4th INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

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Bari, Italy

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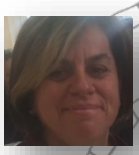

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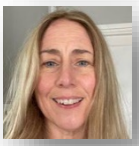

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PROF. BRYAN MARKEY
BRYAN MARKEY IS ASSOCIATE PROFESSOR OF VETERINARY MICROBIOLOGY AT THE DUBLIN SCHOOL OF VETERINARY MEDICINE. HE IS HEAD OF THE SECTION OF VETERINARY PATHOBIOLOGY AND A MEMBER OF THE SCHOOL EXECUTIVE

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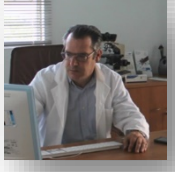
DEPARTMENT OF PATHOBIOLOGY, PHARMACOLOGY AND ZOOLOGICAL MEDICINE

LUCA GUARDABASSI
DVM, PHD, PROFESSOR IN ONE HEALTH AMR AT THE UNIVERSITY OF COPENHAGEN



DR. ANTONIO MARTÍNEZ-MURCIA
PROFESSOR OF MICROBIOLOGY, MIGUEL HERNÁNDEZ UNIVERSITY

Invited Speaker

THE ASF VACCINE IS CLOSER THAN EVER

J. M. Sanchez- Vizcaino
Complutense University of Madrid, Spain

J. M. Sanchez- Vizcaino

ORIGIN AND STUDIES:
Born in Murcia, Spain. He received a bachelor's degree and a doctorate (DVM and Ph.D) from the Faculty of Veterinary Medicine of the Complutense University of Madrid (UCM), subsequently completing postgraduate studies in animal immunology and virology at Cornell University in New York.
PROFESSIONAL EXPERIENCE:
Upon his return to Spain, he joined the National Institute for Agricultural and Food Research (INIA), where he entered as a researcher in 1978 and developed a large part of his professional career as a researcher and director of important departments and centers, such as: Director of the Department of Animal Virology (ASF eradication program in Spain), Director of the Department of Animal Health (control and eradication of ASF and African horse sickness) and Founding Director of the High Biological Safety Center (BSL3 and BSL3+) for Animal Health Research (CISA) Fight against classical and African swine fever, horse sickness, PRRS, BSE, etc. until 2002 when he joined the UCM as Professor.
Since 2002 he has been Full Professor of Animal Health at the Complutense University of Madrid, carrying out his teaching and research work in the Department of Animal Health of the Faculty of Veterinary Medicine and in the VISAVET Center of the UCM, as well as director of the reference laboratory of the World Organization for Animal Health (OIE) for African Swine Fever and African Horse Sickness.
The scientific contributions of Professor Sánchez-Vizcaíno have contributed significantly to the control and eradication of several animal diseases on four continents, including African swine fever, African horse sickness and classical swine fever. All this, thanks to the development of new rapid and sensitive diagnostic methods and reagents, new strategies and epidemiological models, and the development of vaccines. He is currently de coordinator of the EU VACDIVA project: A safe, DIVA and protected vaccine for ASF in wild boar and domestic pigs.
He has given different training courses and seminars on the early detection of infectious diseases, diagnosis of viral diseases and their control and eradication, in several countries on the five continents.
He being appointed since the year 2021 as Emeritus Professor of Animal Health at the UCM.
ACHIEVEMENTS AND PUBLICATIONS:
He has more than 260 scientific publications in high-impact international journals, as well as being the author of 47 chapters in internationally prestigious books (African swine fever in Diseases of swine, African horse sickness in the OIE manual, Trends in Emerging Viral Infections of Swine.) and digital courses dedicated to immunology, infectious diseases, and health simulations.
He has directed more than 150 national and international research projects and 37 doctoral theses on animal infectious diseases, has participated in 80 R&D contracts, in 10 patents and has participated as a speaker in more than 500 congresses, conferences, courses and interviews. (TV, radio, press, video, web) all over the world.
AWARDS AND SCIENTIFIC DISTINCTIONS:
He has several decorations and appointments, among which we highlight: the Commendation of Number of the Order of Agrarian Merit (03/12/99) and of Food Merit (05/05/03), the Cross of Military Merit with white badge (03 /01/03), all for his contributions to animal health and the control of infectious diseases. He received the ANAPORC 1990 national pig research award, the "PORCO BRAVO 1999" International Pork Award, the 2000 First Prize in Pork Health and Production, the 2007 Animal Health Award "The Best of La Verdad 2007" and the 2012 Albéitar Awards in the Scientific Category, as recognition for his contribution to the scientific development of research in Veterinary Science, SEPOR 2019 RESEARCH Award for his lifelong dedication to research in Animal Health and the achievements made.
He has been awarded the Medal of Merit from the World Organization for Animal Health (OIE), in international recognition of his exceptional services to veterinary science (Paris 05/24/09), and has been awarded Doctor Honoris Causa by the University of Murcia (Murcia 04/22/10), as well as named 2013 George C. Poppensiek Visiting Professor in Global Animal Health by Cornell University (Ithaca 09/2013) and Honorary Professor (Adjunct Professor) of the University of Minnesota (since September of 2018).
CURRENT SCIENTIFIC INTEREST
His scientific interest focuses on the study of the epidemiology and preventive medicine of animal infectious diseases, as well as the development of new diagnostic techniques, new generation vaccines and models and new strategies for their control. He is currently PI of several research projects, among which we highlight the European VACDIVA, "H2020-SFS-2019-1, theme: A vaccine against African Swine Fever" endowed with 10 million Euros and the project, financed by the Institute National Health Department, titled the role of COVID 19 in pets.

