Associate Laboratory for Animal and Veterinary Sciences

Emergent Infectious Diseases and Zoonosis - Big Data

ABSTRACTS BOOK

Faculdade de Medicina Veterinária da
Universidade de Lisboa

NOV 29th-30th 2023 | Auditório B

Associate Laboratory for Animal and Veterinary Sciences

Emergent Infectious Diseases and Zoonosis - Big Data

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PROGRAMME

NOV 29th, Wednesday

08:30 **Registration**

09:00 **Opening Session**

Esmeralda Delgado, vice-president of the Faculdade de Medicina Veterinária, Universidade de Lisboa
Susana Guedes Pombo, director of Direção Geral de Alimentação e Veterinária (DGAV)
Luis Lopes-da-Costa, director of AL4AnimnalS
Alexandre Leitão, coordinator of thematic line 2 of AL4AnimnalS

09:30 **SESSION: Image |** Chair: **Andrew Hemphill**, U Bern

Image data analysis and interpretation: machine learning dimensions
Keynote speaker: Adrian Hehl | Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland

10:15 **Free oral communications**

Enhancing research on endometrial physiology and pathophysiology in postpartum dairy cows with laser capture microdissection
Gonçalo Pereira

10:35 **Coffee break and poster viewing**

11:00 **Poster oral short presentations |** Chair: **António Duarte**, ULisboa; **Isabel Pereira da Fonseca**, ULisboa

Decoding the genomic landscape of Staphylococcus aureus isolated from chicken bursitis: unraveling antibiotic resistance and virulence mechanisms
Patricia Poeta

Phenotypic characterization of Enterococcus spp. isolated from chickens for human comsuption
Patricia Poeta

Antibiotic resistance profile in Escherichia coli isolated from livestock: Focus on rabbits and pigs in food animals
Patricia Poeta

Bats, a beacon of hope in antimicrobial resistance
Patricia Poeta

Biofilms in cat-derived Pseudomonas aeruginosa: insights into formation and implications
Patricia Poeta

Bioinformatics designing of an epitope-based vaccine for Bovine Genital Campylobacteriosis
Maria Elisabete Silva

Comparison of Skin Prick Tests (SPT), Intradermal Tests (IDT) and In Vitro Tests in the Characterization of Insect Bite Hypersensitivity (IBH) in a Population of Lusitano Horses: "Contribution for Future Implementation of SPT in IBH Diagnosis".
Vera Pessoa

Ex-Vivo Permeation Of P28, an Azurin Derived Cell-Penetrating Peptide, to Enhance the Ocular Delivery of Erythropoietin Encapsulated into Nanoparticles
Gonçalo Santos

First Evaluation of the Use of Foxim as a Contribute to the Control of Gasterophilus intestinalis Burden in Horses at Pasture: a Pilot Study
Teresa Rodrigues

Production of parasitic recombinant proteins for combination therapy against schistosomiasis
Maria João Gouveia

Climatic factors and parasite infection in dogs and cats of the Azores islands
Romana Teixeira

Detection of Canine Vector-Borne Diseases agents in Galgo Español used as hunting dogs in Alentejo, Portugal
Maria Francisca Esteves

Ticks (Ixodidae) collected on cattle and tick-borne pathogens at traditional housing systems in Huambo, Angola
Esmeralda Inácio

Toxoplasma gondii: experimental infection and study of the immune and inflammatory response using different genotypes
Inês Fançony

Toxoplasma gondii and Mental Health: Reinforcing the Link
Maria Manuel Fernandes

Overview of Cryptosporidium spp. and Giardia spp. in animals in Portugal in a three-year period
Mariana Louro

Searching for Cryptosporidium infection in bats from Brazil
Pedro Andrade

Encephalitozoon Cuniculi Seroprevalence and Analytical Trends in the Population of Pet Rabbits Attending FMV-Ulisboa Veterinary Teaching Hospital
Ana Reisinho

13:00 Lunch

14:30 **SESSION: Omics |** Chair: **Adrian Hehl**, U Zurich; **Telmo P. Nunes**, ULisboa

Microbiome and antibiotic resistance

Keynote speaker: Isabel Gordo | Fundação Calouste Gulbenkian - Instituto Gulbenkian de Ciência, Evolutionary Biology Group

15:15 **Application of differential affinity chromatography coupled to MS and proteomics for the identification of drug targets and for studies on the mode of action of anti-parasitic drugs**

Invited speaker: Andrew Hemphill | Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland

15:35 **Applications of -omics to the study of host-pathogen interactions in animal diseases: ruminant apicomplexans parasites as an example**

Invited speaker: Luis Miguel Ortega-Mora | SALUVET Group, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Spain

15:55 **Genomics for sustainability: identifying rare mutations of interest by the exploration of local genetic resources in Portuguese-speaking African countries**

Invited speaker: Andreia Amaral | Escola de Ciência e Tecnologia, Universidade de Évora; Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon; Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

16:15 **Coffee break and poster viewing**

16:45 **SESSION: Disease surveillance (1st PART) |**
Chair: **Isabel Gordo**, FCG IGC; **Fernanda Dórea**, SVA Sweden

Free oral communications

Interoperability structure for farm data sources and databases to monitor and manage antimicrobial consumption in animal production - the case for USAM SuLei.
João Niza Ribeiro

Environmental Contamination and Staff Colonization by Carbapenem-Resistant Gram-Negative Bacteria in Small Animal Veterinary Practices
Joana Moreira da Silva

Carriage of Carbapenemase and ESBL-producing Escherichia coli in dogs and cats in veterinary care and co-carriage with their owners
Juliana Menezes

Exploring trends of resistance of Klebsiella spp. in companion animal infections: a three-year study
Laura Fernandes

Biological and genetic characterization of Echinococcus implications for dynamics of transmission
António Castro

Encephalitis Project
José Manuel Correia da Costa

19:00 **Dinner**

NOV 30th, Thursday

09:00 **SESSION: Disease surveillance (2nd PART) |**
Chair: **Luis Ortega-Mora**, SALUVET U Complutense Madrid; **Yolanda Vaz**, DGAV Lisboa

Data-driven early disease detection: automated workflows and decision-support in animal health surveillance
Keynote speaker: Fernanda C. Dórea | Global Project Coordinator, FAO & National Veterinary Institute (SVA), Sweden

09:45 **Little Stories About Increasing Quantities of Big Data: A Data Analyst's Perspective**
Invited speaker: Paulo Jorge Nogueira | Escola Nacional de Saúde Pública, Universidade Nova de Lisboa

10:05 **Detection and quantification of excessive mortality events in Portuguese bovine populations**
Invited speaker: Telmo Nunes | Faculdade de Medicina Veterinária, Universidade de Lisboa

10:25 **Coffee break and poster viewing**

10:45 **Free oral communications**

Estimating Lagged Risks of Temperature Exposure: Insights from Hospital Admissions for Infectious and Parasitic Diseases
Maria Oliveira

Feline Immunodeficiency Virus Hospitalization: Insights from a Comprehensive Epidemiological Database using Predictive Modelling
Miguel Maximino

C Reactive Protein/Albumin Ratio as a SRIS Indicator in Dogs with Parvovirus
Diana Lopes

Evaluation of SARS-CoV-2 susceptibility in cats: serological survey for antibodies against the virus
Isa Moutinho

Unravelling (new) neurotropic viruses - AL4animals Project Outline
Líbia Zé-Zé

Examination of a herd experiencing Neospora caninum-associated abortion by a mouse monoclonal antibody-based competitive ELISA
João Crisóstomo

Immunomagnetic separation method for identification of Toxoplasma gondii oocysts in environmental samples
Susana Sousa

Toxoplasma gondii detection in pre-harvested vegetables in the North of Portugal
Cláudia Marques

Uncovering Zoonotic Toxoplasma Gondii Genetic Diversity and its Implication on Toxoplasmosis Pathogenesis – A One Health Approach (Al4animals Project Management Framework)
Cláudia Marques

12:45 **Closing Session**

13:00 **Lunch**

Free oral communications

Enhancing research on endometrial physiology and pathophysiology in postpartum dairy cows with laser capture microdissection

Pereira G. 1,2, Guo Y. 3, Silva E. 1,2, Silva M. F. 1,2, Bevilacqua C. 4, Charpigny G. 5, Humblot P. 3, and Lopes-da-Costa L. 1,2

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The bovine endometrium is a complex and heterogeneous tissue, and quantifying gene transcription from the entire endometrium may not accurately capture cell-specific transcription (Pereira et al., 2022a). Additionally, interactions between different cell types may be overlooked, leading to an incomplete interpretation of molecular data. Laser Capture Microdissection (LCM) emerged as a powerful research tool for isolating specific cell populations, under microscopic guidance (Bevilacqua and Ducos, 2018). One of the key advantages of LCM is the ability to obtain small, single-cell samples within the context of their tissue microenvironment (Espina et al., 2006).

LCM and transcriptomic analysis were combined to evaluate endometrial physiology and pathophysiology of postpartum dairy cows (high vs low plasma progesterone – P4 or presence vs absence of endometrial inflammation). Endometrial biopsies were taken at 44 days postpartum and endometrial cell types (luminal epithelium - LE; glandular epithelium - GE, stroma - ST) were isolated by LCM. Transcriptomic profiles were determined by RNAseq and differentially expressed genes (DEGs) identified by DESeq2 package in R (adjusted p-value of 0.05).

This approach confirms that LE, GE and ST endometrial cells have distinct molecular signatures, which are influenced in a cell type-specific manner by P4 (Pereira et al., 2022b) and inflammation (Pereira et al., 2022a). In both models, over 90 % of the DEGs were cell type specific. As most relevant specific conclusions, high P4 was associated with downregulation of cell cycle genes in GE cells, indicating an anti-proliferative action of P4 in epithelial compartments. High P4 was also linked to transcription profiles of ST cells primarily associated with interactions between epithelial and stromal compartments. In cows with endometritis, LE cells exhibited upregulation of genes related to attraction of leukocytes and protection against inflammatory responses (CCR10 and CCL21), and proteins involved in Eph-ephrin signaling (EPHA4 and EFNA1). In contrast, GE cells displayed downregulation of genes that prevent epithelial damage and induce anti-inflammatory and tolerogenic responses, contributing to a delayed resolution of inflammation. These included TGFβ receptors, IL10RA, and two interleukin-1 receptor-associated kinases (IRAK3 and IRAK4). Also, ST cells showed upregulation of genes involved in tissue regeneration and recruitment of immune cells, (IL17B and IL17D), as well as upregulation of members of the R-spondin family of Wnt modulators (RSPO1 and LGR6).

Altogether, these studies reveal the intricate cell-specific regulation of biological processes in the bovine endometrium, previously unnoticed through whole tissue approaches. Using the LCM tool and considering the cell compartment, as the physiological unit, these studies offer fresh insights into endometrial physiology and pathophysiology and address novel putative biomarkers and therapeutic targets.

