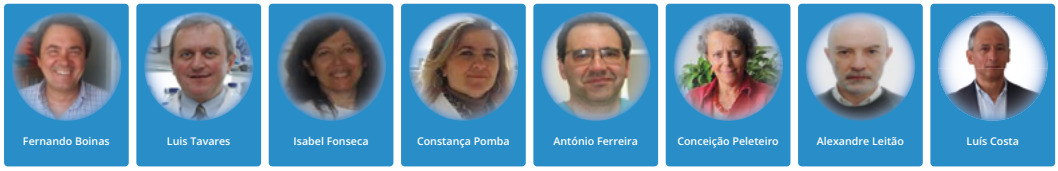
Research Groups and Labs

Animal Science and Food Safety



Animal Genetic Resources | Animal Nutrition and Biotechnology | Animal Production Systems | Animal Behaviour and Welfare | Food Technology and Safety | Quality of Animal Products

Animal Health and Veterinary Medicine



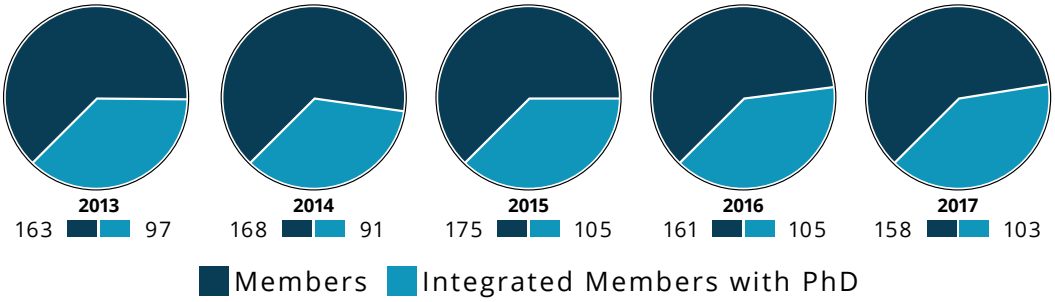
Infectious Diseases | Microbiology and Immunology | Parasitology and Parasitic Diseases | Antibiotic Resistance | Clinical Research | Pathology | Tropical Animal Health and Production | Reproduction and Development

A SNAPSHOT OF CIISA FACTS AND STATS

CIISA IN NUMBERS

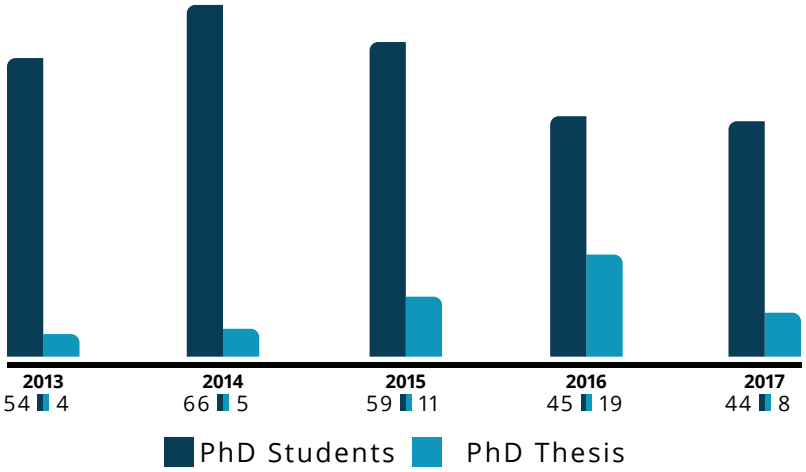
CIISA's Members

(2013-2017)



PhD Students and Thesis

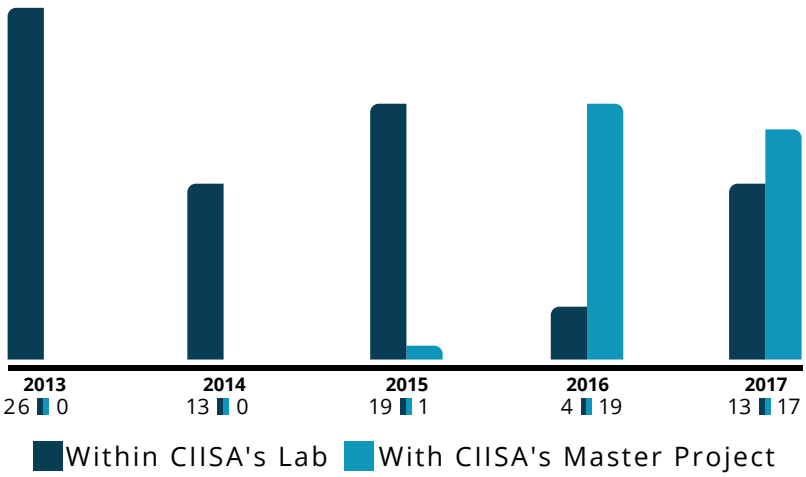
(2013-2017)



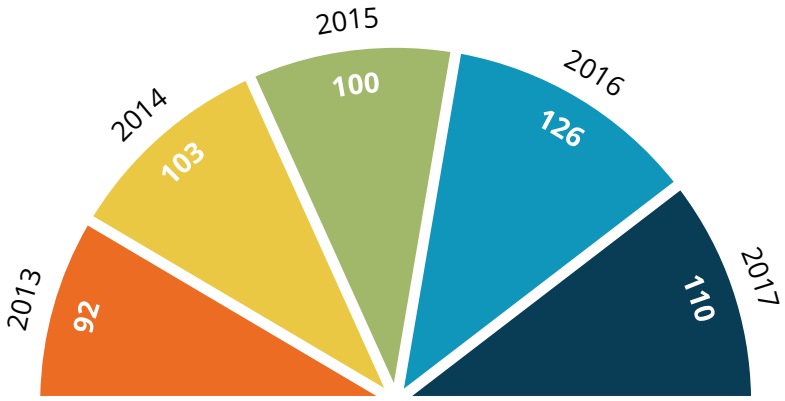
Master Thesis

(within CIISA's Lab and with CIISA's Master Project)

(2013-2017)



International Publications



Total of 531 Publications

Publications in International Peer-Review Journals

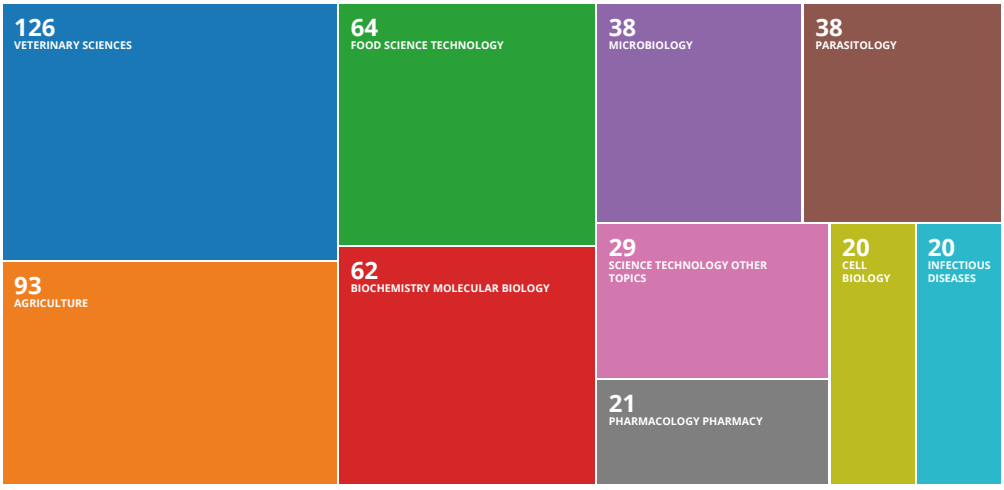
(2013-2017)

Research Group

233	298
Animal Science and Food Safety	Animal Health and Veterinary Medicine

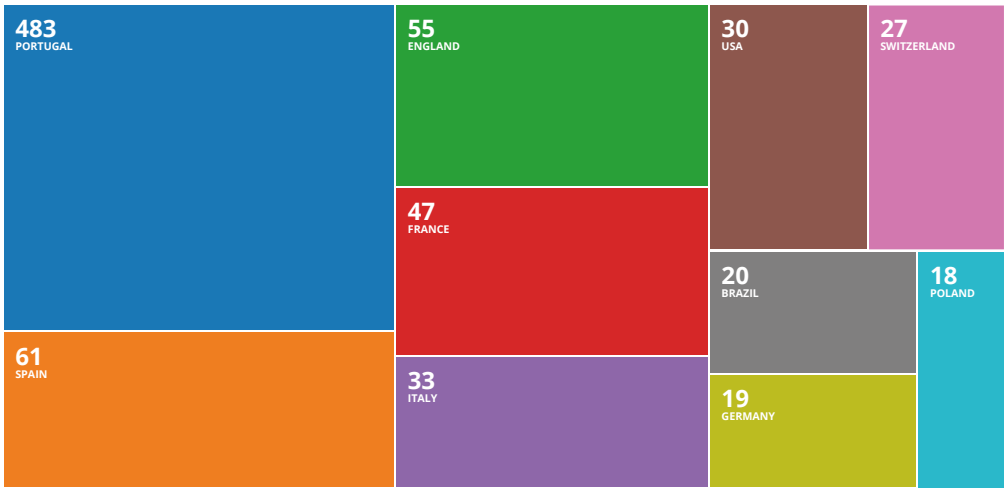
Scientific Areas of CIISA's Publications

(2013-2017)



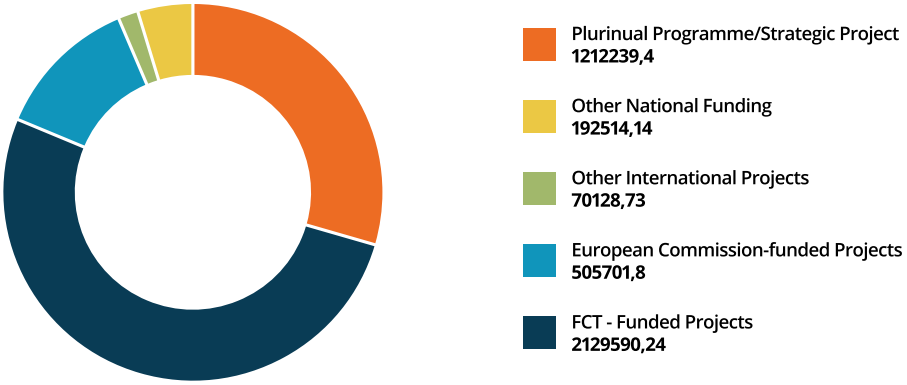
Internationalization of CIISA's publications

(2013-2017)



CIISA's funding sources – bench, missions, logistics

(2013-2017)



RESEARCH GROUPS

Research Groups

CIISA's labs are organized in two research groups: *Animal Science and Food Safety* and *Animal Health and Veterinary Medicine*. Animal Science and Animal Health are arguably two major research fields at the core of critical societal concerns of present times. Animal Science grants the development of novel sustainable systems to secure and provide plentiful, healthy and nutritious food for an increasing Human population. Animal Health directly impacts public health and the global economy and, in essence, it is at the base of the research required to sustain Human Health. The concepts of From *Farm to Fork* and *Sustainable Animal Production*, and of *One Health* and *Translational Medicine* are major pillars of CIISA's research, and the basis of the four major thematic lines developed by CIISA. The *From Farm to Fork* and *Sustainable Animal Production* lines relevantly include nutritional biotechnology, quality and safety of animal products, biodiversity conservation (world-wide and native genetic resources), and animal welfare and wellbeing (in intensive and extensive production systems). The *One Health* and *Translational Medicine* lines mainly consider emergent infectious diseases and zoonosis, such as vector-borne diseases, translational medicine, such as oncobiology, antimicrobial resistance, and comparative medicine aspects, namely animal models of Human disease.

RESEARCH GROUPS

ANIMAL SCIENCE AND FOOD SAFETY

Animal Science and Food Safety

CIISA´s *Animal Science and Food Safety* group performs cutting edge research in animal science and food safety integrated in a diversity of national and international collaborations. Animal Science and Food Safety are two mutually connected scientific fields of major importance to humankind. Globally, we assist to an increasing demand for high quality animal products, supplied from sustainable and environmentally friendly production systems that promote animal welfare and the occupation of the rural areas. In addition, modern food and technology sciences have made enormous contributions to the safety and availability of animal-derived foods worldwide. The group is actively contributing towards the development and the densification of the National Scientific and Technologic System by establishing a diversity of scientific interactions with a cohort of institutions spread from the North to the South of the Portuguese territory, established under the framework of detailed partnership. The group's integration in highly relevant international scientific networks allows it to lead the national effort to develop high impact research in the Animal Science and Food Safety fields, while contributing to the training of the next generation of eminently motivated and well prepared animal science scientists. During 2013-17, the *Animal Science and Food Safety* group raised approximately one million euros in external funding and provided advanced training to well above 50 PosDocs, PhD and MSc students. Major discoveries were reported in 233 papers published in international journals.

Animal Nutrition and Biotechnology

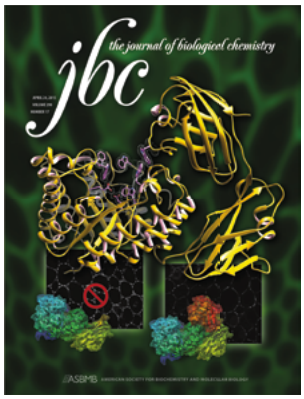
Lab Leader Carlos Fontes | **Researchers** Ana Filipa Sequeira, Joana Brás, Luís Ferreira, Maria José Centeno, Shabir Najmudin, Vânia Fernandes, Victor Alves, Virgínia Rico Pires | **PhD Students** Benedita Andrade, Immacolatta Venditto, Kate Cameron, Luís André, Márcia Correia, Pedro Bule, Vânia Cardoso | **Technicians** Helena Santos **Administrative** Maria Paula Silva

From left to right: Maria Paula, Victor Alves, Joana Brás, Luís André, Virginia Pires, Helena Santos, Mª José Centeno, Patrícia Ponte, Luís Ferreira, José Prates, Immacolatta Venditto, Pedro Silva (visitor), Carlos Fontes, Márcia Correia. Inset: Shabir Najmudin and Pedro Bule.

1. Research Interests and Expertise

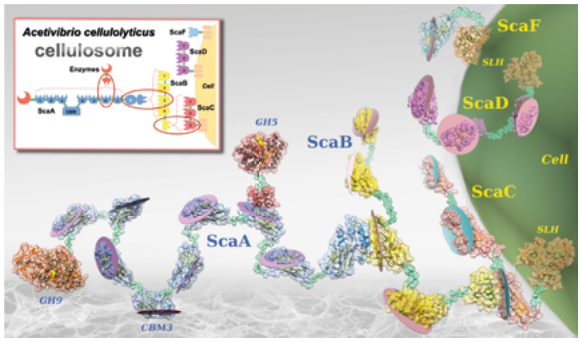
During the last 20 years the Animal Nutrition and Biotechnology (ANB) Lab has been developing cutting edge research to elucidate the role of Carbohydrate-Active enZYmes (CAZYmes) and their ancillary non-catalytic Carbohydrate- Binding Modules (CBMs) in animal nutrition. In nature these microbial enzymes play a major role in the hydrolysis of complex carbohydrates, such as cellulose and hemicellulose, in the gut of herbivores, thus providing a major source of metabolic energy from dietary fiber. Anaerobic microbes that colonize the gastro intestinal tract of mammals organize CAZymes in high molecular mass multi-enzyme complexes, termed Cellulosomes, which represent one of the most elegant and intricate nanomachines evolved by Nature. Work developed at ANB Lab contributed to uncover the mechanism of cellulosome assembly that involves the binding of dockerin domains, located at the C-terminus of cellulases and hemicellulases, to repeated cohesin modules of a structural non-catalytic protein. Thus, highly specific and tight protein:protein interactions modulate the mechanisms of cellulosome organization and cell-surface attachment. Detailed structural, biochemical and molecular studies revealed the intricacies of cellulosomes in different bacteria. More recently these studies culminated in the characterization of a novel mechanism of cellulosome assembly in the major cellulolytic bacterium found in the rumen of mammals, *Ruminococcus flavefaciens*, which combines more than 200 different enzymes (see highlights). Overall these studies indicate that the assembly of CAZymes into cellulosomes potentiates enzyme stability and cooperativity leading to the efficient hydrolysis of recalcitrant polysaccharides. Finally, we have been investigating different biotechnological applications for CAZymes, namely to improve the nutritive value of cereal-based diets for non-herbivore monogastric animals (see highlight). During the 2013-2017 period these studies were published in 43 papers and more than 50 protein structures were deposited in the Protein Data Bank (www.rcsb.org/pdb/index.html). A selection of the 10 most exciting papers published by the ANB lab during 2013-2017 is provided below.

2. Highlights



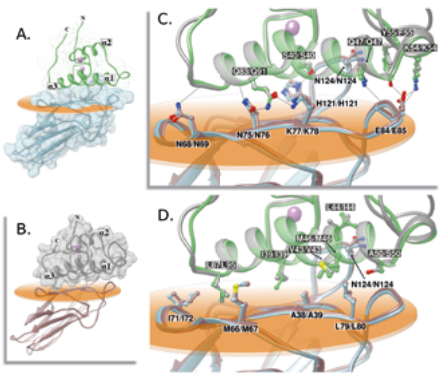
Understanding the role of Family 46 Carbohydrate-binding Modules to the Enzymatic Hydrolysis of different polysaccharides

In collaboration with the group of Harry Gilbert at The University of Newcastle, UK, the ANB Lab revealed that the CBM of endo-β-1,4-glucanase B (BhCel5B) from *Bacillus halodurans* displays a previously unknown function. Strikingly the CBM on its own did not bind soluble or insoluble polysaccharides. The domain was tightly associated with the catalytic module. Depending on the nature of the carbohydrate, the CBM cooperated with the catalytic module to participate in substrate binding or target the enzyme to parts of the cell wall rich in polysaccharides. The data was published in JBC and the paper was selected as 2015 best of JBC papers of the week. BhCel5B structure was selected for the JBC cover (figure on the left).



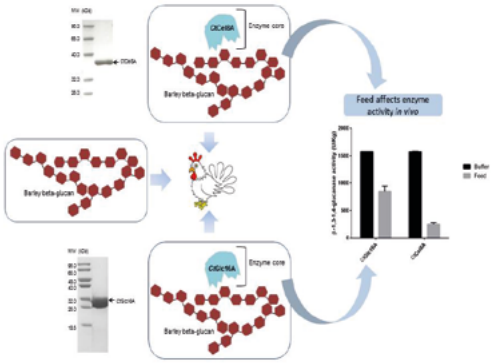
Molecular architecture of *Acetivibrio cellulolyticus* cellulosome

Central to cellulosome assembly is a large non-catalytic protein, termed scaffoldin, that contains several repeated cohesin (Coh) domains that establish high affinity protein- protein interactions with dockerin (Doc) modules located in CAZymes. Thus, scaffoldins provide a molecular platform on to which the enzymes will bind through their appended Doc modules. The above figure represents the highly complex cellulosome of *A. cellulolyticus* where several unique adaptor scaffoldins and anchoring scaffoldins contribute to increase the intricacy of this remarkably elaborate nanomachine.



Structure of the cohesin-dockerin complex of the cellulosome of the major cellulolytic bacteria in the rumen, *Ruminococcus flavefaciens*

In collaboration with groups located at the FCT/UNL (Portugal), the Weismann Institute of Science (Israel), the Universities of Newcastle and York (UK) and the Queen's University (Canada), ANB lab recently solved the structures of two primordial Coh-Doc complexes in *Ruminococcus flavefaciens* cellulosome revealing that, in contrast with the majority of these protein- protein interactions identified in nature, *Ruminococcus* Dockerins display a conserved mechanism of Cohesin recognition involving a single-binding mode (figure above). Thus, this work reveals a novel mechanism of cellulosome assembly and challenges the ubiquitous implication of the dual-binding mode in the acquisition of cellulosome flexibility.



β-glucanases and not cellulases improve the nutritive value of cereal-based diets for poultry.

Addition of CAZymes to diets containing high levels of soluble non-starch polysaccharides, leads to lower digesta viscosity in poultry and pigs, leading to an improved performance. The ANB lab in collaboration with colleagues at the Agronomy Institute ISA-ULisboa revealed that not all enzymes promote a positive effect in animal nutrition. In the particular case of β-glucan rich diets, β-glucanases but not cellulases, which also effectively cleave the anti-nutritive polysaccharide, improve the nutritive value of cereal-based diets for poultry (figure on the left). High levels of cellulose in diets trap cellulases that become unavailable to attack β-glucans.

3. Representative Projects

"WallTraC: The Plant Cell Wall Training Consortium", 2011-2015, project funded by the European Comission (FP7) considering Marie Curie Initial Training Network (ITN) grants. This project involved 9 partner institutions from 6 countries. CIISA was the hosting institution of the PhD students Immacolatta Venditto and Kate Cameron.

PTDC/BIA/MIC-5947-2014, funded by Foundation for Science and Tecnology (FCT); PI - Carlos MGA Fontes

4. Selected Publications

Bule P, Alves VD, Israeli-Ruimy V, Carvalho AL, Ferreira LM, Smith SP, Gilbert HJ, Najmudin S, Bayer EA & Fontes CMGA (2017). Assembly of *Ruminococcus flavefaciens cellulosome* revealed by structures of two cohesin-dockerin complexes. ***Scientific Reports*** 7:759.

Pires VM, Pereira PM, Brás JL, Correia M, Cardoso V, Bule P, Alves VD, Najmudin S, Venditto I, Ferreira LM, Romão MJ, Carvalho AL, Fontes CMGA & Prazeres DM (2017). Stability and ligand promiscuity of type A carbohydrate-binding modules are illustrated by the structure of *Spirochaeta thermophila* StCBM64C. ***Journal of Biological*** **Chemistry** 292, 4847-4860.

Venditto I, Luis AS, Rydahl M, Schückel J, Fernandes VO, Vidal-Melgosa S, Bule P, Goyal A, Pires VM, Dourado CG, Ferreira LM, Coutinho PM, Henrissat B, Knox JP, Baslé A, Najmudin S, Gilbert HJ, Willats WG & Fontes CMGA (2016). Complexity of the *Ruminococcus flavefaciens* cellulosome reflects an expansion in glycan recognition. ***Proceedings of the National Academy of Sciences USA*** 113, 7136-7141.

Bule P, Alves VD, Leitão A, Ferreira LM, Bayer EA, Smith SP, Gilbert HJ, Najmudin S & Fontes CMGA (2016). Single Binding Mode Integration of Hemicellulose-degrading Enzymes via Adaptor Scaffoldins in *Ruminococcus flavefaciens* Cellulosome. ***Journal of Biological Chemistry*** 291, 26658-26669.

Brás JL, Pinheiro BA, Cameron K, Cuskin F, Viegas A, Najmudin S, Bule P, Pires VM, Romão MJ, Bayer EA, Spencer HL, Smith S, Gilbert HJ, Alves VD, Carvalho AL & Fontes CMGA (2016). Diverse specificity of cellulosome attachment to the bacterial cell surface. ***Scientific Reports*** 6:38292.

Fernandes VO, Costa M, Ribeiro T, Serrano L, Cardoso V, Santos H, Lordelo M, Ferreira LMA & Fontes CMGA (2016). β-1,3-1,4-glucanases and not β-1,4-glucanases improve the nutritive value of barley-based diets for broilers. ***Animal Feed Science and Technology*** 211, 153–163.

Venditto I, Najmudin S, Luís AS, Ferreira LMA, Sakka K, Knox JP, Gilbert HJ & Fontes CMGA (2015). Family 46 Carbohydrate-binding Modules Contribute to the Enzymatic Hydrolysis of Xyloglucan and β-1,3- 1,4-Glucans through Distinct Mechanisms. ***The Journal of Biological Chemistry*** 290, 10572-86. (2015 Best JBC Papers of the Week)

Cameron K, Weinstein JY, Zhivin O, Bule P, Fleishman SJ, Alves VD, Gilbert HJ, Ferreira LMA, Fontes CMGA, Bayer EA & Najmudin S (2015). Combined crystal structure of

a type-I cohesin, mutation and affinity- binding studies reveal structural determinants of cohesin-dockerin specificity. ***The Journal of Biological Chemistry*** 290, 16215-16225.

Cameron K, Najmudin S, Alves VD, Bayer EA, Smith SP, Bule P, Waller H, Ferreira LM, Gilbert HJ & Fontes CMGA (2015). Cell-surface attachment of bacterial multi-enzyme complexes involves highly dynamic protein-protein anchors. ***The Journal of Biological Chemistry*** 290, 13578-13590.

Luís AS, Venditto I, Temple M J, Rogowski A, Baslé A, Xue J, Knox JP, Prates JAM, Ferreira LMA, Fontes CMGA, Najmudin SE & Gilbert HJ (2013). Understanding how noncatalytic carbohydrate binding modules can display specificity for xyloglucan. ***The Journal of Biological Chemistry*** 288(7):4799–4809.

5. Collaborations

Over the years the ANB Lab has established a large network of collaborations with scientists in a variety of laboratories, in particular those located in North America (Queen's University, Canada), the European Union (Universities of York and Newcastle, UK; NOVA University of Lisbon, Portugal), Israel (The Weismann Institute of Science) and India (Indian Institute of Technology Guwahati). These network of collaborations allowed exploring shared infrastructure and human resources and to develop structural, biochemical and molecular studies in a complementary and truly efficient way.

6. Outreach

A spin off company was generated from work developed at the ANB Lab, NZYTech (www.nzytech. com). NZYTech is the leading company worldwide in the provision of a large bank of carbohydrate degrading enzymes for R&D. Currently NZYTech provides >1000 highly purified enzymes in the mg scale. These represent a high value enzyme repository for research and development.

7. Advanced Studies (PhD e MSc thesis)

During 2013-2017, 6 PhDs were awarded to young scientists developing their studies at the ANB lab, including 2 Marie Curie European PhDs that developed research under the ITN WallTrac (www.walltrac-itn.eu) supported by an EU FP7 grant.

Animal Production Systems

Lab Leader Rui J.B. Bessa | **Researchers** Alexandra Francisco, José Pedro Lemos, José Santos Silva, Magda Fontes, Renato Nuno Carolino, Rosário Marques, Rui Caldeira
Post-Docs Susana Alves | **PhD Student** Maria Teresa Dentinho

From Left to right: "Acatar", Maria João Fradinho, Rui Bessa, José Pedro Lemos, Rui Caldeira, Magda Fontes, Martin Janicek, Alexandra Francisco, José Santos Silva, Susana Alves, Teresa Dentinho, Chica Manuel, Ana Mónica Godinho, Afonso Rodrigues, "Xahidra"

1. Research Interests and Expertise

The Animal Production Systems (APS) Lab is focused on the study and optimization of animal production systems, in order to increase animal/herd productivity and optimize feeding to improve animal product quality. Ruminants and equines have been our major target species, but our activities include also other farm animals and poultry species. Determinants of meat and milk quality have been our main interest in ruminants, while in equines the adequate development of foals has been the main objective.

The relationships between rumen microbial metabolism and product quality of ruminants have been one of our major research interests. Lipids of ruminants' edible products contain relatively high proportions of saturated and trans fatty acids due to the extensive biohydrogenation (BH) of unsaturated fatty acids carried out by the rumen microbiota. We have been deeply involved on the development of practical production strategies to modulate the biohydrogenation of unsaturated fatty acids that occurs in the rumen in order to improve the fatty acid composition of meat and milk. However, the biology and chemistry of rumen biohydrogenation is still poorly understood and undesirable changes of the usual biohydrogenation pathways can occur in intensively fed ruminants, leading to strong accumulation of trans-10 18:1 in the tissues. Thus, most of our recent efforts have been directed to elucidation the trans-10 shifted biohydrogenation pathways and of the main production and dietary factors that determine its occurrence in intensively finished ruminants.

The valorisation of edible animal products has been pursued by searching objective tools to demonstrate their association to differentiated and/or certified production systems and by modulation of animal metabolism via animal nutrition. Also, we have been evaluating consumer's preferences regarding objective and subjective animal product quality traits and incorporating that knowledge on the optimization of production systems.

Our lab combines the advanced expertise in lipid metabolism and on advanced lipid analytical techniques with expertise on animal production system, management and nutrition.

With equine production, our main research interests are focused on the study of growth and development of the foal, body condition changes and metabolic indicators in the broodmare during the productive cycle, and the relationship between foals' growth patterns and bone quality. We aimed also to investigate the use of new feed materials rich in essential nutrients, which may have beneficial impacts at different metabolic process.

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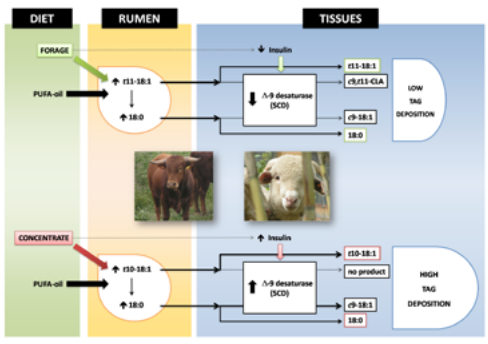
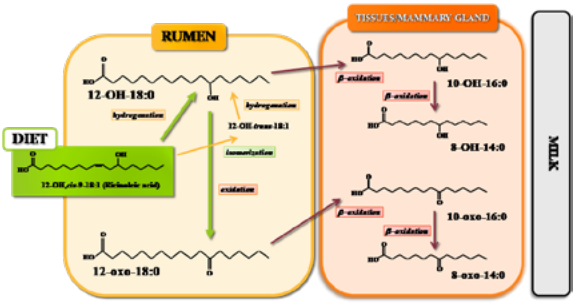
2. Highlights

During the last 5 years our lab achieved a consolidated international position in the area of ruminant animals' lipid metabolism and its resulting impacts on meat quality. Understanding the complexity of the ruminal lipid metabolism provides a solid base to regulate meat and milk lipid composition by the host animal. We have identified novel biohydrogenation intermediates and microbial lipids, refined the biohydrogenation pathways for several unsaturated fatty acids and explored the modulatory effects of tannins on biohydrogenation.

- The main recent research highlights of the lab are: Update and improvement of the analytical techniques to study the rumen microbial plasmalogen lipids.
- Identification for the first time in ruminants digestive contents and tissues the t10,c15-18:2 and its establishment as the missing biohydrogenation intermediate of the postulated trans-10 shifted pathways of the ruminal biohydrogenation of linolenic acid.
- Identification for the first time of the occurrence of conjugated 22:6 isomers during the initial steps of docosapentaenoic acid (DHA) ruminal biohydrogenation.
- Establishment of the metabolization of ricinoleic acid on lactating ruminants.
- Advances in the understanding of the occurrence of the trans-10 shifted biohydrogenation, including the large individual variability and genotype influences.
- Identification for the first time of long branched chain fatty acids as constituents of the rock rose (*Cistus ladanifer*) shrub.
- Establishment of the growth curves of Lusitano foals



Simplified summary of the main drives that could limit the *c*9,*t*11-CLA deposition in meat of ruminants fed high forage or high concentrate diets (adapted from *Bessa et al, Eur. J. Lipid Sci. Technol.* 2015).