Invited Speaker

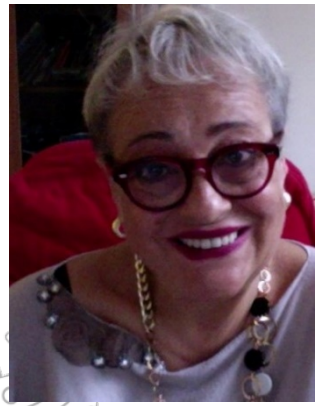
MALASSEZIA YEAST: AN EMERGING PROBLEM IN MEDICAL AND VETERINARY MEDICINE

C. Cafarchia

Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy

Malassezia spp. organisms are lipid-dependent yeasts, inhabiting the skin and mucosa of humans and animals. They are involved in a variety of skin disorders in humans and animals and may cause bloodstream infections in severely immunocompromised patients. Despite a tremendous increase in scientific knowledge of these yeasts during the last two decades, the epidemiology of *Malassezia* spp. infections and fungemia in animals and humans remains largely underestimated. In addition, the pathogenic role of these yeasts species have been suggested only for strains causing skin infections. Since multiple *Malassezia* spp. and/or genotypes with varying antifungal profiles may cause unique or similar pathologies, serious concern about the diagnostic procedures and antifungal treatment have been raised. Despite the attempt to treat these fungal infections, a trend toward the recurrence is often observed in humans and animals with dermatitis. Moreover, the clinical evidence of treatment failure with terbinafine in patients with *Pityriasis versicolor* or with itraconazole in dogs with dermatitis as well as with fluconazole or posaconazole in preventing *M. furfur* fungemia in humans suggested a probable occurrence of drug resistance phenomena in these species. However, *in vitro* susceptibility testing for *Malassezia* spp. has not yet been standardized but the low activity of fluconazole, voriconazole and echinocandins against *Malassezia* spp. seems to be a common finding regardless the testing methods. Herein, the most recent literature on *Malassezia* spp. skin infection and fungemia, pathogenetic mechanisms of *Malassezia* spp, diagnostic procedures, *in vitro* susceptibility testing and therapeutic approaches will be summarizes and discussed.

Claudia Cafarchia



D.BSc, Phd, Associate Professor at the Department of Veterinary Medicine, University of Bari Aldo Moro. She attended the course of Mycology Medical Mycology at The Centraalbureau voor Schimmelcultures (CBS Fungal Biodiversity Centre), Utrecht, The Netherlands. She is a member of the ISHAM-Veterinary Mycology Working Group and of the ISHAM-Malassezia epidemiology and Working Group. Supervisor of Mycology laboratory and Member of the Scientific Committee of Specialization Courses in "Infectious Diseases, Prophylaxis and Veterinary Police" and of the Scientific Committee of the PhD course in “Animal Health and Zoonosis” University of Bari, Italy.

In charge of the courses of Veterinary Mycology at Department of Veterinary Medicine and Supervisor for degree, Phd and specializations theses. Associate Editor of the Medical Mycology, Academic Editor of Antibiotics. Responsible and Component of research projects admitted for funding on the basis of competitive call. The professional and scientific activity is aimed in studying fungi of medical and veterinary concern. Scientific activity consists of an overall production of 202 scientific articles in international and national journals; 90 contributions in international / national conference; 6 book chapters. She is one of two co-editors of a comprehensive "Micologia veterinaria e comparata” Textbook with ISBN published by Aracne Editore pp.1-378.