POSTERS

Decoding the Genomic Landscape of Staphylococcus aureus Isolated from Chicken Bursitis: Unraveling Antibiotic Resistance and Virulence Mechanisms

P1

Silva, V. 1,2,3,4, Teixeira, P. 5, Caniça, M. 5,6,7, Pinto P. 8, Vieira-Pinto, M. 7,8,9, Igrejas, G. 1,2,3, Poeta, P. 4,7,9

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Introduction: Staphylococcus aureus, a bacterium commonly associated with infectious processes, poses significant challenges to poultry health. Chicken bursitis, a prevalent condition, provides a unique context to explore the genetic factors governing antibiotic resistance and virulence in S. aureus strains. Thus, the aim of this study was to isolate S. aureus from chicken bursitis and to characterize isolates antimicrobial resistance and genetic lineages.

Methods: Twenty-four S. aureus isolates were collected from chicken bursitis samples and subjected to Whole Genome Sequencing (WGS). The WGS data was then analysed to identify antibiotic resistance profiles using bioinformatics tools. Multilocus Sequence Typing (MLST) was employed to categorize the isolates into clonal complexes and sequence types. The presence of virulence genes was determined through a thorough examination of the genomic sequences.

Results: Upon WGS analysis, none of the isolates exhibited resistance to methicillin. The isolates were uniformly classified into clonal complex 5 (CC5), predominantly associated with Sequence Type 5 (ST5), with a single isolate designated as ST634. Across all isolates, resistance to tetracycline, quinolones, and fosfomycin was widespread. The genetic determinants of these resistances included tet38, tetK, norA, mepA, fosB, and various regulators influencing resistance gene expression. Isolates resistant to aminoglycosides, macrolides, and lincosamides harbored aph(3')-IIIa, lmrS, erm(C), and aadC genes. The virulence gene profiling identified an array of factors such as hemolysins (hla, hld, hlb, hlgC), leukocidins (lukD, lukE), and enterotoxins (sei, selX, sen, seo, seu).

Conclusions: This WGS-based exploration provides a profound understanding of antibiotic resistance and virulence traits within S. aureus isolates from chicken bursitis. The identified genetic signatures accentuate the significance of ongoing surveillance and targeted interventions, essential for preserving poultry health and mitigating potential risks within the broader agricultural ecosystem. Continuous monitoring is imperative to ensure sustainable and resilient animal health practices.

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Phenotypic characterization of Enterococcus spp. isolated from chickens for human comsuption

P2

Ribeiro, J. 1,2,3, Igrejas, G. 4,5, Heleno, S.A. 3,6, Reis, F.S. 3,6, and Poeta, P. 1,2,7,8

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Enterococci are commensal bacteria in the human and animal gut, but have become important pathogens in recent decades, mainly due to the overuse of antibiotics. In animal production, antibiotics are often used to prevent bacterial infections and, in most developed countries, to promote animal growth, which has contributed to the spread of antibiotic-resistant bacteria and genes, posing a serious threat to public health. The antimicrobial resistant bacteria that have emerged and live in the animal production environment can be transmitted to humans through human-animal contact, consumption of or contact with animal products. In addition, during food processing, when an animal is slaughtered, the muscles are exposed and can be cross-contaminated if the gastrointestinal tract is ruptured or if contaminated instruments and materials are used. Therefore, Enterococcus spp. were isolated from chicken faeces and their phenotypic profile was studied to determine whether they were resistant to antibiotics.

Forty samples of chicken faeces were collected in Savinor (Trofa, Portugal) and all were immersed in BHI broth and incubated (24h/37ºC). For the isolation of enterococci, samples were plated on Slanetz-Bartley agar and incubated (72h/37ºC). Colonies showing pink colour on Slanetz-Bartley agar were plated on Kanamycin Aesculin Azide agar (24h/37ºC) and only those that hydrolysed esculin were considered. All isolates were tested for antimicrobial susceptibility against ten antimicrobial agents. The bacterial isolates were tested for susceptibility to ampicillin (10 µg), vancomycin (30 µg), teicoplanin (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), linezolid (30 µg), quinupristin-dalfopristin (15 µg), imipenem (10 µg), tetracycline (30 µg) and ciprofloxacin (5 µg) by the Kirby-Bauer disc diffusion method. All bacterial isolates were tested according to CLSI (2023) except for imipenem which was tested according to EUCAST (2023). Thirty-one (77.5%) Enterococcus spp. were isolated from the faecal samples and nine were multi-drug resistant as they were resistant to at least three classes of antibiotics. The highest rate of resistance was to quinupristin-dalfopristin (83.9%), followed by tetracycline (48.4%), erythromycin (22.6%), imipenem (22.6%) and ciprofloxacin (6.7%). Our results suggest that chickens can act as vectors for the spread of antimicrobial-resistant enterococci, as they can be transmitted to humans, the environment, or other livestock via faecal material.

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Antibiotic resistance profile in Escherichia coli isolated from livestock: Focus on rabbits and pigs in food animals

P3

Silva A. 1,2,3,4, Silva V. 1,2,3,4, Tavares T. 1,2,3,4,5, Pereira J.E. 6,7, Maltez L. 6,7, Valentão P. 8, Falco V. 9, Igrejas G. 2,3,4 and Poeta P. 1,2,6,7

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Food-producing animals serve as major reservoirs for many foodborne pathogens, including Escherichia coli. These pathogens have the potential to cause disease, ranging from sporadic diseases to chronic complications and even death. The increase in antibiotic resistance in these animals poses a significant public health concern, affecting clinical settings, animal husbandry practices, and the food industry. The main objective of this work was to characterize the antibiotic resistance of E. coli strains isolated from pigs and rabbits farms in Portugal.

Antimicrobial susceptibility was performed by a Kirby–Bauer disk diffusion method against 16 antibiotics, relevant to both human and animal health, according EUCAST (2022) guidelines. Among 46 E. coli strains obtained from pigs, the highest resistance rate was detected in trimethroprim-sulphamethoxazol and ampicillin, in 97.36% of the isolates. Other high resistance rates were detected for streptomycin (73.68%), imipenem (68.42%), gentamicin (44.73%) and amikacin (31.57%). Additionally, 78.94% of these bacterial strains were multi-resistant and were therefore considered resistance to more than three different antibiotic classes. From 48 E. coli strains from rabbits, all isolates presented a multi-resistance profile, with high levels of resistance to β-lactams (100%), aminoglycosides (93.75%), cephalosporines (100%) and tetracycline (91.6%). In both cases, there is a worrying rate of multidrug resistance. In terms of specific antibiotic resistance rates, pig and rabbit isolates showed high resistance to β-lactams and aminoglycosides. These results show concerning rates of resistance to some important and very important antibiotics in humans and veterinary medicine. Despite similarities in resistance patterns, differences between specific antibiotics and their resistance rates highlight the complexity of antibiotic resistance in different animal species.

This comparison highlights the need for a comprehensive approach to antibiotic management in pigs and rabbits farms, given the common and unique challenges posed by antibiotic resistance in these animals.

Acknowledgments: This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry—LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). Adriana Silva is grateful to FCT (Fundação para a Ciência e a Tecnologia) for financial support through the PhD grant SFRH/ BD/04576/2020.

Bats, a beacon of hope in antimicrobial resistance

P4

Sabença, C.1-4, Barros, P. 5,6, Cabral, J.A. 5, Igrejas, G.2-4, Torres, C.7 and Poeta, P. 1,4,8,9

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Exploring antimicrobial resistance in bats provides a unique window into the dynamics of microbial interactions in these nocturnal creatures. Investigating the microbial communities within bat populations offer insights into the evolution and spread of antibiotic resistance and their implications for public health. For this work, we studied 42 Klebsiella spp. isolates from seven different species of bats (Nyctalus leisleri, Rhinolophus ferrumequinum, Pipistrellus pipistrellus, Tadaria teniotis, Rhinolophus mehelyi, Plecotus auritus and Plecotus austriacus) recovered in HiCrome Klebsiella Selective Agar Base media. Antibiograms were performed for 17 antibiotics according to EUCAST guidelines. Extended-spectrum β-lactamases (ESBL) production was tested by the double-disc synergy test, but all Klebsiella spp. isolates were negative for this test. Klebsiella spp. isolates only showed resistance to ampicillin (37/42), gentamicin (29/42) and amikacin (4/42). Only four Klebsiella spp. isolates were susceptible to all antibiotics. We can conclude that antibiotic resistance is present Klebsiella isolates of different species of bats; however, the isolates of this study, obtained without previous selection, were susceptible to most of the antibiotics tested, with the exception of aminoglycosides (69% of resistance) and ampicillin. K. pneumoniae is intrinsically resistant to ampicillin which could justify the high levels of resistance to this antibiotic in our isolates, therefore, the future step of this work is to identify the species of the Klebsiella isolates. The presence of isolates that are susceptible to a broad spectrum of antibiotics is encouraging, as it implies that in this ecosystem antimicrobial resistance is not widely disseminated. However, it is crucial to continue monitoring antibiotic resistance trends over time, as resistance patterns can evolve. Regular surveillance helps in adapting treatment strategies and maintaining the effectiveness of available antibiotics.

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Biofilms in cat-derived Pseudomonas aeruginosa: insights into formation and implications

P5

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The formation of biofilms by P. aeruginosa in cats is a contributing factor to the worsening of chronic infections, such as respiratory, urinary tract, and ear infections. The matrix produced by biofilm-forming microbial cells forms a protective layer that reduces antibiotic penetration and shields bacteria from the immune system, complicating the treatment of these infections. The main objective of this study was to investigate the biofilm-formation capacity of P. aeruginosa isolated from domestics' cats. The strains were isolated using the selective medium Pseudomonas CN selective agar. The crystal violet method in 96-well plates was used to assess the ability of strains to form biofilms. Among the 29 isolated strains, only one was unable to form any detectable biofilm using this method, while the vast majority ofmost strains tested (n = 21) produced a biofilm qualified as intermediate, and 7 isolates were considered as weak biofilm producers .producers. Genetic variability and environmental factors play crucial roles in the biofilm-forming capabilities of P. aeruginosa and our results on a panel of isolates show ana significant phenotypic diversity. In conclusion, the characterization of biofilm formation capacity by P. aeruginosa strains is important because it can influence antibiotic selection in treatment as biofilms are known to increase P. aeruginosa resistance to antibiotics. In this way, knowledge of this property could assist veterinarians in choosing more effective antimicrobial treatments.

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Bioinformatics designing of an epitope-based vaccine for Bovine Genital Campylobacteriosis

P6

Marta F Silva 1,2,3, Gonçalo Pereira 1,2, Luísa Mateus 1,2, Luís Lopes-da-Costa 1,2, Elisabete Silva 1,2

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Bovine Genital Campylobacteriosis (BGC) is a worldwide distributed venereal disease caused by Campylobacter fetus subsp. venerealis (Cfv) resulting in significant reproductive losses and economic impact. However, there are no effective vaccines globally available for BGC control. This study explored reverse vaccinology and bioinformatic strategies to design a multi-epitope vaccine against Cfv.