Proposed metabolism of ricinoleic acid (12-OH,cis-9–18:1) in dairy ruminants. Thick arrows represent the main metabolic pathways and thin arrows the putative pathways including all the oxo-fatty acids (FA) and hydroxy-FA detected in milk fat of goats fed castor oil, whereas the dashed arrows represent putative pathways including the hydroxy-FA detected in incubations of castor oil with strained rumen fluid (adapted from *Alves et al., J. Dairy Sci. 2017*)

3. Representative Projects

PTDC/CVT/120122/2010 - Modulation of rumen biohydrogenation through polyunsaturated fatty acid overload and tannins. PI: Rui Bessa (FMV-ULisboa), Partners: ICETA-Porto/REQUIMTE/ UP. (2012-2015)

PRODER/IFADAP/2011/PA Nº 23957 - New processes in the Lusitano horse production: The optimization of feeding as the base of the system productivity and the quality of foals. PI: Rui Caldeira. Partners: Coudelaria Henrique Abecasis, Sociedade Agrícola Santa Joana, CIISA/ FMV/ULisboa e UTAD/CECAV. (2011-2014)

COOPERAÇÃO CIENTÍFICA E TECNOLÓGICA FCT/CNPq - 2014/2015 Procº 4.4.1.00. Impacto do sistema de alimentação sobre a qualidade do leite e do queijo de caprinos no semiárido Brasileiro. PI (Portugal): Rui Bessa (FMV-ULisboa), PI (Brasil): Marta Madruga (UFPB). (2014-2015)

Red CYTED 116RT0503 "Marcas de qualidade de carne e produtos cárneos ibero americanos". PI: Instituto Politécnico de Bragança. (2016-2019)

FAPEMA Edital Nº 031/2016 "Pecularidades digestivas e metabólicas de ovinos rabo largo". PI: Universidade Federal do Maranhão (2017-2019)

ALT20-03-0145-FEDER-000023 CistusRumen - Utilização Sustentável de Esteva (Cistus Ladanifer L.) em pequenos ruminantes – Aumento da competitividade e redução do impacto ambiental. PI: Eliana Jerónimo (Cebal). Partners: CEBAL; INIAV; Univ. Évora; FMV-ULISBOA. (2016-2019)

ALT20-03-0145-FEDER-000040 ValRuMeat - Valorização da Carne de Ruminantes em Sistemas Intensivos de Produção. PI: José Santos Silva (INIAV) Partners: INIAV, FMV-ULISBOA, CEBAL (2016-2019)

4. Selected Publications

Alves, S.P., Santos-Silva J., Cabrita A.R.J., Fonseca, A.J.M., Bessa, R.J.B. 2013. Detailed dimethylacetal and fatty acid composition of rumen content from lambs fed lucerne or concentrate supplemented with soybean oil. ***PLoS ONE*** 8(3):e58386.

Rosa, H.J.D., Rego, O.A., Silva, C.C.G., Alves, S.P., Alfaia, C.M.M., Prates, J.A.M., Bessa, R.J.B. 2014. Effect of corn supplementation of grass finishing of Holstein bulls on fatty acid composition of meat lipids.***J.Anim. Sci.*** 92:3701-3714.

Alves, S.P., Bessa, R.J.B. 2014. The trans-10,cis-15 18:2: a missing intermediate of trans-10 shifted rumen biohydrogenation pathway? ***Lipids*** 49:527-541.

Viegas, I., Nunes, L. C., Madureira, L., Fontes, M. A., Santos, J. L. 2014. Beef credence attributes: Implications of substitution effects on consumers´ WTP. ***J. Agric. Econ.*** 65:600-615.

Bessa, R. J. B., Alves, S. P., Santos-Silva, J. 2015. Constraints and potentials for the nutritional modulation of the fatty acid composition of ruminant meat. ***Eur. J. Lipid Sci. Technol.*** 117:1325-1344.

Fradinho, M.J., R.J.B. Bessa, G. Ferreira-Dias, R.M. Caldeira. 2016. Growth and development of the Lusitano horse managed on grazing systems. ***Livest. Sci.*** 186: 22-28.

Harris, P.A., Ellis, A.D, Fradinho, M.J., Jansson, A., Julliand, V., Luthersson, N., Santos, A.S., Vervuert, I. 2017. Review: Feeding conserved forage to horses: recent advances and recommendations. ***Animal*** 11: 958–967.

Alves, S. P., Araujo, C. M., Queiroga, R. C., Madruga, M. S., Parente, M. O. M., Medeiros, A. N., Bessa, R. J. B. 2017 New insights on the metabolism of ricinoleic acid in ruminants. ***J. Dairy Sci.*** 100:8018-8032.

Costa, M., Alves, S. P., Francisco, A., Almeida, J., Alfaia, C. M., Martins, S. V., Prates, J. A. M., Santos-Silva, J., Doran, O., Bessa, R. J. B. 2017. The reduction of starch in finishing diets supplemented with oil does not prevent the accumulation of trans-10 18:1 in lamb meat. ***J. Anim. Sci.*** 95:3745-3761.

Aldai, N., Delmonte, P., Alves, S.P., Bessa, R.J.B., Kramer, J. F. K. 2018. Evidence for the initial steps of DHA biohydrogenation by mixed ruminal microorganisms from sheep involves formation of conjugated fatty acids. ***J. Agric. Food Chem.*** 66:842-855.

5. Collaborations

The Animal Production System lab is open to collaborations with other research teams. In fact, fruitful collaborations were established with teams from University of Bask Country (Dr. Noelia Aldai Lab), University of Pisa (Profº Marcelo Mele lab), University of Firenzi (Profª Arianna Buccioni lab), University of Toulouse (Profª Annabelle Meynadier lab), Leibniz Institute for Farm Animal Biology (Dr. Dirk Dannenberger), Agriculture and Agri-Food Canada (Dr. Tim MacAllister, Dr. John Kramer, Dr. Mike Dugan labs), US Food and Drug Administration – FDA (Dr. Pier Delmonte lab), Universidade Federal da Paraíba (Prof. Ariosvaldo Medeiros & Rita Queiroga), Universidade Federal do Maranhão (Profª Michele Maia), Universidade Federal da Grande Dourados (Prof. Fernando Vargas Júnior), Universidade Federal do Ceará (Profª Elzania Pereira)

6. Outreach

CIISA scientists from the Animal Production Systems lab were awarded with the Elsevier Prize for the best poster at 15th Euro Fed Lipid Congress that was held in Uppsala, Sweden from 27 to 30 of August of 2017. The work was presented by Susana Alves with the title "The trans-10 shifted ruminal biohydrogenation pathway of α-linolenic acid: quantitative associations with t10,c15-18:2 and t10-18:1" and the following authors: Susana P. Alves, Alexandra Francisco, José Santos-Silva and Rui J.B. Bessa

7. Advanced Studies (PhD e MSc thesis)

During this period 4 PhDs were awarded to researchers developing their studies at the APS Lab.

Quality of Animal Products

Lab Leader José Prates | **Researchers** Cristina Alfaia, Mário Quaresma
Post-Docs José Pestana, Paula Lopes, Susana Martins, Marta Madeira
PhD Students Anabela Lopes, Cátia Martins, Diogo Coelho, Eva Rolo, Sofia Costa
Master Students Breno Puerta, Marta Jesus, Victor Major | **Technicians** Ana Ferreiro

From Left to right: Cátia Martins, Diogo Coelho, Marta Jesus, Victor Major, Cristina Alfaia, José Pestana, Marta Madeira, Breno Puerta, José Prates, Mário Quaresma, Paula Lopes and Ana Ferreiro

1. Research Interests and Expertise

The Quality of Animal Products (QAP) Lab is devoted to manipulate fat deposition in livestock species aiming at improving production efficiency and sustainability, meat quality and human health. Our work is organized in two research lines: (1) Development of novel feeding strategies to improve fat partitioning and products quality; and (2) Valorization of bioactive lipids to reduce animal adiposity and improve human health.

Our research team has consolidated expertise in animal lipid metabolism and meat quality, as well as on molecular, genetic and cellular mechanisms underlying signalling pathways that regulate the deposition of adipose tissue and fatty acids. Our research work uses advanced analytical and instrumental methodologies, including HPLC-ECD, HPLC-DAD/FD, GC-FID, GC-MS, FPLC, ELISA, Luminex, RIA, Blotting, microarrays, miRNAs and RT-qPCR.

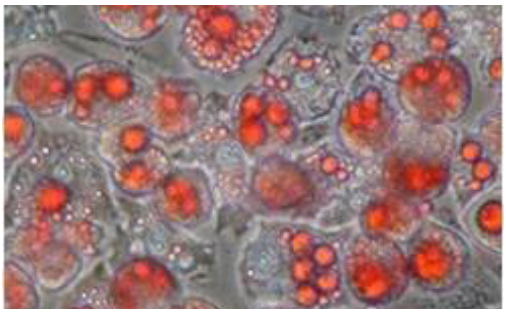
2. Highlights

Notable outcomes on the period 2013-2017 have been achieved by our group for both research lines, as follows:

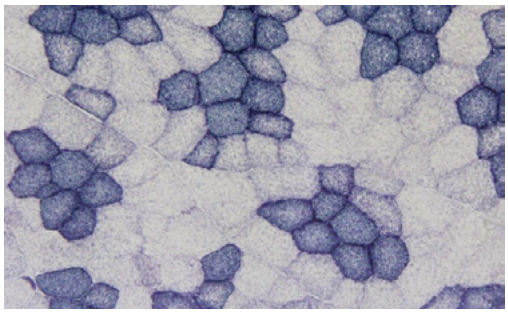
Development of effective nutritional strategies to improve fat partitioning and disclosed that intramuscular fat deposition in pigs is mediated by shifting the metabolic properties of muscle fibers, by higher fluidity of adipose membranes and by the up-regulation of key lipogenic enzymes and transcription factors. Moreover, we firstly elucidated that dietary lysine restriction improves fat partitioning in lean pig but not in fatty pig genotypes.

The thorough investigation of biological effects of conjugated linoleic acid (CLA), in several mammalian models, contributed to explain some of the reported health benefits of these isomers (the reducing weight properties of CLA are due to adipocyte apoptosis and increased adipocyte plasma membrane glycerol fluxes; CLA is able to influence the biosynthesis of bioactive endocannabinoids that may contribute to ameliorate fatty liver). In addition, novel anti-adipogenic properties of the individual trans8, cis10 CLA isomer has been firstly described by us.

It was also elucidated that the combination of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oil, but not from microalgae, can counteract the undesirable health effects of saturated fat based diets and benefit neurological function. We also have shown that the incorporation of DHA in tissues is higher when it is ingested as triacylglycerols (TAG) structured at the sn-2 position, which enhances efficacy and health benefits of dietary DHA *n*-3 supplementation.



Red oil (RO) staining of 3T3-L1 adipocytes



Succinate dehydrogenase (SDH) staining of muscle cell fibers.

3. Representative Projects

COST Action-FA1401/oc-2015-2-20148. IPEMA - Innovative approaches in pork production with entire males. (J.A.M. Prates - Management Committee Member). Partners: University of Bonn (DE), University of Hokenheim (DE), Institute for Agricultural and Fisheries Research (BE), KU Leuven (BE), Agricultural Institute (BG), University of Guelph (CA), Agroscope (CH), University of Life Sciences of Prague (CZ), Danish Meat Research Institute (DK), University of Copenhagen (DK), Institut de Recerca i Tecnologia Agroalimentàries (SP), University of Lleida (SP), IFIP Institut du Porc (FR), INRA (FR), University of the West of England (UK), JSR Genetics (UK), University of Bristol (UK), University of Sassari (IT), Norwegian Institute of Food (NO), University of Life Sciences in Poznań (PO), Swedish University of Agricultural Sciences (SW). (2016-2018)

P2020/08/SI/3399/2015 - Valorização das microalgas para a produção de suínos e melhoria da qualidade da sua carne com base numa nova geração de enzimas comerciais. PI: Carlos Fontes (FMV/ULisboa). Partners: Nzytech, INIAV, IPMA and FMV-ULisboa. (2016-2019)

PTDC/CVT/5931/2014 - Developing novel feed enzymes for improving the nutritional value of microalgae for monogastrics. PI: José A.M. Prates (FMV/ULisboa). Partners: INIAV, IPMA and FMV-ULisboa. (2016-2019).

PTDC/CVT/103934/2008 - Nutritional strategies to increase bioactive fatty acids proportion in lambs eatable fat. PI: José Santos Silva (INIAV, CIISA). Partners: INIAV and FMV-ULisboa. (2011 – 2014)

AGL2011-25807 (Spanish Government Funding) - Metabolic fate and health properties of structured triacylglycerols rich in *n*-3 long chain polyunsaturated fatty acids, eicosapentaenoic (EPA, 20:5*n*-3) and docosahexaenoic (DHA, 22:6*n*-3). PI: José L.G. Guerrero (Univ. Almeria Spain) Partners: Univ. Almeria (Spain), IPMA–Portugal, FMV-Ulisboa. (2012-2014)

COST-STSM-FA1002-17034 (Farm Animal Proteomics) - Proteomic changes underlying the improved fat partitioning in commercial pigs. Partners: CIISA-FMV-ULisboa and Univ. York (UK). PI: José A.M. Prates (FMV/ULisboa). (2014)

COST Action-FA1401/oc-2013-2-16276. PiGutNet - European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs. (J.A.M. Prates - Management Committee Member). Partners: Univ. Bologna (IT); Univ. Copenhagen (DK); Univ. Automona Barcelona (SP), Univ. Bristol (UK); Univ. Thessaloniki (GR); Univ. College Dublin (IR); Gembloux Agro-Biotech (B); Univ. Helsinki (FI); INRA (F); Wageningen Univ. (NL) (2014-2018)

PTDC/CVT/111744/2009 - Changes in muscle gene expression as response to selection for high growth and to growth path. PI: Paulo Costa (CIISA), Partners: CIISA/FMV/ULisboa; INIAV. (2011-2013)

CAPES 2009 - PROBIO-CÓBIA - Desenvolvimento sustentável do cultivo de bijupirá (Rachycentron canadum): implementação de um plano de imunização, uso de probióticos e dietas vegetais. PI: Julieta R. Engrácia (UNESP – Univ. Estadual Paulista Júlio de Mesquita Filho, Brasil), Partners: UNESP, Univ. Fed. Rural Pernambuco; CIIMAR). (2010-2014)

PTDC/CVT/99210/2008 - Improving fat partitioning and nutritional quality in pigs: developing feeding strategies and providing insights into genetic and metabolic mechanisms. PI: José A.M. Prates (FMV/ULisboa). Partners: CIISA/FMV/ULisboa; INIAV). (2010-2013)

4. Selected Publications

Lopes, P.A., Bandarra, N.M., Martins, S.V., Martinho, J., Alfaia, C.M., Madeira, M.S., Cardoso, C., Afonso, C., Paulo, M.C., Pinto, R.M.A., Guil-Guerrero, J.L., Prates, J.A.M., (2017). Markers of neuroprotection of combined EPA and DHA provided by fish oil are higher than those of EPA (*Nannochloropsis*) and DHA (*Schizochytrium*) from microalgae oils in Wistar rats. ***Nutr. Metabol.*** 14:62.

Madeira, M.S., Lopes, P.A., Costa, P., Coelho, D., Alfaia, C.M., Prates, J.A.M., (2017). Reduced protein diets increase intramuscular fat of psoas major, a red muscle, in lean and fatty pig genotypes. ***Animal*** 11:2094-2102.

Bandarra, N.M., Lopes, P.A., Martins, S.V., Ferreira, J., Alfaia, C.M., Rolo, E.R., Correia, J.J., Pinto, R.M.A., Ramos-Bueno, R.P., Batista, I., Prates, J.A.M., Guil-Guerrero, J.L. (2016). DHA at the sn-2 position of structured triacylglycerols improved *n*-3 polyunsaturated fatty acid assimilation in tissues of hamsters. ***Nutr. Res.*** 36:452-463.

Madeira, M.S., Rolo, E. A., Alfaia, C. M., Pires, V. R., Luxton, R., Doran, O., Bessa, R. J. B., Prates, J. A. M. (2016). Influence of betaine and arginine supplementation of reduced protein diets on fatty acid composition and gene expression in the muscle and subcutaneous adipose tissue of crossbred pigs. ***Br. J. Nutr.*** 115:937-950.

Pires, V.M.R., Madeira, M.S., Dowle, A.A., Thomas, J., Almeida, A.M., Prates, J.A.M., (2016). Increased intramuscular fat induced by reduced dietary protein in finishing pigs: effects on the longissimus lumborum muscle proteome. ***Molecular Biosystems*** 12: 2447-2457.

Madeira, M. S., Alfaia, C. M., Costa, P., Lopes, P. A., Martins, S. V., Lemos, J. P. C., Moreira, O., Santos-Silva, J., Bessa, R. J. B., Prates, J. A. M. (2015). Effect of betaine and arginine in lysine-deficient diets on growth, carcass traits and pork quality. ***J. Anim. Sci.*** 93:4721-4733.

Piras, A., Carta, G., Murru, E., Lopes, P.A., Martins, S.V., Prates, J.A.M., Banni, S. (2015). Effects of dietary CLA on n-3 HUFA score and N-acylethanolamides biosynthesis in the liver of obese Zucker rats. ***Prostaglandins, Leukotrienes Essent. Fatty Acids*** 98:15-19.

Rodrigues, P.O., Martins, S.V., Lopes, P.A., Ramos, C., Miguéis, S., Alfaia, C.M., Pinto, R.M.A., Rolo, E.A., Bispo, P., Batista, I., Bandarra, N.M., Prates, J.A.M. (2014). Influence of feeding graded levels of canned sardines on the inflammatory markers and tissue fatty acid composition of Wistar rats. ***Br. J. Nutr.*** 112:309-319.

Lopes, P.A., Costa, A.S.H., Costa, P., Pires, V.M.R., Madeira, M.S., Achega, F., Pinto, R.M.A., Prates, J.A.M. (2014). Contrasting cellularity on fat deposition in the subcutaneous adipose tissue and longissimus lumborum muscle from lean and fat pigs under dietary protein reduction. ***Animal*** 8:629-637.

Madeira, M.S., Pires, V.M.R., Alfaia, C.M., Luxton, R., Doran, O., Bessa, R.J.B., Prates, J.A.M. (2014). Combined effects of dietary arginine, leucine and protein levels on fatty acid composition and gene expression in the muscle and subcutaneous adipose tissue of crossbred pigs. ***Br. J. Nutr.*** 111:1521-1535.

5. Collaborations

European Network COST - IPEMA

José A.M. Prates is a Management Committee Member of COST action 2015 IPEMA (oc-2015-2-20148), involving 29 Institutions and Companies from 20 countries, including German, English, Italian, Spanish, Swedish, Switzerland and Canadian Universities.

The COST action IPEMA aims at facilitating the development of integrated solutions for the development of alternatives to the surgical castration of piglets, namely raising entire males and immunocastration.

European Network COST - PIGUTNET

José A.M. Prates is a Management Committee Member of COST action 2014 PiGutNet (oc-2013-2-16276), involving 55 Institutions and Companies from 17 countries, including Italian, Dutch, Polish, Swedish and English Universities.

PiGutNet
European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs.

- Fidel Toldrá, Institute of Agrochemistry and Food Technology (Valencia, Spain)
- Francisco Pérez, University Autonoma de Barcelona (Spain)
- Guil-Guerrero, University of Almeria (Spain)
- Julieta Engrácia, University Mesquita Filho (São Paulo, Brasil)
- Olena Doran, University of The West of England (Bristol, UK)
- Paolo Trevisi, University of Bologna (Italy)
- Sebastiano Banni, University of Cagliari (Italy)
- Tristan Chalvondemer, Ajinomoto (Japan)
- Ulrike Weiler, University of Hohenheim (Stuttgart, Germany)

6. Outreach

Workshops for High School Students (Verão na ULisboa), annually from 2013 to 2017

7. Advanced Studies (PhD e MSc thesis)

In the period of 2013-2017, 5 PhD and 15 MSc were awarded to young scientists working in the lab under the supervision of members of the team.

Food Technology and Safety

Lab Leader António Salvador Barreto | **Researchers** Ana Rita Henriques, Esther Baptista Gabriela Veloso, João Cota, Maria João Fraqueza, Miguel Cardo, Rui Seixas | **Post-Docs** Teresa Lemsadek, Marta Laranjo | **PhD Students** Ana Borges | **Master Students** André Rocha Margarida Traça, Joana Andrade, Rita Bernardes, André Rocha, Andreia Aires, Margarida Ruivo **Technicians** Helena Fernandes, Maria José Fernandes

From left to right: Ana Borges, Maria João Fraqueza, Gabriela Veloso, João Cota, Marília Ferreira, Maria José Fernandes, Teresa Lemsadek, Helena Fernandes, Antonio Barreto, Esther Baptista, Ana Rita Henriques,Joana Andrade, Rita Bernardes, André Rocha, Andreia Aires and Margarida Ruivo

1. Research Interests and Expertise

The FoodTech&Safe (FTS) Lab team main interests are related with the development of novel technologies and processes improving food quality and managing proactively the health risks associated to biological and chemical hazards arising from the food chain, namely zoonotic agents (bacteria and parasites) and food chemical hazards (dioxins and other undesirable substances, residues and contaminants), particularly on animal origin-foods.

The team develops scientifically based strategies in order to respond to the technological and research needs of food industry aiming food safety, quality improvement and consumers' satisfaction. The FTS Lab research include the development of new food processing technologies, improving preservation for better shelf life, food authenticity, quality and safety with emergent technologies. Some specific ongoing research lines include:

1. Strategies to reduce the formation of carcinogenic chemicals in Dry Cured Meat Products;
2. Traditional foods shelf life improvement by the application of emergent technologies (Isostatic High Pressure, UV pulsed light, ultrasounds) and by the development of new ingredients and processes;
3. Selection of bacteria with technological and/or probiotic potential from traditional fermented products of animal origin, namely meat sausages and cheese. Quality and safety improvement of traditional fermented meat products by the use of protective microbiota or bacteriocins nanoparticles under the concept of biopreservation;
4. Control of *Campylobacter* and *Campylobacter* -like organisms on poultry meat and products;
5. Ready to eat products: Pathogenomics and control of *Listeria monocytogenes*;
6. Pathogenomics of bacterial foodborne pathogens Enterococci, *Salmonella* and *Aeromonas*.

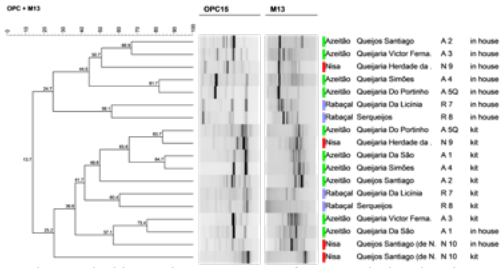
The FTS Lab team is experienced in food microbiology (OMIC technologies), chemistry and food processes optimization. Our expertise is related to safety preventive measures and food quality and safety management systems. The team work is linked to the pathogenomics of *Campylobacter* and *Campylobacter-like* organisms, *Listeria monocytogenes*, *Salmonella*, *Aeromonas* and with the genomic diversity and interactions of Lactic acid bacteria and *Staphylococcus* spp.

The FoodTech&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 offering the possibility of new food and processes development.

2. Highlights

- Studies reported a high level of *Campylobacter* contamination in poultry and also provided evidence that antimicrobial resistance (particularly to fluoroquinolones) is common among strains isolated from organic and intensive poultry production systems in Portugal. These results reinforce the need to develop strategies to reduce *Campylobacter* in chickens, and the necessity to reduce the use of antimicrobials in the poultry sector and implement specific control procedures to decrease the level of contamination in poultry meat by *Campylobacter* spp. Multi drug resistance (MDR) *C. coli* and two of the four MDR *C. jejuni* isolates studied presented polymorphisms at the cmeABC locus, strongly suggesting the role of this efflux pump in the MDR phenotype, in addition to the previously described point mutations in specific molecular targets. Also, *C. coli* were dominant with a high frequency of resistance to fluoroquinolones, tetracycline and ampicillin among quail flocks and producers studied. The fact that quail could be submitted to the same therapeutics continuously, inducing selectivity due to antibiotic stress pressure, seems to diminish the strains genetic diversity with the prevalence of a single pulsotype among quail's producers. *C. coli* contamination in quails could be a source of exposure to multidrug-resistant isolates for consumers. Presently, the group in an ongoing project will develop strategies at producing and industry level for *Campylobacter* control on poultry meat and meat products.

- Dry cured meat sausages are very popular ready-to-eat meat based products. Strategies to improve quality and safety of cured meat products have been studied by the group. The use of hybrid genotype meat in the manufacture of these products, traditionally made exclusively of Alentejano pig meat, does not affect their sensory properties. Moreover, salt reduction does not negatively affect the quality and acceptability of sausages. The application of emergent technologies such as HPP on this much appreciated traditional products showed that this technology can be successfully applied without losses of sensory and nutritional characteristics. The modelling and optimization of the HPP process applied on dry fermented sausages are an advantage to industry efficiency. The utilization of HPP by the industry can significantly increase dry fermented meat sausage shelf life and safety, providing an opportunity to reach the global market. The application of essential oils in the manufacture of dry cured sausage resulted in an interesting strategy to improve its safety against *Salmonella* spp, *L. monocytogenes* and *S. aureus*, but with sensory limitations, that does not allow its use in high concentrations, that are those more interesting for pathogen inhibition.

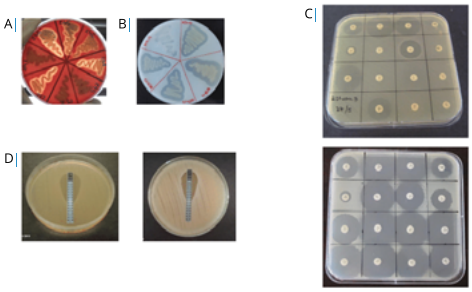


Dendrogram build using the Bionumerics software (Applied Maths), showing similarity levels between total DNA isolated from distinct food samples.

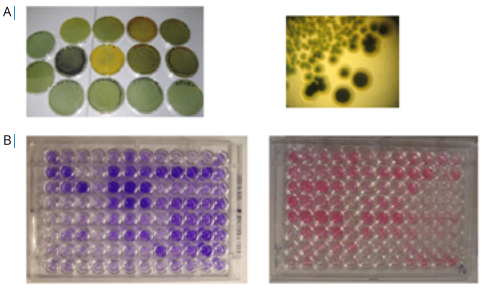
Industries with a high hygienic audit classification revealed higher probability to present *L. monocytogenes* serogroups more commonly associated to human disease, which could be the result of a previous identification of the pathogen, with an enforcement of the hygiene program without recognizing the real source of contamination. This reinforces the general assumption that a conjoined diagnosis using audit data and microbiological testing offers a more comprehensive insight and strengthens the Food safety management systems assessment conclusions.

The selection of lactic acid bacteria (LAB) and coagulase negative staphylococci (CNS) to be used as starters on meat products have been done by the group. Genomically diverse isolates were

selected. Autochthonous LAB and CNS showing low antibioresistance and low biogenic amines producing were selected as potential starters for future use in the production of dry fermented meat sausages.



A) Evaluation of hemolysin production, a known virulence factor, using Columbia agar supplemented with blood
B) Evaluation of gelatinase production, a known virulence factor, using culture medium supplemented with gelatin
C) Use of the agar diffusion method for evaluation of antimicrobial susceptibility
D) Evaluation of antimicrobial susceptibility using the E-test



A) Isolation of food-related aeromonads
B) *In vitro* assays for biofilm forming ability using microtiter plates

3. Representative Projects

"Farm2Fork-Identification of key steps on Antibiotic resistance dissemination through the food chain from farm-to-fork"; Project n.º 29510 IC&DT – AAC n.º 02/ SAICT/2017**.** PI at FMV: Maria João Fraqueza; Partners: ITQB Universidade Nova & Faculdade de Medicina Veterinária.

"*Campyfree*: Strategies for *Campylobacter* control on poultry meat and meat products"; PDR2020-101-031254. PI at FMV: Maria João Fraqueza. Partners: Faculdade de Medicina, Lusiaves-Industria E Comercio Agro-Alimentar Sa, Hiperfrango - Produção Avicola, Lda , Ass. Nac. Dos Centros De Abate e Ind. Tranf. De Carne De Aves - ANCAVE, Universidade Católica Portuguesa. 2017 – 2021. Project funded by IFAP. Site: www.campyfree.com

"Strategies for meat products quality and safety with the reduction of potential carcinogenic hazards"; PDR2020-1.0.1-FEADER-031359. PI at FMV: Maria João Fraqueza; Partners: Faculdade De Medicina Veterinária, Cooperativa Agricola de Boticas Capolib C.R.L., Assoc. Nac. Criad. Suinos Raca Bisara, Paladares Alentejanos - Soc. Pro. e Com. De Produtos Alimentares, Lda., Irmãos Monteiro, SA, Cercica-Coop. Educação E Reabilit. Cidadãos Inadaptados Cascais, C.R.L., Universidade Católica Portuguesa, Universidade de Évora, Universidade de Trás Os Montes E Alto Douro. 2017 – 2021. Project funded by IFAP. Site: www.safemeatprod.com

"Novos produtos derivados da alheira". Pedido de apoio nº 56345, Proder – Medida 4.1 – Cooperação para a Inovação. PI at FMV: Maria João Fraqueza; Partners: Bôfumeiro, Lda (promotor), Faculdade Medicina Veterinária, ESAB-Instituto Politécnico de Bragança. 2015 – 2018. Project funded by PRODER/IAPMEI.

"Portuguese traditional meat products: strategies to improve safety and quality". PTDC/AGR-ALI/119075/2010, PI: Maria João Fraqueza. 2012-2015

4. Selected Publications

Alves, S. P., Alfaia, C. M., Škrbić, B. D., Zivancev, J. R., Fernandes, *M. J.,* Bessa, R. J. B., Fraqueza, M. J. (2017). Screening chemical hazards of dry fermented sausages from distinct origins: Biogenic amines, polycyclic aromatic hydrocarbons and heavy elements. ***Journal of Food Composition and Analysis***, 59: 124–131.

Henriques A. R., Telo Da Gama L., Fraqueza M. J. (2017). Tracking *Listeria monocytogenes* contamination and virulence in the ready-to-eat meat-based food products industry according to the hygiene level. ***International Journal of Food Microbiology,*** 242: 101-106.

Laranjo, M., Gomes, A., Agulheiro-Santos, A. C., Potes, M. E., Cabrita, M. J., Garcia, R., Rocha, J. M., Roseiro, L. C., Fernandes. M. J., Fraqueza, M. J., Elias, M. (2017). Impact of salt reduction on biogenic amines, fatty acids, microbiota, texture and sensory profile in traditional blood dry-cured sausages. ***Food Chemistry***, 218: 129-136.

Santos, S. C., Fraqueza M. J., Elias, M., Barreto, A. S., Semedo-Lemsaddek, T. (2017). Traditional dry smoked fermented meat sausages: Characterization of autochthonous enterococci. LWT ***Food Science and Technology***, 79: 410-415.

Fraqueza, M.J., Patarata, L., Lauková, A. (2016). Protective cultures and bacteriocines in fermented meats. Chapter 11 *In*: ***Fermented meat products: Health aspects***. Editor: Nevijo Zdolec. CRC Press Taylor & Francis Group, NW, USA, ISBN:978-1-4987-3304-5. pp. 228-269.

Cardo, M., Martins, A., Raminhos, C., Campos, M., Bernardo, F. (2016). Determination of PCDD/F levels in wood shavings used as bedding material for poultry production. ***Journal of Environmental Protection***, **7**, 2047-2055.

Semedo-Lemsaddek, T., Carvalho, L., Tempera, C., Fernandes, M. H., Fernandes, M. J., Elias, M., Barreto, A. S., Fraqueza, M. J. (2016). Characterization and technological features of autochthonous coagulase-negative staphylococci as potential starters for Portuguese dry fermented sausages. ***Journal of Food Science***, 81: M1198-M1202.