Invited Speaker

IMPACT OF ANIMAL ROTAVIRUSES ON HUMAN HEALTH

V. Martella
Unversity of Bari Aldo Moro, Department of Veterinary Medicine, Valenzano, Italy

Rotaviruses are important enteric pathogens of humans and animals. Group A rotaviruses (RVAs) account for up to 1 million children deaths each year, chiefly in developing countries. Both mono-valent or poly-valent vaccines targeting the main VP7 and VP4 antigens (G and P type, respectively) are now available for prevention of acute gastro-enteritis (AGE) in children. RVA-associated enteritis is also a major problem in young calves, piglets and foals. Early in the epidemiological studies for RVAs in humans, either sporadic cases or epidemics by atypical, animal-like RVA strains were described. Complete genome sequencing of human and animal RVA strains has revealed a massive genetic heterogeneity in the 11 double stranded RNA segments across different RVA strains and has provided evidence for frequent intersections between the evolution of human and animal RVAs, as a result of multiple, repeated events of interspecies transmission and subsequent adaptation. After 2015, a pandemic horse-like G3 strain emerged in human population worldwide. This strain was predominant in several epidemiological studies. The virus differed in the VP7 gene and in the genome constellation from classical G3 human viruses, demonstrating the potential impact of this phenomenon on a global scale.

Vito Martella

Vito Martella received his veterinary degree in 1996 from the Faculty of Veterinary Medicine in Bari, Italy. Between 1997 and 2000, he completed a PhD on infectious diseases of small animals, and from 2000 he was a researcher working on various aspects of virology and diagnostics. From 2001 to 2017, he was Associate Professor and since 2017 he is Full Professor at the Department of Veterinary Medicine of Bari, Italy. From 2014 to 2018 Coordinator of the PhD program in Animal Health and Zoonoses. From 2018 to 2022, coordinator of the Master course in Safety of food of animal origin and health (LM86). From 2019 to present, head of Section IV of the Italian Supreme Health Council. His current research focus is primarily on enteric viruses at the interface between animals and humans, virus discovery and characterization and optimization of diagnostic tests. He is member of the RCWG (rotavirus classification working group) and of the ICVT (International Committee for Virus Taxonomy) calicivirus study group. He has more than 400 publications in peer-reviewed scientific journal, with more than 15000 citations and a H index of 61 in Scopus.

Invited Speaker

BROADENING THE UNDERSTANDING OF THE MOLECULAR BASIS FOR SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* COLONIZATION IN CATTLE

S.A. Barth, C. Berens, M. Weber, C. Menge
Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany

Many cattle are colonized by Shiga toxin (Stx)-producing *Escherichia coli* (STEC), making them a major source of human enterohemorrhagic *E. coli* (EHEC) infections. The highly virulent *E. coli* O104:H4 strain having caused the largest outbreak of hemolytic uremic syndrome in 2011 possessed a blended virulence profile of human adapted enteroaggregative (EAEC) and EHEC. Experimental infection of calves proved that cattle can even carry such unusual EHEC strains at least transiently. Harbouring STEC in their intestinal tract without clinical symptoms, cattle were long believed to resist the detrimental effects of the Stxs, the principal virulence factors in the pathogenesis of human EHEC-associated diseases. However, different types of target cells for Stxs, primarily belonging to the adaptive arm of the immune system, exist in cattle and depressed cellular immune responses foster intestinal STEC colonization, an effect that can be mitigated by Shigatoxoid vaccination. Nevertheless, even classical STEC strains differ in their ability to colonize cattle more persistently ($STEC^{per}$) or only sporadically ($STEC^{spo}$). This difference is primarily encoded in the accessory rather than the core genome but phenotypic traits beyond classical virulence factors also correlate with the realization of a specific colonization pattern. $STEC^{per}$ have abandoned some metabolic traits, while $STEC^{spo}$ conserved properties leveraging survival in the environment. The ability to produce Stx, therefore, seems to be sufficient to qualify pathogenic *E. coli* strains for both, being successful colonizers in ruminants as well as emerging human pathogens while a range of STEC/EHEC strains exist that have further adopted to cattle by metabolic adjustment.