The proteome of Cfv strain NCTC 10354 was analyzed to select the extracellular and outer membrane proteins potentially involved in virulence. The proteins classified as antigenic, non-allergenic, hydrophilic, thermal stable, and with only one transmembrane helix were selected for epitope analysis. For this, the linear B-cell epitopes were predicted and the sequences of linear B-cell epitopes were used to predict MHC type I and II binding peptides. The epitopes with the ability to bind to several MHC alleles and good antigenicity scores were used to design the multi-epitope vaccine. The vaccine tertiary structure was predicted and subjected to model refinement, and stability was improved through disulfide engineering. Codon optimization was performed for an efficient expression in Escherichia coli K12 and cloning was simulated in silico using a pET-30a(+) vector. The immune simulation was performed using the C-ImmSim server.

A total of 1849 proteins were analyzed, and of these 11 non-allergenic proteins were selected as virulence factors with extracellular/outer membrane localization. Nine of these proteins fulfilled the physicochemical criteria and had only one transmembrane helix. One outer membrane (OmpA) and a flagellar (FliK) protein were selected for the prediction of B- and T-cell epitopes. A total of 15 epitopes were selected for further analysis (MHC class I: 8 from protein FliK and 1 from OmpA; MHC class II: 5 from FliK and 1 from OmpA). These epitopes, conserved among the 31 Cfv strains deposited in GenBank, were integrated into the vaccine candidate to form a multi-epitope fragment, which included 2 epitopes from OmpA and 13 from FliK linked together by GPGPG linkers, and connected to the cholera toxin subunit B by an EAAAK linker. This vaccine candidate has 370 amino acid residues, a molecular weight of 38550.51, and a theoretical isoelectric point of 9.05. This construct was predicted to be antigenic, non-toxic, non-allergenic, and soluble upon overexpression. The protein structure was predicted and optimized and the sequence was successfully cloned in silico into a pET-30a(+) vector. The immune simulation showed the ability to induce an immune response through increased B and T-cell populations and immunoglobulin titers. Overall, this study developed a novel vaccine candidate suitable for further in vitro and in vivo experimental validation, which may become a useful tool for the control of BGC.

This study was supported by FCT (Project PTDC/CVT-CVT/30145/2017), CIISA (UIDB/00276/2020), and Associate Laboratory AL4AnimalS (LA/P/0059/2020).

Comparison of Skin Prick Tests (SPT), Intradermal Tests (IDT) and In Vitro Tests in the Characterization of Insect Bite Hypersensitivity (IBH) in a Population of Lusitano Horses: "Contribution for Future Implementation of SPT in IBH Diagnosis".

P7

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Introduction: Insect Bite Hypersensitivity (IBH) prevalence in Portugal, in Lusitano horses, is not known, but environmental characteristics are favorable to the activity of Culicoides and common knowledge shows high occurrence in Lusitano stud farms.

Aim of the study: This study aimed at comparing skin tests and in vitro allergy tests for the diagnosis of IBH in Lusitano horses.

Material and Methods: Thirty controls (C) and 30 IBH-affected (T) Lusitano horses were evaluated. T horses were included based on anamnesis and physical examination, supported by questionnaires. All horses were submitted to skin tests, Intrademal (IDT) and Skin Prick Tests (SPT), on the neck with 14 specific allergens, 13 recombinant proteins (r-proteins) from Culicoides nubeculosus (Cul n) and Culicoides obsoletus (Cul o) salivary glands and Culicoides nubeculosus Whole Body Extract (Cul n WBE). Addicionally, a cluster of six T and six C horses were also tested with Cul n 3 and Cul n 4 produced in insect cells and barley, as well as E. coli produced Cul o 3 and Cul o WBE. Allergen concentrations were 10 µg/mL for IDT and 100 µg/mL for SPT, and wheal diameters were assessed at 20 min, 6 and 48 h. IDTs were considered positive when wheal diameter was ≥50% of the histamine wheal and SPTs ≥ 0.9 cm. In vitro tests, allergen-specific serum IgE evaluation and sulfidoleukotriene (sLT) release assay were also carried out.

Results: Results showed that Cul n WBE, Cul n 7, 8, 9, Cul o1P and Cul o 2P were the best performing allergens for SPTs ($p \leq 0.0001$) in the 1st allergen panel and Cul o WBE, Cul n 3 Bar and Cul n 4 Bac ($p \leq 0.05$) in the 2nd, presenting a higher discriminatory diagnostic potential than IDTs, at a concentration of 100 µg/mL, with readings assessed at 20 min. Regarding in vitro tests overall, the sLT release assay performed best.

Conclusion: Our study showed that skin prick tests presented the highest discriminatory diagnostic potential in IBH diagnosis. This increases our knowledge about IBH in Lusitano horses and could represent a step forward in the future development of specific immunotherapy.

Keywords: Insect Bite Hypersensitivity (IBH); Allergens; Intradermal tests (IDT); Skin Prick tests (SPT); In vitro tests.

Ex-Vivo Permeation Of P28, an Azurin Derived Cell-Penetrating Peptide, to Enhance the Ocular Delivery of Erythropoietin Encapsulated into Nanoparticles

P8

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Glaucoma is the leading cause of irreversible blindness worldwide. This optic neuropathy is characterized by progressive degeneration of retinal ganglion cells and vision loss. Recently, neuroprotection in this type of disease has received special attention and, as erythropoietin and its recombinant forms like erythropoietin beta (EPOβ) have already been proven to exert neuroprotective and neuroregenerative effects on the retina, attempts have been made to develop nano-formulations that would facilitate its ocular permeation.

Cell-penetrating peptides (CPPs) are a class of peptides that can translocate through cell membranes without compromising their integrity. Azurin, a 128 amino acid periplasmic copper-containing protein produced by Pseudomonas aeruginosa, has the capacity to permeate through the cellular membrane of several cell lines. The segment of 28 amino acids from 50 to 77 is essential for the efficient internalization of the protein, acting as a CPP. This 28-amino acid peptide sequence is called p28.

This study aimed to evaluate the permeation of p28, through the main ocular layers, and developing peptide-functionalized nanoparticle system of hyaluronic acid (HA) and chitosan (CH) for ocular delivery of erythropoietin (EPO). Ex vivo permeation assays were studied in fresh porcine corneas, scleras, and conjunctivas using Franz-type diffusion cells and fluorescent labelled peptide (P28_Alexa 488). The nanoparticles were prepared by ionic gelation technique with chitosan and hyaluronic acid, and their functionalization with P28 was done by adsorption. The nanoparticles were characterized in terms of size, zeta potential (ZP), and polydispersity index (PI). Percentage of functionalization was determined. Permeation assays of EPO from nanoparticles functionalized with P28 were evaluated in ex vivo permeation assay. Ex vivo permeation data showed that P28 permeates through the three ocular layers, with the following permeability coefficients: conjunctiva, 7.55±0.30x10^-6 cm s−1; sclera, 6.04±0.33x10^-6 cm s−1; and cornea 3.27±0.36x10^-6 cm s−1. The efficiency of peptide nanoparticles association was 89%, and nanoparticles presented a mean size in the range of 240-260nm, a positive ZP (+37 - +39mV) and low PI (0.104-0.155). These parameters were not significantly different for the several produced nanoparticles ($P>0.05$). The peptide-functionalized EPO nanoparticles permeated amount was 50% higher through the conjunctiva than that observed in our previous study using 100 IU/200 µL of EPOβ into NPs of CS/HA. In the sclera and cornea, the amounts were not significantly different.

In conclusion, coating chitosan/hyaluronic acid nanoparticles with P28 could result in a promising nano-formulation characterized by increased retention time and enhanced permeation through ocular anatomical barriers, namely the conjunctiva, which can lead to better bioavailability of EPO in the retina.

First Evaluation of the Use of Foxim as a Contribute to the Control of Gasterophilus intestinalis Burden in Horses at Pasture: a Pilot Study

P9

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Introduction: Gasterophilosis (mostly by Gasterophilus intestinalis) is one of the most frequent gastrointestinal infections in horses, showing a worldwide distribution and occurring often in Portugal, especially in horses that spend most of their time at pasture during the summer. Foxim is an organophosphate mostly used in the treatment of the poultry red mite Dermanyssus gallinae. The postsynaptic accumulation of acetylcholine interferes with the transmission of normal impulses in the arthropod nervous system, leading to paralysis and death of the parasite. This study aimed to assess the efficacy of foxim as a new approach to control G. intestinalis. eggs from the coat of horses.

Material and Methods: Twelve horses aged 1-2 years and 2 mature horses entered the study, all from the same PS Lusitano stud farm and infested with G. intestinalis eggs deposited on their coats. A sample of hair (5cm x 5cm) was shaved with a blade from the forelimbs of these horses into individual plastic containers, before (BF) and 24 hours after applying foxim (AF). One litre of a solution, obtained by adding 1 ml Byemite® (500 mg Foxim/ml) to 1 litre of water at 40ºC, was applied with a hard sponge on the forelimbs of each horse. All BF and AF samples were observed under a stereo microscope Olympus SZ51 on days 1, 3, 7 and 10 and all eggs were observed and registered.

Results: On day 1, the BF samples presented an average of 21.14 +/- 14.24 eggs, whereas the AF samples presented an average of 15.36 +/- 11.88 eggs.

In the AF samples there were some transparent egg shells, some transparent operculums, as well as dead and some even damaged larvae inside open eggs. In this group, most intact eggs remained closed on day 10 and were then opened with fine tweezers under the microscope to find dead larvae inside.

In the BF samples, some larvae were found outside the eggs and some eggs had live larvae starting to crawl out. The eggs that remained closed on day 10, were also opened to find mostly live larvae.

Discussion: With all the limitations of this being a pilot field study, still the predominance of egg changes and of dead larvae in the samples collected after applying foxim suggest the relevance to carry out further investigation of its use as a predictably strong contribute for the control of G. intestinalis in horses at pasture. Considering the heavy burdens presented by horses, in the summer months, we believe this could be an enormous contribute for the prevention of gastric ulcers and colic in horses.

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Production of parasitic recombinant proteins for combination therapy against schistosomiasis

P10

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Schistosomiasis is one of the major neglected tropical diseases affecting millions of people worldwide. Importantly, the infection caused by Schistosoma haematobium is directly associated with the development of squamous cell carcinoma of the bladder despite its molecular and/or cellular mechanism remains unknown. Previously, a postulate emerged hypothesizing that metabolites produced by the parasite might trigger the carcinogenesis-infection associated. The treatment relies on a single drug, praziquantel (PZQ), and therefore, there is a reasonable concern about drug resistance. Moreover, the drug itself is not capable of counteracting infection-associated disease lesions, thus, there is a pressing for novel therapies. In our point of view, the treatment should not only eliminate the parasite but also ameliorate the infection-associated pathologies, ultimately counteracting the carcinogenesis associated with Schistosoma haematobium infection. Studies have shown that the use of antioxidants in combination with other drugs can be helpful in enhancing the antihelminthic activity of drugs and inhibiting in vitro the formation of metabolites that could contribute to the development of cancer. Since the success of any chemotherapeutic regimen often depends on the host immune response during the infection, among other factors, we hypothesized that the use of relevant parasite recombinant proteins as immunotherapy combined with drugs might be beneficial to treat schistosomiasis.