Fraqueza, M. J. (2015). Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. ***International Journal of Food Microbiology***, 212: 76-88.

Fraqueza, M.J., Barreto, A.S. (2014). HACCP: Hazard Analysis and Critical Control Points. Chapter 53. *In*: ***Handbook of Fermented Meat and Poultry***: Second Edition. Editors: Fidel Toldrá, Y. H. Hui, Iciar Astiasarán, Joseph G. Sebranek and Règine Talon. John Wiley & Sons, Ltd, Chichester, UK.

5. Collaborations

- Faculty of Technology, University of Novi Sad, 21000 Novi Sad, Serbia
- IBET-Instituto de Biologia Experimental e Tecnologica
- INSA- Instituto Nacional de Saúde Doutor Ricardo Jorge
- Institut de Recerca i Tecnologia Agroalimentàries, IRTA, Espanha
- Institut National de la Recherche Agronomique, Saint-Genès Champanelle, France.
- Institute of Animal Physiology, Kosice, Slovakia.
- Instituto Tecnológico Agrario. Junta de Castilla y León. Estación Tecnológica de la Carne. Guijuelo (Salamanca) .
- ITQB- Instituto de Tecnologia Química e Biológica-Universidade Nova
- Universidad de Castilla-La Mancha
- Universidade Federal de Alagoas, Campus Arapiraca, Pólo Viçosa, Brasil.
- Vilnius Gediminas Technical University, Department of Chemistry and Bioengineering, Vilnius, Lithuania.

Seven National Universities, Polytechnics Institutes Cooperatives and Private Food Producting Companies

6. Outreach

Team researchers are aware of the benefits of outreach activities for a broader impact of their activity. Aiming to engage industry and public with research work developed at FTS Lab, the team researchers are involved in various outreach actions including open days, school visits, workshops, public talks, networks.

7. Advanced Studies (PhD e MSc thesis)

During the period 2013-2017, 3 PhD and 23 Master degrees were awarded to students working in the lab.

Animal Genetic Resources

Lab Leader Luís Telo da Gama | **Researchers** Andreia J. Amaral, Maria Cristina Bressan



From left to right: Luís Telo da Gama, Andreia J. Amaral and Maria Cristina Bressan

1. Research Interests and Expertise

Characterization, conservation and management of Animal Genetic Resources, using demographic, molecular and genomic information

Genetic structure of Iberian and American Creole populations and relationships with other European breeds

Genetic improvement programs using both conventional selection and incorporation of genomic information (estimation of genetic parameters, prediction of breeding values, optimization of selection strategies, etc.).

Investigation of genetic markers associated with various traits of economic importance in Portuguese breeds of livestock.

Genetics of meat quality in various species, using quantitative and molecular approaches.

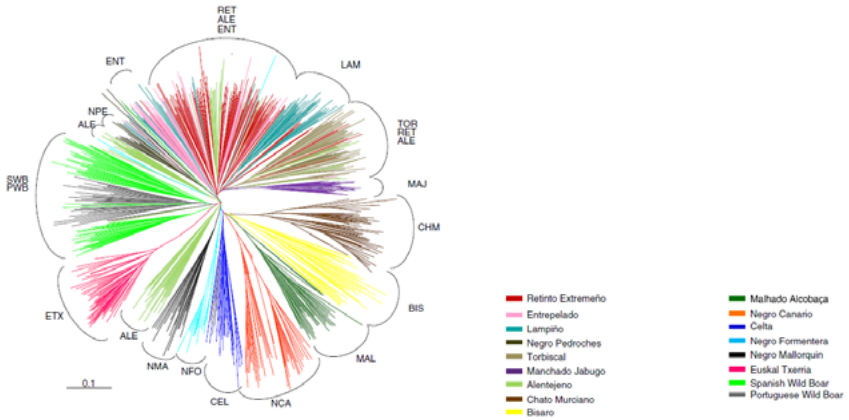
Novel statistical-genetic models for analysis of complex traits.

2. Highlights

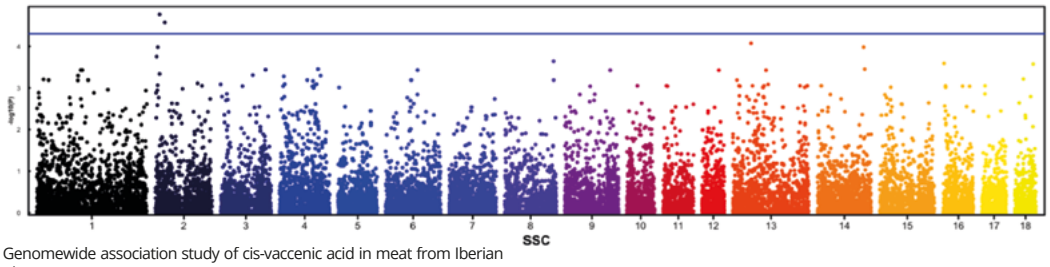
Characterization of the genetic diversity and structure of Creole cattle, horse, donkey, pig and goat breeds, published in various scientific journals and transferred to the local stakeholders.

In-depth analyses of meat, carcass and sensory traits in Iberian pigs, and investigation of the underlying genetic influences.

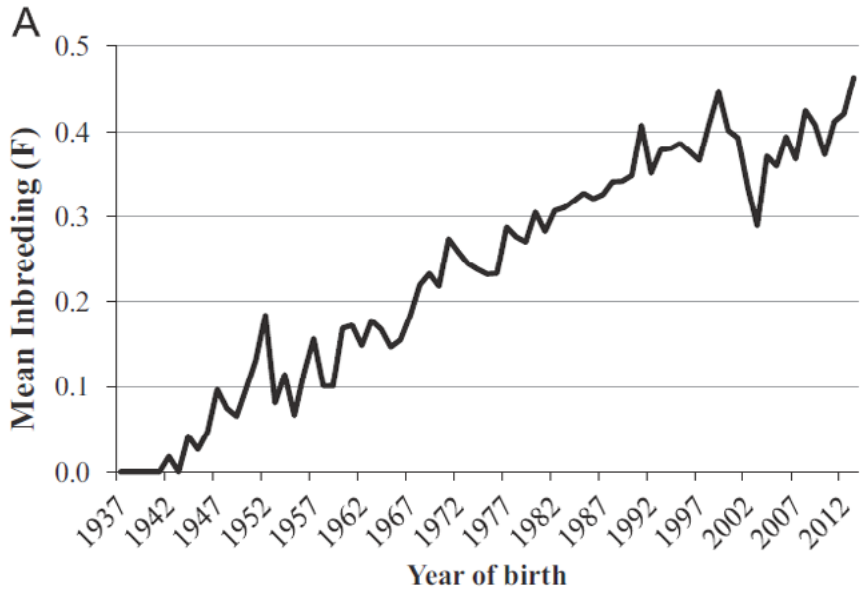
Solid networking established with the stronger research groups working in characterization, conservation and management of animal genetic diversity, both in Europe and Latin America



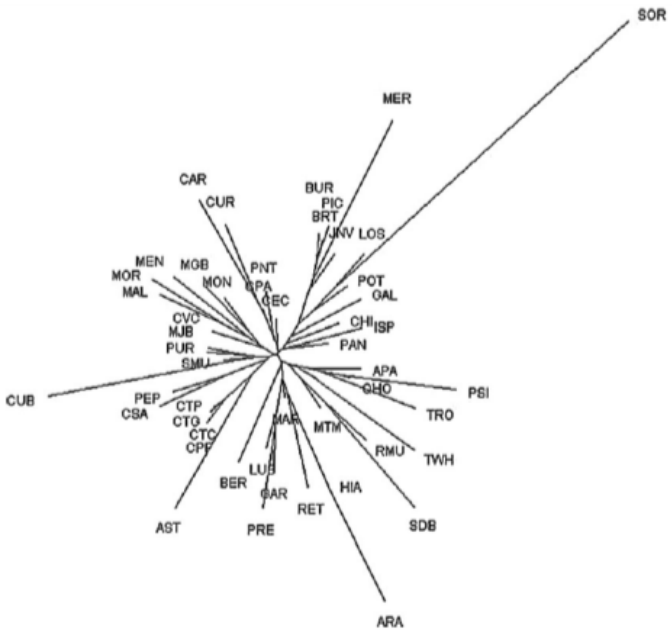
Genetic distance between individuals of 17 pig breeds from the Iberian Peninsula



Genomewide association study of cis-vaccenic acid in meat from Iberian pigs



Evolution of mean inbreeding by year of birth in Sorraia horse.



Genetic distance between 50 horse breeds from America and the Iberian Peninsula

3. Representative Projects

PTDC/CVT/116729 "Genetics of meat quality in Iberian pigs". PI: Maria Cristina Bressan. (finished 2014).

COST Action CA15112: "Functional Annotation of Animal Genomes - European network (FAANG-Europe)". Coordination: Alan Archibald (Roslin Institute, UK). Participant: Luis T. Gama (underway).

Project H2020-SFS-2015-2 "IMAGE – Innovative Management of Animal Genetic Resources". Coordination: Michèle Tixier-Boichard (INRA – France). Coordination of Work Package 7 (Outreach): Luís T. Gama. (underway).

Project 02/SAICT/2017 "MERINOparasite - Identification of genetic markers underlying genetic resistance to internal parasites in the Merina Branca sheep breed using the OvineSNP50 Beadchip array. Coordination: Helga Waap (INIAV). Participant: Luis T. Gama (underway).

4. Selected Publications

Gama, L.T., M.C. Bressan, E.C. Rodrigues, L.V. Rossato, O.C. Moreira, S.P. Alves, R.J.B. Bessa. (2013). Heterosis for meat quality and fatty acid profiles in crosses among *B. indicus* and *B. taurus* finished on pasture or grain. ***Meat Science*** 93:98.

Gama, L.T., A. Martínez-Martínez, M.I. Carolino, V. Landi, J.V. Delgado, A.P. Vicente, J.L. Vega-Pla, O. Cortés, C.O. Sousa and the BIOPIG Consortium. (2013). Genetic structure, relationships and admixture with wild relatives in native pig breeds from Iberia and its islands. ***Genetics, Selection and Evolution*** 45:18

Ginja, C., L.T. Gama, O. Cortes, J.V. Delgado, S. Dunner, D García, V. Landi, I. Martín-Burriel, A. Martínez-Martínez, M.C.T. Penedo, C. Rodellar, P. Zaragoza, J. Cañon, BioBovis Consortium. (2013). Analysis of conservation priorities of Iberoamerican cattle based on autosomal microsatellite markers. ***Genetics, Selection and Evolution*** 45:35

Piccoli, M.L., J. Braccini Neto, F.V. Brito, L.T. Campos, C.D. Bértoli, G.S. Campos, J.A. Cobuci, C.M. MacManus, J.O.J. Barcellos, L.T. Gama. (2014). Origins and genetic diversity of British cattle breeds in Brazil assessed by pedigree analyses. ***Journal of Animal Science***, 92:1920-1930.

Martínez, A.M., L.T. Gama, J.V. Delgado, J. Cañón, M. Amills, C.B. Sousa, C. Ginja, P. Zaragoza, A. Manunza, V. Landi, N. Sevane and BioGoat Consortium. (2015). The Southwestern fringe of Europe as an important reservoir of caprine biodiversity. ***Genetics, Selection and Evolution.*** 47:86.

Gómez, M.M., L.T. Gama, J.M. León, J. Fernández, S. A. Attalla, J.V. Delgado. (2016). Genetic parameters for harmony and movements in Spanish-Arab horses estimated by REML and Bayesian procedures. ***Livestock Science*** 188:159-165.

Cortés, O., A.M. Martinez, J. Cañon, N. Sevane, L.T. Gama, C. Ginja, V. Landi, P. Zaragoza, N. Carolino, P. Sponenberg, A. Vicente, J.V. Delgado and the BioPig Consortium. 2016. Conservation priorities of Iberoamerican pig breeds and their ancestors based on microsatellite information. ***Heredity.*** 117:14-24.

Bressan, M. C., J. Almeida, J. Santos Silva, C. Bettencourt, O. Moreira, A. T. Belo, A. Francisco, L. T. Gama. (2016). Carcass characteristics and fat depots in Iberian and F1 Large White x Landrace pigs finished intensively or raised outdoors in oak-tree forests. ***Journal of Animal Science*** 94:2592–2602.

Cortés, O., J. Cañón, L.T. Gama, A.M. Martínez, J.V. Delgado, C. Ginja, L.M. Jiménez, J. Jordana, C. Luis, M.M. Oom, P. Sponenberg, P. Zaragoza, Biohorse Consortium and J.L.Vega-Plá. (2017). The legacy of Columbus in American horse populations. ***J. Animal Breeding and Genetics.*** 134:340-350.

5. Collaborations

Membership of the Conbiand network and close collaboration with its members on research work aimed at assessing the genetic diversity and structure of livestock breeds in Iberoamerica.

Membership of the Strategic Advisory Board of the International Center for Advanced Mediterranean Agronomic Studies (CIHEAM).

6. Outreach

Collaboration with various breeder associations in their selection and conservation programs

Collaboration with the Administration (Ministry of Agriculture, Autonomous Regions, etc.) in the development of legislation and rules to be followed and applied at the national level and negotiated with the EU.

Participation in the stakeholders meeting of the IMAGE research project, discussing issues related to conservation of Animal Genetic Resources

Membership of the Ibero-American Programme on Science and Technology for Development (CYTED), participating as an expert member of its Agrofood Committee.

Course on Mixed Models and Genomic Selection (Corrientes, Argentina)

Course on Conservation of Animal Genetic Resources (Balcarce, Argentina)

7. Advanced Studies (PhD e MSc thesis)

Over the period considered, supervision of 7 Master and 7 PhD students.

Animal Behaviour and Welfare

Lab Leader George Stilwell | **Researchers** Ilda Rosa, Inês Figueiredo, Manuel Sant'Ana
PhD Students Ana Vieira, Inês Ajuda

From left to right: George Stilwell, Manuel Sant'Ana

1. Research Interests and Expertise

Pain management in farm animals – how to identify, measure, prevent and treat pain in disease and surgical procedures. Our main areas of research have been disbudding, castration and lameness (trimming).

How to effectively use behaviour for adequate and early recognition of disease and pain in farm animals.

Disease and its impact on animals' welfare and performance. Our research focus mainly on how disease can affect the wellbeing of animals.

Development of animal welfare assessment protocols to be applied on intensive and extensive systems of animal production. The protocols already developed are for intensively kept dairy cattle and dairy goats. The protocols for other production systems are currently being developed, including for dairy cows permanently on pasture (Azores Islands).

Humans' attitudes and actions may have a critical effect on animal welfare. Some of our studies include looking on how management, handling or training animals can impact their behaviour and welfare.

Consumers' interest in knowing how, where and by whom food is produced and how animals are handled from "farm to fork", has increased tremendously. By translating welfare science and ethics, through education and communication with farmers, veterinarians and consumers, we aim to improve standards for care and production of animals.

Pain evaluation and control in calves' disbudding.

Use of an algometer to grade pain in hoof lesions of dairy cows

2. Highlights

The work done on pain in dehorning and disbudding is considered a reference in the scientific community. Current studies on pain alleviation during trimming, castration and dehorning, using topic local anaesthetics, are equally important because of their application in practice.

The Animal Behaviour and Welfare (ABW) Lab has been involved in some very important European projects on farm animal welfare. These include, for example, AWIN – Animal Welfare Indicators (2011-2015) and the ongoing ANICARE projects. The protocol for the welfare assessment of dairy goats is available both in print and as a digital application (AWINGoat App), as is a smartphone application for the management of lameness in dairy goats (Welgoat App).

The ANICARE project aims to produce didactic material to promote debate on animal welfare within the animal production community (farmers, veterinarians and students). These videos will present several features of animal husbandry and handling and discuss how they may affect welfare. The final material (videos) will be published and public available.

The ABW Lab has also been involved in the development of a protocol to assess the welfare of dairy cows at pasture as part of a programme called "Milk from Happy Cows" (S. Miguel, Azores). This activity has included training of assessors (including students from FMV), workshops with farmers and results' analysis.

AWINGOAT – application for smartphones for on-farm welfare assessment of dairy goats (developed in partnership with University of Milan).

WELLGOAT - application for smartphones for training and management in dairy goat lameness.

3. Representative Projects

"Development, integration and dissemination of animal-based welfare indicators, including pain, in commercially important husbandry species, with special emphasis on small ruminants, equidae & turkeys" – AWIN-Animal Welfare Indicators FP7-KBBE-2010-4

"Educate animal welfare as a farming opportunity" – ANICARE n° 2017-1-FR01-KA202-037287 under the Erasmus+ Programme Key Action.

"Use of Trisolfen to manage pain during hoof trimming of dairy cows" - DGAV 49/ECVPT/2017.

4. Selected Publications

Stilwell G., Schubert H., Broom D.M. (2014) Effects of analgesic use postcalving on cow welfare and production. ***Journal of Dairy Science***. 97(2): 888-91.

Vieira A., Brandão S., Monteiro A., Ajuda I., Stilwell G. (2015) Development and validation of a visual body condition scoring system for dairy goats with picture-based training, ***Journal of Dairy Science***. 98 (9): 6597–6608. Selected as Editor´s choice in September 2015.

Vieira, A., Oliveira, M.D., Nunes, T., Stilwell, G. (2015) Making the case for developing alternative lameness scoring systems for dairy goats. ***Applied Animal Behaviour Science***. 171: 94-100.

Krug C, Haskell MJ, Nunes T, Stilwell G (2015) Creating a model to detect dairy cattle farms with poor welfare using a national database. ***Preventive Veterinary Medicine.*** 122 (3): 280-286.

Stilwell G (2016) Small ruminants' welfare assessment dairy goat as an example. ***Small Ruminant Research.*** 142: 51- 54

Saraiva S., Saraiva C., Stilwell G. (2016) Feather conditions and clinical scores as indicators of broilers welfare at the slaughterhouse. ***Research in Veterinary Science.*** 107: 75-79.

Vieira A., Oliveira M.D., Nunes T., Stilwell G. (2016) Design and test of a web-survey for collecting observer's ratings on dairy goats' behavioural data. ***Applied Animal Behaviour Science.*** 185: 52-58.

Can E., Vieira A., Battini M., Mattiello S., Stilwell G. (2016) On-farm welfare assessment of dairy goat farms using animal-based indicators: the example of 30 commercial farms in Portugal, ***Acta Agriculturae Scandinavica, Section A — Animal Science***, 66: 1, 43-55.

Pereira-Figueiredo I., Costa H., Carro J., Stilwell G., Rosa I. (2017) Behavioural changes induced by handling at different timeframes in Lusitano yearling horses. ***Applied Animal Behaviour Science.*** 196: 36-43.

Can E., Vieira A., Battini M., Mattiello S., Stilwell G. (2017) Consistency over time of animal-based welfare indicators as a further step for developing a welfare assessment monitoring scheme: The case of the Animal Welfare Indicators protocol for dairy goats. ***Journal of Dairy Science***. 100 (11): 9194-9204.

5. Collaborations

Along the years we have developed very close cooperation with numerous national and foreign research teams and institutions, namely: University of Cambridge (Prof. Donald Broom), University of S. Paulo (Prof. Adroaldo Zanella), Scotland's Rural College (SRUC) (Prof. Cathy Dwyer) University of Milan (Prof. Silvana Mattiello and Prof. Elisabetta Canalli), University of Sydney (Prof. Peter Windsor), University of Guelph (Prof. Todd Duffield), University of British Columbia (Prof. Anne Marie de Passille and Prof. Jeff Rushen), Universidade Trás-os-Montes e Alto Douro and Estação Zootécnica Nacional.

Collaboration was also strong with all institutions belonging to the AWIN consortium: Scottish Agricultural College; Norwegian University of Life Sciences; University of Milan, Department of Animal Science; NEIKER – Instituto Vasco de Investigacion y Desarrollo Agrario; Universidade Positivo, Curitiba, Brasil; Pferdeklinik Havelland, Equine Clinic; University of Cambridge; Institute of Animal Science, Uhrineves, Prague; Indiana University.

Ongoing close collaboration includes the ANICARE consortium: EPLEFPA de la Côte Saint André, France; Instituto Superior Técnico, Portugal; Escola Universitária Vasco de Gama, Portugal; Cooperativa Agrícola dos Agricultores do Vale do Lima, Portugal; Ruralidade Verde, Portugal; Ommpu Tonttu Luomutila, Finland; Federacion EFA de Galicia, Spain; Sens (Sciences, Energies, Nature, Santé), Belgium.

Researchers from our lab working with horses also collaborate with the association TheKidsFellows® Research Group in Anthrozoology.

As Member of the Management Committee of the COST ACTION FA1308 DairyCare, focused on dairy animal health and welfare, our lab also collaborated with animal science and technology researchers from almost all European countries.

There has been also research cooperation with commercial companies such as Animal Ethics (Australia), Tiveritas (UK), Zoetis, Bayer, Elanco and others.

Last but not least, because the lab's main objectives are on the area of applied research on behaviour and welfare of farm animals, there has been always a very close cooperation with farmers and practitioners.

6. Outreach

Collaboration with various breeder associations in their selection and conservation programs

Members of the Animal Behaviour and Welfare Research Lab are frequently involved in workshops and meetings with the purpose of training farmers, vets and students in various issues related with farm animal welfare. These include:

- Training of evaluators and farmers on farm application of welfare assessment protocols.
- Training on pain management during farm routine procedures.
- Talks on welfare and farm profitability.
- Discussion of bioethics and welfare issues with veterinarian, farmers, retailers or consumers.

Training for dairy goat welfare assessment

7. Advanced Studies (PhD e MSc thesis)

Over the period considered, 2 PhD and 6 Master degrees were awarded to young scientists developing their studies at the ABW Lab.

RESEARCH GROUPS

ANIMAL HEALTH AND VETERINARY MEDICINE

Animal Health and Veterinary Medicine

The *Animal Health and Veterinary Medicine* group is a leading partner enrolled in a prominent national and international task-force that develops biomedical research under the concepts of *One Health* (Animal + Human + Environment) and T*ranslational Medicine*. Under the scope of *One Health*, research is directed to animal diseases of major veterinary relevance, including zoonoses and emergent vector-borne diseases that are of global concern. The fruitful cooperation between clinical and fundamental researchers, arising from the medical, veterinary, pharma and biotechnological fields, is pivotal for the emerging concept of T*ranslational Medicine*, which is fully explored by the. *Translational Medicine* involves the fusion of basic science and clinical research on programs that catalyze biomedical innovations into practical clinical solutions, for both animals and humans. Ultimately, this will increase our rate of innovation and the speed at which we develop and introduce superior products and services into the clinical arena. In the above contexts, animal health and medicine sciences are particularly well positioned to establish sound research bonds with other biomedical sciences, thus positioning the group at a leading role in the national and international scenarios. This spirit is well expressed in the level of internationalization fostered by a highly complementary network of national collaborators, which is explored to provide a sustainable research framework allowing extensive mobility and training for students. During 2013-17, the *Animal Health and Veterinary Medicine* group was awarded with over 1.75 million euros external funding, authored 298 articles in indexed journals and supervised 25 PhD theses.

Mainstream to the group's activities are the Faculty Teaching Hospital and the recently renovated animal facility. The group Teaching Hospital provides an invaluable source of clinical cases of companion and farm animals, allowing research in a reference clinical asset. The animal facility supports biomedical research using different animal models, which is a major tool in collaborative networks with medical and pharma schools, hospitals (human and veterinary), and the biotech and pharma industry. Animal handling and experimentation is subjected to EU and national legislation and good-practice codes, executed by certified personnel, and advised and monitored by an institutional Animal Welfare Body.

Infectious Diseases

Leader Fernando Bóinas | **Researchers** Alexandre Leitão, Fernando Ferreira, Paula Viana, Carlos Martins, Telmo Nunes, Isabel Neto, Virgílio Almeida | **PhD students** João Coelho, Gonçalo Frouco, Telmo Nunes, Sara Madeira, Ferdinando Freitas, Margarida Simões | **Technicians** Rui Perestrelo, Ju Silva

African Swine Fever Unit
Left to Right Standing: Fernando Ferreira, Rui Perestrelo, Ju Silva, João Coelho and Gonçalo Frouco | *Seating:* Alexandre Leitão, Paula Viana, Carlos Martins, Margarida Simões and Ferdinando Freitas,

Epidemiology Unit
Left to Right: Fernando Boinas, Telmo Nunes, Sara Madeira, Isabel Neto and Virgílio Almeida

1. Research Interests and Expertise

Research at the Infectious Diseases (ID) Laboratory has aimed at deepening the knowledge on different aspects of African swine fever virus (ASFV) biology towards development of vaccine candidates, and evaluating relevant epidemiological aspects of infectious diseases namely zoonosis and emergent diseases affecting livestock, companion animals and wildlife, and development of animal health information systems and risk analysis assessments.

Ornithodoros erraticus: vector of ASFV

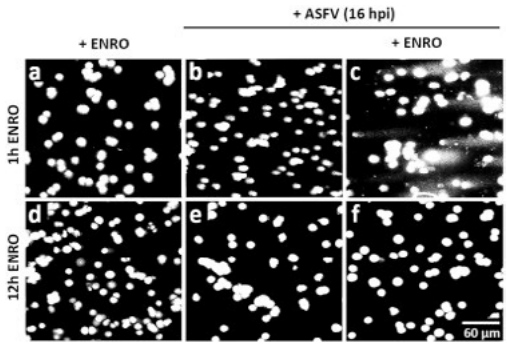
Epidemiologial studies of *Mycobacterium* bovis infection in free-ranging wild boar

2. Highlights

Research at the ID Lab has been conducted in the African swine fever and on the Epidemiology Units.

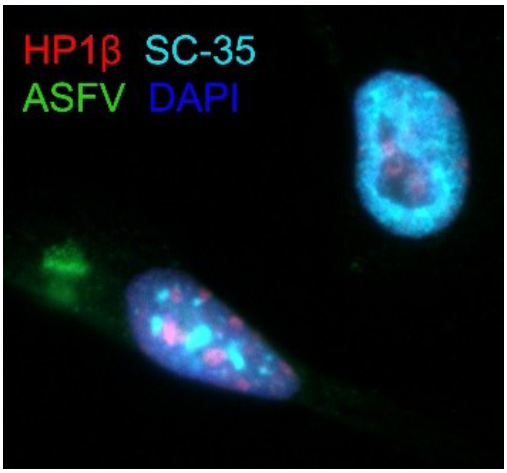
1. **The African swine fever (ASF) unit**, developed research regarding different aspects of African swine fever virus (ASFV) infection towards the development of vaccine models against the disease and on the role of arthropod vectors of the etiological agent. ASF is a devastating disease affecting domestic and wild pigs, caused by a complex virus belonging to the *Asfarviridae* family, able to cause significant socio-economic losses in affected countries, being a major threat for the global pig industry. Due to the fact that no vaccine has been obtained, and to the complexity of the different epidemiological scenarios involving domestic pigs, wild boars and vectors (argasids, *Ornithodororos* spp), the prevention, control,

and eradication of ASF is mainly based on its early detection and implementation of strict (and challenging) sanitary measures., After its eradication in early 90´s in Portugal and Spain, nowadays the disease is enzootic in the great majority of Sub-Saharan countries and in the Island of Sardinia (Italy). In 2007 it was declared in Georgia where from it rapidly spread to other Eastern European countries (Armenia, Azerbaijan, Russian Federation, Ukraine, and Belarus), and to EU since 2014, namely to Lithuania, Poland, Latvia, Estonia, Check Republic, Romania and recently to Hungary. During the period covered by this report, researchers of the ASF unit have developed comprehensive studies on the dynamics and role of *Ornithodoros erraticus* in the epidemiology of the disease (1 scientific paper), on virus-cell interactions and on functional characterization of several ASFV genes involved in viral transcription and replication, towards the development of rational design of antiviral molecules and efficient and safe vaccines, namely by the generation of defective infectious single cycle (DISC) viral particles. Besides the studies on the spatiotemporal of ASFV DNA replication and on dynamics of subnuclear domains and chromatin epigenetic markers to better understand the role of the host cell nucleus during the early phase of infection (5 Scientific papers, 1 PhD), the biological activity of several viral proteins has been characterized *in vitro* and the corresponding viral mutants generated (A104R, I215L, QP509L, Q706L, P1192R (8 Scientific papers, 2 PhD, 1 MSc), Enrofloxacin induces viral DNA breaks, thus acting as an ASFV-topoisomerase poison (Freitas et al., 2016).

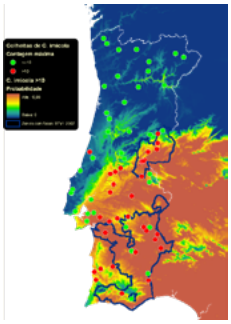


2. The Epidemiology Unit research (EpU) focus on applied studies of diseases in animal populations and on factors determining its occurrence over time aiming at identifying opportunities for enhancement of animal and public health, namely on zoonosis, and also on animal productivity and welfare. The EpU follows the *One Health* transdisciplinary approach with collaborations with national and international laboratories, official and private veterinary services. Its main research areas of activity are:

- Epidemiological studies on Animal Health and Welfare and in Public Health (11 scientific papers, a book chapter, a PhD thesis, 15 MSc)
- Investigating the role of the wild fauna-livestock-environment interface at several ecosystem levels on the maintenance or reemergence of pathogens relevant in Public Health (5 scientific papers, a book chapter, a PhD thesis, 2 MSc)
- Studying the ecology and modelling the spatial distribution of disease vectors in relation with arboviral diseases (3 scientific papers)
- Use of epidemiological methodology to support scientific studies in veterinary sciences (5 scientific papers in parasitology and 5 papers in clinical studies)



Redistribution of speckles (blue) to heterochromatic regions (red) in ASFV-infected cell (green)



The EpU is a partner in the development of the national animal health information and decision support system for the national animal health authority (DGAV) and also supports official and private veterinary development of surveillance, control and eradication programs for animal diseases, such as the *Bluetongue* Disease national surveillance plan, the *Aujeszky's* Disease national eradication programme, or the *Infectious Bovine Rhinotracheitis* and *Bovine Viral Diarrhoea voluntary herd control programmes*. The Unit participates actively in the training of official and private veterinarians in animal health workshops.

Epidemiological risk map for the presence of Culicoides vectors of Bluetongue Viruses

3. Representative Projects

European projects:

ASFORCE - Targeted research effort on African swine fever (FP7/2007-2013; EU/FP7-KBBE.2012.1.3-02; Grant Agreement n° 311931). Coordination FMV-ULisboa, Carlos Martins. Project duration: from 01-10-2012 to 30-09-2015. (www.asforce.org)

European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents VectorNet (OC/EFSA/AHAW/2013/02): Project duration: from 1-5-2014 to 30-4-2018

ASF-STOP - Understanding and combating African swine fever in Europe. COST Action - OC-2015-1-19550. Project duration: from 03-05-2016 to 02-05-2020. (www.asf-stop.com)

National projects:

- FCT - PTDC/CVT/112707/2009: Role of African swine fever virus topoisomerase II in viral DNA replication - a possible target for antiviral therapy. PI: Carlos Martins. Project duration: from 01-03-2010 to 31-08-2013.

4. Selected Publications

Simões, M., Martins, C., Ferreira, F. (2015). Alterations of nuclear architecture and epigenetic mechanisms during African swine fever virus infection. ***Viruses*** 7(9): 4978-4996.

Coelho, J., Martins, C., Ferreira, F., Leitão, A. (2015). African swine fever virus ORF P1192R codes for a functional type II DNA topoisomerase. ***Virology,*** 474: 82–93.

Freitas, F.B., Frouco, G., Martins, C., Leitão, A., Ferreira, F. (2016). In vitro antiviral activity of fluoroquinolones against African swine fever virus. ***Antiviral Research*** 134: 34–41.

Frouco, G., Freitas, F.B., Coelho, J., Leitão, A., Martins, C., Ferreira, F (2017). DNA-binding properties of the African swine fever virus pA104R, a histone-like protein involved in viral replication and transcription. ***Journal of Virology*** JVI.02498-16.

Ribeiro, R., Otte, J., Madeira, S., Hutchings, G.H., Boinas, F. Experimental Infection of *Ornithodoros erraticus* sensu stricto with Two Portuguese African Swine Fever Virus Strains. Study of Factors Involved in the Dynamics of Infection in Ticks. ***PLoS One***. 2015 Sep 14;10(9):e0137718.

Wilson, A.J., Ribeiro, R., Boinas, F. Use of a Bayesian network model to identify factors associated with the presence of the tick *Ornithodoros erraticus* on pig farms in southern Portugal. Prev Vet Med. 2013 May 15;110(1):45-53.

Ribeiro R, Wilson Aj, Nunes T, Ramilo Dw, Amador R, Madeira S, Baptista Fm,Harrup Le, Lucientes J, Boinas F. (2015)Spatial and temporal distribution of Culicoides species in mainland Portugal (2005-2010). Results of the Portuguese Entomological Surveillance Programme. ***PLoS One.*** Apr 23;10(4):e0124019.

Caetano Mc, Afonso F, Ribeiro R, Fonseca Ap, Abernethy Da, Boinas F. (2014) Control of Bovine Brucellosis from Persistently Infected Holdings Using RB51 Vaccination with Test-and-Slaughter: A Comparative Case Report from a High Incidence Area in Portugal. ***Transbound Emerg Dis***. Apr 17.

Nuno Santos, Almeida, V., Gortázar, V., Correia-Neves, M. (2015). Patterns of *Mycobacterium tuberculosis*-complex excretion and characterization of super-shedders in naturally-infected wild boar and red deer**.** ***Veterinary Research*** **(**46**)** 129.

Seixas, R., Nunes, T., Machado, J., Tavares, L., Owen, S. P., Bernardo, F., & Oliveira, M. (2017). Demographic characterization and spatial cluster analysis of human Salmonella 1, 4, [5], 12: i: - infections in Portugal: A 10 year study. ***Journal of Infection and Public Health***, 18(0), 187–197.

5. Collaborations

International

Agence Nationale de Securite Sanitaire de L'Alimentation, de L'Environnement et du Travail (ANSES), Ploufragan, França, Agencia Estatal Consejo Superior de Investigaciones Cientificas (CSIC), Madrid, Espanha; Bulgarian Food Safety Agency (BFSA), Sofia, Bulgaria; Centre de Cooperation International en Recherche Agronomique Pour Le Developpement (CIRAD), Paris, França; Danish Meat Association, Copenhagen, Denmark; Diomune SL,Madrid, Spain; Friedrich Loeffler Institut (FLI), Bundesforschungsinstitut Fuer Tiergesundheit, Greifswald-Insel Riems, Germany; Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (CISA-INIA), Madrid , Spain; Istituto Zooprofilatico Sperimentale Dell'umbria e Delle Marche (IZS-UM), Perugia, Italy; Instituto de Investigación en Recursos Cinegéticos da Universidad de Castilla-La Mancha, Espanha; Pfizer Olot, Spain; State Research Institution National Research Institute For Veterinary Virology And Microbiology of Russia (VNIIVViM), Pokrov, Russian Federation; The Pirbright Institute (PIR), Pirbright, United Kingdom; The Royal Veterinary College (RVC), London, United Kingdom; Universidad Complutense de Madrid (UCM),Madrid , Spain; Inmunologia y Genetica Aplicada SA (INGENASA), Madrid,Spain; SAFOSO AG (Safe Food Solutions), Bern, Switzerland; Technical University of Denmark, Copenhagen, Denmark. Institute of Molecular Biology of NAS – Armenia. Faculty of Veterinary Medicine, University of Pretoria, South Africa; Department for Environment, Food and Rural Affairs (DEFRA).

National

Boehringer Ingelheim Portugal, CardioID Technologies and MSD Animal Health Portugal (clinical epidemiology cooperative research projects on companion animal infectious diseases); Department of Veterinary Medicine, University of Évora; National Animal Health Authority (DGAV); Life and Health Sciences Research Institute, University of Minho; National Institute for Agrarian and Veterinary Research (INIAV).

6. Outreach

International

DG Sante (Tutoring in risk assessment courses "Better Trainning for Better Food"); European Scientific Counsel Companion Animal Parasites (Developing and translation of guidelines for the treatment and control of parasites in pet animals); Faculty of Veterinary Medicine, University of Pretoria (Training workshops on animal brucellosis); FAO- UN (Tutoring for advanced courses on GIS for animal disease control); Technical University of Denmark (two-tier traineeships for Master and PhD students). Training and knowledge transfer on relevant aspects of ASF conducted under the development of ASFORCE Project through the organization of workshops attended by around 400 veterinarians including CVOs from EU and non-EU countries;

National

Associação de Criadores do Sul (ACOS)/ Évora University (collaboration on a rural extension project); ASAE-*Economic* and *Food* Safety Authority (participation on the Scientific Council); Faculty of Biology, Pharmacy, Medicine and IST Técnico of the University of Lisbon (collaboration on the master degrees of Microbiology and Clinical Microbiology and Emerging Infectious Diseases); DGAV-National Animal Health Authority (evaluation and support for the development of prevention, control and eradication programmes, contingency plans, animal health information systems expert advice, advanced courses for official and private veterinarians); SOCLA/ COPRAPEC Animal health producer organizations (consultancy services and technical support); University of Azores (partnership agreement for the preparatory studies of the Integrated Master Degree in Veterinary Medicine).

International training workshops in epidemiological methods

7. Advanced Studies (PhD e MSc thesis)

5 PhDs were awarded to researchers working in the lab during this period.

Microbiology and Immunology

Lab Leader Luís Tavares | **Researchers** Ana Duarte, Manuela Oliveira, Solange Gil, Frederico Aires da Silva, Ricardo Bexiga | **Post-Docs** Sandra Aguiar | **PhD Students** Ana André, Carla Mottola, Joana Dias, Maria Carolina Bento, Miguel Grilo, Raquel Santos, Rui Seixas | **Technicians** Carla Carneiro, Clara Cartaxeiro

From left to right front row: Ana André, Joana Dias, Ricardo Bexiga, Luís Tavares, Raquel Santos, Miguel Grilo, Diana Martins, F. Aires da Silva;
Back row: Carla Carneiro, Sandra Aguiar, Clara Cartaxeiro, Manuela Oliveira, Eva Cunha, Ana Duarte, Solange Gil.

1. Research Interest and Expertise

The Microbiology and Immunology (MI) Lab aims at studying patterns of bacterial, viral and oncogenic diseases affecting both animals and humans, and to develop novel therapeutic and control strategies for a sustainable health, including vaccines, phage therapy and antimicrobial peptides. It also focus on microbial surveillance in shared ecosystems, on the development of new diagnostic tools based on biosensors and on the characterization of virulence mechanisms of emerging zoonotic pathogens affecting health quality under the wide concept of "One Health".

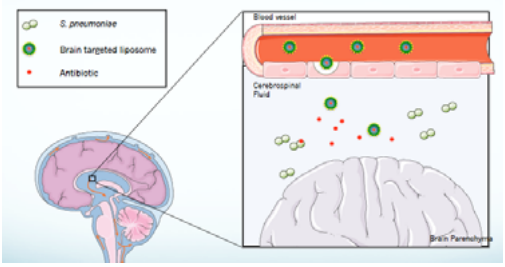
The main contributions to health quality of animals and humans occur namely through studying:

- Small animal viral, inflammatory and oncogenic diseases using animal models to assess novel immunology based therapeutic strategies such as the use of monoclonal antibodies and immunomodulatory molecules to treat lymphoma, immunodeficiency, SIRS and Sepsis;
- Antimicrobial resistance and virulence mechanisms of emerging bacterial isolates, from animal and human origins and possible communication routes with impact on public health. Novel strategies for controlling these strains are being developed, focusing in the use of compounds that interfere with bacterial viability and/or inhibit Quorum-Sensing systems, such as antimicrobial peptides;
- The collateral effects of mastitis on the spread of virulent agents and in the quality of milk as an important public health determinant and the impact of current dairy farm management practices on Public Health and Animal Health Economics;
- Microbial Surveillance in wild and free ranging animals, including cetaceans, Eurasian and Neotropical otters, sea turtles and Critically Endangered cyprinids, and its impact in ecosystems shared by domestic livestock, wildlife and humans,

2. Highlights

Overcoming the Brain Drug Delivery Bottleneck: Development of recombinant antibodies and peptides for brain targeting and drug delivery across the Blood-Brain Barrier

Central nervous system (CNS) disorders affect up to 1 billion people worldwide, which corresponds to around 13% of the global health burden, surpassing both cardiovascular disease and cancer. The treatment of brain diseases has been a major challenge since a long time. Nevertheless, in spite of the major advances in neuroscience and drug development, many potential therapeutics are still unable to get into the CNS due to the blood-brain barrier (BBB), a physical and selective metabolic barrier between the brain and the systemic circulation. The BBB is therefore the bottleneck in brain drug development and the most important factor limiting the future growth of therapeutics for treatment of major unmet neurodegenerative disorders. Within this context, we have been exploring novel screening approaches and molecules that can target endogenous BBB transport systems into the brain. With this approach we have been creating a new generation of small antibody fragments and peptide scaffolds that can provide a more selectively and effective drug delivery into the brain, which may be used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's, brain cancers and meningitis.



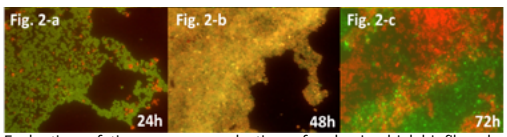
Mechanism of immunoliposome brain delivery in a pneumococcal meningitis model.

Polyphasic validation of antimicrobial peptides as alternative treatment for diabetic foot infections

There is a crucial need to develop new and effective strategies to treat Diabetic Foot Infections (DFI), a major complication of Diabetes *mellitus* (DM), especially considering: a) DM increasing prevalence worldwide; b) its higher incidence on individuals aged over 60 years, impairing their quality of life; c) DFI evolution, leading to lower limb amputation; d) the association between DFI and *Staphylococcus aureus*, particularly biofilm-producing and antimicrobial resistant strains such as Methicillin-Resistant *S. aureus* (MRSA); and e) WHO recent classification of MRSA as high priority agents for developing new therapeutic protocols.

Antimicrobial Peptides (AMP) are potential substitutes for conventional antibiotics. In fact, one of the best characterized AMP, nisin, has been broadly used as a food preservative to control gram-positive bacteria. Our research team already demonstrated the antimicrobial activity of nisin against *S. aureus* DFI isolates collected in Lisbon Medical Centers and evaluated the potential of a Guar gum biogel to be used as a topical delivery system for this AMP. It was observed that nisin can rapidly diffuse in the gel and eradicate staphylococcal planktonic cells and established biofilms.

This project aims to validate the potential of the developed nisin-biogel as an alternative treatment for DFI. This innovative therapeutic strategy may in the future substitute or complement antibiotherapy for an effective DFI treatment, contributing to reduce amputations frequency and increase diabetic patients' quality of life. It will also prompt a decrease in resistance dissemination in the hospital and community settings. Validation of the Guar gum biogel as a delivery system for antimicrobials will also allow developing new topical therapeutics for other skin infections.



Evaluation of time-course production of polymicrobial biofilms by Diabetic Foot Isolates (DFI) using Fluorescent *In Situ* Hybridization - Polymicrobial biofilm formed by *Acinetobacter* sp. and *Staphylococcus* aureus

Decreasing the impact of mastitis

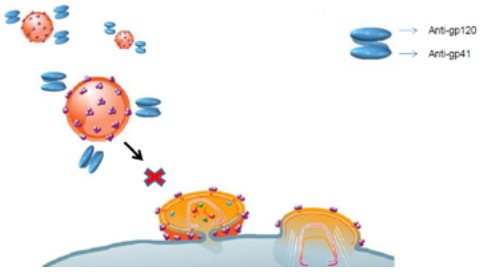
Mastitis is the most costly disease for dairy production and the first reason for antibiotic use in dairy cattle. It has a multitude of consequences, ranging from direct economic impact due to decreased milk quality and quantity, to indirect but far-reaching effects that include decreased fertility, increased infectious disease transmission and potential increase in antimicrobial resistance. This research line aims at characterizing the extent of such impact and to develop forms to improve mastitis diagnosis and optimize antimicrobial use. To achieve this it is important to understand how mastitis pathogens reach the host, colonize its tissues, evade its defenses and resist antimicrobial destruction. We are currently characterizing the epidemiology of infection for relevant mastitis pathogens including *Staphylococcus* spp. and *Streptococcus* spp., determining their antimicrobial resistance patterns and mechanisms, as well as virulence determinants, which could help mitigate their impact on dairy cattle health and productivity.

Waste milk resulting from mastitis treatments on 31 farms has been shown by this group to contain Mycobacterium avium paratuberculosis, and to contribute to the emergence of antimicrobial resistance, thus being posing a significant threat to the future sustainability of dairy farms.

Development of therapeutic approaches for Feline and Human Immunodeficiency virus.

RFeIFN-ω therapy is common in retroviral infections, although there are not so many studies that support its use. In this research line, we tried to extend the current knowledge about this compound, in order to support and clarify the evident clinical improvement previously described. The presented experimental work relied on two distinct clinical trials. This work innovated the extension of the main therapeutic properties of the licensed rFeIFN-ω protocol in naturally retroviral infected cats, explored its immune modulation properties and validated a new oral protocol.

Human immunodeficiency virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) infection represent one of the main public health problems worldwide. In this context, several stages of the HIV replicative cycle have been identified as targets for therapeutic intervention. One of the stages of considerable interest that has attracted the attention of several research groups is the HIV entry / fusion process in the host cell. In this context, we are developing a novel therapeutic approach through bispecific recombinant antibodies with the ability to block the process of HIV entry into the host cell. In this project we intend to develop small recombinant antibodies against the viral proteins gp120 and gp41 of HIV-1. With this strategy we aim to develop a double-acting recombinant antibody in the virus entry process that will contribute to the identification of promising new molecules in HIV/ AIDS eradication.

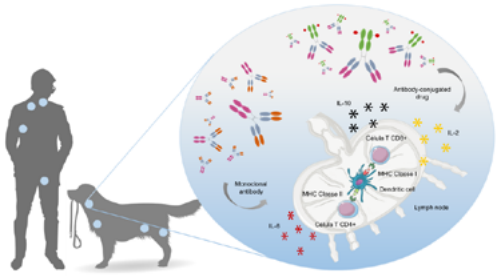


Bispecific antibodies for HIV infection treatment

Clinical and immunological characterization of naturally occurring canine lymphoma: Development of recombinant antibodies and small molecules compounds for diagnosis and therapeutic applications

Naturally occurring tumors in dog mimic features of those observed in humans, difficult to reproduce with other models. This represents a unique opportunity to study long-term efficacy and toxicity of cancer immunotherapies. To overcome limitations associated with conventional preclinical models, we validate the naturally occurring canine B-cell lymphoma as an animal model for the development of immunotherapeutic strategies and new diagnostic tools for non-Hodgkin lymphoma (NHL). A multidisciplinary

approach is being used for (1) construction of canine and human lymphoma biobanks, (2) comparative immunotherapeutic targets characterization, (3) selection and characterization of innovative therapeutic molecules such as recombinant antibodies and small molecules compounds against clinically validated targets (i.e.,CD20) and novel receptor targets associated with lymphomas, (4) efficacy and safety studies of the selected therapeutic candidate molecules on xenograft mouse models.



The scope of the immunotherapy approach against lymphoma

3. Representative Projects

PGG/015/2016 - Development of bispecific antibodies against gp120/gp41 for treatment of HIV-1 infection. PI: Frederico Aires da Silva (FMV-UL). Program Gilead GÉNESE 2016 (2016-2018).

PTDC/BBBBIO/0508/2014 - Overcoming the Brain Drug Delivery Bottleneck: Development of single domain antibodies for brain targeting and drug delivery across the Blood-Brain Barrier. PI: Frederico Aires da Silva (FMV-UL). Partners: IMM/FM/UL, IST-ITN-UL, TechnoPhage SA. FCT funding (2016-2019).

PTDC/QEQMED/5512/2014 - Alzheimer3D: Trifunctional Conjugates for the Selective Drug Delivery in Alzheimer's disease. PI: Rudi Oliveira (FF-UL). Partners: FMV-UL, IMM/FM/UL, CEDOC, TechnoPhage SA. FCT funding (2016-2019).

PTDC/BBBNAN/1578/2014 - Peptides for blood brain barrier transmigration and drug delivery novel therapies for the central nervous system. PI: Vera Neves (IMM/FM/UL). Partners: FMV/UL, IST-ITN-UL, TechnoPhage SA. FCT funding (2016-2019).

Virbac Portugal - "Infective endocarditis following periodontal disease in dogs: an experimental approach towards prevention, based on antimicrobial peptides", PI: Manuela Oliveira, Partners: Virbac Portugal, Virbac Saúde Animal, Portugal. (2015-2020).

4. Selected Publications

Leão, C., Botelho, A., Martins, E., Aguiar, C., Rebelo, I., Nunes, T., Bexiga, R. (2017). Presence of *Mycobacterium avium* subs. *paratuberculosis* DNA in milk used to feed calves in Portugal. ***Journal of Dairy Research*** 84(2): 124-127.

Neves, V., Aires-Da-Silva, F., Morais, M., Gano, L., Ribeiro, E., Pinto, A., Aguiar, S., Gaspar, D., Fernandes, C., Correia, J.D., Castanho, M.A. (2017). Novel Peptides Derived from Dengue Virus Capsid Protein Translocate Reversibly the Blood-Brain Barrier through a Receptor-Free Mechanism. ***ACS Chemical Biolology*** 12(5):1257-1268.

Cantante C, Lourenço S, Morais M, Leandro J, Gano L, Silva N, Leandro P, Serrano M, Henriques AO, Andre A, Cunha-Santos C, Fontes C, Correia JDG, Aires-da-Silva F, Goncalves J. (2017) Albumin-binding domain from Streptococcus zooepidemicus protein Zag as a novel strategy to improve the half-life of therapeutic proteins. ***J. Biotechnol.*** 253:23-33.

Cunha-Santos, C., Figueira, T.N., Borrego, P., Oliveira, S.S., Rocha, C., Couto, A., Cantante, C., Santos-Costa, Q., Azevedo-Pereira, J.M., Fontes, C.M., Taveira, N., Aires-Da-Silva, F., Castanho, M.A., Veiga, A.S., Goncalves, J. (2016). Development of synthetic light-chain antibodies as novel and potent HIV fusion inhibitors. ***AIDS*** 30:1691-1701.

Mottola, C., Semedo-Lemsaddek, T., Mendes, J.J., Melo-Cristino, J., Tavares, L., Cavaco-Silva, P., Oliveira, M. (2016). Molecular typing, virulence traits and antimicrobial resistance of diabetic foot staphylococci. ***Journal of Biomedical Science*** 23: 33.

Neves, V., Aires-da-Silva, F., Corte-Real, S., Castanho, M.A.R.B. (2016). Antibody Approaches To Treat Brain Diseases. ***Trends in Biotechnology*** 34(1): 36-48.

Gil, S., Leal, R., McGahie, D., Sepúlveda, N., Duarte, A., Niza MMRE., Tavares L. (2014). Oral recombinant feline interferon-omega as an alternative immune modulation therapy in FIV positive cats: clinical and laboratorial evaluation. ***Research in Veterinary Science*** 96(1):79-85.

Bento, M.C., Eira, C.I., Vingada, J.V., Marçalo, A.L., Ferreira, M.C.T., Fernandez, A.L., Tavares, L.M.M.,

Duarte, A.I.S.P., (2016). New insight into dolphin morbillivirus phylogeny and epidemiology in the northeast Atlantic: opportunistic study in cetaceans stranded along the Portuguese and Galician coasts. ***BMC Veterinary Research*** 12: 1-12.

Oliveira, M., Serrano, I., Santos, J.P., Bilocq, F., Pereira, N., Loureiro, N.S., Tavares, L., Pirnay J.P., De Vos, D. (2017). Pseudomonads from wild free-living sea turtles in Príncipe Island, Gulf of Guinea. ***Ecological Indicators*** 91: 260-264.

5. Collaborations

- Instituto de Medicina Molecular
- Instituto Superior Técnico, Lisbon
- Instituto Português de Oncologia, Lisbon, Portugal
- Institut Pasteur, Paris, France
- Faculty of Veterinary Medicine, University of Glasgow, Glasgow, Scotland
- Queen Astrid Military Hospital, Brussels, Belgium
- Technophage S.A, Lisbon, Portugal
- University Medical Center, Gottingen, Germany
- University of Veterinary Medicine, Vienna, Austria.

6. Outreach

The work on canine lymphoma won the STARTHealth@ULisboa 2016 - 2nd prize for best start-up project.

7. Advanced Studies (PhD e MSc thesis)

During the period 2013-2017, 4 PhD and 7 Master degrees were awarded to students working in the lab.

Parasitology and Parasitic Diseases

Lab Leader Isabel Fonseca | **Researchers** Graça Pires, José Meireles, Luís Carvalho **Post-Docs** David Ramilo | **PhD Students** Marco Santos, Margarida Alho, Jacinto Gomes | **Technicians** Lídia Gomes

From Left to right: Margarida Simões (collaborator), Marco Santos, David Ramilo, Lídia Gomes, Graça Pires, Isabel Fonseca, Luís Carvalho, Margarida Alho and Jacinto Gomes. *Inset:* José Meireles .

1. Research Interests and Expertise

The Parasitology and Parasitic Diseases (PPD) Lab research interests refers to endo/ectoparasites of Small/Exotic Pets, Horses/Livestock, Wildlife, Vector and Food Borne Diseases under One Health concept, Forensic Entomology, Bee Health and parasites identification using morphological, serological and molecular methods.

Team members' expertise has been related to: a) Epidemiology, immune response, diagnosis and treatment of canine leishmaniosis; b) horse/donkey strongyle control using alternative/complementary methods namely targeted treatment and parasiticide fungi; c) Bioecological, morphological, molecular and risk analysis studies of *Culicoides* genus; d) detection of public spaces contamination by *Toxocara canis*, *T. cati* and cat lungworms and other co-infective parasites regarding animal health and Public Health risk; e) epidemiological aspects and distribution of *Dirofilaria immitis* and *Angiostrongylus vasorum* in dog, wild and zoo animals; f) studies about CVBD agents in vertebrates and in ixodids in Portugal; g) Studies about inter-transmissibility of gastrointestinal helminths and other agents in captive/wild marine mammals and domestic/feral/wild carnivores and ungulates, their importance in Public/Animal Health as well as biological tags in Wildlife Diseases monitoring; h) Cattle breed resistance to tropical theileriosis; i) Bee health.

2. Highlights

- Studies in *Leishmania infantum*: a) in dog: neutrophils effector mechanisms, immunomodulation in canine Kupffer cells by meglumine antimoniate, modulation of memory cell subsets of liver resident T lymphocyte; b) in cat: combination of allopurinol and N-methyl-glucamine antimoniate for the treatment of feline leishmaniosis.
- Description of a new *Culicoides* species for science - *Culicoides paradoxalis* sp. nov. and first

reference of 20 *Culicoides* species in Portugal

- Development of molecular assays and studies in population genetics for detection of *Theileria annulata* in cattle
- Occurrence of gastrointestinal helminths and chewing lice in wild birds in southern Portugal
- First reference of *Avispora mochogalegoi* n. sp. in the little owl (*Athene noctua*) and *Caryospora peneireiroi* n. sp. in the common kestrel (*Falco tinnunculus*) in mainland Portugal
- Parasitological survey of shelter cats/dogs and wildlife animals - from the 6 different groups/ species of endoparasites detected, 4 present zoonotic potential.
- Prevalence and seasonal variations of canine Dirofilariosis in Portugal - higher prevalence of dirofilariosis with an increasing rate of infection in southern areas of Portugal attributed to bioclimatic/ecological factors.
- Patchy distribution of *Angiostrongylus vasorum* in dogs all over the country, while *A. abstrusus*, showed a higher prevalence northwards regarding the cats.
- Environmental contamination with *Toxocara* spp. eggs in two thirds of the sampled soil of public parks and playground sandpits of Lisbon The molecular analysis showed contamination with *T. cati* and *T. canis* eggs.
- Targeted selective treatment approach by administration of nematophagous fungi - larvicidal *Duddingtonia flagrans* and ovicidal *Mucor circinelloides*- in water suspension, industrially manufactured pellets or gelatins for horses EPG control.

E-SOVE 2016 -Training course groups at FMV/ULisboa

Insect trap placement during Two-day Training Course on Vector Control, October 2016.

3. Representative Projects

Consequences of the interaction between neutrophils and *Leishmania infantum* in canine leishmaniosis establishment (PI Gabriela Santos-Gomes, IHMT and co-PI I. M. Pereira da Fonseca, FMV), PTDC/CVT/113121/2009. 2010-2013.

Ecoepidemiology of *Dirofilaria* spp.: molecular characterization, potential vectors and transmission dynamics (ECOEPIDIRO) (PI Silvana Belo, IHMT, Luís Carvalho, FMV). 2011-2015. Project FCT PTDC/SAL-SAP/113523/2009.

Regulatory immune response in dogs with leishmaniosis at various clinical stages and undergoing different therapeutic protocols. (PI Isabel Pereira da Fonseca and co-PI Gabriela Santos-Gomes, CMDT/IHMT/UNL). FCT, PTDC/CVT/118566/2010. 2011-2015.

Project STOP - Simple Test of Parasitic Eggs (FMVUL/Merial Portugal Saúde Animal, Sanofi Group) (PI Luis Carvalho). 2011-2017

European network for neglected vectors and vector-borne infections (EURNEGVEC) - COST Action TD 1303 (PI Andrei Mihalca, Cluj Napoca, Romenia). 2013 – 2017.

European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents(VECTORNET).OC/EFSA/AHAW/2013/02.

Efficacy of a novel topical combination product containing eprinomectin (Broadline®) against feline lungworms: a multi-centric study. (FMV/UL, University of Bari and Merial Animal Health) (PI Alessio Giannelli). 2015- 2016.

4. Selected Publications

Ramilo DW, Nunes T, Madeira S, Boinas F, Pereira da Fonseca I. (2017). Geographical distribution of *Culicoides* (Diptera: Ceratopogonidae) in mainland Portugal: presence/absence modelling of vector and potential vector species. ***PLoS ONE*** 12(7): e0180606.

Alho, A.M., Marcelino, I., Colella, V., Flanagan, C., Silva, N., Correia, J.J., Latrofa, M.S., Otranto, D., Madeira de Carvalho, L. (2017) Dirofilaria immitis in pinnipeds and a new host record. ***Parasites & Vectors,*** 10:142-147.

Pereira M, Valério-Bolas A, Santos-Mateus D, Alexandre-Pires G, Santos M, Rodrigues A, Rocha H, Santos A, Martins C, Tomás A, Passero F, Pereira da Fonseca I, Santos-Gomes G. (2017) Canine neutrophils activate effector mechanisms in response to *Leishmania infantum*. ***Veterinary Parasitology***, 248, 10-20.

Alho, A.M., Silva, J., Fonseca, M.J., Santos, F., Nunes, C., Madeira de Carvalho, L.M., Rodrigues, M., Cardoso, L. (2016). First report of *Cytauxzoon* sp. infection in a domestic cat from Portugal ***Parasites & Vectors***, 9 (1): 220-224.

Alho, A.M., Pita, J., Amaro, A., Amaro, F., Schnyder, M., Grimm, F., Custódio, A.C., Cardoso, L., Deplazes, P., Madeira de Carvalho, L.M. (2016). Seroprevalence of vector-borne pathogens and molecular detection of *Borrelia afzelii* in military dogs from Portugal. ***Parasites & Vectors***, 9(1): 225-230.

Jacquet, S., Garros, C., Lombaert, E., Walton, C., Restrepo, J., Allene, X., Baldet, T., Cetre-Sossah, C.,; Chaskopoulou, A., Delecolle, J.-C., Desvars, A. Djerbal, M., Fall, M., Gardes, L., de Garine-Wichatitsky, M., Goffredo, M., Gottlieb, Y., Gueye-Fall, A., Kasina, M., Labuschagne, K., Lhor, Y., Lucientes, J., Martin, T., Mathieu, B., Miranda, M., Pages, N., Pereira Da Fonseca, I., Ramilo, D., Segard, A., Setier-Rio, M.L., Stachurski, F., Tabbabi, A., Talla Seck, M., Balenghien, T., Guis, H., Chevillon, C., Bouyer, J., Huber, K. (2015) Colonization of the Mediterranean Basin by the vector biting midge species *Culicoides imicola*: an old story. ***Molecular Ecology***, 24 (22): 5707-5725.

Jacquet, S., Huber, K., Pages, N., Talavera, S., Burgin, L.E., Carpenter, S., Sanders, C., Djerbal, M., Lhor, Y., Lucientes, J., Miranda, M., Pereira Da Fonseca, I., Ramilo, D., Setier-Rio, M.L. (2015). Range expansion of the Bluetongue vector *Culicoides imicola* in continental France thanks to meteorological events. ***Scientific Reports*** 6:27247.

Alho, A.M, Landum, M., Ferreira, C., Meireles, J., Gonçalves, L., Madeira de Carvalho, L.M., Belo, S. (2014) Prevalence and seasonal variations of canine Dirofilariosis in Portugal. ***Veterinary Parasitology***, 206: 99-105.

Ramilo, D., Garros, C., Mathieu, B., Benedet, C., Allene, X., Silva, E., Alexandre-Pires, G., Pereira da Fonseca, I., Carpenter, S., Radorva, J., Delecolle, J.C. (2013). Description of *Culicoides paradoxalis* sp. nov. from France and Portugal (Diptera: Ceratopogonidae). ***Zootaxa*** 3745 (2): 243-256.

5. Collaborations

International Universities/Scientific Research Institutes/ Organizations

- CIRAD, Contrôle des maladies animales exotiques et emergentes. Campus Inter. Baillarguet. Montpellier, France.
- European Centre for Diseases Prevention a nd Control.
- European Food Safety Agency.
- Facultad de Veterinaria Universidad de Zaragoza, Spain.
- Iceland Food and Veterinary Authority (MAST). Iceland.
- Institute of Parasitology, Zurich University, Switzerland.
- Laboratory of Entomology, Institute of Parasitology and Tropical Pathology, Faculty of Medicine, Strasbourg, France
- Norwegian Veterinary Institute (SVA), Norway
- Research Institute of Food Safety, Animal Health and Environment/BIOR, Department of Parasitology, Latvia.
- Technical University of Denmark. National Veterinary Institute. Denmark.
- University of Bari, Faculty of Veterinary Medicine, Italy.
- University of Nápoles Frederico II, Faculty of Veterinary Medicine, Italy.