Due to their function and characteristics, we focus on the following S. mansoni proteins: thioredoxins (Trx1, Trx2), peroxiredoxins (Prx1, Prx2, Prx3, Prx4), thioredoxin glutathione reductase (TGR), tegumental protein (Sm8) and fatty acid binding protein (FABP). To their produce, we first obtained adult worms after liver perfusion of infected CD1 to prepare cDNA. Through PCR we amplify the gene of interest, subcloned into pGEM-T Easy Vector and pQE30 and sequence. The S. mansoni recombinant proteins were expressed in E. coli (JM109 or M15) strains system following purification and quantification (Bradford method). So far, we have successfully produced and purified some of the recombinant proteins, namely rSmPrx3, rSmTGR, rSmTrx1, rSmTrx2, and rSmFABP. At the moment, we are conducting functional characterization of the parasitic recombinant proteins through several enzymatic assays. Following that, we will assess their immunological features in vitro and in vivo using the recombinant proteins alone, as well as in combination with PZQ.

The close association between chemotherapy and immunotherapy suggests that a dual therapeutic approach would be more effective in targeting the parasite and boosting immunity to prevent and/or reverse associated pathologies.

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Climatic factors and parasite infection in dogs and cats of the Azores islands

P11

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Introduction: The climate of the Azores archipelago is characterized by high levels of air humidity, mild temperatures, regular and abundant rainfalls and by a regime of vigorous and strong winds. The present study aimed to evaluate the relationship between a set of climatic factors and the presence of parasitism in this peculiar insular territory.

Methods: In this case study, 598 fecal samples were gathered from five different islands (Corvo, Flores, Santa Maria, São Miguel and Terceira). 296 from dogs (49,5%) and 302 (50,5%) from cats, being examined with the mini-FLOTAC and Baermann methods. Data was collected from the European Climate Assessment & Dataset (ECA&D) from the period of January, 2023 to November, 2023 using the closest geographic stations. Here average temperature of the last 21 days and accumulated rainfall in the last 30 days prior to the sample collection are the parameters taken into consideration as factors that influence parasite infection.

Results: Using the Wilcoxon test, accumulated rainfall was correlated with the presence of infection in dogs with the fallowing parasites: Ancylostomatidae ($p = 0.01562$); Cystoisospora spp. ($p = 0.03116$). In dogs, average temperature was only correlated to parasite infection in individuals positive to Trichuris vulpis ($p = 0.002743$). In cats, only average temperature was correlated with the presence of parasitic infection: Toxocara cati ($p = 0.002112$); Aelurostrongylus abstrusus ($p = 0.002563$).

Discussion: This study indicates that climatic factors such as temperature and rainfall are correlated with the presence of certain parasites, in dogs of the Azores islands. The same happens with cats when it comes to the clime temperature.

Detection of Canine Vector-Borne Diseases agents in Galgo Español used as hunting dogs in Alentejo, Portugal

P12

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Agents of CVBD pose a substantial threat to both canine and human health, with some of these having zoonotic potential. The rising prevalence of these diseases has raised them to the level of global concern. Although there have been many research on the occurrence of CVBD in companion dogs, there have been fewer data on hunting dogs who live in close contact with humans and wildlife.

In Portugal, Galgo Español, particularly those used for hare hunting, are frequently exposed to the arthropod vectors of these agents. In order to assess the rate of infection of CVBD agents in this population, a study was conducted involving collection of blood samples from 34 Galgo Español dogs living in Municipality of Évora, Portugal. A questionnaire was also elaborated about the living conditions of the animals, to assess possible risk factors.

Diagnostic techniques used were blood smears, modified Knott's technique, immunochromatography and IFAT. A questionnaire was also performed to assess for possible CVBD risk factors in Galgo Español dogs.

The findings revealed a high incidence of CVBD agents, with 33 of 34 dogs (97.06%), revealing exposure to several agents. Of the 8 male dogs in this study, all had positive results to at least 1 agent as well as 25 of 26 females. Overall R. conorii was the most prevalent pathogen (82%-28/34), followed by R. rickettsii (79%-27/34), A. phagocytophilum (68%-23/34), B. canis (44%-15/34) and E. canis (3% - 1/34). Blood smear examination indicated the following prevalences: Babesia spp. (6% - 2/34), Hepatozoon spp. (6% - 2/34), Mycoplasma spp. (3% - 1/34) and Rickettsiales forms (21%-7/34). No seroprevalence was detected for L. infantum and there were no findings of D. immitis antigens or microfilariae.

The statistical analysis revealed several significant associations between the collected information on each dog and the outcomes of the diagnostic techniques employed. The risk factors identified in the study include the age of the dogs, the type of housing they reside in, the number of dogs sharing living space, the introduction of new dogs into the pack, the type and frequency of deworming practices and the post-training/hunting care provided.

The study's findings emphasize the relevance of identifying the rate of infection of CVBD in various dog populations, as well as the necessity for improved knowledge and dedication to proper prophylaxis to protect hunting dogs and reduce the risk of CVBD. There is also the issue of these agents spreading to other animals, such as wolves, foxes, and even hares in the case of Leishmania. This highlights the importance of comprehensive CVBD prevention efforts for both domestic dogs and wildlife.

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Ticks (Ixodidae) collected on cattle and tick-borne pathogens at traditional housing systems in Huambo, Angola

P13

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Hard ticks (Acari: Ixodidae) are the obligate hematophagous ectoparasites with the highest economic impact infesting cattle. One of the main consequences of tick parasitism is the decrease in body weight gain directly from blood spoliation. However, tick-borne pathogens represent the major causes of losses in cattle production. Many of these pathogens are zoonotic and act as important health threats to farming communities, particularly at tropical regions.

A total of 345 tick specimens were collected from 25 bovines (4 males and 21 females) belonging to different small farmers in Huambo, Angola. Tick specimens were morphologically identified and 137 were processed for genomic DNA extraction and are being screened for the presence of tick-borne pathogens belonging to different genus, namely, Babesia and Theileria (rRNA 18S gene as target) as well as for Anaplasma and Ehrlichia (rRNA 16S gene as target).

The large majority (97%) of the ticks collected belongs to the genus Rhipicephalus, with 75.4% belonging to the sub-genus Boophilus and 22.3% to the sub-genus Rhipicephalus. A small percentage of 1.4% of the specimens collected were identified as Amblyomma and only 0.9% as Hyalomma. Although, molecular pathogen screening is still in process, at the moment were already identified the following species with importance in veterinary medicine, on R. Boophilus specimens: Babesia bigemina (2), Babesia bovis (1), Theileria mutans (5), Theileria velifera (1), anaplasma marginale (3), Anaplasma capra (2) and anaplasma platys (3).

All tick genus identified are reported in Angola, however, to the best of our knowledge this is the first report on B. bovis in Angola, and also the first report on the amplification of A. capra DNA in R. B. decoloratus ticks.

Studies on tick and tick-borne pathogens distribution are crucial to determine vector capacity and ongoing work on our tick collection addressing the diversity of tick-borne pathogens should contribute to strategically implement sustainable control measures to protect both human and animal health.

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Toxoplasma gondii: experimental infection and study of the immune and inflammatory response using different genotypes

P14

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Toxoplasmosis is a zoonosis caused by the obligate intracellular protozoan, Toxoplasma gondii. This microorganism can affect warm-blooded animals (intermediate hosts) and felines (definitive hosts). Systematic genetic characterization of Toxoplasma gondii strains isolated from different hosts and with different geographical origins has revealed high genetic diversity: three clonal lines predominate in Europe and non-clonal lines (also called atypical) predominate in South America. Infection with this parasite is often asymptomatic. Severe clinical symptoms can occur in congenital infections, eye infections, encephalitis in immunosuppressed patients and multivisceral toxoplasmosis (associated with atypical genotypes). A possible relationship between the genotype of Toxoplasma gondii and the pathogenesis of the infection has been postulated.

The objective of this work is to investigate how the genotype of the infecting strain regulates the host's immune response, in particular the inflammatory response. To achieve this, we propose to analyse, in experimental infections of cell lines with different genotypes (I, II and III), some phenotypes associated with inflammation, such as cell proliferation, apoptosis and cell migration, as well as intracellular mechanisms of the host cell against the parasite, more specifically the lysosomal system. The main techniques to be used will be immunocytochemistry, ELISA and microscopy techniques (fluorescence, TEM).

It is expected that the different genotypes will induce a different immune response on the part of the host cell and a different pathology.

Keywords: T. gondii, genotypes, immune response, inflammation, experimental infection

Toxoplasma gondii and Mental Health: Reinforcing the Link

P15

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With worldwide distribution, Toxoplasma gondii is an obligate intracellular parasite capable of infecting humans and other warm-blooded animals. The associated infection - toxoplasmosis - is one of the most common, and it is estimated that approximately one-third of the world's population is chronically infected by this parasite. Given its tendency to form tissue cysts during its latent stage, predominantly in the brain, T. gondii has also been linked to neuropsychiatric disorders, suggesting that latent infection may have subtle neurological effects. T. gondii seropositivity has been associated with several mental disorders, such as schizophrenia, suicidal tendencies, OCD, bipolar disorder, and depression. It has also been linked to other neuropsychiatric diseases such as migraines, epilepsy, Alzheimer's and Parkinson's disease.

As the global concern with and prevalence of mental health disorders grows, we aim to reinforce the link suggested between this parasite and neuropsychiatric disorders by understanding the mechanisms behind these associations. We plan to do so through a seroepidemiologic survey of patients diagnosed with schizophrenia or secondary epilepsy in Portugal. Simultaneously, try to understand the mechanisms that modulate the infection outcomes, specifically, how the parasite genotype influences the expression of neurotransmitters, the host's immune response and the behaviour of infected animal models. Furthermore, the effect of currently prescribed anti-psychotic medications on T. gondii will be evaluated through a cell assay, as some published studies have reported that this could suppress parasite replication and lower anti-Toxoplasma antibody titers.

We expect to observe a higher seroprevalence of T.gondii in neuropsychiatric patients, and different host's immune responses and the behaviours of different T.gondii genotypes.

Keywords: Toxoplasma gondii; mental health; genotypes, infection, animal models

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Overview of Cryptosporidium spp. and Giardia spp. in animals in Portugal in a three-year period

P16

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Cryptosporidium and Giardia are parasitic protozoa that cause intestinal infections in humans and animals, typically transmitted through contaminated food or water. Identifying their presence in Portugal is crucial for preventing water-borne outbreaks and contamination in animal farms. The study aims to clarify their epidemiology in Portugal.

Researchers analysed 1270 faecal samples from 139 animal species between 2021 and 2023, using modified Ziehl-Nelseen stain and direct immunofluorescence assay. The results showed that 15.4% (196/1270) of the samples were positive for at least one parasite. Specifically, 6.1% (78/1270) were positive for Cryptosporidium spp., and 10.1% (128/1270) were positive for Giardia spp.. Only 0.9% (11/1270) had co-infections. Birds and aquatic animals tested negative for both parasites.