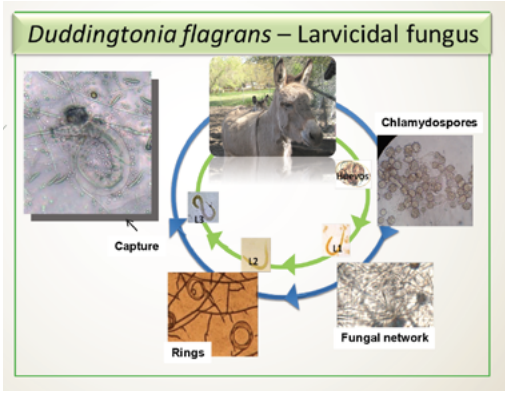
National Universities/Scientific Research Institutes

- Faculdade de Ciências Médicas da Universidade Nova de Lisboa. FCT,
- Faculdade de Ciências, Universidade de Lisboa.
- Instituto Gulbenkian de Ciência (IGC) - FCT
- Instituto de Higiene e de Medicina Tropical, Universidade Nova de Lisboa.
- Jardim Zoológico e de Aclimação de Lisboa.
- RIAS - Centro de Recuperação e Investigação de Animais Silvestres, Ria Formosa, Olhão.
- Universidade de Trás-os Montes e Alto Douro.

6. Outreach

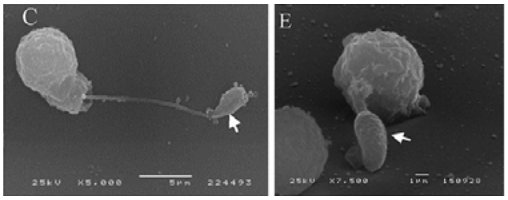
PPD Lab team organized seminars and international congresses. Researchers have been invited as trainers for international and national workshops and as consultors and opinion leaders for pharmaceutical companies (Bayer Saúde Animal, Merial Animal Health Europe, Merck, Sharp and Dohme, VIRBAC and Boehringer Ingelheim Animal Health).

Head of Cukicoides paradoxalis sp. nov. (Ramilo et al., 2013).



Targeted selective treatment approach by administration of nematophagous fungi - larvicidal *Duddingtonia flagrans*


L3 larval stage trapped by fungal hyphae of *Duddingtonia flagrans* (400X).



Dog PMN attaches (C) to and phagocytes (E) a L. *infantum* promastigote (Pereira et al., 2017).

7. Advanced Studies (PhD e MSc thesis)

PPD Lab research activities allowed the production of 5 PhDs and 69 Master thesis, as well as national and international internships (European and non-European).

Tropical Animal Health and Production

Lab Leader Alexandre Leitão | **Researchers** Alfaro Cardoso | **Post-Docs** Sofia Nolasco, Dulce Santos | **PhD Students** Helga Waap, Sofia van Harten , Eduardo Marcelino, Samuel Francisco, Inês Delgado, Sara Zúquete

From Left to right: Alfaro Cardoso, Inês Delgado, Sara Zúquete, Sofia Nolasco, Dulce Metelo and Alexandre Leitão

1. Research Interests and Expertise

The Tropical Animal Health and Production (TAHP) Lab was started in 2016, after the fusion of Instituto de Investigação Científica Tropical (IICT) with the University of Lisbon. The University assumed IICT mission and, within this frame, the Lab is focused on developing scientific 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.

The lab has developed research work on *Toxoplasma gondii*, an apicomplexan parasite of high impact on animal and human health that, in addition, constitutes a relevant model for the study of other cyst forming coccidian parasites. This work has been focused on the parasite interaction with the host cell and on the role of proteins that we postulate to be involved in invasion, and in the regulation of the parasite replication and cyst formation. The work on *Besnoitia besnoiti*, a scarcely studied coccidian parasite of cattle emerging in Europe, has been mainly focused on the interaction with the host cell tubulin cytoskeleton, centrosome and Golgi apparatus and on improving diagnostic tests and developing epidemiological studies.

Ticks and tick borne diseases are a major problem for animal production in tropical areas and control measures must be based on updated knowledge on tick epidemiology and on animal management practices at the local level. Within this period, we have studied these aspects in Huambo province, Angola and Geba River basin, Guinea-Bissau. In an attempt to contribute for the development of novel tick control approaches, we have been studying the tick-host adhesion complex so as to understand its structural changes occurring during the tick life cycle, allowing its detachment and enabling it to fall from the host. Understanding cement degradation may lead to a strategy to induce tick precocious fall out from the the host, interrupting its life cycle.

Recently, we initiated a research line concerning anti-*Trypanosoma* defense systems in *Glossina* flies in collaboration with Instituto de Higiene e

Medicina Tropical, Universidade Nova de Lisboa.

For many infectious agents there are no suitable vaccines and some of the vaccines currently in use show lower efficacy when applied in vaccination programmes in tropical climates. Aiming to contribute for the development of better vaccines, we have been studying the modulation of the immune response by targeting TLR2, alone or in combination with other innate stimuli.

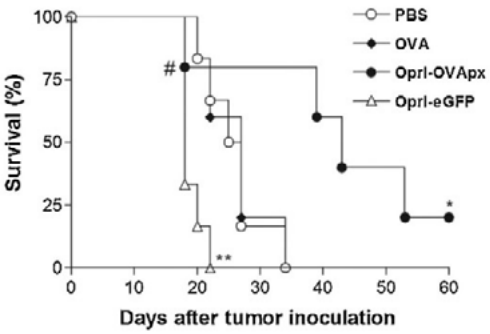
Within the Animal Production Area, the studies involve the identification of genetic and functional profile of animal and plant (pastures and forages) adaptation to drought, hyperthermia and seasonal feed restriction regarding meat and milk production.

Rural development projects are being implemented in Portuguese speaking African countries, in partnership with local and Portuguese NGOs, dealing with agriculture and livestock trading and production systems, aiming to enhance farmers organizations structures, livestock production and trading technical assistance and capacity building.

2. Highlights

Exploring TLR2 stimulation for programming the adaptive immune response

We have previously established an *E. coli* cloning system for the expression of antigens in fusion with the OprI lipoprotein, a TLR2/1 ligand, to be used in immunogenic formulations (Basto et al., 2012, J Biotechnol 157:50-63). Now, we have established an efficient depyrogenation method for recombinant bacterial outer membrane lipoproteins, solving one of the main constraints to their use in vaccine research. With this protocol we have consistently obtained recombinant OprI fusion antigens with LPS contents below 0.02 endotoxin units/µg of protein without affecting their biological activity (Basto et al., 2014, Protein Expr Purif, 98:10-17). Subsequently, we have been characterizing the immune response profile elicited *in vivo* by the fusion antigens using the ovalbumin antigen model (Basto et al., 2015, Mol Immunol, 64:36-45) and, in collaboration with the Institute for Parasitology, Vetsuisse Faculty, University of Berne, using a *Neospora caninum* vertical transmission mouse model with significant reductions in parasite burdens and vertical transmission rates (unpublished).



Survival rates after B16-OVA tumor challenge of mice immunized ovalbumin (OVA), OVA expressed in fusion with OprI (OprI-OVApx) or OprI-eGFP. PBS was used as control (Basto et al., 2015).

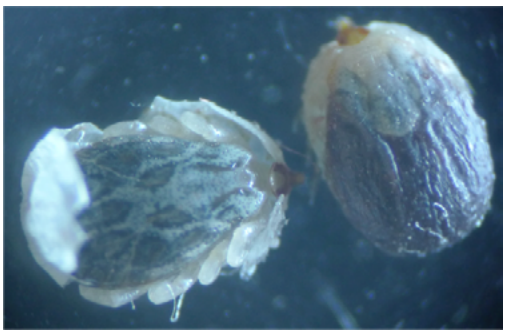
Mammary gland in bovines acclimated to a tropical environment

Ongoing climate changes have a significant impact on agricultural production. This has prompted us to study the adaptation to abiotic stress of plants and animals, namely regarding increase of milk production in temperate and tropical regions. Our work aims at studying the eventual acclimatization of Holstein animals to tropical environment with specific incidence of mammary gland gene expression profile, comparing it with two different genetic groups and with Holstein bovines submitted to temperate environments but with the same management system and nutritional levels. We identified 14 genes with differential expression in the mammary gland in at least one of the Holstein Brasil, Gyrolando and Gyr (tropical Brazil) experimental groups, associated with the function of mammary gland development, thermal stress tolerance and milk composition. On Holstein cattle, we identified 12 genes with differential associated with milk composition, mammary gland development and heat stress

tolerance. These findings are compatible with an acclimatization process to tropical environment. Transcripts that can have a relevant role in this process were identified (Wetzel-Gastal et al., 2016, South African Journal of Animal Science, 46: 1-13; Wetzel-Gastal et al., 2018, Trop Anim Health Prod, 50:187-195).

Tick (Acari: Ixodidae) infestations in cattle

We have studied the tick infestation in cattle along the Geba River basin, Guinea-Bissau, during the dry and rainy seasons. This led to a deeper characterization of *Rhipicephalus sulcatus* and a *Rhipicephalus sanguineus*-like specimen, exploring their genetic relationship with other *R. sanguineus*, which supports their classification as distinct species within *R. sanguineus* group (Zúquete et al., Ticks Tick Borne Dis 8:161-169). Similar studies on tick infestations and on the bovine production system were conducted in Huambo province, Angola (unpublished).



Dermacentor marginatus molt adult.

3. Representative Projects

Research projects

EXPL/CVT-EPI/1945/2013 - Mob1 protein: a critical factor in Toxoplasma gondii replication. PI: Sofia Nolasco. Partners: Fundação da Faculdade de Ciências-ULisboa, IICT/MNE. FCT Funding (2014 –2015).

PTDC/CVT-EPI/3460/2012 - Let's make them fall: a genomic, proteomic and immune-histologic approach on the involution of tick aggregation cement. PI: Luis Alfaro Cardoso. Partners: IHMT; BIOCANT; Pró-INSA. Funding FCT (2013-2015)

PTDC/CVT/113889/2009 - Modulation of acquired immunity by bacterial cell wall-derived formulations containing heterologous antigens expressed by a new OprI based system. PI: Dulce Santos, FCT (2011 – 2014).

PTDC/CVT/105470/2008 - The katanin role in Mt cytoskeleton remodeling during host cell invasion by Toxoplasma gondii. PI: Sofia Nolasco. Principal Contractor: Fundação Calouste Gulbenkian, Instituto Gulbenkian de Ciência. Partners: Instituto de Investigação Científica Tropical. (2010 – 2013).

Rural Development Projects

Cabinda (Angola) Integrated Agricultural Development Study. Promoted by the Government of Angola, financed by the Bank of African Development. In cooperation with CESO/COBA partnership. (July – November 2016).

Food Security and Nutrition in Quinara, Guiné- Bissau. In cooperation with NGO ACEP and ATA (Portugal), RA (Guiné-Bissau). Financed by Fundação Calouste Gulbenkian. (April 2014 – December 2015).

Project PAANE (EU) – Quinara, Guiné-Bissau: Rural Development. In cooperation with NGO ACEP and ATA (Portugal), RA (Guiné-Bissau). Financed by EU and Portuguese Cooperation. (January 2013 – March 2014).

4. Selected Publications

Aguado-Martínez, A., Basto, A.P., Leitão, A., Hemphill, A. (2017). *Neospora caninum* in non-pregnant and pregnant mouse models: cross-talk between infection and immunity. ***International Journal for Parasitology***, 47(12): 723-735.

Cardoso, L.A., Ferrão, J., Fernandes, T.H. (2017). Malnutrition in Developing Countries: Role of Agriculture and Trading. ***Journal of Food Security***, 5(6): 248-258.

Zúquete, S.T., Coelho, J., Rosa, F., Vaz, Y., Cassamá, B., Padre, L., Santos, D., Basto, A.P., Leitão, A. (2017). Tick (Acari: Ixodidae) infestations in cattle along Geba River basin in Guinea-Bissau. ***Ticks and Tick-borne Diseases***, 8(1): 161-169.

Wetzel-Gastal, D., Feitor, F., van Harten, S., Sebastiana, M., Sousa, L.M.R., Cardoso, L.A. (2016). Genomic study of the mammary gland in bovines acclimated to a tropical environment. ***South African Journal of Animal Science***, 46(1): 1-13.

Almeida, A.M., Palhinhas, R.G., Kilminster, T., Scanlon, T., van Harten, S., Milton, J., Blache, D., Greeff, J., Oldham,

C., Coelho, A.V., Cardoso, L.A. (2016). The Effect of Weight Loss on the Muscle Proteome in the Damara, Dorper and Australian Merino Ovine Breeds. ***PLOS ONE*** 11(2):e0146367.

Basto, A.P., Badenes, M., Almeida, S.C.P. Martins, C., Duarte, A., Santos, D.M., Leitão, A. (2015). Immune response profile elicited by the model antigen ovalbumin expressed in fusion with the bacterial OprI lipoprotein. ***Molecular Immunology***, 64: 36-45.

Waap, H., Nunes, T., Cortes, H., Leitão, A., Vaz, Y. (2014). Prevalence and geographic distribution of *Besnoitia besnoiti* infection in cattle herds in Portugal. ***Parasitology Research***, 113: 3703-3711.

Basto, A.P., Leitão, A. (2014). Targeting TLR2 for vaccine development. ***Journal of Immunology Research***, 2014: 619410.

Cardoso, R., Nolasco, S., Gonçalves, J., Cortes, H.C., Leitão, A., Soares, H. 2014. *Besnoitia besnoiti* and *Toxoplasma gondii*: Two apicomplexan strategies to manipulate the host cell centrosome and Golgi apparatus. ***Parasitology***, 141:1436-1454.

Van Harten, S., Brito, R., Almeida, A.M., Scanlon, T., Kilminster, T., Milton, J., Greeff, J., Oldham, C., Cardoso, L.A. (2013). Gene expression of regulatory enzymes involved in the intermediate metabolism of sheep subjected to feed restriction. ***Animal*** 7: 439-445.

5. Collaborations

International institutions

- Faculdade de Medicina Veterinária, Universidade José Eduardo dos Santos, Huambo, Angola.
- Faculty of Medicine, University of Cantabria, Santander, Spain.
- Institute of Parasitology, Vetsuisse Faculty, University of Bern.
- Institute of Parasitology, Vetsuisse and Faculty of Medicine, University of Zurich.
- Chair for Experimental Parasitology Faculty of Veterinary Medicine, Ludwig-Maximilians-University of Munich (LMU), Munich, Germany.

National institutions

- Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa.
- Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa. Colaboração científica e Instituição Participante. Sofia Nolasco
- Laboratório de Parasitologia, Instituto Nacional de Investigação Agrária e Veterinária.
- Laboratório de Parasitologia Victor Caeiro, Departamento de Medicina Veterinária, Universidade de Évora.
- Plant Cell Biotechnology Laboratory, Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa.

Official services

- Direcção Geral de Alimentação e Veterinária (DGAV)

Networking and consortiums

- ApicoWplexa: Apicomplexa in farm animals. Informal network launched in 2012 (http://www.apicowplexa.net/)
- COST Action CA15116 - Understanding and combating African swine fever in Europe (ASF-STOP).

6. Outreach

- European Researchers Night, 2016
- EXPO FCT open day 2017
- Descobre a ULisboa, 2017
- Verão na ULisboa 2015, 2016

7. Advanced Studies (PhD e MSc thesis)

During the period 2013-2017, 7 PhD and 3 MSc degrees were awarded to students working in the lab.

Pathology

Lab Leader Maria da Conceição Peleteiro | **Researchers** Jesús Ruberte, Jorge Jesus Correia, Mário Pinho, Luísa Mendes Jorge, Carlos Augusto Pinto, Fernando Afonso, José Ferreira da Silva, Fernando Garcia e Costa | **Post-Docs** Cláudia Marques, David Ramos Gonzalez | **PhD Students** Andreia Valença, Andreia Gameiro, João Bettencourt Cota, Rute Noiva, Maria João Soares, Cátia Martins | **Master Students** Fábio Santos **Technicians** Hugo Pissarra, Sandra Carvalho

From Left to right- Front row: Fábio Santos, Andreia Valença, Hugo Pissarra.
Back row: Ferreira da Silva, Mário Pinho, Jorge Correia, Maria da Conceição Peleteiro, Sandra Carvalho, Luísa Jorge and Fernando Afonso.

1. Research Interests and Expertise

By definition, a Pathology Laboratory is a structure of human and technical resources that addresses four components of disease: the aetiology, the pathogenesis, the structural alterations of organs, tissues and cells and the consequences of the changes produced in an organism that may or may not cause its death.

In Veterinary Medicine the object of the work of the pathologists is very variable, from mammals, to birds, fish, or even invertebrates. In the mammals group many species can be delt with, from the large bovine and horses, to the minute lab mouse, including all sorts of median size cats and dogs, and zoological or exotic species.

As far as research is concerned, the Pathology Lab/Team should be seen as a platform where the most common activities can be summarized as follows:

Diagnosis for internal and external clients, with the major client being the Small Animal Hospital of the Faculty. This part of the activity, going on for more than three decades, provides the large experience this Lab/Team can claim as major asset.

Teaching of undergraduates and postgraduates (residents). The Pathology Lab/Team is registered as "Qualified for Residency Programs" by the European College of Veterinary Pathology, since 2000.

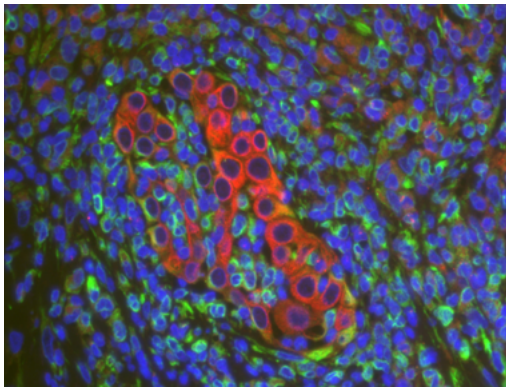
Support to various research projects internal and external. This support is materialized by processing samples for histological observation; performing immunohistochemistry and histochemistry techniques; providing in most cases the adequate reports and registering the results by microphotography.

2. Highlights

- Oncology and oncological markers for diagnosis and treatment. This subject has been primarily dealing with canine and feline mammary tumors, pursuing markers that are thought to be useful for the establishment of accurate prognosis and more efficient treatments. Other tumors have also been studied such as canine

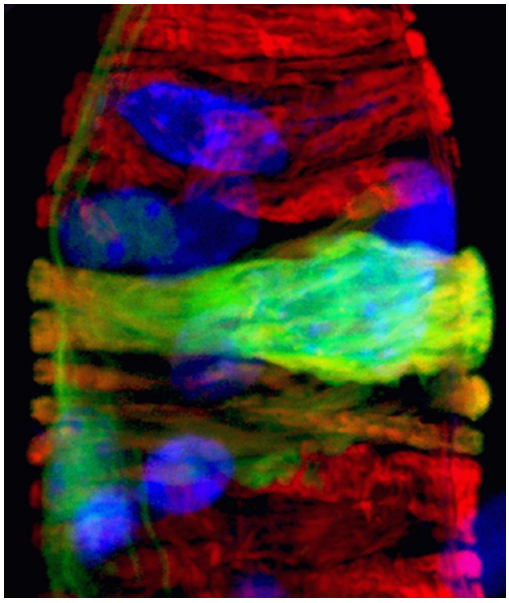
oral tumors and tumor growth in genetically modified mice.

- Mammalian and avian pathogenesis of infectious and toxic diseases. This subject has been particularly active in the study of the new variant of the Rabbit Haemorrhagic Disease Virus.
- Stem cells differentiation and use. The study of the fate and effect of the application of stem cells in the evolution of necrotic lesions as the ones induced by myocardial infarction are part of an important partnership, including the MIT. Other projects have also been developed involving stem cells in bioscaffolds.
- Retinal changes associated with disease and ageing. This work has been done in close cooperation with CBATEG (Centre de Biotecnologia Animal i de Teràpia Gènica), Universidad Autónoma de Barcelona, Spain
- Quantitative and semi-quantitative histology. Projects on antimicrobial effect of feeds in animal production have been achieved in collaboration with researchers from the Institute of Agronomy (ULisboa), using image analysis tools.
- Aquaculture. Projects have been performed in collaboration with various research institutions involving the sanitary monitorization of bivalves in Portugal.



Retromammary lymph node of a female cat with mammary carcinoma. Immunofluorescence analysis of CXCR4 and CXCL12 expression. Photomicrographs show a niche of neoplastic cells overexpressing CXCR4-Alexa Fluor® 594 (red staining in cytoplasmic membrane and cytoplasm) and CXCL12-Alexa Fluor® 488 (green staining mainly observed at the cytoplasmic membrane). DNA is identified by DAPI staining (blue). Photomicrography was taken in the high-powered magnification (× 400).

Studies on the effect of environmental conditions over incubation - celosomy in a 14 day old chick embryo.



Whole mount retina from B-actin EGFP transgenic mice and analyzed under confocal laser scanning microscopy. Blood vessel: smooth muscle cells are labelled with phalloidin (red). Two highly green fluorescent cell populations are observed within the vascular wall: interstitial cells and synthetic smooth muscle cells. Nuclei are counterstained with Hoechst

3. Representative Projects

Concluded Projects

EXPL/BIM-MED/1970/2013 – CARDIOSAFER – Development of a safer and more effective mesenchymal stem/stromal cell (MSC)-based therapy for myocardial regeneration. 2014 - 2015. Partners: Associação do Instituto Superior Técnico para a Investigação e o Desenvolvimento (IST-ID); Faculdade de Medicina Veterinária, Universidade de Lisboa; Centro Hospitalar de Lisboa Central, EPE (CHLC), Hospital de Santa Marta. Principal Investigator – Cláudia Lobato (IST/ID).

Instituto de Salud Carlos III, Espanha. (PS09/01152) - Senescencia celular en los vasos sanguíneos de la retina: implicaciones de P16 en el cross-linking enzimático del colágeno de la membrana basal durante el envejecimiento. Principal Investigator: Jesús Ruberte. 2010 -2013.

Instituto de Salud Carlos III, Espanha (PI12/00605) - Analysis of SIRT1 effects in vascular microaneurysm formation during aging. Principal Investigator: Jesús Ruberte. 2012-2015.

PTDC/SAU-ORG/110856/2009 - Analysis of a new pathway to import iron into the retina: role of ferritin as iron carrier and implications in radical detoxification. Principal Investigator: Jesús Ruberte. CIISA/FMV/ULisboa. 2011-2013

Ongoing projects

MITP-TB/ECE/0013/2013 – CARDIOSTEM – Engineered cardiac tissues and stem cell-based therapies for cardiovascular applications. 2014 - 2017, extended to May 2018. Partners: Centro de Neurociências e Biologia Celular (CNBC/UC); Associação do Instituto Superior Técnico para a Investigação e o Desenvolvimento (IST-ID); Cell2B - Advanced Therapeutics SA (Cell2B); Centro Hospitalar de Lisboa Central, EPE (CHLC), Hospital de Santa Marta; Crioestaminal, Saúde e Tecnologia SA (Crioestaminal); Faculdade de Medicina Veterinária, Universidade de Lisboa; Instituto de Biologia Experimental e Tecnológica (IBET); Instituto Nacional de Engenharia Biomédica (INEB Porto). Principal Investigator: Lino da Silva Ferreira (CNBC/UC).

Instituto de Salud Carlos III, Espanha. (PI16/00719) - Retinal iron uptake through TIM2: implication in diabetic macular oedema. Principal investigator: Jesús Ruberte. CIISA/FMV/ULisboa. Start: 2016

PTDC/CVT-EPI/3638/2014: "Molecular evaluation of HER2 and Topoisomerases in feline mammary carcinoma - Developing rational strategies for effective diagnosis and cancer chemoimmunotherapy". Principal investigator: Fernando Ferreira. CIISA/FMV/ULisboa. Start: 2016

4. Selected Publications

López-Luppo, M., Nacher, V., Ramos, D., Catita, J., Navarro, M., Carretero, A., Rodriguez-Baeza, A., Mendes-Jorge, L., Ruberte, J. (2017). Blood Vessel Basement Membrane Alterations in Human Retinal Microaneurysms During Aging. ***J. Invest Ophthalmol Vis Sci***; 58(2):1116-1131.

Lisa A. Mestrinho, Hugo Pissarra, Sandra Carvalho, Maria C. Peleteiro, Jerzy Gawor and Maria M. R. E. Niza (2017). Comparison of Histological and Proliferation Features of Canine Oral Squamous Cell Carcinoma Based on Intraoral Location: 36 Cases. ***Journal of Veterinary Dentistry***, Vol. 34(2) 92-99

Gil da Costa, R. M., Peleteiro, M. C., Pires, M. A., DiMaio, D. (2017). An Update on Canine, Feline and Bovine Papillomaviruses. ***Transboundary and Emerging Diseases***, 64(5):1371-1379.

Soares, M., Ribeiro, R., Najmudin, S., Gameiro, A., Rodrigues, R., Cardoso, F. & Ferreira, F. (2016). Serum HER2 levels are increased in cats with mammary carcinomas and predict tissue HER2 status. ***Oncotarget***, 7(14):17314-26.

Soares, M., Madeira, S., Correia, J., Peleteiro, M., Cardoso, F. & Ferreira, F. (2016). Molecular based subtyping of feline mammary carcinomas and clinicopathological characterization. ***The Breast Journal***, 27, 44-51.

Neves Simões, I., Vale, P., Soker, S., Atala, A., Keller, D., Noiva, R., Carvalho, S., Peleteiro, C., Cabral, J., Eberli, D., Lobato da Silva, C., Batista, P. (2016). Acellular Urethra Bioscaffold: Decellularization of Whole Urethras for Tissue Engineering Applications. ***Scientific Reports***, 6, 7: 41934.

Lopes, A.M., Correia, J., Abrantes, J., Melo, P., Ramada, M., Magalhães, M.J., Alves, P.C. & Esteves, P.J. (2015). Is the New Variant RHDV Replacing Genogroup 1 in Portuguese Wild Rabbit Populations? ***Viruses***, 7, 27-36.

Djokovic, D., Trindade, A., Gigante, J., Pinho, M., Harris, A.L., Duarte, A. (2015). Moderate Dll4/Notch Signaling Inhibition Promotes Productive Angiogenesis and Growth of Skin Papillomas. ***BMC Cancer***, 15:608-616.

Mendes-Jorge, L., Ramos, D., Valença, A., López-Luppo, M., Pires, V.M., Catita, J., Nacher, V., Navarro, M., Carretero, A., Rodriguez-Baeza, A., Ruberte, J. (2014). L-ferritin binding to scara5: a new iron traffic pathway potentially implicated in retinopathy. ***PLoS One***. 26;9(9):e106974.

5. Collaborations

The Pathology Lab recognizes as major partners in its research all institutions considered project associates, namely:

- CEBATEG (Centre for Animal Biotechnology and Gene Therapy), Universidad Autonòma de Barcelona, Spain
- IPMA - Instituto Português do Mar e da Atmosfera. Lisbon
- IPMA – Estação Piloto de Piscicultura de Olhão, Algarve, Portugal
- Vasco da Gama Aquarium, Lisbon
- Faculty of Fisheries, Hue University of Agriculture and Forestry, Hue city, Vietnam.
- National Prosecutors Office and Courts of Law on matters regarding Forensic Pathology
- Portuguese Institute for Nature Conservation and Forests on projects regarding poisoning of wild birds
- Lisbon School of Health Technology (Polytechnic Institute of Lisbon) on assistance in the training of graduates of Clinical Analysis in histology and immunohistochemistry.
- High Studies Cooperative Egas Moniz, Monte da Caparica, on assistance in the training of graduates of Clinical Analysis in histology and immunohistochemistry.
- High Studies Cooperative Egas Moniz, Monte da Caparica, on assistance in the training of graduates of Clinical Analysis in histology and immunohistochemistry.

6. Outreach

The Pathology Lab Team has been involved in activities involving various societies and scientific and non-scientific institutions:

Portuguese Society of Animal Pathology, Seminars on Forensic Pathology

Fauna Group (AEFMV-ULisbon), various courses and seminars on pathology of wild animals

Organization of the 16th Technical Congress of Pathological, Cytological and Thanatological Anatomy, Faculty of Veterinary Medicine, University of Lisbon, 8-10th May, 2015.

From 2013 to 2017, organization of several Annual Meetings of the Portuguese Society of Animal Pathology (XVIII, XIX, XX and XXII).

From 2013 to 2017, the Pathology Lab Team has supported histological processing and analysis of materials from projects involving non CIISA teams in other institutions, such as:

- Faculty of Pharmacy, University of Lisbon
- Faculty of Medicine, University of Lisbon
- Department of Bioengineering, Instituto Superior Técnico (Técnico Lisboa), University of Lisbon

7. Advanced Studies (PhD e MSc thesis)

Within the period 2013-2017, 5 PhD and 45 Master thesis were produced whose work was developed in the Pathology Lab under the supervision or co-supervision of members of the Pathology Team.

Antimicrobial Resistance

Lab Leader Constança Pomba | **Researchers** Rodolfo Oliveira Leal
PhD Students Adriana Belas, Cátia Marques, Pamela Valente | **Master Students** Catarina Aboim, Cláudia Marconi, Pedro Guilherme | **Junior Fellow** Juliana Menezes

From Left to right: Juliana Menezes, Luís Telo da Gama (collaborator), Constança Pomba, Rudolfo Leal, Adriana Belas, Pamela Valente, Pedro Guilherme, Cátia Marques, Cláudia Marconi and Catarina Aboim.

1. Research Interests and Expertise

Our research focuses on the study of the antimicrobial treatment of multidrug-resistant bacterial infections in animals and the public health repercussions of the clonal dispersal of these pathogens, their resistance and virulence genes. By studying the transmission dynamics (animal-to-human and vice-versa) we want to determine if and how this transmission occurs. This knowledge will deliver measures that can be applied to break the transmission cycle and thus support new antimicrobial stewardship polices. Moreover, the group is also interested in host-pathogen interactions, with the aim of identifying new potential targets for the treatment of bacterial infections. We use conventional microbiological and molecular methods, namely next generation sequencing, to:

- Characterize the resistome and microbiome of animals and humans in contact;
- Assess the epidemiological relatedness of animal and human bacterial isolates by whole-genome sequencing; determine risk factors for infection/colonization by multidrug-resistant bacteria in animals;
- Update the surveillance of antimicrobial and biocide resistance in pathogenic bacteria;
- Develop new therapeutic approaches for the treatment of multidrug-resistant bacteria infections.

2. Highlights

Antimicrobial-resistance in Gram-negative bacteria.

We described, for the first time, the OXA-23 carbapenemase in *Acinetobacter baumanni* ST2 from a cat with urinary tract infection (Pomba *et al.*, 2014). Some variation within the *E. coli* ST131 lineage have been associated with clonal commonality across strains isolated from humans and pets (Pomba *et al*., 2014). A multicenter study comprising 16 clinical microbiology laboratories from 14 European countries revealed significant differences in the antimicrobial resistance frequencies of bacteria causing urinary tract infection in companion animals across Europe (Marques *et al*., 2017). A cross sectional study revealed that prior antimicrobial use (last year)

in healthy dogs is a risk factor for colonization by ESBL/AmpC-producing *E. coli* (Belas *et al.*, 2014).

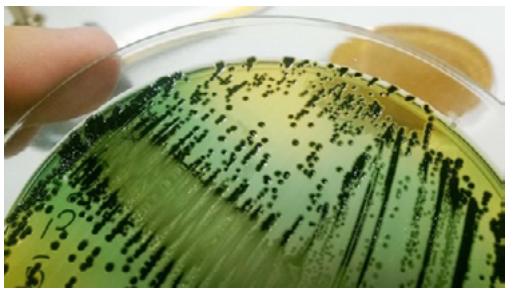
Antimicrobial-resistance in Gram-positive bacteria.

The antimicrobial resistance of staphylococci from companion animal infections increased significantly over the last 16 years (Couto *et al*., 2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complex 5 were described in animals from Portugal for the first time (Couto *et al.*, 2015). Acquisition of the *fexA* and *cfr* gene in *S. pseudintermedius* was observed during florfenicol treatment in a dog (Couto *et al*., 2016).

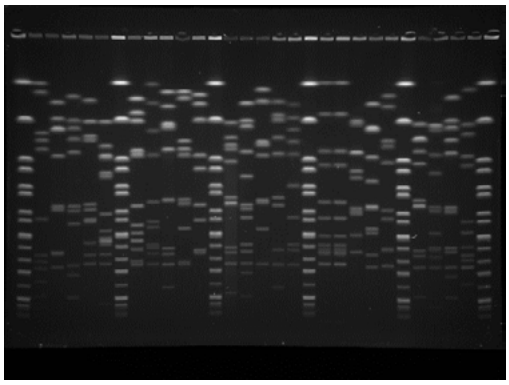
Advances in profilaxis

We identified 4 highly immunogenic proteins that seem promising vaccine candidate antigens of *Staphylococcus pseudintermedius* by serological proteome analysis (Couto *et al*., 2016).

MacConkey agar plate with Enterobacteriaceae



Hektoen Enteric agar plate with *Proteus mirabilis*



Pulse Field Gel Electrophoresis

3. Representative Projects

"Risk of companion animal to human transmission of antimicrobial resistance during different types of animal infection." Consortium PET-RISK JPIAMR/0002/2016 (2017-2020) Constança Pomba (PI) Partners: UK - Small Animal Medicine and Surgery Group The Royal Veterinary College; Canada- Ontario Veterinary College at the University of Guelph, Alemanha- Institute of Microbiology and Epizootics Centre of Infection Medicine Department of Veterinary Medicine Freie Universität Berlin; Switzerland- Institute of Veterinary Bacteriology, University of Bern.

"Immunogenicity of vaccine candidate antigens from *Staphylococcus pseudintermedius* in dogs." PTDC/CVT-EPI/4345/2012 (2012 – 2015) Constança Pomba (PI), Partners: ITQB/UNL.

4. Selected Publications

Marques C ., Gama, L.T., Belas, A., Bergström, K., Beurlet, S., Briend-Marchal, A., Broens, E.M., Costa, M., Criel, D., Damborg, P., van Dijk, M.A., van Dongen, A.M., Dorsch, R., Espada, C.M., Gerber, B., Kritsepi-Konstantinou, M., Loncaric, I., Mion, D., Misic, D., Movilla, R., Overesch, G., Perreten, V., Roura, X., Steenbergen, J., Timofte, D., Wolf, G., Zanoni, R.G., Schmitt, S., Guardabassi, L. & Pomba C (2016). European multicenter study on antimicrobial resistance in bacteria isolated from companion animal urinary tract infections. ***BMC Veterinary Research*** 12: 213.

Fernandes L Centeno, M.M., Couto, N., Nunes, T., Almeida, V., Alban, L. & Pomba, C. (2016). Longitudinal characterization of monophasic Salmonella Typhimurium throughout the pig's life cycle. ***Veterinary Microbiology*** 192:231-237.

Couto N Martins, J., Lourenço, A.M., Pomba, C. & Varela Coelho, A. (2016). Identification of vaccine candidate antigens of *Staphylococcus pseudintermedius* by whole proteome characterization and serological proteomic analyses. ***Journal of Proteomics.*** 133:113-124.

Couto, N., Monchique, C., Belas, A., Marques, C., Gama, L.T. & Pomba, C. (2016). Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period. ***Journal of Antimicrobial Chemotherapy*** 71:1479-87

Couto, N., Belas, A., Rodrigues, C., Schwarz, S. & Pomba, C. (2016) Acquisition of the fexA and cfr genes in *Staphylococcus pseudintermedius* during florfenicol treatment of canine pyoderma. ***Journal of Global Antimicrobial Resistance*** 7:126-127.

Catry, B., Cavaleri, M., Baptiste, K., Grave, K., Grein, K., Holm, A., Jukes, H., Liebana, E., Lopez Navas, A., Mackay, D., Magiorakos, A.P., Moreno Romo, M.A., Moulin, G., Muñoz Madero, C., Matias Ferreira Pomba, M.C., Powell, M., Pyörälä, S., Rantala, M., Ružauskas, M., Sanders, P., Teale, C., Threlfall, E.J., Törneke, K., van Duijkeren, E. & Torren Edo, J. (2015). Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. ***International Journal Antimicrobial Agents*** 46:297-306.

Pomba, C., Endimiani, A., Rossano, A., Saial, D., Couto, N. & Perreten, V. 2014. First report of OXA-23-mediated carbapenem resistance in sequence type 2 multidrug-resistant *Acinetobacter baumannii* associated with urinary tract infection in a cat. ***Antimicrobial Agents Chemotherapy*** 58:1267-1268.

Pomba, C., López-Cerero, L., Bellido, M., Serrano, L., Belas, A., Couto, N., Cavaco-Silva, P., Rodríguez-Baño, J. & Pascual, A. 2014. Within-lineage variability of ST131 Escherichia coli isolates from humans and companion animals in the south of Europe. ***Journal of Antimicrobial Chemotherapy*** 69:271-273.

Belas, A., Salazar, A.S., Gama, L.T., Couto, N. & Pomba, C. 2014. Risk factors for faecal colonisation with Escherichia coli producing extended-spectrum and plasmid-mediated AmpC β-lactamases in dogs. ***Veterinary Record*** 175: 202.

5. Collaborations

- Annete Loeffer- UK - Small Animal Medicine and Surgery Group, The Royal Veterinary College
- Scott Weese-Canada- Ontario Veterinary College at the University of Guelph
- Stefan Swartz - Alemanha- Institute of Microbiology and Epizootics Centre of Infection Medicine Department of Veterinary Medicine Freie Universität Berlin
- Vicent Perreten- Suiça- Institute of Veterinary Bacteriology, University of Bern.
- José Melo-Cristino, Mário Ramirez, João Carriço - Instituto de Medicina Molecular, Universidade de Lisboa, Portugal.
- Gabriela da Silva, Faculdade de Farmácia, Universidade de Coimbra, Portugal.
- Patrícia Cavaco-Silva- Centro de Investigação Interdisciplinar Egas Moniz Instituto Universitário Egas Moniz, Portugal.
- Miguel Viveiros, Isabel Couto - Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal.

6. Outreach

- European Medicine Agency, London, United Kingdom: AWP/CVMP – Antimicrobial Working Party of the Committee for Medicinal Products for Veterinary Use. Constança Pomba, member (2007-at present) and former vice-chair (2013-2016).
- European Medi cine Agency, London, United Kingdom: Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the Committee For Medicinal Products For Veterinary Use, former member 2014-2016
- European Society in Clinical Microbiology and Infectious Diseases / Study Group for Veterinary Microbiology – ESCMID / ESGVM. Constança Pomba, Treasurer (2017-at present), former Postgraduate education responsable (2015-2016)
- ESCMID Postgraduate Education Course "Antimicrobial Stewardship in Veterinary Medicine." Gothenburg, Sweden, 10.- 11.09.2016; Organiser(s): ESGVM, ESGAP, University of Copenhagen, University of Lisbon and University of Gothenburg, International Society for Companion Animal Infectious Diseases (ISCAID), European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA) Constança Pomba (Course co-coordinator)
- 1st Post-graduate course "Antibioterapia de animais de companhia, aves e exóticos", Faculty of Veterinary Medicine, University of Lisbon (2015). Constança Pomba (Course coordinator)
- International Seminar "Use of antimicrobials in animal production – need for change?" 3th of October at the Faculty of Veterinary Medicine Lisbon 2013. Constança Pomba (Course coordinator)

- Round tables on antimicrobial resistance in companion animals (2016)
- C. Marques, C. Pomba (2016). Os Desafios da Resistência aos Antibióticos em Animais de Companhia. Vet Talks CEVA. Novembro 2016, Lisboa, Portugal
- C. Marques, C. Pomba (2016). Resistência aos antimicrobianos em animais de companhia. Vetsummit. Maio 2016, Oeiras, Portugal

AR Lab researchers at the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 2015, Copenhagen, Denmark. From left to right: Adriana Belas, Constança Pomba, Cátia Marques.

AR Lab awarded the "Prémio Investigação nos Prémios Veterinária Atual 2016" for work developed on a vaccine against *Staphylococcus pseudintermedius* in dogs. (http://www.veterinaria-atual.pt/na-clinica/premios-veterinaria-atual-laboratorio-de-resistencia-aos-antibioticos-e-biocidas-fmvul-vence-premio-investigacao/)

Prize for best poster presentation at the 26th Congress of Veterinary Internal Medicine congress (ECVIM, 2016) awarded to AR Lab member.

AR researcher awarded with prize for best oral presentation at the XII Congresso Hospital Veterinário Montenegro (2016)

AR Lab PhD student awarded the "Second best oral presentation" by the European Society of Veterinary Nephrology and Urology (ESVNU) at the 25th European Congress of Veterinary Internal Medicine, (ECVIM, 2015).

Cátia Marques and her supervisor Constança Pomba at the24th European Congress of Veterinary Internal Medicine (ECVIM), 2014, Mainz, Germany.

AR Lab PhD student Cátia Marques wins prize for "Best Oral Presentation" at the 24th European Congress of Veterinary Internal Medicine (ECVIM, 2014)

AR Lab scientist awarded with prize for best oral presentation at the XXII APMVEAC National Congress (2014).

AR team studies recognized in the international press (https://www.sciencenews.org/article/fido-and-fluffy-could-unleash-drug-resistant-microbes) Studies presented by PhD students Cátia Marques and Adriana Belas at the joint meeting of the American Society for Microbiology Microbe 2016 and the Interscience Conference on Antimicrobial Agents and Chemotherapy in Boston found a rising in antimicrobial-resistance trend in bacteria causing urinary tract infections in pets.

7. Advanced Studies (PhD e MSc thesis)

Over the period considered 1 PhD and 10 Master thesis were produced in the lab.

Clinical Research

Lab Leader António Ferreira | **Researchers** Anabela Moreira, Berta São-Braz, Esmeralda Delgado, José Duarte Correia, José Sales Luis, Luís Lamas, LIsa Mestrinho, Mafalda Lourenço, Manuela Espada Niza, Miguel Saraiva Lima, Paula Tilley, Rita Fonseca, Sandra Jesus, Teresa Vila de Brito | **PhD Students** Ana Paula Resende, Joana Simões, Nuno Félix, Rudolfo Leal, Vera Pessoa | **Master Students** Ana Catarina Rodrigues, Ana Xavier, Joana Oliveira, Rita Correia, Ruben Dinis, Silvia Spínola | **Technicians** Belmira Carrapiço

From left to right: Manuela Espada Niza, Berta São-Braz, Lisa Mestrinho, Esmeralda Delgado, Rita Fonseca, António Ferreira, Paula Tilley, Teresa Vila de Brito, J.P. Sales Luis, J.H. Duarte Correia and Sandra Jesus.

1. Research Interest and Expertise

The Clinical Research Lab congregates the research efforts of several clinical teams, mainly including veterinary surgeons, which perform clinical teaching and medical and surgical care services at the Teaching Hospital of FMV-ULisboa. Although scientific research is not the main objective of the Teaching Hospital, it generates large pools of clinical cases on which research studies on multiple scientific areas can be carried out. During the 2013-2017 period, the most relevant contributions were achieved in the areas of ophthalmology, allergy and immunology, pharmacology, dentistry and animal production medicine.

2. Highlights

The Ophthalmology team developed experimental ophthalmic microsurgery in laboratory the rat and rabbit animal models. These developments included the episcleral vein coagulation for glaucoma induction and the intravitreal microinjection of proteins for assessment of crossover of hemato-ocular barriers. The team also evaluated ocular drug delivery and the immune response in allergic conjunctivitis.

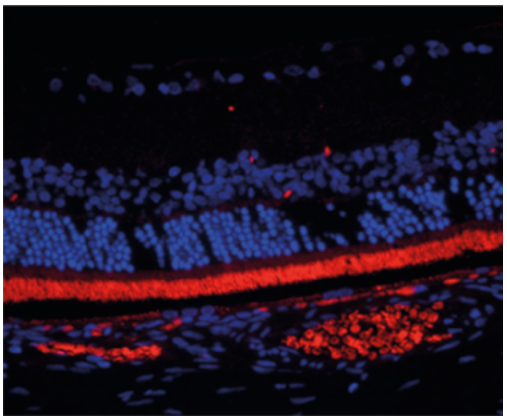
The Dermatology team expanded knowledge on atopic dermatitis and allergic conjunctivitis in the canine model, evaluating aeroallergens and developing novel diagnostic test for this condition. The team also evaluated a medical grade honey based cream in the treatment of suppurative otitis, as an alternative to conventional antibiotic therapy.

The Pharmacology and Toxicology team focused research on pharmacokinetics of novel drugs directed to ocular diseases, and on alternative therapeutic approaches, namely immunotherapy and phytotherapy. In the Forensic Veterinary Medicine scope, the team studied the societal perception of animal maltreatment and the link between animal abuse and family and community violence.

The Dentistry team focuses studied canine oral squamous cell carcinoma, a spontaneous tumor in pet dogs with potential interest for translational research. The team also addressed their attention

to acute inflammatory proteins in oral disease and oral anomalies in brachycephalic breeds.

The Animal Production Medicine team focused research on ruminant metabolic diseases, namely pregnancy toxemia in goats, and in respiratory diseases of the horse, namely Equine Asthma Syndrome and laryngeal abnormalities.



Immunohistochemistry for cell nucleus marker of retina (DAPI, blue) and EPO antibody (red).

3. Representative Projects

Research Grant ESVO 006-2014 (European Society of Veterinary Ophthalmology) **"**Osmolarity tear film in canine keratoconjunctivitis sicca" (2014); PI - Esmeralda Delgado.

Research Grant ESVO 2015 (European Society of Veterinary Ophthalmology) "In vitro ocular permeation of EPO protein in pig cadaveric eyes" (2015); PI - Esmeralda Delgado.

4. Selected Publications

Resende AP, Silva B, São-Braz B, Nunes T, Gonçalves L, Delgado E (2017). Ex vivo permeation of erythropoietin through porcine conjunctiva, cornea, and sclera***. Drug Delivery and Translational Resesearch.*** 7: 625.

Mestrinho LA, Faísca P, Peleteiro MC, Niza MMRE (2017). PCNA and grade in 13 canine oral squamous cell carcinomas: association with prognosis. ***Veterinary and Comparative Oncology*** 15: 18-24.

Félix NM, Leal RO, Goy-Thollot I, Walton RS, Gil SA, Mateus LM, Matos AS, Niza MMRE (2017). Effects of buprenorphine in the adrenal, thyroid, and cytokine intra-operative responses in a rat model (*Rattus norvegicus*): a preliminary study. ***Iranian Journal of Basic Medical Sciences***, 20: 368-379.

Resende AP, São-Braz B, Delgado E (2016) Ocular erythopoietin penetration after subconjunctival administration in glaucomatous rats. ***Ophthalmic Research*** 56:104-110.

Lima MS, Silveira JM, Carolino N, Lamas LP, Pascoal RA, Hjerpe CA (2016). Usefulness of clinical observations and blood chemistry values for predicting clinical outcomes in dairy goats with pregnancy toxaemia. ***Irish Veterinary Journal*** 69: 16.

Lima MS, Cota JB, Vaz YM, Ajuda IG, Pascoal RA, Carolino N, Hjerpe CA (2016). Glucose intolerance in dairy goats with pregnancy toxemia: Lack of correlation between blood pH and beta hydroxybutyric acid values. ***Canadian Veterinary Journal*** 57: 635-40.

Mestrinho LA, Pissarra H, Faísca PB, Bragança M, Peleteiro MC, Niza MMRE (2015). p63 and E-cadherin expression in canine oral squamous cell carcinoma. ***Veterinary Pathology*** 52: 614-20.

Mestrinho LA, Faísca PB, Niza MM (2014). Parotid salivary duct stenosis following caudal maxillectomy. ***Journal of Veterinary Dentistry*** 31: 40-3.

Resende AP, São-Braz B, Delgado E (2013). Alternative route for erythropoietin ocular administration. ***Graefe's Archive for Clinical and Experimental Ophthalmology*** 251: 2051-2059.

Delgado E, Marques-Neves C, Rocha I, Sales Luís JP, Silva-Carvalho L (2013). Myogenic oscillations in rabbit ocular vasculature are very low frequency. ***Ophthalmic Research***, 50: 123-128.

5. Collaborations

- Direcção Geral de Alimentação e Veterinária (DGAV)
- IMM-ULisboa,
- Pharmacy Faculty, ULisboa
- Structure and Motion Laboratory, Royal Veterinary College, University of London, UK
- Vetsuisse-Fakultät, University of Bern, Switzerland

6. Outreach

The clinical research lab works with and in the community as an extension of their research, supporting the overall mission of providing state of the art care for veterinary patients. With regards to small animal practice, cutting edge techniques have been attempted in challenging cases, resulting in various publications.

Important scientific meetings, co-organized by members of the clinical research lab, were held in Portugal during 2013-2017:

Annual Scientific Meeting of the European College of Veterinary Surgeons, 7-9th July 2016 in Lisbon – Luís Lamas was a co-organizer together with a Portuguese team;

Annual Meeting of the European Society of Veterinary Ophthalmology, 1-4th October 2015 in Lisbon – Esmeralda Delgado was the Scientific officer.

The European Veterinary Dental Society (EVDS) aims to promote clinical dental research with benefit to animals and people. Lisa Mestrinho enrolled the EVDS board in 2012, as President Elect (2012-2014), President (2014-2016) and Past President (2016-2018)**.**

The **European Society of Veterinary Ophthalmology** (ESVO) **supports** scientific activities and continuing education in veterinary ophthalmology. Esmeralda Delgado enrolled the ESVO executive board as a scientific officer.

4th June 2013 – Board of the European Veterinary Dental Society (2012-2014).

20th October 2013 - ESVO's Executive Board.

7. Advanced Studies (PhD e MSc thesis)

During the period 2013-2017, 4 PhD and 18 Master degrees were awarded to students working in the lab.

Reproduction and Development

Lab Leader Luis Lopes da Costa | **Researchers** Ana Catarina Torres, António Duarte, Elisabete Silva, Graça Ferreira Dias, João Pedro Barbas, Jorge Pimenta, Luísa Mateus, Nestor Chagas e Silva, Rosa Lino Neto Pereira | **Post-Docs** Alexandre Trindade | **PhD Students** Ana Amaral, Gonçalo Pereira, Carina Fernandes, Maria Rosa Rebordão, Mariana Batista, Marta Silva, Patrícia Diniz, Pedro Pinto Bravo | **Master Students** Ana Murta, Beatriz Lourenço, Carolina Encarnação, Inês Machado, Maria Inês Romeiras, Marta Pinto

Upper photo, from left to right: João Pedro Barbas, Rosa Lino Neto Pereira and Jorge Pimenta. *Lower photo front row, from left to right:* Patrícia Diniz, Marta, Luísa Mateus,Elisabete Silva, Maria Rosa Rebordão, Graça Ferreira Dias, Ana Catarina Torres. *Back row:* Nestor Chagas e Silva, Gonçalo, Alexandre Trindade, Luís Lopes da Costa, António Duarte, Ana Amaral, Marta Pinto and Carina Fernandes.

1. Research Interests and Expertise

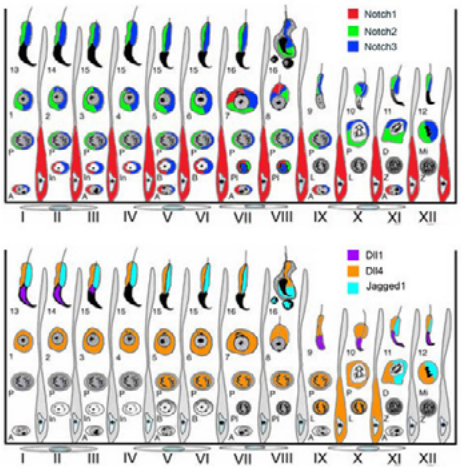
The Reproduction and Development (R&D) Lab develops research, knowledge transfer and consulting services in biology, biotechnology and medicine of reproductive and developmental processes in animals, including lab comparative medicine models for other animal species and humans. The R&D Lab performs fundamental, applied and translational research, involving a high level of interdisciplinary collaboration within the lab, with CIISA's and other national labs, and of internationalization. This interdisciplinary and translational atmosphere is well reflected in the broad academic backgrounds of R&D Lab team members, PhD students and postdoctoral fellows (veterinary doctors, medical doctors, animal scientists, biologists, biotechnologists, microbiologists and health technicians). The R&D Lab displays state-of-art facilities and expertise in reproductive assessment and technologies at the animal, endocrine, tissue, cellular and molecular levels. The R&D Lab runs a transgenic mouse setting, involving several own transgenic and genetic knock-out and knock-in mutants, which are ancillary tools in embryonic and postnatal normal and abnormal development, including cancer. The R&D Lab is also in the front of research on the interactions between host and infectious agents causing reproductive failure in animal species. Technology transfer, scientific and technical consulting and formation are provided to other national and international labs, pharmaceutic companies, farmer's cooperatives and animal breeders

2. Highlights

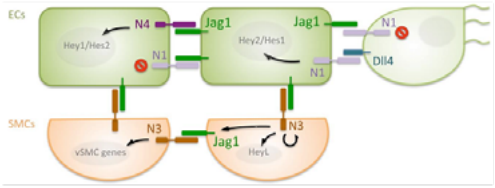
Unraveling Notch Signaling

Since starting this pioneering research line back in 2004 (Duarte et al. 2004, Genes and Development, 18, 2474-8), the R&D Lab developed a world leading expertise in the unraveling of Notch signaling in reproductive, developmental

and cancer biology, publishing over 30 Notch-related papers in high impact journals (including *Nature*, *PNAS*, *Developmental Cell*, *J.Exp.Med*.). Notch is a cell signaling pathway which acts as a critical regulator of cell fate decisions. The team evaluated this regulatory role in relevant events of gametogenesis, embryonic and tumor development. In spermatogenesis, Notch proved to be a key-regulator of the pace of the spermatogenic cycle. Cancer development often employs mechanistic pathways that are relevant during embryonic development, which occurs in tumor neoangiogenesis and the various phases of cancer progression. The team bridged knowledge obtained from Notch signalling functions in developmental angiogenesis and organ development to homologous functions in tumour neoangiogenesis and in various phases of cancer progression (cancer growth and metastatic process) and performed pre-clinical studies into the possibility of targeting Notch proteins as a form of therapy in several types of cancer.



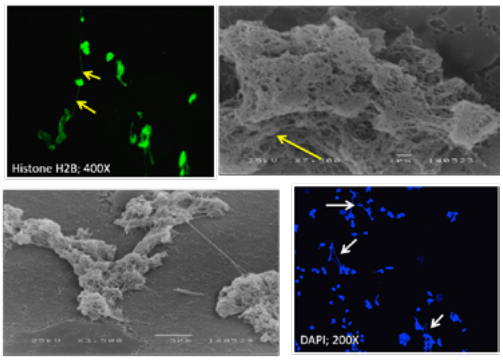
Schematic representation of expression of Notch proteins during the spermatogenic cycle of the mouse (Murta et al., 2013).



Proposed model for Jagged1/Notch signaling in angiogenesis (Pedrosa et al., 2015).

Understanding the physiopathology of fibrosis in the uterus and oviduct

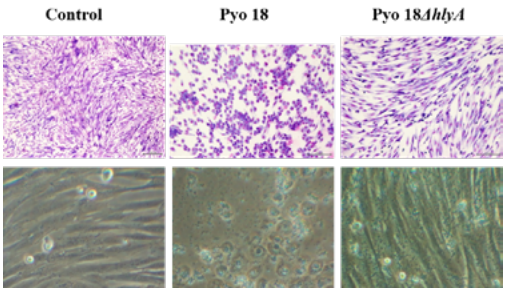
Age and pathologic associated uterine fibrotic degenerative changes (endometrosis) are relevant causes of infertility in the mare. Using this species as a model, the team has been studying the mechanisms behind the establishment of fibrotic lesions, being first one to show: (i) the presence of side-population cells (stem-like cells) in mare endometrium; (ii) the ability of equine neutrophils to form Neutrophil Extracellular Traps (NETs) in mare endometrium in vivo, in response to pathogenic bacteria; as well as (iii) the role of NETs proteases on collagen deposition in the endometrium, mediated by prostaglandin pathways. The inhibition of NETs components cathepsin G and elastase may reduce the establishment of mare endometrial fibrosis by stimulating the production of anti-fibrotic PGE2 and inhibiting pro-fibrotic PGF2α. The team also documented oviduct fibrosis associated to endometrosis.



NETs in the uterus of mares with *E. coli* and *S. equi spp zooepidemicus* endometritis

Deciphering host-pathogen interactions in pyometra pathogenesis

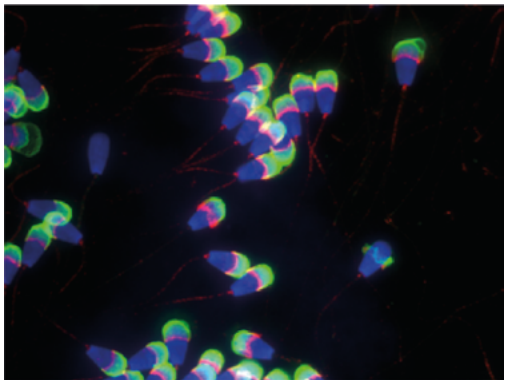
Pyometra, a common and serious uterine disease of dogs, develops from a complex interaction of host-pathogen factors. Involved in most cases, the bacterium *Escherichia coli* is associated with severe systemic signs (endotoxemia, sepsis) and life-threatening cases. Using virulence factors loss-of-function mutant *Escherichia coli*, the team documented that endometrial stromal cell damage induced by Hemolysin A (HlyA) is a potential relevant step of *Escherichia coli* virulence in the pathogenesis of pyometra in the bitch. The HlyA-induced decrease of cytokine transcription by stromal cells, which may occur during the early stages of infection, may allow bacteria to establish a niche prior to the activation of an adequate innate immune response.



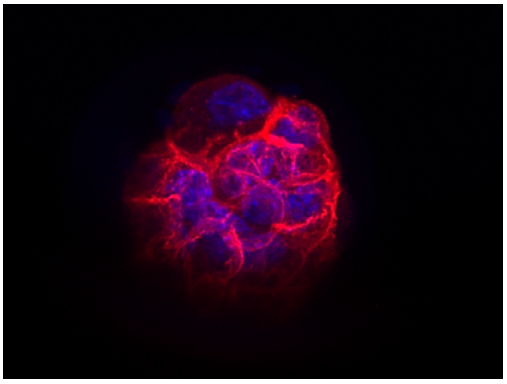
Effect of *E. coli* Pyo18 isogenic *hlyA* deletion mutation (Pyo18ΔhlyA) in endometrial cell culture. Morphology of endometrial cells after 1h (Giemsa staining, top) and 4 h phase-contrast, bottom).

Reproductive and developmental biotechnologies directed to germplasm preservation

The preservation and sustainable management of animal genetic resources become an international priority due to the threatening global decline of biodiversity. The cryopreservation of germplasm, namely embryos and gametes is a crucial tool for the maintenance of the biodiversity of the animal species and the preservation of the local breeds many of them considered endangered of extinction. The team addressed studies on lipid metabolism, gene transcription and protein expression in gametes and embryos, and on cryobiology in order to improve the fertility rates after insemination and/or embryo transfer.



DLL4 protein relocation in bovine spermatozoa surface undertaking the acrossome reaction. DLL4 in green. Acrossome status: (NR) Non-reacted;(R) Reacting; (AR) Acrossome reacted.



Immunolocalization of β-Catenin in a mouse embryo (compact morula).

Insights into the physiologic role of prion like genes and proteins in reproductive function

During past decades, prion proteins, the pathological agents causing sporadic, genetic and infectious neurodegenerative disorders, have constituted major concerns for public health authorities. Although the invariably fatal outcome of prion derived diseases in humans and animals has overshadowed the physiological action of prions, there is increasing evidence that support the essential role of prion and prion-like proteins

in reproductive physiology. The team examined the roles played by prion (PrPC), prion-like testis specific (Prt), Shadoo and Doppel genes and proteins, in spermatogenesis, sperm motility, zona binding and 3D NMR structure and fertilization. Also, the team has been evaluating the genetic regulation of these genes in granulosa cells, using novel techniques of gene silencing (siRNAs) and epigenetic analysis (methylation).

3. Representative Projects

PTDC/CVT/105022/2008 "The Notch-Delta signaling pathway in the reproductive function of the mouse and cow"(2009-2013), PI:Luís Lopes da Costa. FCT

PTDC/CVT/115703/2009 **"**Characterization of the role of a novel anti-angiogenic biopharmaceutical, Dll4-Fc, in the control of tumour growth in animal models" (2010-2014**)**, PI: Alexandre Trindade. FCT

EXPL/CVT-REP/2289/2013 "Notch-wnt signaling cross-talk in the regulation of pre-implantation embryo development (2014-2015) PI: Elisabete Silva. FCT

PTDC/CVT-REP/4202/2014 "How to solve infertility: Is fighting neutrophil extracellular traps, cytokines or prostaglandins a solution?" (2016 - 2019). PI: Graça Ferreira-Dias. FCT

PTDC/CVT/121805/2010 "Neutrophil Extracellular Traps and cytokines in the endometrium: *Friends or Foes*?" (2012 – 2015); PI - Graça Ferreira-Dias, FCT.

SAICTPAC/0019/2015 LISBOA-01-0145-FEDER-016405: "POINT4PAC – Precision oncology and innovative therapies and technologies" (2017-2019); PI - Cecília Rodrigues (iMed.ULisboa); PI (CIISA) - António Duarte, PAC-Compete 2020.

ALT20-03-0246-FEDER-000021 - ALT-BiotechRepGen Animal Genetic Resources and Biotechnologies: looking forward to the future. PI: Rosa M. Lino Neto Pereira (UISBRG – INIAV); (2017-2019)

4. Selected Publications

Murta, D., Batista, M., Silva, E., Trindade, A., Henrique, D., Duarte, A., Lopes da Costa, L. (2013). Dynamics of Notch pathway expression during mouse testis post-natal development and along the spermatogenic cycle. ***PLoS ONE*** 8(8): e72767

Galvão A., L. Valente, D.J. Skarzynski, A. Szóstek, K.K. Piotrowska-Tomala, D. Ramilo, M.R. Rebordão, L. Mateus, G. Ferreira-Dias (2013). Effect of cytokines and ovarian steroids on equine endometrium: an in vitro study. ***Reproduction, Fertility and Development***, 25(7):985-997.

Pimenta, J Sardinha, CC Marques, A Domingos, MC Baptista, JP Barbas, IC Domingos, P. Mesquita, P Pessa, R Soares, A Viegas, E Cabrita, A.E.M. Horta, CA Fontes, JAM Prates, RMLN Pereira (2013). Inhibition of ovine in vitro fertilization by anti-Prt antibody. Hypothetical model for Prt-ZP interaction. ***Reproductive Biology and Endocrinology***, 11:25.

Murta, D., Batista, M., Trindade, A., Silva, E., Henrique, D., Duarte, A., Lopes-da-Costa, L. (2014). In vivo Notch signaling blockade induces abnormal spermatogenesis in the mouse. ***PLoS ONE*** 9(11): e113365

Henriques S, Silva E, Lemsaddek A, Lopes-da-Costa L, Mateus L. (2014). Genotypic and phenotypic comparison of Escherichia coli from uterine infections with different outcomes: clinical metritis in the cow and pyometra in the bitch, ***Veterinary Microbiology***, 170:109-116.