Regarding the type of feeding, herbivores had the highest number of positive samples for either parasite, followed by carnivores and omnivores. Giardia spp. and Cryptosporidium spp. were mainly found in mammal species, with 14.6% (125/854) and 7.3% (62/854) positive results, respectively. The reptile samples positive for Giardia spp. (3/277) are believed to be pseudo-parasitism from ingesting contaminated prey. In contrast, 5.8% (16/277) of samples tested positive for Cryptosporidium spp. and were mostly species-specific. Therefore, it is crucial to distinguish between species-specific and non-species-specific Cryptosporidium infections, especially in predatory species like snakes, which can also ingest contaminated prey.

When looking at the role of the species, it becomes clear that Giardia spp. mainly affects farm and domestic animals, with 22.4% (54/241) and 20.2% (18/89) of infections found within these groups, respectively. Wild animals also play an essential role in spreading Giardia spp., with 7.5% (28/374) of positive cases found among them. On the other hand, Cryptosporidium mainly affects farm animals, with 23.7% (57/241) of infections found within this group. Exotic and domestic animals had a lower infection rate, with 5.6% (16/288) and 2.3% (2/89) of infections, respectively. Wild animals were found to have a lower risk of Cryptosporidium infection.

The results indicate that farm animals pose the highest risk of spreading these parasites to the environment, other animals, and humans. Domestic animals are also at risk of Giardia spp. infection, which can be transmitted to their owners. In addition, exotic animals with Cryptosporidium spp. are of concern due to their increasing popularity as companion animals. As some of these animals are predators, it is crucial to accurately identify positive cases, given their resistance to treatment and potential for pseudo-parasitism.

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Searching for Cryptosporidium infection in bats from Brazil

P17

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Cryptosporidium is an obligate enteric protozoan causing cryptosporidiosis in a wide variety of vertebrates being a major cause of death by diarrhea in humans younger than 5 years. Some Cryptosporidium species can infect humans, however, most human infections are caused by C. parvum and C. hominis, with young calves as main zoonotic reservoir of C. parvum causing large economical loss. Wild ungulate can also be a reservoir for C. parvum being a threat for water courses and public health, as well as dogs and cats which are often infected by Cryptosporidium.

In wild, Cryptosporidium has been found in bat feces. The huge diversity, over 1300 bat species associated with the ability to fly long distances and interact with humans and animals makes these animals potential zoonotic reservoirs for viruses, bacteria and protozoa.

In this study, 190 intestine samples collected during bat necropsy were used. Of these, 94 samples were from hematophagous bats and 96 samples from non-hematophagous bats – insectivorous and fruit bats. The Ethics Committee for Animal Use of School of Veterinary Medicine and Zootechny, São Paulo State University, Brazil (protocol number 0259/2022) approved this study. Environmental authorization through the Biodiversity Authorization and Information System (SISBIO) from the Chico Mendes Institute for Biodiversity Conservation (ICMBio), protocol number 85973-1 was also obtained.

DNA was then extracted and quantified. High concentrated samples were diluted in order to obtain samples with DNA concentration between 4 and 100 ng/µg. Pools of 5 samples each were made and used for testing the presence of Cryptosporidium DNA by conventional PCR for the 18S rRNA gene.

All samples tested were negative for Cryptosporidium DNA. Despite the negative results, bats are possible carriers of Cryptosporidium spp and may disseminate oocysts in the environment, a relevant aspect in One Health context.

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Encephalitozoon Cuniculi Seroprevalence and Analytical Trends in the Population of Pet Rabbits Attending FMV-Ulisboa Veterinary Teaching Hospital

P18

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Introduction: Encephalitozoon cuniculi is an ubiquitarian Microsporidian, widespread in rabbit population, considered opportunist to immunosuppressed Humans. In rabbits, the infection can range from no clinical signs to severe eye, kidney, or neurologic disease. Both clinically healthy and affected rabbits shed infectious spores in the urine. The prevalence of infection varies between publications and data from the Portuguese rabbit population is scarce.

In this study, on E. cuniculi in rabbits attending Lisbon's Teaching Hospital/FMV, we have gathered information about seroprevalence, epidemiological data and analytical factors linked to the infection and its severity.

Materials and Methods: Rabbits aged over 6 months, observed from Aug-Nov 2023, were included, regardless clinical status, upon owners written consent and approval by FMV-ULisboa's Research and Teaching Ethics Committee.

A questionnaire was filled, including other pets in contact and the composition of the Human family. Clinical examination was performed and 2 ml of blood were collected from the saphenous vein or the cranial vena cava. Samples were submitted to complete blood count, biochemical analysis and titration of IgG and IgM against E. cuniculi.

Results: Thirty-six rabbits were included in the study, 16 females and 20 males, aged from 6 months to 9 years old and weighting 0,9 - 4,9 kg. Although only 2 animals showed clinical signs of encephalitozoonosis (one neurologic and one ocular disease), 18 were seropositive for E. cuniculi, indicating a seroprevalence of 47% (16/34) in clinically unsuspected rabbits. Eight were IgM and IgG positive and 10 were only IgG positive.

No statistically significative differences were observed between seropositive and negative rabbits. However, some tendencies arise: 11/18 seropositive animals live with other pets, vs 8/18 seronegative. All the rabbits in the household have the same serological status. We have found that 10/36 rabbits lived with children and one with immunosuppressed individuals. Regarding laboratory analysis, 6/18 seropositive animals showed leukocyte counts below 4100 x103/µl, whereas the same was observed in 2/18 seronegative rabbits.

Discussion: A seroprevalence of 50% for E cuniculi agrees with many studies performed with pet rabbits all over the World. The identification of E. cuniculi infection in 47% of the rabbits without clinical signs, that were involved in this survey, could rise a One Health concern regarding exposure to E. cuniculi in children or immunocompromised individuals. Also, the fact that all rabbits in the same household share the same serological status leads to the recommendation of testing rabbits before the introduction of new animals in a group.

The data gathered so far rise the possibility of association between leukopenia and E. cuniculi infection. Ence, low leucocyte counts in rabbits without signs of disease could raise the suspicion of infection and be regarded as an indication for testing.

Invited Communications

Application of differential affinity chromatography coupled to MS and proteomics for the identification of drug targets and for studies on the mode of action of anti-parasitic drugs.

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Toxoplasmosis and neosporosis constitute important challenges for public health, animal production, and animal welfare. While T. gondii also affects humans, and causes abortion and/or fetal malformation upon primary infection during pregnancy and can cause severe disease in immunosuppressed individuals, N. caninum is of economic importance by causing abortion in cattle and other ruminants. So far, only a limited panel of drugs has been marketed for clinical applications against toxoplasmosis, none for neosporosis.

Classical screening approaches using mostly drug repurposing, and investigations on unique targets of the parasite (target-based approaches) have led to the identification of novel drug candidates for the treatment of toxoplasmosis and neosporosis. Especially studies on essential proteins of these two parasites as potential drug targets has fostered the hope of identifying novel and useful compounds. An example of such a target-based approach are bumped kinase inhibitors (BKIs), inhibitors of calcium-dependent protein kinase 1 (CDPK1), which is essential for host cell invasion and egress. However, despite good efficacies in vitro, only a few compound classes were shown to be effective in suitable rodent models or larger animal species. This shows that for both, target-based as well as classical screening-based drug discovery, off-target effects and adverse side effects in the hosts must be considered.

We have applied differential affinity chromatography coupled to MS and proteomics (DAC-MS-proteomics) using a panel of different drug classes such as BKIs and ruthenium-based organometallic compounds, and identified parasite- and host-derived proteins that physically interacted with these drugs. Especially for BKIs we could show that they most likely affect numerous other targets besides CDPK1. However, not all of these drug-binding proteins are actual targets per se, and further characterization using other techniques is required. DAC-MS-proteomics can also inform on potential off-targeting of compounds that might affect host cells or tissues, and we postulate that this could be used to predict adverse events in vivo prior to carrying out in vivo studies, thus avoiding unnecessary animal experimentation. Another proteomics-based strategy that can shed light on the mode of action of drugs is to monitor the protein expression patterns of drug-treated parasites or of drug-resistant parasite strains. This is done by continuous breeding in the presence of increasing drug amounts or by mutagenesis followed by selection of resistant populations or clones in the presence of lethal concentrations of the drug. The resistant strains are then compared to the corresponding non-resistant strains on the genomic, transcriptomic, and/or proteomic levels, and the selective up- or downregulated expression of proteins can inform on relevant physiological changes that occur during drug treatment or resistance formation.

Invited Communications

Applications of omics approaches to the study of host-pathogen interactions in animal diseases: ruminant cyst-forming apicomplexan parasites as an example

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In recent years, the concept of "omics", which refers to the study of various biological molecules such as DNA, RNA, proteins, and metabolites together with the use of high throughput techniques, have profoundly changed biomedical research. The area of "integrated omics" has made possible the combination of various methodologies, allowing the use of comprehensive genome-wide association investigations and techniques to better understand the complexities of host-pathogen interactions and infection dynamics. Understanding such interactions is paramount for further developing of effective control strategies.

Our research group (SALUVET-UCM; https://ucm.es/saluvet) is focused on the pathogenesis of infections by apicomplexan parasites and their ruminant hosts. Specifically, two of them Neospora caninum and Toxoplasma gondii are relevant because they are a frequent cause of reproductive failure in cattle and sheep, respectively. One of our recent aims has been the use of omics to study the factors that determine the outcome of infection. In this abstract, we summarize some studies carried out following this approach.

Neospora caninum is responsible for a worldwide infectious disease that causes reproductive failure in cattle. Despite the substantial economic losses due to bovine neosporosis, very little is still known about the molecular mechanisms triggering abortion and foetal death in cows infected with this parasite. In a recent study, placentas from heifers challenged with the high-virulence isolate Nc-Spain7 exhibited focal necrosis and inflammatory infiltrates as soon as 10 days post-infection (dpi), although parasite detection was minimal. These lesions were more frequent at 20 dpi, coinciding with higher rates of parasite detection and the occurrence of foetal death in some animals. In contrast, such lesions were not observed in placentas from animals infected with the low-virulence isolate Nc-Spain1H, where the parasite was detected only in placenta from one animal at 20 dpi. Whole-transcriptome analyses supports the hypothesis that an intense immune response probably triggered by parasite multiplication could be a key contributor to abortion.

In T. gondii, short-term laboratory (in vitro) adaptation has been demonstrated for canonical type II and III strains, modifying their phenotypic traits in terms of cystogenesis and virulence in mice. Preliminary results showed remarkable changes in gene expressions during the adaptation process including groups of genes associated with the stage of the parasite and others directly related to virulence.

In future studies, novel and powerful techniques such as single-cell RNAseq and long-read next generation sequencing will allow more in-depth studies of all the intimate mechanisms of interaction between these pathogens and their hosts.