Pedrosa AR, Trindade A, Carvalho C, Graça J, Carvalho S, Peleteiro MC, Adams RH and Duarte A. (2015). Endothelial Jagged1 promotes solid tumor growth through both pro-angiogenic and angiocrine functions. ***Oncotarget***, 6(27), 24404-24423.

Pedrosa AR, Trindade A, Fernandes AC, Carvalho C, Gigante J, Tavares AT, Diéguez-Hurtado R, Yagita H, Adams RH and Duarte A. (2015). Endothelial JAGGED1 antagonizes dll4 regulation of endothelial branching and promotes vascular maturation downstream of dll4/notch1. ***Arteriosclerosis, thrombosis, and vascular biology*** 35(5), 1134-1146.

Henriques S., Silva E., Silva M.F., Carvalho S., Diniz P., Lopes-da-Costa L., Mateus L. (2016). Immunomodulation in the canine endometrium by uteropathogenic *Escherichia coli*. ***Veterinary Research*** 47:114.

Ferreira LM, Garcia-Herreros M, Domingos A, Marques CC, Mesquita P, Barbas JP, Baptista MC, Pimenta J, Horta AEM, Prates JAM, Pereira RMLN (2017) Prion protein 2 (dublet) gene (PRND): role in ovine semen capacitation, cryopreservation and fertility. ***Reproduction, Fertility and Development***, 29(5) 985-997

Torres A.C., Boruszewska D., Batista M., Kowalczyk-Zieba I., Diniz P., Sinderewicz E., Saulnier-Blache J.S., Woclawek-Potocka I., Lopes-da-Costa L. (2014). Lysophosphatidic acid signaling in late cleavage and blastocyst stage bovine embryos. ***Mediators of Inflammation*** 2014:678968.

5. Collaborations

- Department of Obstetrics, Reproduction and Herd Health, Faculty of Veterinary, University of Gent, Belgium.
- Faculty of Veterinary, University of Murcia, Spain
- Instituto de Investigação do Medicamento (iMed, ULisboa), Portugal
- Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Portugal
- Polish Academy of Science, Olsztyn, Poland
- SLU Swedish University of Agricultural Sciences, Uppsala, Sweden

6. Outreach

1rst Prize- Polish Academy of Science (Olsztyn, Poland) "Nagroda Wydziału II Nauk Biologicznych i Rolniczych PAN 2016" – for Scientific merit in research on the physiopathology of Reproduction in the horse – Polish Academy of Science, Olsztyn, Poland. 2016. (G. Ferreira-Dias)

Advance training to veterinary surgeons 2014-2016: Training Courses on "Official Supervision as Regards Semen, Ova and Embryos" funded by the BETTER TRAINING FOR SAFER FOOD INITUATIVE of the European Union Programme 2014 – 2016. AETS Consortium (AETS/ Agrotec) – Contract n° 2013 96 16. This training was addressed to official veterinarians from local veterinary services responsible for official supervision of semen collection and storage centers and of embryos collection and production teams, and to officials from central Competent Veterinary Authorities in charge of this field of regulation.

R&D Lab team have organized training courses in reproductive technologies and provided consultancy to breeder's associations, cooperatives and private companies.

Lab members organized the 20th Annual European Society for Domestic Animal Reproduction (ESDAR) Conference, 2016, Lisbon, Portugal.

7. Advanced Studies (PhD e MSc thesis)

During the period 2013-2017, 8 PhD and 23 Master degrees were awarded to students working in the lab.

ADVANCED TRAINING AND SCIENTIFIC CAREER DEVELOPMENT

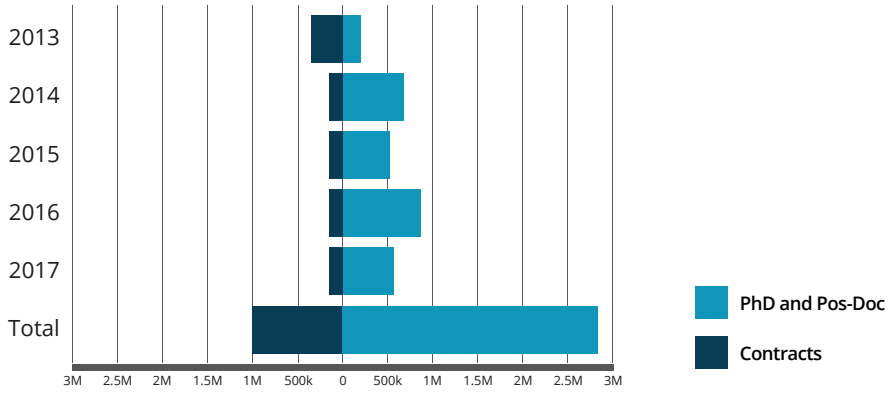
Advanced Training and Scientific Career Development

CIISA Coordination and researchers are committed to advanced training, including Post-Doc, PhD and Masters programmes for a variety of scientific backgrounds. CIISA is enrolled in the Doctoral Programme in Veterinary Sciences through its host institution FMV-ULisboa. This PhD program was accredited in 2007 and includes 5 specialities: Animal Production, Food Safety, Animal Health, Clinics and Biological and Biomedical Sciences. PhD grants for students were awarded by the Foundation for Science and Technology (FCT), by the EU through ITN grants (Marie Curie PhD fellowships), or by projects lead by CIISA's researchers. Other collaborations in advanced training within the University of Lisbon include the PhD degree in Sustainability Science – Resources, Food and Society (FMV and 11 other schools), the Bachelor and Master degrees in Zootechnical Engineering (FMV and ISA), the Master degree in Microbiology (FMV and 3 other schools) and the Master degree in Clinical Microbiology and Emerging Infectious Diseases (Medical Faculty and FMV).

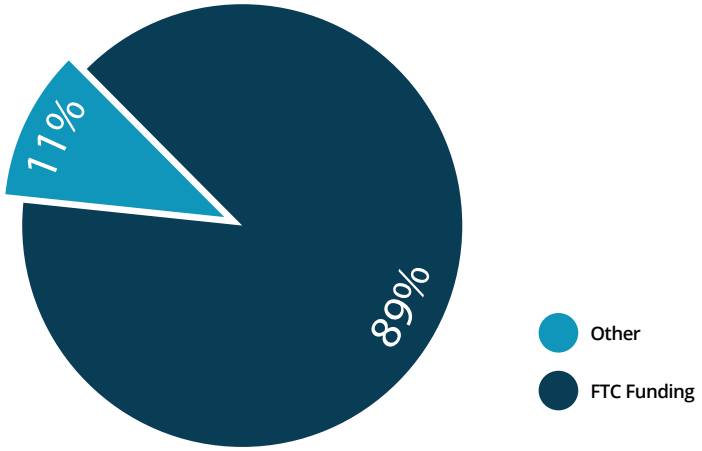
At FMV-ULisboa the stimulus for veterinary students to develop their scientific interests, starts early in the 2nd year, when they visit research labs in small groups and meet researchers and the research lines of CIISA. During the ensuing years and on the curricular traineeship, students have the opportunity to perform research activities and carry out final year research projects within CIISA's labs. Since 2015, CIISA annually launches internal competitive research project grants for Masters students to undertake their thesis under a research environment. This program aims to encourage the most promising students, at an early stage of their careers, to progress into a scientific environment and to gain independence skills. This program has gained a remarkable success among students and their CIISA's supervisors, and in 2017 already 20% of final year students enrolled themselves in CIISA's Masters grants. Since 2015, CIISA annually launches competitive calls for project grants addressed mainly to PhD students and junior Post-Docs. These projects, termed INOV, are intended to explore innovative ideas and to allow obtaining preliminary data in cutting edge areas of research that may sustain the preparation of solid applications to external funding. INOV projects, with one year of duration, have a maximum budget of €5.000, and represent an investment both in novel ideas and the development of junior scientific careers. This investment in the stimulus of scientific career development and entrepreneurism represented 62% of the Pluriannual funding of the Foundation for Science and Technology (FCT) to CIISA.

CIISA's funding sources for human resources – fellowships and contracts

(2013-2017)

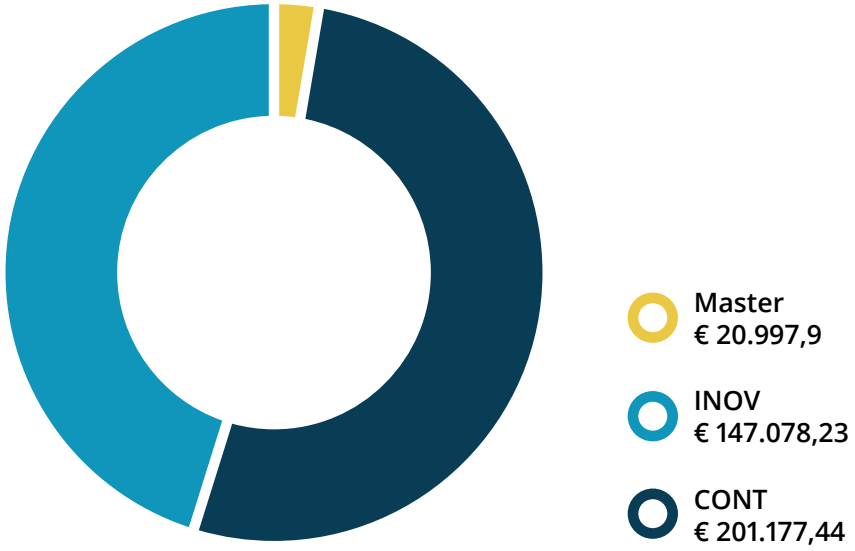


Allocation for funding for Fellowships and contracts



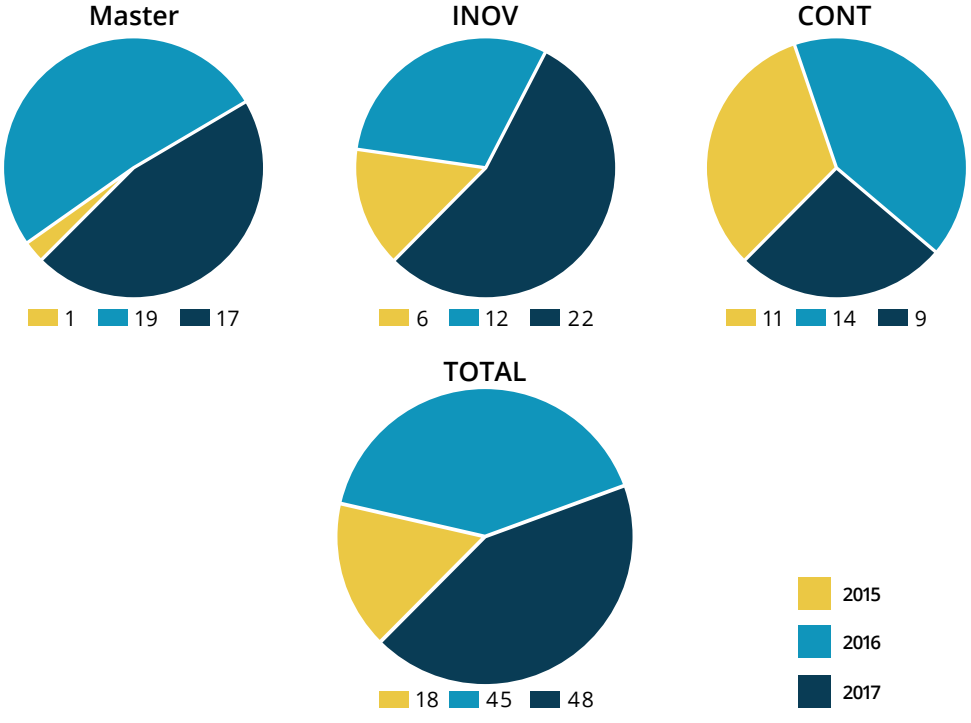
Budget for CIISA's Internal Projects

(2015-2017)



Projects Master Degree, INOV and CONT

(2015-2017)



Master Thesis Projects
(2015-2017)

2017

Jessica Monteiro "Surveillance and Molecular Diagnostic of Fibropapillomatosis and Chlamydiaceae in Green Turtles (Chelonia mydas) from Guiné-Bissau, West Africa". Supervisor: Ana Duarte; Co-supervisor Ana Rita Patricio

Catarina Costa "Sero-surveillance and Molecular Diagnostic of West Nile Virus, Avian Influenza Virus and Chlamydiaceae in Wild Birds of Portugal". Supervisor: Sílvia Barros (INIAV); Co-supervisor: Ana Duarte

Cátia Silva "Evaluation of food safety concepts and procedures in school canteens". Supervisor: Ana Rita Henriques; Co-supervisor António Barreto

Maria Cristina Barroso "Detection of MDR1 gene mutation in portuguese dog breeds". Supervisor : Berta São Braz

Catarina Aboim Alexandre Gomes "Gut microbiome assessment in dogs with spontaneous Hyperadrenocorticism – a pilot study". Supervisor: Constança Pomba; Co-supervisor: Rodolfo Leal

Mariana Nunes "Study of IL31 profile in canine allergic conjunctivitisco" Supervisor Esmeralda Delgado

Mafalda Iria de Sousa "Study of the antiparasitic effect of the Leucaena leucocephala leguminous in goats from São Tomé and Príncipe" Supervisor: Isabel Fonseca co-supervisor: Maria Ludovina Padre

Patrícia Ramalho T"he role of serum amyloid A in clinical monitoring of chronic gingivostomatitis in the cat" Supervisor Lisa Mestrinho

Maria Inês Antunes "Diagnosis of anthelmintic efficacy in sheep and goats dewormed with an association of closantel and mebendazole" Supervisor: Luís Carvalho Co-supervisor: Miguel Saraiva Lima, George Stilwell

Catarina Barros "Acute inflammatory markers in equine piroplasmosis" Supervisor : Luis Lamas

Ana Paula Martins "Evaluation of renal dysfunction and/or peritonitis in female dogs with pyometra" Supervisor: Luisa Mateus

Viktor Nikonov "Molecular diagnostic of dermatophytosis in dogs and cats" Supervisor: Manuela Oliveira

Sara Isidoro "Assessing the dissemination of antimicrobial resistance in highly anthropogenic-pressured freshwater ecosystems using cyprinid species as indicators" Supervisor: Manuela Oliveira

Cecília Cruz "Evaluation of the degree of stress, dehidration and exhaustion in horses used for urban tourism – contribution to the implementation of animal welfare rules as a means of promoting quality turism" Supervisor: Paula Tilley

Patrícia da Silva Caldeira "Ultrasound evaluation of Right Dorsal Colitis in Horses"Supervisor: Rita FonsecaCo-supervisor Hugo Rosa, José Tomas

Claudia Almeida "Characterization of B-cell lymphoma receptors with comparative immunotherapy target potential" Supervisor: Solange Gil

Claudia Verdial "Preventing Nosocomial Bacterial Infections In The Infectious Diseases Isolation Unit Of The Veterinary Teaching Hospital"Supervisor: Solange Gil

2016

Mara Alexandra Batista Nunes "Characterization of reference intervals of thyroid (tT4 e TSH) in Water Portuguese Dog" Supervisor Ana Mafalda Lourenço

Joana Ferreira "Assessing Listeria monocytogenes challenge testing in ready-to-eat foods**"**Supervisor Ana Rita Henriques

Joana Nariano Vieira "Wearable devices as a non-invasive monitoring approach on hospitalized canine and feline patients at a teaching veterinary hospital and IDIU" Supervisor: António Ferreira co-supervisor Solange Gil

Bárbara Abrantes "Detection of early renal toxicity through microalbuminuria determination in cats after doxorubicin chemotherapy treatment" Supervisor: Berta São Braz co-supervisor Gonçalo Vicente

Cláudia Varandas "Study of the immune response in canine allergic conjunctivitis" Supervisor: Esmeralda Delgado co-supervisor: Solange Gil

Maria do Carmo Vassalo Santos "Prevalence of Iron Storage Disease in captive lemurs" Supervisor George Stilwell

Frederico Alves "Correlation study between cardiacal and respiratory frequences and survival rates in hospitalized patients with infectious disease" Supervisor: Solange Gil

Filipa Trigo da Roza "Spatiotemporal bacterial evolution to biocides (Evolução espácio-temporal da resistência bacteriana aos biocidas)Supervisor: Luis Lamas co-supervisor: Manuela Oliveira

Beatriz Soares; Raquel Dourado "Determination of parturition date in Labrador bitches using ultrasonographic fetometry as compared to hormonal and cytological methods" Supervisor: Luisa Mateus

Luis Belo "Evaluation and comparison of the efficacy of pre-surgical asepsis with chlorhexidine-alcohol vs iodopovidone in the prevention of bacterial contamination of internal organs after skin incision" Supervisor Miguel Carreira co-supervisor: Manuela Oliveira

Inês Mendes Andrade "Effect of the maternal pH in goats with pregnancy toxemia on the survival rate of newborn kids" Supervisor: Miguel Saraiva Lima

Francisca Gonçalves "Preliminary study in order to identify potential predictors of laminitis in horses aged ≥ 15 years with a phenotype for pituitary pars intermedia dysfunction (PPID)" Supervisor: Paula Tilley

2015

Isabel Melo Ferreira "Caponization versus immunocastration of broilers" Supervisor: Mário Quaresma, Co-supervisor: Rui Bessa

Fábio Abade dos Santos "Pilot Study on the transmission dynamics of Wild Rabbit Haemorrhagic Disease virus; spatiotemporal analysis of RHDV2 in wild rabbits, mesocarnivores, rodents and insects." Supervisor: Co-supervisor: Conceição Peleteiro, Maria Margarida Duarte

Joana Domingues "Study of Besnoitiosis clinical and epidemiologic characteristics in beef cattle farms" Supervisor: George Stilwell

Rita Mourão Rosa "Measurement of alpha-2-acid glycoprotein in cats with gingivostomatitis" Supervisor: Lisa Mestrinho

Tiago Silva "Development of technique of viable sperm selection by colloid single layer centrifugation in domestic dog as an experimental model for wild carnivores" Supervisor: Luisa Mateus

Joana Catarina Andrade; Rita Dias Bernardo "Susceptibility to food-grade sanitizers of Listeria spp. and Listeria monocytogenes in the planktonic form and in mixed-species biofilms: cases from the ready-to-eat food products food chain" Supervisor: Ana Rita Henriques

Vanessa Félix "Isolation of bioactive compounds from Arbutus unedo "medronheiro" and evaluation of antimicrobial properties against bacteria from food related environments" Supervisor: Teresa Lemsaddek

Maria Sara Santos "Pain reduction in dairy goat kids' disbudding" Supervisor: George Stilwell

INOV Projects
(2015-2017)

2017

"Evaluation of the bovine COCs as a target for PGF2α action" PI: Ana Catarina Torres

"Efficacy of UV pulsed light for shelf-life extension and inactivation of Listeria monocytogenes on sliced and packaged traditional Portuguese cured meat products" PI: Ana Cristina Borges

"Endotypes in canine atopic dermatitis: identification of patient clusters based on serum biomarker analysis" PI: Ana Mafalda Lourenço

"Tracking Listeria monocytogenes in Portuguese ready-to-eat food industries and virulence traits characterization using PFGE and MLVA subtyping" PI: Ana Rita Henriques

"Role of biofilm and mutant selection window concentrations in the circulation of antibiotic resistance genes in a dog dental plaque model" PI: Eva Cunha

"Epidemiological evaluation of mosquito vectors of emergent arboviruses important for human and animal health in rural, urban and zoo habitats" PI: Fernando Boinas

"Bovine Viral Diarrohea and Bovine Genital Campylobacteriosis in Portugal, a prevalence survey and impact assessment" PI: Gonçalo Pereira

"Differential Gene Expression in Alentejana cattle under Theileria annulata in vitro infection of monocyte-derived macrophages" PI: Jacinto Gomes

"A novel strategy of in vivo selection of tumor specific antibodies against canine lymphoma" PI: Joana Dias

"Meat from pig carcasses with suppurative vertebral osteomyelitis – safe or not?" PI : João Bettencourt Cota

"Xenografting of bovine ovarian tissue as a model for testing new drugs and examining angiogenesis, follicle premature depletion and dynamics" PI: Jorge Pimenta

"Spirulina microalga as a potential source of carotenoids in broiler meat" PI: José Pestana

"Monitoring of Schmallenberg virus in Culicoides (Diptera: Ceratopogonidae) genus from mainland Portugal" PI: Margarida Simões

"Antioxidant potential of dietary supplementation with Spirulina as a feeding strategy for the pig industry" PI: Marta Madeira

"Development of cell culture models to study Campylobacter fetus subsp. venerealis-host cells interactions" PI: Marta Silva

"Development of an ELISA for the detection of bornavirus antibodies in psittacine birds" PI: Miguel Fevereiro

"Characterization of transmembrane protein channels in the small intestine of piglets fed on amino acids enriched diets: a translational model to humans" PI: Paula Lopes

"Is the microalgae Nannochloropsis oceanica a source of natural rumen protected n-3 polyunsaturated fatty acids? " PI: Rui Bessa

"Exploring new feed materials: the inclusion of Nannochlopsis oceanica in horse diets". PI: Rui Caldeira

"Definition and optimization of a new PCR-based methodology for blood meal analysis and molecular identification of mosquito species". PI: Sara Madeira

"The effect of lipid moieties on the extent of lipolysis in the rumen" PI: Susana Alves

"Art Imitates Microbes: Insights, challenges and opportunities of Lignocellulosic-degrading strategies revealed by anaerobic Fungi" PI Virginia Pires

2016

"An in vitro tridimensional matrigel culture system to evaluate early maternal communications: role of steroids and prostanoids" PI: Ana Catarina Torres

"First national survey of gastrointestinal parasites infecting dogs, cats and wild carnivores in Portugal using FLOTAC" PI: Ana Margarida Alho

"Functional and molecular evaluation of the chemokine receptor CXCR4 in feline mammary carcinoma and its role as a prognostic oncomarker" PI: Cláudia Marques

"Improving lipid extraction from microalgae and assessing suitability of de-fatted biomass as animal feed" PI: Cristina Alfaia

"Communication skills: are exosomes messengers of infection of dendritic cells by Besnoitia or Toxoplasma?" PI: Dulce Santos

"Validation of circulating miRNAs as diagnostic and prognostic biomarkers for feline mammary carcinomas" PI: Fernando Ferreira

"Histone deacetylase inhibitors – a novel therapy for canine lymphoma" PI: Joana Dias

"Genotypic and phenotypic characterization of pig carcass Salmonella at the slaughterhouse level" PI :João Cota

"Bovine genital campilobacteriosis: analysis of the Campylobacter fetus venerealis virulence potential" PI: Luisa Mateus

"Modulation of aquaporins gene expression by n-3 polyunsaturated fatty acids (PUFA) lipid structures in white and brown adipose tissue from hamsters"
PI: Paula Lopes

"Dietary induction of stearoylCoA desaturase activity to improve meat fatty acid profile in lambs" PI: Paulo Costa

"Development of a new immunoliposome drug delivery system for treatment of pneumococcal meningitis" PI: Sandra Aguiar

2015

"WntPG pathways communication in the murine embryo development and competence" PI: Ana Catarina Torres

"Gamete-maternal communication in the bovine oviduct: implication of the Notch signaling pathway" PI: Elisabete Silva

"Contribution to the evaluation of the discriminative power of some variables involved in the staging of equine Recurrent Airway Obstruction (RAO)" PI: Joana Simões

"The application of high pressure technology to oilseeds and their effect on fatty acid rumen metabolism" PI: Rui Bessa

"Neuroprotective effects of individual n-3 long-chain polyunsaturated fatty acids" PI: Susana Martins

"Food ID-card: Microbial barcoding for Portuguese traditional fermented food products"PI: Teresa Lemsaddek

CONT Projects
(2015-2017)

2017

"Developmental versus oncogenic Notch function in the prostate" PI: Alexandre Trindade

"First national survey of gastrointestinal parasites infecting dogs, cats and wild carnivores in Portugal using FLOTAC" PI: Ana Margarida Alho

"Proceeding studies on Culicoides (Diptera: Ceratopogonidae) genus: morphological, molecular, risk analysis and insecticide control approaches" PI: David Ramilo

"Identification of bovine sperm acrosomal proteins that interact with DLL4 and JAG1 to regulate acrosome reaction" PI: Elisabete Silva

"Ocular administration of erythropoietin in a colloidal drug delivery system in glaucoma" PI: Esmeralda Delgado

"Relationship between Escherichia coli strains causing pyometra in female dogs and the fecal E. coli population of the host" PI: Luisa Mateus

"Effect of a nisin-biogel on the Diabetic Foot Infections microenvironment" PI: Manuela Oliveira

"A Pilot Study on the transmission dynamics of Wild Rabbit Haemorrhagic Disease virus; spatiotemporal analysis of RHDV2 in wild rabbits, mesocarnivores, rodents and insects" PI: Maria da Conceição Peleteiro

"In vitro efficacy of a brain targeted antibiotic encapsulated liposome for treatment of basterial meningitis" PI: Sandra Aguiar

2016

"Programming the type and localization of adaptive immunity by targeting TLR2: a strategy for the development of novel veterinary vaccines " PI: Afonso Basto

"Notch pathway function in oncology recapitulates ontogeny and represents a potential therapeutic target for prostate cancer" PI: Alexandre Trindade

"Development of an ELISA assay for the serological surveillance of dolphin morbillivirus infection in stranded cetaceans" PI: Ana Duarte

"Study on the role of African swine fever virus (ASFV) ubiquitin-conjugating e2-like enzyme towards vaccine development against ASF" PI : Carlos Martins

"Interplay of Notch pathway ligands Dll4 and Jagged1 in the modulation of mammalian spermatogenesis" PI: Daniel Murta

"Proceeding studies on Culicoides (Diptera: Ceratopogonidae) genus: morphological, molecular, risk analysis and insecticide control approaches" PI: David Ramilo

"Notch signaling: a regulator of sperm function?" PI: Elisabete Silva

"Ocular administration of erythropoietin in a colloidal drug delivery system in glaucoma"PI: Esmeralda Delgado

"Equine Oviduct Specific Glycoprotein – Is there a cross talk with sperm cells in the oviduct?" PI : Graça Dias

"Role of the sprn and prnp prion family genes in bovine follicular granulosa cells steroidogenesis" PI: Jorge Pimenta

"Contribution of linolenic acid to the accumulation of t10-18:1 in tissues of lambs fed cereal based diets supplemented with oil" PI: Rui Bessa

"Screening of the Mob1 interactome" PI: Sofia Nolasco

"New insights on ruminal biohydrogenation of long-chain highly unsaturated fatty acids" PI: Susana Alves

"Therapeutics for Diabetic Foot Ulcers: combined action of antimicrobial peptides and antiseptics versus conventional antibiotherapy": Tânia Raquel Santos

2015

"Notch components expression during prostatic development and maturation" Pi: Alexandre Trindade

"Ocular administration of erythropoietin in a colloidal drug delivery system in glaucoma" PI : Esmeralda Delgado

"New insights in equine oviduct function" PI: Graça Dias

"Evaluation of the importance of dogs as a zoonotic reservoir of Uropathogenic Escherichia coli strains" PI: Luísa Mateus

"Sharing of resistant/virulent Klebsiella pneumoniae and Proteus mirabilis clones among household members of companion animals with UTI" PI: Constança Pomba

"Understanding the structural determinants of cohesin-dockerin specificity in Ruminococcus flavefaciens cellulosomes" PI: Pedro Bule

"New insights on ruminal biohydrogenation of α-linolenic acid" PI: Susana Alves

"New strategies for an efficient vaccine design on African Swine Fever Virus (ASFV) – development of ASFV-A104R-DISC mutants" PI: Carlos Martins

"Unraveling new therapies for diabetic foot ulcer: antimicrobial peptides and probiotics"PI: Manuela Oliveira

"Mob1, critical factor in Toxoplasma gondii replication" PI: Sofia Nolasco

"Clinical and immunological characterization of naturally occurring canine lymphoma"PI: Solange Gil

ADVANCED TRAINING AND SCIENTIFIC CAREER DEVELOPMENT

PHD THESIS

2017

Miguel José Sardinha de Oliveira Cardo - "Study of OCDD 1,2,3,4,6,7,8, - HPCDD, 1,2,3,6,7,8-HXCDD and 1,2,3,7,8, - PECDD Congeners of Dioxin Absorption in Poultry"; PhD Veterinary Sciences, Food Safety; Supervisor: Fernando Manuel D´Almeida Bernardo

Pedro Miguel Bule Gomes - "Divergent Cellulosome Architecture in Rumen Bacteria: Structure and Function Studies in Cohesin-Dockerin Complexes of Ruminococcus Flavefaciens", PhD Veterinary Sciences, Animal Production; Supervisor: Carlos Mendes Godinho de Andrade Fontes, Co-Supervisor: Shabir Najmudin

Mónica Mendes da Costa - "Modulation of ruminal biohydrogenation in sheep through dietary tannins or energy sources", PhD Veterinary Sciences, Animal Production; Supervisor: Rui José Branquinho de Bessa

Ana Margarida Pignateli Vasconcelos de Assunção Alho - "Dirofilia immitis and angiostrongylus vasorum: epidemiology and impact of major heratworms in carnivores in Portugal", PhD Veterinary Sciences, Animal Health; Supervisor: Luís Manuel Madeira de Carvalho, Co-Supervisor: Silvana Maria Duarte Belo

Carla Mottola - "Virulence Characterization and Antimicrobial Resistance of Major Bacterial Genera from Diabetic Foot Infections", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Maria Manuela Castilho Monteiro de Oliveira, Co-Supervisor: Patrícia Maria Cavaco Silva de Sá Montez

Maria Alexandra Sobral Pessoa de Oliveira - "Nutritional strategies in the modulation of ruminal biohydrogenation to improve the fatty acid profile of ruminant's meat", PhD Veterinary Sciences, Animal Production; Supervisor: Rui José Branquinho de Bessa

Rui Emanuel Antunes Seixas - "Virulence of Salmonella Typhimurium 1,4,[5], 12:i - The New Emergent Strain", PhD Veterinary Sciences, Animal Health; Supervisor: Maria Manuela Castilho Monteiro de Oliveira, Co-Supervisor: Fernando M A Bernardo

Nuno Manuel Mira Flor Santos Félix - "Critical Illness-Related Corticosteroid Insufficiency in Hemorrhagic Shock- an experimental study about its pathophysiologic mechanisms in a rat model (Rattus norvegicus)", PhD Veterinary Sciences, Clinics; Supervisor: Maria Manuela Grave Rodeia Espada Niza

2016

Ana Rita Barroso Cunha de Sá Henriques - "Listeria monocytogenes in the ready-to.eat food chain: characterization and preventive control measures", PhD Veterinary Sciences, Food Safety; Supervisor: Maria João dos Ramos Fraqueza

Maria Madalena Sá Carvalho Cristovão - "Study of tick infestation and the Cattle Production System of the Municipality of Ecunha (Huambo, Angola)", PhD Veterinary Sciences, Animal Health; Supervisor: José Alexandre da Costa Perdigão e Cameira Leitão, Co-Supervisor: António B. Alicerces Chivinda Eduardo

Ana Filipa Pereira Sequeira - "Development of a novel platform for high-troughput gene design and artificial gene synthesis to produce large libraries of recombinat venom peptides for drug discovery", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Carlos Mendes Godinho de Andrade Fontes, Co-Supervisor: Catarina Isabel Proença Duarte Guerreiro

Catarina Isabel Cruz de Carvalho – "Use of mice in the study of new targets for anti-angiogenic cancer therapy", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: António José de Freitas Duarte

Lisa Alexandra Pereira Mestrinho - "Contribution to the study of prognostic factors in canine oral squamous cell carcinoma", PhD Veterinary Sciences, Clinics; Supervisor: Maria Manuela Grave Rodeia Espada Niza, Co-Supervisor: Maria da Conceição da Cunha e Vasconcelos Peleteiro

Carla Margarida Pinheiro Cardoso Duarte - "Portable lab-on-chip platform for bovine mastitis diagnosis in raw milk", PhD Veterinary Sciences, Animal Health; Supervisor: José Ricardo Dias Bexiga, Co-Supervisor: Paulo Jorge Peixeiro de Freitas

Sofia Correia Rosa de Barros Henriques - "Escherichia coli- host interactions in the pathogenesis of canine pyometra", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Luísa Maria Freire Leal Mateus, Co-Supervisor: Maria Elisabete Silva

Maria João da Costa Soares - "Clinical and Molecular Characterization of Feline Mammary Carcinomas Overexpressing HER2 Proto-Oncogene (FMC-HER2+) - New Strategies for Effective Diagnostic and Cancer Therapy", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Fernando António da Costa Ferreira, Co-Supervisor: Jorge Manuel de Jesus Correia

Eduardo Miguel Baptista Ferreira Marcelino - "Protein disulfide isomerase as a target for besnoitiosis therapy. Molecular characterization and studies of its role in infection and host imune response", PhD Veterinary Sciences, Animal Health; Supervisor: José Alexandre da Costa Perdigão e Cameira Leitão, Co-Supervisor: Carlos Manuel Mendes Novo

Alexandra Eduarda Amador de Oliveira Francisco "Enhancing bioactive fatty acids of the meat from lambs reared in intensive systems through nutritional modulation," PhD Veterinary Sciences, Animal Production; Supervisor: Rui José Branquinho Bessa, Co-Supervisor: José Manuel Bento Santos Silva

Rute Marina Garcia da Noiva - "Eggs and chiken embryos as potencial sentinels for flock and hatchery health", PhD Veterinary Sciences, Animal Health; Supervisor: Maria da Conceição da Cunha e Vasconcelos Peleteiro, Co-Supervisor: Hulimangala Lingareddy Shivaprasad

Maria João de Sousa Ferreira Martelo Fradinho - "Growth and development of the lusitano foal on extensive systems", PhD Veterinary Sciences, Animal Production; Supervisor: Graça Maria Leitão Ferreira Dias, Co-Supervisor: Rui Manuel de Vasconcelos e Horta e Caldeira

David Wilson Russo Ramilo - "Phenotypic and genetic characterization of Culicoides (Diptera: Ceratopogonidae) in Portugal and comparison of the effect of insecticide molecules in their control", PhD Veterinary Sciences, Animal Health; Supervisor: Isabel Maria Soares Pereira da Fonseca de Sampaio, Co-Supervisor: Javier Lucientes Curdi

João Bettencourt Barcelos Cota - "Bovine papillomavirus type 2 infection in the pathogenesis of the Bovine Enzootic Hematuria-related oncological process", PhD Veterinary Sciences, Animal Health; Supervisor: Maria da Conceição da Cunha e Vasconcelos Peleteiro, Co-Supervisor: Ana Isabel Simões Pereira Duarte

Sérgio Eduardo Ramalho de Sousa - "Gastrointestinal parasitism in Miranda donkeys. Epidemiology and selective control of strongyles infection", PhD Veterinary Sciences, Animal Health; Supervisor: Luis Manuel Madeira de Carvalho, Co-Supervicor: Adolfo Paz Silva

Marina Martins Badenes - "In vivo evaluation of the role of Delta-like 4/Notch signaling in the development of intestinal tumors", PhD Veterinary Sciences, Biological and Biomedical Sciences, Supervisor: António José de Freitas Duarte, Co-Supervisor: Luís Filipe Lopes da Costa

Natacha Monge Gomes do Couto - "Insights into the dynamics of methicillin-resistant staphylococci in animals - a focus on staphylococcus pseudintermedius in dogs", PhD Veterinary Sciences, Clinics; Supervisor: Maria Constança Matias Ferreira Pomba, Co-Supervisor: Ana Maria Jesus Bispo Varela Coelho

Ana Rita Ponce Álvares de Águeda Pedrosa - "The role of Jagged 1 in adult angiogenesis and in solid tumor development", PhD Veterinary Sciences, Biological and Biomedical Sciences, Supervisor: António José de Freitas Duarte, Co-Supervisor: Alexandre José Neto Trindade

João Nuno Santos Coelho - "Molecular Characterization and Functional Analysis of ORF P1192R from african swine fever vírus", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: José Alexandre da Costa Perdigão e Cameira Leitão, Co-Supervisor: Fernando António da Costa Ferreira

2015

Anabela Ferreira Lopes - "The effect of different cooking methods on the nutritional value of veal - experimental study on Barrosã and Mertolenga", PhD Veterinary Sciences, Food Safety; Supervisor: José António Mestre Prates, Co-Supervisor: José Pedro da Costa Cardoso de Lemos

Ana Catarina Lopes Vieira Godinho de Matos "Development and integration of animal-based welfare indicators, including pain, in goat farms in Portugal", PhD Veterinary Sciences, Clinics; Supervisor: George Thomas Stilwell, Co-Supervisor: Mónica Duarte Correia de Oliveira

Margarida Pires Simões - "Spatiotemporal study of morpho-functional modifications on cell nucleus during african swine fever vírus early phase of infection", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Fernando António da Costa Ferreira, Co-Supervisor: Carlos Manuel Lopes Vieira Martins

Helga Marlene Cardoso Waap - "Epidemiologia e diagnóstico da besnoitiose bovina em Portugal" PhD Veterinary, Animal Health; Supervisor: Yolanda Maria Vaz, Co-Supervisor: José Alexandre da Costa Perdigão e Cameira Leitão

Alexandra Jorge Tavares - "The role of MOB proteins in protozoan cell cycle regulation", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Maria Helena Antunes Soares, Co-Supervisor: José Alexandre da Costa Perdigão e Cameira Leitão

Daniela Wetzel Gastal - "Characteristics of the genetic adaptation of lactopoiesis under tropical conditions of cattle exploitation", PhD Veterinary Sciences, Animal Health; Supervisor: Luís Granger Alfaro Cardoso, Co-Supervisor: Deodália Maria Antunes Dias

Vânia Ondina Pedro Fernandes - "Discovering novel carbohydrate-active enzymes in the cellulosome of anaerobic bactéria", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Carlos Mendes Godinho de Andrade Fontes and Catarina Isabel Proença Duarte Guerreiro, Co-Supervisor: Luís Manuel dos Anjos Ferreira

António Pedro Andrade Vicente - "Characterization and selection of the lusitano horse breed", PhD Veterinary Sciences, Animal Production; Supervisor: Luís Lavadinho Telo da Gama, Co-Supervisor: Nuno Renato Pimentel Carolino

Maria Inês Alves de Carvalho Martins Carolino - "Genetic influences on carcass and meat characteristics in cattle", PhD Veterinary Sciences, Animal Production; Supervisor: José António dos Santos Pereira de Matos, Co-Supervisor: José Manuel Bento Santos Silva

Immacolata Venditto - "Structure and function of novel Carbohydrate-Active Enzymes (CAZYmes) and Carbohydrate-Binding Modules (CBMs) involved in Plant Cell Wall hydrolysis", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Carlos Mendes Godinho de Andrade Fontes, Co-Supervisor: Shabir Najmudin

Kate Cameron - "Structure and function relationships in novel cohesin-dockerin complexes", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Carlos Mendes Godinho de Andrade Fontes, Co-Supervisor: Shabir Najmudin

2014

Daniel José de Moura Carita Dinis Murta - "Notch signaling, genital remodeling and reproductive function", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: Luís Filipe Lopes da Costa

Deolinda Paulino Camarada Embaló - "Study of lactic microbiota in fermented milk consumed in the south of Angola" PhD Veterinary Sciences, Food Safety; Supervisor: Maria Gabriela Lopes Veloso, Co-Supervisor: Joaquim Morais

Jacinto José Carneiro Gomes - "Epidemiology, molecular diagnostics and population genetics of Theileria annulata in Portugal" PhD Veterinary Sciences, Animal Health; Supervisor: Gabriela Santos Gomes, Co-Supervisor: Isabel Maria Soares Pereira da Fonseca de Sampaio

Rita Isabel de Amorim Cardoso - "Besnoitia besnoiti and Toxoplasma gondii invasion : the role of the parasite's tubulin folding pathway and manipulation of host cell organization", PhD Veterinary Sciences, Biological and Biomedical Sciences; Supervisor: José Alexandre da Costa Perdigão e Cameira Leitão, Co-Supervisor: Carlos Manuel Lopes Vieira Martins

Rodolfo Assis Oliveira Leal - "Recombinant feline interferon omega therapy in cats naturally infected with Feline Immunodeficiency Virus : clinical, viral and immunological relevance" PhD Veterinary Sciences, Clinics; Supervisor: Maria Manuela Grave Rodeia Espada Niza, Co-Supervisor: António José de Almeida Ferreira

2013

Ana Sofia Henriques da Costa – "Genetic, Metabolic and Endocrine Regulation of Fatty Acid Deposition in Autochthonous Bovine Breeds with Distinct Genetic Backgrounds", PhD Veterinary Sciences, Food Safety; Supervisor: José António Mestre Prates, Co-Supervisor: Carlos Mendes Godinho de Andrade Fontes

Inês Ferreira Viegas - "Consumers' willingness to pay for safer, cleaner and animal friendlier beef", PhD Veterinary Sciences, Animal Production; Supervisor: Magda Alexandra Aguiar Fontes and José Manuel Lima Santos, Co-Supervisor: Maria Madalena Barreira

Marta Sofia Morgado dos Santos Madeira - "Feeding Strategies to Improve Fat Partioning and Meat Quality in Pigs", PhD Veterinary Sciences, Food Safety; Supervisor: José António Mestre Prates, Co-Supervisor: Rui José Branquinho de Bessa

Jorge Manuel Botelho Garcia Andrade Pimenta - "Insights into the role of "prion-Like" genes and proteins on scrapie suceptibility and ram fertility", PhD Veterinary Sciences, Animal Production, Supervisor: Rosa Maria Lino Neto Pereira, Co-Supervisor: José António Mestre Prates

ADVANCED TRAINING AND SCIENTIFIC CAREER DEVELOPMENT

MASTER THESIS

2017

Anabela Moreira (2017), Crimes contra animais de companhia. Perceção, tipificação e relação com outros ilícitos penais em medicina veterinária forense.

Ana Catarina Baião de Sá Rodrigues (2017), Estudo dos efeitos ecocardiográficos antes e depois da sedação com doses baixas de alfaxalona em associação com o butorfanol, em cães – estudo piloto.

Ana do Espírito Santo da Silveira Botelho (2017), Alterações anátomo-patológicas em aves de rapina – estudo comparativo entre a eletrocução e outras causas de ingresso em centros de recuperação.

Ana Catarina Baião de Sá Rodrigues (2017), Estudo dos efeitos ecocardiográficos antes e depois da sedação com doses baixas de alfaxalona em associação com o butorfanol, em cães – estudo piloto.

Ana Ribeiro (2017), Perfil nutricional da fracção lipídica da carne de aves cinegéticas (perdiz, faisão, pato).

André Santana (2017), Análise da conformação com base em métodos objectivos no Cavalo Puro Sangue Lusitano.

Beatriz Lourenço (2017), Analisis of the populational structure of the *Escherichia coli* population in fecal and vaginal samples in the bitch.

Carla Loução (2017), Seasonal influence in the succession of entomological fauna on carrions of *Canis familiaris*.

Carolina Afonso e Cunha (2017), Avaliação dos efeitos sobre o bem-estar do sobrecrescimento das unhas de cabras leiteiras – estudos preliminares.

Cristina Oliveira (2017), Reabilitação física de cães com doença ortopédica no membro pélvico.

Irene Antunes (2017), Improvac na imunocastração de frangos: efeito de diferentes protocolos de imunocastração na composição e qualidade da carne.

Isabel Ferreira (2017), Imunocastração de frangos, uma alternativa não cirúrgica à produção de capão: avaliação do efeito do improvac.

Íris Teixeira Carvalho (2017), Rastreio de parasitas gastrointestinais e pulmonares em gatos de gatis nos distritos de Lisboa e Setúbal.

Inês Machado (2017), Piometra in the bitch and in the queen: differences and similarities.

Joana Raquel Andrade de Oliveira (2017), Avaliação retrospectiva do tratamento de gengivoestomatite crónica felina em 80 casos clínicos.

Joana Sofia Fernandes (2017), Maneio da dor através da fisioterapia e acupuntura em cães geriátricos com osteoartrite.

Marta Freitas (2017), Produção leiteira e curvas de lactação em ovelhas Assaf.

Rita Alexandra Martins Correia (2017), Factores de risco em lesões de reabsorção dentária no gato.

Ruben Miguel de Sousa Dinis (2017), Avaliação de factores de prognóstico associados ao fibrossarcoma no gato – estudo retrospectivo.

Maria Inês Baptista Romeira (2017), Abordagem da endometrite num centro de reprodução equina: presença de fluido uterino como parâmetro indicador da inflamação

Mariana Pereira de Jesus (2017), O rácio neutrófilos/linfocitos no linfoma B difuso de células grandes no Cão.

Marta Isabel Fragoso Lampreia Braga (2017), Prevalência e sazonalidade de parasitoses gastrointestinais, cardiovasculares e hemáticas em canídeos do distrito de Beja, Portugal.

Marta Sofia Cerveira Pinto (2017), Interação das vias de sinalização WNT-PG do desenvolvimento e competência do embrião murino.

Nádia Torres Cabral (2017), Aplicação das técnicas de TAC e MRI no diagnóstico de hérnia discal em Cão.

Patrícia Susana T. Antunes Gomes (2017), Influência do maleato de oclacitinib no hematócrito de cães atópicos.

Rute Martins (2017), Modulation of aquaporins gene expression by *n*-3 PUFA lipid structures in white and brown adipose tissue from hamsters.

Sinclair Patrick Owen (2017), The first epidemiological study on the prevalence of cardiopulmonary and gastrointestinal parasites in cats and dogs from the Algarve region of Portugal using the FLOTAC technique.

Susana Lopes Afonso (2017), Contribuição para o estudo dos efeitos benéficos da fisioterapia em cães com displasia da anca.

Sofia Faes Morazzo (2017), A via do Nodal na fibrose do endométrio da égua.

Tatiana Ferreira (2017), Efeito da suplementação da dieta com feno de aveia e cenoura nos índices zootécnicos e fracção lipídica da carne de coelho.

2016

Ana Carolina Ribeiro (2016), Maneio do cão geriátrico nas 48 horas pós-cirúrgicas.

Ana Catarina Neves (2016), Reabilitação física de cães com hérnias de disco toracolombares.

Ana Sofia Bonito de Figueiredo Valente (2016), Helmintofauna gastrointestinal e hepática do ratinho-caseiro (*Mus musculus domesticus*) do arquipélago da Madeira: potencial zoonótico e importância em sanidade animal.

Andreia Granjeiro Jacinto (2016), Parasitological survey of Tritrichomonas foetus in cats.

Angelina Gonçalves Pedrosa (2016), Valor prognóstico de características clinicopatológicas e imunofenotípicas em tumores mamários caninos.

Bárbara de Miranda Matos (2016), Parasitoses gastrointestinais e pulmonares em felinos domésticos no Minho.

Beatriz Santos (2016), Survey of metastrongyloid lungworms in domestic cats (*Felis silvestris catus*) from the metropolitan area of Lisbon, Portugal.

Gonçalo Mendes Morgado (2016), Parasitoses internas e frequência de desparasitação em cães do concelho de Vila Franca de Xira, Portugal.

Inês da Silva Soleiro Nunes Marcelino (2016), Estudo sobre parasitoses cardiopulmonares e gastrointestinais em pinípedes em contexto zoológico.

Ivo Tiago dos Santos (2016), Conservação *ex situ* do lince ibérico (*Lynx pardinus*): determinação de valores de referência para análises sanguíneas e análise da morfometria da população do Centro Nacional de Reprodução do Lince ibérico.

Joana Martinho (2016), Neurobiological effects of long chain *n*-3 polyunsaturated fatty acids.

Mª Inês Canavarro (2016), Aplicação do protocolo AWIN em explorações de pequenos ruminantes.

Maria Gomes (2016), Efeito da relação valina:lisina em dietas de baixa proteína na performance de leitões entre os 7- 20 kg de peso vivo.

Maria Eduarda Soares Lopes Fernandes Morais (2016), Gastrointestinal parasite screening in pet reptiles in the area of Perth, Western Australia.

Marta Rodrigues Tavares (2016), Relevância clínica dos níveis séricos do VEGF em gatas com tumores mamários.

Miguel Mendes Veiga (2016), Population Viability Analysis of endangered Saharawi Dorcas Gazelle (*Gazella Dorcas neglecta*).

Pedro Miguel Gouveia Feliciano (2016), Análise da expressão de topoisomerase IIα em linfoma B difuso de células grandes em cão

Rita Vaz Rodrigues (2016), Otimização da caracterização de fibras musculares estriadas em cão (*Canis familiaris*) por histoquímica e imunohistoquímica, com potencial aplicação ao diagnóstico.

Susana Paula de Oliveira Carrega (2016), Parasitismo gastrointestinal em aves de rapina num Centro de Recuperação de Animais Silvestres.

Tiago Miguel Pinto (2016), Reabilitação física de cães com doenças articulares no membro torácico.

2015

Ana Sofia Ribeiro (2015), Evaluation of two serological techniques (IFAT and ELISA) and one molecular technique (qPCR in skin sample) in the diagnosis of Leishmania infantum infection in dogs.

Ana Sofia Xavier (2015), Bile peritonitis: clinical characterization, underlying causes and surgical outcome in 26 dogs and 1 cat.

Bernardo Maria de Melo Franco Gaspar Cabaço (2015), Epidemiologia e controlo de helmintes intestinais em éguas e poldros Puro-Sangue Lusitano em regime de maneio extensivo no Ribatejo e no Baixo Alentejo.

Catarina Isabel Gomes Arranja (2015), Estudo da resposta inflamatória em tumores mamários de cadela.

Catarina Flaspoehler B. Gomes Cardoso (2015), Abordagem da pancreatite canina e felina: do diagnóstico clínico ao diagnóstico histopatológico.

David do Espírito Santo Otero Pureza (2015), Prevalência, grau de contaminação e viabilidade de ovos de *Toxocara* spp. em parques públicos da área da grande Lisboa.

Diana Osório Lopes (2015), Caracterização produtiva e reprodutiva de uma exploração intensiva de suínos.

Edna Maria de Andrade Vieira Can (2015), Welfare assessment in portuguese dairy goat farms: on-farm overall feasibility of an international prototype.

Guilherme Mocica (2015), Efeito da suplementação com forragem hidropónica nos parâmetros produtivos e na qualidade da carne de bovinos da raça blonde d'aquitaine.

Inês Peixoto Barão (2015), Utilização da condição corporal e dos níveis de glucose e β-hidroxibutirato sanguíneos como indicadores precoces de toxémia de gestação em cabras.

João Diogo Bernardo Bragança Ferreira (2015), Presence of *Cryptosporidium baileyi* in different Red Grouse (*Lagopus lagopus scoticus*) moors and its relation with agents of other avian diseases.

João Paulo Vistas André (2015), Análise da expressão de marcadores da matriz extracelular e de angiogénese em tumores mamários de gata.

Luís Miguel de Jesus Mota Fernandes Lajas (2015), Survey of parasitic diseases in african-lions (*Panthera leo*) from Niassa National Reserve, Mozambique.

Maria João Carvalho Teives (2015), Detecting infection by *Babesia* spp., *Hepatozoon* spp., *Leishmania* spp., *Ehrlichia* spp. and *Dirofilaria immitis* in felines (*Felis catus domesticus*) in the Alcochete District, using parasitological and serological techniques.

Marlene Delgado (2015), Deteção de alto nível de resistência a aminoglicosídeos em estirpes clínicas de Enterococci.

Marta Pereira (2015), Avaliação dos níveis serológicos de HER2 – Relevância clínica em cadelas com tumores mamários malignos.

Octávio Pereira (2015), Fluoroquinolonas como inibidoras das topoisomerases de *Leishmania infantum*.

Rita Magalhães da Cruz (2015), Actividade de fungos sobre ovos, larvas e oocistos de parasitas de ungulados silvestres e do cão.

Sérgio Henrique Faria (2015), Estudo retrospectivo da dirofilariose cardiopulmonar numa população de canídeos do litoral Alentejano, Portugal.

Sílvia Maria Ferreira Leal (2015), Prevalência *de Cryptosporidium* spp. e de *Giardia* spp. em Cães do Distrito de Bragança, Portugal Continental.

Susana Carolina Martins Ferreira (2015), Parasite ecology in the Spotted Hyena in Serengeti National Park in Tanzania.

Marta Barradas (2015), Estudo das características físico-químicas e nutricionais de carne de raça brava de lide.

Nuno Milharadas (2015), Efeito da suplementação da dieta com vitamina e sobre a estabilidade oxidativa da carne de bovino.

2014

Adelaide Teixeira (2014), Otimização da obtenção de plasma rico em plaquetas no Gato.

Ana Oliveira (2014), Linfoma canino e felino – Revisão bibliográfica e estudo de 3 casos clínicos.

Andreia Isabel Mendes Dias (2014), Identificação e isolamento de células com o fenótipo "Side Population" no endométrio da égua.

Ana Rita (2014), Estudo morfométrico do rádio de cão.

Cristiana Correia (2014), Valorização nutricional do repiso de tomate para a melhoria da qualidade da carne de suíno.

Daniela Sharon de Souza Nunes (2014), Patologia clínica de gatas com tumores mamários submetidas a quimioterapia com doxorrubicina.

Inês Santos (2014), Desenvolvimento e avaliação preliminar de um sistema de libertação de antibiótico de quitosano para utilização em infeções oculares superficiais.

Joana Peixoto (2014), Infeção do tracto urinário em felinos com doença renal crónica.

Letícia Marcelino (2014), Doença articular degenerativa em geriatria felina – uso do meloxicam em doses subterapêuticas.

Mariana Yuan Ribeiro Couto (2014), Estudo do comportamento alimentar e análise do parasitismo gastrointestinal do Burro raça de Miranda (*Equus asinus*) em sistema de pastoreio livre.

Marta Silva (2014), Interaction between uteropathogenic Escherichia coli and epithelial and stromal cells of the canine endometrium.

Patrícia Carvalho (2014), Reabilitação física do paciente neurológico pós-cirúrgico.

Uriel Jorge Iria Mateus (2014), Caracterização do comportamento de vitelos em regime intensivo na procura de factores de risco para cross-sucking.

2013

Ana Catarina Machado Pires Augusto (2013), Caracterização anatomo-patológica de nódulos cutâneos em animais exóticos.

Ana Catarina Mateus Murta (2013), Apoptose e proliferação celular na hiperplasia quística do endométrio em cadela.

Ana Marta Fonseca Lopes Cabrita (2013), Curvas de lactação em cabras Saanen, Alpinas e cruzadas.

Ana Sofia de Pinho Lopes (2013), Implementação do Projeto Rastreio STOP (Simples Teste de Ovos Parasitários) em equídeos em Portugal.

André Garcia Beselga (2013), Avaliação da expressão do receptor HER-2 em carcinomas mamários caninos.

Beatriz Rosa Fernandes Duarte da Silva (2013), Neoplasias oculares em cães e gatos: estudo retrospetivo 2001-2012

Carolina Maria Pimenta Lopes (2013), Estudo dos parasitas gastrointestinais do sacarrabos (*Herpestes ichneumon*) e outros carnívoros silvestres coabitantes, com relevância em Portugal.

Carolina Páramos Merino Faria Encarnação (2013), Comparison of virulence profiles of *E. coli* strains isolated from canine pyometra, cistitis and feaces.

Catarina Krug M. Silva (2013), Identification of welfare indicators in portuguese dairy cattle farms.

Cláudia Monchique (2013), Evolução da resistência aos antibióticos em *Staphylococcus* spp.: 1999 a 2006.

Daniela Cristina Sobreiro Marques (2013), Avaliação da expressão da COX2 em tumores mamários de cadela.

Daniela Erica de H. e G. Ribeiro da Cunha (2013), Avaliação da expressão de COX2 em tumores mamários de gata por imunohistoquímica – Correlação com aspetos clínicopatológicos, classificação histopatológica e possíveis implicações clínicas.

Diogo João Franco dos Santos (2013), Caracterização do Parasitismo de Ungulados Silvestres e aspectos da sua Epidemiologia na Tapada Nacional de Mafra, Concelho de Mafra, Portugal.

Dolores Saial (2013), Use of antimicrobials and cephamicin resistance in companion animals.

Gisele Fernandes (2013), Caviar pérola, estudo da estabilidade físico-química e microbiológica do produto.

João Catela (2013), Comparação do perfil de ácidos gordos em carne de suíno produzida com diferentes objetivos comerciais.

José Costa (2013), Impacto do transporte e do tempo na abegoaria no ph das carcaças de vitela, em condições comerciais.

Laura Fernandes (2013), Transmissão de *Salmonella typhimurium* Monofásica após o Nascimento em Suínos.

Mariana Silva Lopes Santos Matos (2013), Hábitos de desparasitação em animais de companhia: inquérito a proprietários de cães e gatos, da região de Lisboa, Portugal.

Mafalda Borges Mendes (2013), Caracterização demográfica da raça suína Alentejana.

Marta Silva (2013), Utilidade dos ácidos gordos dos eritrócitos como biomarcadores dos ácidos gordos do músculo.

Rita Sofia Dias Teixeira (2013), Ocorrência de parasitas gastrointestinais em dois grupos de *Chinchilla lanigera* no Norte de Portugal.

Rui André Carvalho Vidal (2013), Rastreio de Doenças de Transmissão Vectorial numa População de Cães com funções militares e policiais.

Sílvia Góis Spínola (2013), Estudo observacional de alterações dentárias em canídeos de raça Chihuahua.

Susana Franco (2013), Avaliação dos efeitos antiproliferativos de fármacos inibidores de topoisomerases do tipo II em promastigotas de Leishmania infantum.

Teresa Cordovil (2013), Uveíte recorrente equina e leptospirose: estudo de variáveis em cavalos sujeitos a vitrectomia, na região de Hannover.

PUBLICATIONS

LIST OF PUBLICATIONS

2017

Aguado-Martinez A, Basto A P, Leitao A, Hemphill A (2017) *Neospora caninum* in non-pregnant and pregnant mouse models: cross-talk between infection and immunity. *International Journal for Parasitology* 47:723-735. doi: 10.1016/j.ijpara.2017.09.001

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OUTREACH

Outreach

Outreach activities were directed to a broad spectrum of stakeholders, at the international and national levels, including academia, biotechnology, pharma and food industries, hospitals and clinics (veterinary and Human), companies, breeder´s associations, cooperatives, state offices (Ministries of Agriculture and of Health), and the general public. These outreach activities considered several forms, namely invited conferences, presentations at congresses and other scientific events, participation and organization of seminars, symposia and colloquia, consulting and advising, technology transfer, and participation in divulgation events to the general public. Below follows, as an example, the major impacts at the economic and societal levels, of CIISA's outreach activities directed to stakeholders of the farm animal industries, including consumers, covering a broad range of expertise areas:

Collaboration with breeders associations in their selection and conservation programs, and with the Administration (Ministry of Agriculture, Autonomous Regions) in the development of legislation and rules to be followed and applied at the national level and negotiated with the EU. At the international consortia Biobovis, Biovis, Biogoat, Biopig, Biohorse, and Biodonkey and through the international project IMAGE, discussing issues related to conservation of Animal Genetic Resources.

Education of farmers and counselling of the Administration to implement welfare evaluation programs at the farm level. Through the international projects AWIN and ANICARE, production of APPs for welfare evaluation and of educational material to the animal production chain.

Collaboration with farmers and the dairy industry production chain in the implementation of mastitis control programmes and promotion of milk quality up to the consumer level. Advanced training to veterinary surgeons through the Training Courses on "Official Supervision as Regards Semen, Ova and Embryos" funded by the BETTER TRAINING FOR SAFER FOOD INITIATIVE of the European Union Programme 2014 – 2016, AETS Consortium (AETS/Agrotec) – Contract n° 2013 96 16. Also, several courses on reproductive technologies and evaluation were addressed to veterinarians, technicians and farmers.

Training and knowledge transfer on relevant aspects of African Swine Fever conducted under the development of ASFORCE Project through the organization of workshops attended by around 400 veterinarians including CVOs from EU and non-EU countries.

Tutoring in advanced courses on GIS for animal disease control within FAO - UN scope, and tutoring in risk assessment courses "Better Trainning for Better Food" within DG Santé scope.

Collaboration with ASAE-Economic and Food Safety Authority (participation on the Scientific Council) and with DGAV-National Animal Health Authority (evaluation and support for the development of prevention, control and

eradication programmes, contingency plans, animal health information systems expert advice, advanced courses for official and private veterinarians).

Integration of the European Medicine Agency, AWP/CVMP – Antimicrobial Working Party of the Committee for Medicinal Products for Veterinary Use, and the Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the Committee For Medicinal Products For Veterinary Use.

Integration of the European Society in Clinical Microbiology and Infectious Diseases / Study Group for Veterinary Microbiology – ESCMID / ESGVM.

Life-long learning programmes

CIISA researchers are involved in the Life Long Learning program at FMV-UL. This is organized taking into account suggestions or external requests from professional or scientific organizations, suggestions from colleagues or alumni and the initiative of teachers and researchers. Over 30 courses were offered in the 2013-2017 period. The international 2-week intensive course "Animal Experimentation", also included in the Doctoral Programme in Veterinary Sciences, is regularly organized by CIISA. This course enrols a multidisciplinary team of veterinary researchers with longstanding expertise in animal experimentation, focused on biomedical research using different animal models, and further CIISA's role as an international leader in animal welfare and experimentation science education and training.

CIISA research seminars are organized on a regular basis throughout the year. Speakers are invited among our researchers and outside collaborators. In addition, CIISA supports the mandatory discipline of FMV's Doctoral Program "Research Seminars", where PhD students regularly present their work.

Entrepreneurship

CIISA stimulates the entrepreneurial spirit of its students and researchers to develop new technologies and products, and to intellectually protect potential data aiming to generate spin-off companies to explore or license their discoveries in Veterinary and Human settings. During 2013-17, CIISA had several examples of success such as the first prize award in the Innovation and Entrepreneurship contest of the Crédito Agricola Bank (Daniel Murta, former PhD student, now researcher with PhD), the 2nd prize for the best start-up project (VetPharma) of the STARTHealth@ULisboa2016 (Joana Dias, PhD student), or a patent application filed in 2017 to intellectually protect a brain targeting and drug delivery technology.

A spin off company was generated from work developed at the ANB lab, NZYTech (www.nzytech.com). Worldwide, NZYTech is the leading company in the provision of a large bank of carbohydrate degrading enzymes for R&D. Currently NZYTech provides >1000 highly purified enzymes in the mg scale. These represent a high value enzyme repository for research and development.

Events for the general public

CIISA develops events for the general public, namely participates in the annual "Night of the European Researcher". CIISA is actively involved in the Summer at ULisboa initiative**.** Annually, during

the first fortnight in July, CIISA hosts secondary students enrolled in this University-wide event. The participants are accompanied by our undergraduate students to interact with scientists in the different laboratories, being introduced to the research activities taking place.

SUMMER AT ULISBOA

EUROPEAN'S RESEARCHERS NIGHT

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