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Genomics for sustainability: identifying rare mutations of interest by the exploration of local genetic resources in Portuguese-speaking African countries

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Sustainability is defined by 'the ability to sustain'. Since the last decades, the concept of sustainability was broadened into the concept of sustainable development. In 1972, the first UN conference on Human environment was held and several important documents were produced such as "The limits of growth" and "Blueprint for survival", creating the path for the increase of awareness for the fact that limits exist for the use of natural resources. The domestication of livestock species has been a process through which human populations have selected from an original gene pool of a species, ecotypes, which are the possible combinations of gene forms adapted to an environment. During the 20th century, with the development of animal breeding practices, these ecotypes originated pure lines with defined breeding goals. Thus, resulting in the loss of genetic diversity of livestock species. The combination of sustainability and genomics is a concept of multiple dimensions with the use of complex technology in which, genomic researchers must focus into the translation of their activities into sustainable traits. The exploration of the animal genetic resources that still subsist in remote regions of Portuguese-speaking countries in Africa using genomic approaches presents an opportunity to discover alleles that provide abilities such as feed efficiency, and disease resilience, much appreciated in a context of climate change. Genomics can therefore be the tool for discovery and increase of genetic diversity promoting food security for human populations.

Free oral communications

Interoperability structure for farm data sources and databases to monitor and manage antimicrobial consumption in animal production - the case for USAM SuLei.

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Antimicrobial Resistance (AMR) is a current and escalating major threat to human and animal health. Global response policies are based on Strategic Framework for Collaboration on AMR (WHO, WAHO, FAO, EPA; 2022) and are supposed to be deployed top-down. The European Union (EU) developed a OH strategy for AMR contentions (2016). The Green Deal imposes a target of 50% reduction in AMC before 2030. AM consumption (AMC) per animal body has decreased in the last decade in EU, but human AMC not. Portuguese AMC in animal production remains among the highest in EU. It needs to be reduced. This presentation aims to report USAM SuLei pilot project, designed to develop and implement farm level standardized action plans (AP) coupled with decision support tools, web based, aimed to reduce AMC in pig and in dairy farms. Assumption supporting the project are: 1) improving animal health and welfare and assuring enhanced biosecurity at farm level will ensure a sustainable reduction of AMC and improve economic performance; 2) it exists within and outside the farms enough data registries sparse in different databases to generate comprehensive knowledge to assist enhanced monitoring and decision support tools in the domains above mentioned. This presentation discusses the structure of the web-based platform designed to monitor and support decision making regarding animal health and AMC in farms, addressing specifically the case for dairy farms.

The system is based in three pillars: 1) a farm tailored AP; 2) integrated GXP; 3) web-based system. Particular focus on AMC. Communication infrastructure and information technologies currently available make it possible escalate to new levels data bases interoperability and management making easy, less costly, and faster data use. USAM SuLei involves several partners as data providers and users: farms and assistant veterinarians. Breeders' associations (ANABLE). Authorities like DGAV or IFAP. Data exists in milking systems, unifeed sytems, registries of the herd stock, veterinary drugs prescription and application. Outside farms is the ANABLE database (Bovinfor) recording production and genetic data at cow level, official Eletronic Prescription (PEMV). Data gathering tools to collect such data are necessary, preferably based on webservices, computers or electronic portable devices used at farms. Standardized data and information collection is therefore possible. The case for the dairy farms, contemplates five animal phases. Data from nutrition and feeding, milking, environment, animal behavior and pathologies are collected. Prescription and treatment information (cow level) is recorded. Data cleaning, transforming, and treating is the intermediary step before data reporting is produced. Data and information are collected and shared among users according to their needs. Reporting about cost and efficiency-based performance data structure is offered through standard or customized interactive boards.

Environmental Contamination and Staff Colonization by Carbapenem-Resistant Gram-Negative Bacteria in Small Animal Veterinary Practices

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Background: Small animal veterinary practices (SAVPs) face the same challenges as human healthcare facilities regarding the dissemination of multidrug-resistant (MDR) bacteria. The aim of this study was to determine the prevalence of MDR bacteria in four SAVPs from the same business group (SAVP-A, SAVP-B, SAVP-C and SAVP-P).

Methods: Environmental swab samples from critical surfaces were collected each SAVP. Nasal (n=37) and rectal swabs (n=29) were voluntarily obtained from staff. Samples were cultured in selective agar plates for the detection of beta-lactam resistance and methicillin resistance. Gram-negative isolates were screened by PCR for the presence of beta-lactamases. WGS was performed for carbapenem-resistant strains.

Results: A total of 144 environmental samples were collected, of which 44.4% (n=64) were positive for growth of Gram positive and Gram negative bacteria. Of these, 11% (n=7) had growth in selective media for carbapenem-resistant bacteria. Stenotrophomonas maltophila (ST39, ST5 and the newly typed ST967) were identified in one sink from the consultation room in SAVP-B and two sinks from different consultation rooms in SAVP-C. This species is intrinsically resistant to different antibiotic classes, including carbapenems and common in human hospitals environment. Furthermore, Pseudomonas aeruginosa ST267 was identified in the sink of SAVP-A. It had a mutation on porin channel OprD (S278P), which confers resistance to imipenem (MIC> 8 mg/mL)1. Four rectal swabs (14%) also tested positive for S. maltophila (n=2) (ST317 and ST84) and P. aeruginosa (n=2) (ST244 and ST274). P. aeruginosa ST274 showed several mutation on OprD, together with mutation on nalC (G71E) of the MexAB-OprM efflux pump, making it resistant to imipenem (MIC> 8 mg/mL) and susceptible to increase exposure to meropenem (MIC= 8 mg/mL). Fourteen individuals (37.8%) harboured methicillin-resistant Staphylococcus spp. in their noses, of which four individuals were positive for methicillin-resistant Staphylococcus aureus. Additionally, one nasal swab tested positive for P. aeruginosa ST244.

Conclusions: The finding of carbapenem-resistant isolates on critical surfaces of SAVPs and their working force, highlights the need for implementing infection, prevention and control (IPC) guidelines tailored to Veterinary Medicine.

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Carriage of Carbapenemase and ESBL-producing Escherichia coli in dogs and cats in veterinary care and co-carriage with their owners

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Background: Companion animals contribute to the maintenance and dissemination of clinically important antimicrobial-resistant pathogens. The aim of this study was to evaluate the possible sharing of ESBL/AmpC- and carbapenemase-producing Escherichia coli between animals under antibiotic treatment and their human household members.

Methods: A prospective longitudinal study was performed from 2018 to 2021 in Portugal and the UK. Faecal samples and nasal swabs from dogs and cats with skin and soft tissue or urinary tract infections, and their cohabiting humans, were inoculated on MacConkey agar plates supplemented with 1.5 µg/mL cefotaxime and 1.0 µg/mL meropenem. Bacterial species and the presence of beta-lactamase genes were confirmed by PCR. Antimicrobial susceptibility was assessed by microdilution testing with MicroScan® Neg MIC Panel Type 44 (Siemens, US) and results interpreted according to EUCAST. Isolates' clonality was assessed by rep-PCR. WGS (Illumina NovaSeq), producing pair-end libraries (150 bp), was performed for animal/owner-derived paired strains with the same rep-PCR pattern to estimate genetic relatedness.

Results: In Portuguese households, one dog (2.3%, n=43) was colonised by a multidrug-resistant OXA-181-producing extraintestinal pathogenic Escherichia coli. Twenty-one companion animals (48.8%, n=43) and 22 humans (28.2%, n=78) harboured ESBL-producing E. coli in at least one timepoint.

In UK households, one dog (12.5%, n=8) was colonised by a multidrug-resistant E. coli co-producing NDM-5 and CTX-M-15 beta-lactamases. ESBL-producing E. coli's were isolated from six animals (75%, n=8) and one human (11.1%, n=9).

Identical rep-PCR pattern was observed for animal/owner paired strains from five Portuguese (11.6%) and two UK household (25%). WGS SNPs analysis confirmed longitudinal sharing of E. coli strains in all households.

Conclusions: This study provides evidence of companion animal-human sharing of ESBL- and/or carriage of carbapenemase producing Enterobacterales within households. These results emphasize the importance of the companion animal-human unit in any efforts to mitigate the spread of antimicrobial resistance and further highlights the value of a One Health approach, integrating human and animal health.

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Exploring trends of resistance of Klebsiella spp. in companion animal infections: a three-year study

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The emergence of hypervirulent Klebsiella spp. exhibiting both invasive capacity and multidrug resistance, poses a growing concern in Human and Animal Health, especially considering the more limited therapeutic choices available for treatment of infections in Veterinary Medicine. This retrospective study spanning January 2020 to June 2023 explores the trends in Klebsiella spp. resistance in companion animal (CA) infections.

Clinical samples from CA infections were cultured on standard and ESBL-selective media, with a total of 4275 samples being identified as Enterobacterales and 906 (21.2%) presenting as ESBL-producing Enterobacterales (ESBL-E). For all Enterobacterales positive-samples, antimicrobial susceptibility was performed, following CLSI and EUCAST guidelines, covering beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines, and folate pathway inhibitors.

While the most prevalent isolated species was Escherichia coli with 2714 total isolates (63.5% of Enterobacterales), Klebsiella spp. isolates (12.6% of Enterobacterales, n=537) were comparatively a much higher proportion of the ESBL-E isolates (53,4%, n=287) versus ESBL-producing E. coli (12.0%, n=325). Of the ESBL-producing Klebsiella spp. infections, 41.5% were UTI and 58.5% were non-UTI. Invasive infections by ESBL-producing Klebsiella spp. fluctuated, with an increase from 2020 (9.8%, n=5) to 2021 (21.3%, n=13), decrease in 2022 (11.7%, n=11) and new increase in the 2023 isolates (18.5%, n=15), despite only encompassing half of the year so far. ESBL-E percentages across the years remained relatively stable.

Regarding susceptibility to the other four antimicrobial classes for Klebsiella spp., prevalence of resistance was mostly over 47% for all studied classes over the course of the study, with an average of 49.4% isolates resistant to fluoroquinolones, 42.4% resistant to aminoglycosides, 52.1% to tetracyclines and 53.7% to folate pathway inhibitors, using as markers, respectively for each class, enrofloxacin, gentamicin and amikacin, tetracycline, and trimethoprim-sulfamethoxazole. Interestingly, although the proportion of Klebsiella spp. resistant to each class marker was generally higher in comparison with E. coli, for these four antimicrobial classes resistance seems to be falling over the three and a half years, requiring further studies to assess the causes behind this effect.

Although ESBL-E numbers presented little variation across the years, the relatively high percentage of ESBL-producers in some genus, such as Klebsiella spp., pose not only a challenge regarding therapeutic choices but may also signify an increase in more virulent, potentially untreatable infections, as the prevalence of resistance to not only first-line antimicrobials in veterinary medicine like trimethoprim-sulfamethoxazole, but also critically important antimicrobials for human medicine such as fluoroquinolones seems to be generally higher in Klebsiella spp. than Enterobacterales overall.

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Biological and genetic characterization of Echinococcus implications for dynamics of transmission

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The Echinococcus granulosus complex exhibits high intra-specific genetic variability, which has implications for dynamics of parasite transmission and pathogenesis. Based on official records of notifiable diseases in Portugal, human hydatidosis presents a dual distribution in Portugal: incidence rates higher to south of the Tagus River, with epicentre of the disease in the municipality of Évora, and lower incidence north of the river. Earlier reports implicated E. granulosus s. s. genotype G1 as the predominant form. Here we describe a study of 53 cystic hydatid samples, most of which were collected at sites north of the Tagus River. The findings clarify our understanding of the hitherto reported dual distribution of hydatidosis in Portugal. In addition, they suggesta a possible role of Echinococcus canadensis in human infections.

Encephalitis Project

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Infections caused by FLA - free-living amoebae are usually included in Emergent and Neglected Diseases. Encephalitis is the main clinical features of FLA infections. The amoebic encephalitis are of concern, since mortality is high and children are often victims. The high mortality is in large part the result of clinicians' lack of familiarity with these amoebic diseases, delay in diagnosis, and the lack of optimal antimicrobial therapy. It can be assumed that many more cases occur on a global scale in humans as well as animals that go undiagnosed and unreported.

Why are there so few cases of balamuthiasis when human contact with soil is inescapable? It is worth noting that the FLA diseases have been recognized only within the past 40 years. Retrospective diagnoses have been made from pathology specimens and hospital records of cases going back to the start of the 20th century; thus, one might wonder why it took so long to identify these diseases. Human progress has facilitated their occurrence in the population; i) the popularity of contact lenses; ii) availability of immunosuppressive drugs that enable organ transplantation; iii) medications that prolong human life to a point at which the immune system is less able to deal with amoebic and other infections; iv) energy saving measures that seal off the interior of a building making the occupants vulnerable to amoebic endocytobionts in the ventilation system. Portugal can be a representative country of this scenario.

The first clinical case of Balamuthia Amoebic Encephalitis (BAE) in Portugal was described in 2006. Later, more cases of Balamuthia mandrillaris and Acanthamoeba sp. infections in humans have been detected, retrospectively, by the study of unexplained cases of encephalitis.

Balamuthia encephalitis is a rare clinical occurrence with a dramatic clinical outcome in the absence of a specific treatment. Moreover, clinicians and veterinarians in Portugal are not aware of the characteristic profile of disease presentation. We hypothesize that the majority of the BAE cases remains identified as "encephalitis of unknown origin" and possibly, they can be much more important than expected in Portugal. So, learning with the experience of Frederick L. Schuster et al., and the California Encephalitis Project, we present this program aiming: (1) to identify and evaluate cases of encephalitis due to Balamuthia Acanthamoeba and Naegleria species and (2) to make clinicians and laboratorians aware of the signs and symptoms of FLA infections. It is expected also, by the present program, to promote among professionals in Portugal the knowledge about the diagnosis of the Free-Living Amoebas including: isolation from environmental samples (soil and water) or human or animal tissues, axenic culture, direct diagnostic after staining, serology and PCR.

SESSION: Disease surveillance|
Chair: Luis Ortega-Mora, SALUVET U Complutense Madrid; Yolanda Vaz, DGAV Lisboa

Data-driven early disease detection: automated workflows and decision-support in animal health surveillance

Keynote speaker: Fernanda C. Dórea | Global Project Coordinator, FAO & National Veterinary Institute (SVA), Sweden

Invited Communications

Little Stories About Increasing Quantities of Big Data: A Data Analyst's Perspective

Paulo Jorge Nogueira

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CIDNUR—Centro de Investigação, Inovação e Desenvolvimento em Enfermagem de Lisboa, Escola Superior de Enfermagem

In my 30-year career as a statistician, epidemiologist, and information specialist, I have observed a significant evolution in data analysis. While my earlier work predominantly involved small data sets, I have increasingly encountered and navigated the complex realm of big data. This presentation shares personal anecdotes and insights, illustrating the unique challenges and enduring issues in utilizing big data, and highlighting how these challenges have evolved yet remained consistent over the decades.

Invited Communications

Detection and quantification of excessive mortality events in Portuguese bovine populations

Telmo P. Nunes

Faculdade de Medicina Veterinária, Universidade de Lisboa

Estimating Lagged Risks of Temperature Exposure: Insights from Hospital Admissions for Infectious and Parasitic Diseases

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Background: The intersection of climate change and infectious diseases is a growing public health concern. Approximately 60% of infectious diseases in humans are zoonoses, with three-quarters of emerging diseases originating from animals. With global temperatures rising, there's an altered geographical distribution of climate-sensitive diseases, which emphasizes the need for encompassing early warning systems based on a One Health approach, i.e., integrating human, animal, and environmental health.

Methods: This study analyzed daily maximum temperature data from the extended summer months (May to September), between 2000 and 2018 in the Lisbon district, obtained from an European-level gridded observations dataset. Human hospital admissions for infectious and parasitic diseases were sourced from the Hospital Morbidity Database, using Major Diagnostic Categories (MDC 18). Population data was acquired from Statistics Portugal. Employing a quasi-Poisson regression, we constructed a distributed lag non-linear model to investigate the lagged impact of temperature exposure on hospital admissions, with a focus on a maximum lag of 10 days. This model allowed for estimating a non-linear exposure-response relationship and assessing non-linear lag effects.

Results: Our findings indicate an overall 16% significant increase in risk of hospital admissions due to infectious and parasitic diseases associated with extreme temperatures (at the 99th percentile temperature of 38ºC). Notably, lags 2, 6, 7 and 8 days post-temperature peaks showed a substantial risk increase (55%, 19%, 22% and 14% respectively), while a decreased risk of 46% was observed at lag 1.

Implications: These results highlight the urgent need for early warning systems that use daily maximum temperature data to predict human hospital admissions for infectious and parasitic diseases. By extending this approach to animal health data, we can further assess the risks to animal populations, paving the way for a comprehensive One Health early warning system.

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Feline Immunodeficiency Virus Hospitalization: Insights from a Comprehensive Epidemiological Database using Predictive Modelling

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Feline Immunodeficiency Virus (FIV) is endemic in cat populations worldwide, transmitted mainly through bite wounds, often leading to the development of immunodeficiency, neoplasia, and opportunistic infections. The Biological Isolation and Containment Unit (BICU) at the Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon, specializes in the hospitalization of animals suspected or confirmed with infectious diseases. This study aimed to identify FIV risk factors in hospitalized cats to improve early triage and optimize the clinical management of these patients.

A case-control study using data from 134 FIV-infected cats and matched controls was conducted. First, a simple regression analysis investigated potential risk factors: breed, age group, sex and neuter status, lifestyle, presence of cohabitants' cats and concomitants disorders, classification of leukocytes, and haematocrit. The following variables with a p-value <0.05 were included in a multiple logistic regression model: breed, age group, sex and neuter status, lifestyle and concomitant disorders. To enhance the model's robustness and mitigate the risk of overfitting, a 5-fold cross-validation was done. The discriminative abilities of both the initial and cross-validated models were quantified by calculating the Area Under the Receiver Operating Characteristic (AUC) curve, along with their respective sensitivity and specificity.

Adult and senior cats (≥ 2 & < 10 and ≥ 10 years), intact male cats with outdoor access and with concomitant disorders were found at a high risk of hospitalization with FIV ($p<0.05$). The final logistic regression model without cross-validation had an estimated AUC of 0.71. The sensitivity and specificity estimates were respectively 0.63 and 0.69. When employing cross-validation techniques, the model's performance varied, however, the same risk factors remained. With 5-fold cross-validation, the AUC slightly decreased to 0.69, with a sensitivity of 0.86 and a specificity of 0.32.

This study identified that intact male cats, with outdoor access, presenting concomitant disorders, and being adult and senior were key risk factors for being hospitalized with FIV. Our multiple logistic regression model, reinforced by 5-fold cross-validation, provided moderate predictive accuracy with an AUC of 0.69 post-validation, reinforcing the value of cross-validation in the reliability of epidemiological models. Furthermore, these findings can significantly aid clinicians in formulating pointed questions during anamnesis. Recognizing these risk factors enables veterinarians to more swiftly suspect of FIV infection leading to an earlier diagnosis and overall better management.

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C Reactive Protein/Albumin Ratio as a SRIS Indicator in Dogs with Parvovirus

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Parvovirus is a highly contagious infectious disease that compromises the intestinal barrier, allowing bacteria and toxins to enter the bloodstream. Animals with Parvovirosis are therefore more susceptible to develop secondary infections and Systemic Inflammatory Response Syndrome (SIRS). The C-Reactive Protein/ Albumin ratio (CAR) is positively correlated with inflammation, proving to be more sensitive than the use of C-Reactive Protein (CRP) and/or albumin alone.

The objective of this study was to identify a specific CAR value that could serve as an indicator for SIRS in dogs infected with parvovirus. Additionally, the study aimed to assess the potential of this value as a diagnostic, monitoring, and prognostic tool in the context of the disease.

Blood samples from 45 dogs with confirmed parvovirosis were analysed using the Catalyst CRP Test (IDEXX®). The albumin values utilized in this study were obtained from the animal's analytical assessment conducted 48 hours after their admission to the hospital. The animals were categorized into two groups: those exhibiting SIRS and those not showing SIRS, based on their CRP (C-reactive protein) values within the range provided by IDEXX.Statistical analyses, including ROC curve analysis performed in R®, were used to determine the CAR value.

The study showed a positive correlation between CRP levels and the presence of Systemic Inflammatory Response Syndrome (SIRS) with a correlation coefficient of p=0.30. The calculated CAR value was 1.6. With a sensitivity of 93.5% and specificity of 85.7%, this value indicates that a dog diagnosed with parvovirus is likely experiencing SIRS.

The results of this study indicate that CAR can be a valuable addition to the analytical panel for diagnosing parvovirus cases. This ratio has the potential to function as an early diagnostic tool, aiding in monitoring treatment progress, and offering prognostic insights into disease outcomes.

Keywords: Parvovirus, Systemic Inflammatory Response Syndrome, C Reactive Protein/ Albumin Ratio

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Evaluation of SARS-CoV-2 susceptibility in cats: serological survey for antibodies against the virus

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Introduction: The emergence of SARS-CoV-2 and the subsequent outbreak of COVID-19, sparked an unprecedented global health and economic crisis. Since then, antibodies against SARS-CoV-2 have been detected in several species. Cats have been a subject of particular concern because of their close contact with humans and their high susceptibility to SARS-CoV-2. According to published studies, cats exhibited rapid recovery and a low incidence of severe disease, suggesting the development of a potent immune response against the virus. In this study, we aimed to characterize the humoral immune response of cats to SARS-CoV-2 to develop potent and broadly neutralizing antibody fragments for treatment and prevention of COVID-19 disease.

Materials and Methods: A biobank of serum samples was constructed from cats admitted to the Veterinary School Hospital (HEV) of FMV-ULisboa. These samples were tested against SARS-CoV-2 Spike-S and RBD proteins from alpha, delta and omicron variants by ELISA (Enzyme-Linked Immunosorbent Assay). Positive samples were subjected to a Surrogate Virus Neutralization Test (sVNT). Finally, serum samples with high neutralizing activity were tested in an infection assay against viral particles pseudotyped with the S protein from different SARS-CoV-2 variants.

Results: Of the 614 cat serum samples collected, 97 (15.8%) tested positive in the ELISA assay. These 97 samples were tested in the sVNT and 21 (21.6%) presented neutralizing antibodies. Four serum samples were identified as simultaneously having neutralizing antibodies against alpha, delta and omicron variants, even before the omicron variant was detected in Portugal. The serum samples highlighted in the ELISA and sVNT assays as having a higher antibody titer showed a greater ability to inhibit SARS-CoV-2 infection in pseudotyped virus assays.

Conclusions and Discussion: Our study attests to the susceptibility of cats to SARS-CoV-2 infection and the development of potent antibodies against the virus in these animals. Based on our results, cats naturally infected with SARS-CoV-2 are a promising source for the development of a new generation of antibodies against COVID-19. This novel therapeutic strategy will be a valuable tool not only for humans, but also for the broad spectrum of susceptible species, particularly feline populations. Furthermore, this study demonstrates the potential of cats in comparative and translational studies of SARS-CoV-2 to provide valuable insights into virus transmission, immune response, and potential treatments. Our results highlight the importance of implementing a one-health approach to control COVID-19 and future zoonotic threats.

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Unravelling (new) neurotropic viruses - AL4animals Project Outline

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The emergence of new viruses as human pathogens is a reality sponsored by globalization, climate change, alterations in animal and livestock habitats and the growing numbers of immunocompromised people. However, in several clinical syndromes in which viruses play an important role, as in neurotropic infections, the conventional diagnostic techniques fail in uncovering the etiologic agent. Diagnostic tests are limited by their sensitivity and, in many cases, by the need for a priori knowledge of the target viruses for PCR, leading to the fact that in a significant percentage of reported febrile syndromes, the etiologic agent remains unknown. Metagenomic Next-Generation Sequencing (mNGS) methodology is ideal to address these challenges and has been successfully employed in the discovery of novel viruses, including those present at low concentrations, as in cerebrospinal fluid samples and in neuroinvasive infections. There is no concrete estimation in Portugal, but at the National Reference Laboratory (NRL, INSA), the diagnostic requests of CNS infections are about 1000 analysis/year being more than 60% negative for all neurotropic viruses tested. Moreover, working in the NRL for vector-borne diseases we are aware that most of the undiagnosed cases are never tested for arthropod- and rodent-borne viruses. This group of viruses includes some of the most relevant for public health in the Mediterranean basin, such as Flaviviruses (West Nile, WNV and Tick-borne encephalitis, TBEV), Phleboviruses (Toscana virus, TOSV) and Arenaviruses (Lymphocytic choriomeningitis virus, LCMV). Moreover, in surveillance studies in vector species we proved the presence of other potentially neuropathogenic RNA viruses in Portugal, as Alcube and several Massilia phlebovirus (as Arrabida virus).

By an One-Health approach, potential emerging neurotropic viruses are being screened by RT-PCR using primers for general viruses group (e.g. pan-flavivirus) in unspecified clinical human specimens of neuroinfections and in animal samples (with compatible symptoms or potential hosts and reservoirs of these viruses). Sera samples of animal host species for known neurotropic viruses (as horses for WNV) are also being analysed for the presence of specific virus antibodies. Positive samples by RT-PCR and undiganosed samples with confirmed CNS clinical signs will be analysed through mNGS. Preliminary results regarding the ongoing analysis will be presented.

With this project we aim to identify novel neurotropic viruses contributing to the scientific knowledge about neuroinvase pathogens, and to the clarification of the epidemiological dynamics and the impact of these infections in Public and Veterinary Health in Portugal. The goal is to provide a real improvement in neurotropic viral infections diagnostics in order to better respond to the clinicians and patients' expectations.

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Examination of a herd experiencing Neospora caninum-associated abortion by a mouse monoclonal antibody-based competitive ELISA

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Neospora caninum is a cyst-forming coccidian parasite which primarily infects cattle and dogs. A major issue in the validation of diagnostic tests for N. caninum is the absence of a true "gold standard" test. A combination of different techniques (using the result of the majority of tests) is frequently used to overcome this problem and differentiate infected from non-infected animals. Alternatively, methods of latent class analysis (such as the Bayesian latent class analysis (BLCA)) can be applied to evaluate the accuracy of diagnostic tests when only an imperfect gold standard is available. Monoclonal antibody (mAb)-based competitive inhibition ELISAs (cELISAs) can potentially improve the specificity of serological techniques and additionally detect antibodies in multiple species without species-specific conjugates. In the present study, we aimed to develop a sensitive and specific cELISA for detecting bovine antibodies against N. caninum using a mAb (Nc-cELISA3.10.5). After validation, the suitability of novel Nc-cELISA3.10.5 to examine a herd with abortion problems was investigated.

Relative to a reference standard, which combined the results obtained by the reference serological tests (majority), the novel Nc-cELISA3.10.5 showed a sensitivity of 98.1% (95% CI: 95.1%- 99.5%) and specificity of 97.7% (95% CI: 95.8% - 99.9%). The second method to define the diagnostic characteristics of the Nc-cELISA3.10.5 using the Gibbs sampler revealed a diagnostic sensitivity of 98.5% (95% CI: 96.5%- 99.9%) and a diagnostic specificity of 98.4% (95% CI: 97.1% - 99.4%) when a cut-off which maximized the Youden's index was applied. In addition, we simulated additional cut-off points that could be utilized for diverse purposes. The cELISA3.10.5 was used to analyse cattle sera from a herd with suspected N. caninum-associated abortions and determine whether these abortions could be attributed to neosporosis. The predominant pattern of abortion and the possible sources of infection were investigated.

In conclusion, we developed and validated a novel Nc-cELISA3.10.5 which may constitute a valuable tool for the diagnosis of N. caninum infections, with the potential to be applied in different host species, and besides the contribution for the perception of the problem, may aid the veterinarians on the identification of the adequate control measures at the herd level or regional level.

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Immunomagnetic separation method for identification of Toxoplasma gondii oocysts in environmental samples

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Toxoplasmosis is the most reported parasitic zoonosis in Europe. Toxoplasma gondii is the third most important contributor to health burden caused by foodborne illness. Ingestion of tissue cysts from undercooked meat is an important source of horizontal transmission to humans. However, there is an increasingly awareness for the consumption of water, fresh vegetables and fruits, as a possible source for oocysts transmission. Detection of Toxoplasma oocysts is complex, and no standardized methods are available. The approach followed was based on our own laboratorial experience with Method 1623.1/EPA for Cryptosporidium oocysts and Giardia cysts. This led us to the hypothesis that a similar method for T. gondii oocysts detection could be developed. A major problem was present: the need for antibodies specific for T. gondii oocysts. T. gondii oocyst wall protein 1 (TgOWP1) integrates a family of proteins, consensually assumed as specific antigens of T. gondii oocyst stage, located in the outer layer of the oocyst wall. A recombinant antigen, rTgOWP1-f was expressed derived from a fragment selected on basis of its structural homology with Plasmodium MSP1-19. Polyclonal antibodies anti-rTgOWP1-f evidence ability for specific identification of environmental T. gondii oocysts. We assume, rTgOWP1-f, as a possible biomarker of oocysts, and developed an immunofluorescence assay for T. gondii oocysts identification. Based on that, a immunomagnetic separation method has being developed, similar to method 1623.1/EPA, using a chicken polyclonal antibody anti-rTgOWP1-f. We believe that such method is able to be standardized, and with potential to become the gold standard method for detection of T. gondii oocysts in environmental samples.

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Toxoplasma gondii detection in pre-harvested vegetables in the North of Portugal

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Fresh produce such as vegetables, might be contaminated with human parasites such as oocysts of Toxoplama gondii. Toxoplasmosis could have severe consequences in women shortly before or during pregnancy and, in people with compromised immune system.

In this work, fresh vegetables such as lettuce and spinach with no previous washing, from 20 farmers from the North of Portugal, were analysed for the presence of Toxoplasma gondii, in order to understand if this parasite might be, specifically a hazard for vegetables in the pre-harvest of these farmers and consequently a potential risk to the consumer.

Toxoplasma gondii oocysts were recovered from vegetables' samples after washing the samples, filtration, and immunomagnetic separation, by combining the knowledge gained with Method 1623.1/EPA for the detection of the Cryptosporidium oocysts and Giardia cysts. Toxoplasma gondii detection was performed after DNA extraction and conventional PCR using specific primers for a 183-bp sequence of the T. gondii repetitive DNA region. Positive DNA samples purified and sequenced. The presence of the parasite was observed by fluorescent microscopy, taking advantage of the oocysts autofluorescence under UV light.

Three of the farmer's produce were positive for Toxoplasma gondii, which indicates that pre-harvest cultures might be contaminated with this parasite, and which highlights the need for a proper vegetables' washing process before eating.

Uncovering Zoonotic Toxoplasma Gondii Genetic Diversity and its Implication on Toxoplasmosis Pathogenesis – A One Health Approach (Al4animals Project Management Framework)

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Toxoplasmosis is a global zoonosis with devastating and costly health impacts for humans, domestic and wild fauna. Protozoan Toxoplasma gondii infects nearly one-third of the world's human population and can infect potentially all homeothermic vertebrates, causing reproductive losses in ruminants across the world.

The main way of transmission is associated with consuming raw and undercooked meat or other animal products containing T. gondii cysts. Ingestion of water, vegetables and fruits contaminated with sporulated oocysts is also of importance.

T. gondii was initially categorized into three main clonal types (I, II, and III). Each of these canonical T. gondii lineages has different patterns of virulence, as empirically determined in mice and supported by restriction length polymorphisms, isoenzyme analysis, large-scale genome sequencing and transcriptomics. However, T. gondii strains have now been categorized into more than 200 different genotypes using PCR-RFLP or into 16 haplogroups using whole-genome sequencing, with haplogroups 1 to 3 corresponding to the three main clonal types and haplogroups 4 to 16 to atypical genotypes.

The impact of the parasite strain on pathogenesis of toxoplasmosis in humans and animals has not yet been fully understood. Importantly, strain-specific differences in how T. gondii regulates host's immunity, transcriptome and inflammasome, could also be responsible for, at least in part, the distinct pathogenicity caused by these parasites during infection and it is an essential issue to address.

Therefore, the overall aim of this proposal is to analyse potential markers responsible for parasite virulence and host resistance/susceptibility to Portuguese strain isolates, using in vitro and in vivo models. A high resolution genetic tool for a deeper characterization of T. gondii and its evasion mechanisms is also an expected outcome of this proposal. Some preliminary results regarding the ongoing work will be presented.

Overall, the obtained data could contribute to the development of specific tools to control the transmission of the parasite based on the unique genetic features of each strain and host and, ultimately, help to prevent disease both in humans and livestock centered on the global missions of the ONE HEALTH Development Initiative.

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