Christian Menge

Prof. Dr. med. vet. Christian Menge
Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut - Bundesforschungsinstitut für Tiergesundheit /, Federal Research Institute for Animal Health
As a licensed DVM, Prof. Menge possesses broad knowledge and expertise in surveillance, diagnosis, control and eradication of epizootic diseases in accordance with rules laid down in national and international animal health legislation. He has particular expertise in the analysis of bacterial host-interactions at the intestinal mucosal surface of livestock. Using in vitro culture systems and experimental infection studies in the target species his group elucidated the mechanisms by which enterohaemorrhagic *Escherichia coli* (EHEC) manipulates its host´s immune system. He also has substantial expertise in clinical immunology and the immunobiology of other bacterial pathogens, e.g., tuberculous and non-tuberculous Mycobacteria and Coxiella, viruses and parasites in large and companion animal species. His recent research focusses on drivers and dynamics of host-to-host and cross-species transmission of bacteria exhibiting antimicrobial resistance (AMR), also deploying innovative sampling strategies from a One Health perspective.

Invited Speaker

HEPATITIS E VIRUS AS A ZOONOSIS WITH DOMESTIC AND WILD RESERVOIRS

J. R. Mesquita
Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Epidemiology Research Unit (EPIUnit), Instituto de Saúde Pública da Universidade do Porto, Porto,Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal

Hepatitis E virus (HEV) is a member of the Hepeviridae family, Orthohepevirus genus, which can be further subdivided into four species: Orthohepevirus A that can infect humans and a wide variety of different animals, Orthohepevirus B that circulates in birds, Orthohepevirus C found in rodents and ferrets, and Orthohepevirus D in bats. The Orthohepevirus A species has 8 different genotypes (HEV 1-8), with types 1 and 2 infecting exclusively humans; 3, 4, 7 and 8 with zoonotic transmission; and 5 and 6 infecting exclusively animals. Among those infecting humans, genotype 3 is treated as an emerging zoonosis of public health importance, being found in industrialized countries, including those in Europe. In these countries, consumption of undercooked or raw infected pork, wild boar and deer meat are major sources of exposure to HEV genotype 3. This presentation will provide an overview of the current knowledge on zoonotic hepatitis E virus, providing information on domestic and wild reservoirs for infection.

João Mesquita

João Mesquita (Mesquita JR) holds a bachelor in Veterinary Medicine, a MSc and a PhD in Virology. He is a Diplomate of the European College of Veterinary Microbiology since 2019. He has been teaching since 2006 and is currently affiliated to Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto, Portugal. Since 2006 he has been involved in several projects related to infectious diseases epidemiology. His research interests are focused particularly on human and animal infectious diseases, particularly on zoonotic agents, with special emphasis on surveillance and epidemiological tools

Invited Speaker

CURRENT AND FUTURE POSSIBILITIES OF MALDI-TOF MS AND NANOPORE SEQUENCING FOR BACTERIAL IDENTIFICATION AND SUSCEPTIBILITY TESTING

F. Boyen

Department of Pathobiology, Pharmacology and Wildlife Medicine, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium.

MALDI-TOF MS has revolutionized bacterial identification in both human and veterinary medicine. It is a simple, rapid, cost-effective and robust tool for the identification of most bacteria and fungi to the species level and sometimes even beyond. Even though MALDI-TOF MS is mainly used for identification, it can also be used to detect antimicrobial resistance, using different approaches. First of all, specific proteins (peaks) can be identified that are linked with a certain resistant subpopulation. This peak profiling often has no direct link with the resistance mechanism, so may only predict resistance in specific subgroups of a bacterial species in regions where these subgroups are highly prevalent. Secondly, specific kits allow the detection of strains able to enzymatically degrade antibiotics (for example ESBL or carbapenemase producing Enterobacteriaceae) in a +/- 2hour protocol. Finally, protocols have been described able to discriminate susceptible from resistant strains for various antibiotics in various bacterial species after short incubation with the respective antimicrobial agents using 1 to 6 hour protocols (Idelevich and Becker, 2021). Increasing quantity and quality of database entries will ensure that identification of bacteria will ameliorate continuously. In addition, machine learning may increase possibilities related to identification, AMR detection and, to some extent, strain typing and detection of host biomarkers (Mortier et al., 2021, Weis et al., 2022). Third generation sequencing (3GS) allows high-throughput native DNA or RNA sequencing that can be processed in real-time, providing an important opportunity to reduce sample-to-result time. Even though there are various providers on 3GS, Oxford Nanopore Technologies currently are most probable to break through in veterinary medicine. Apart from identification of pathogens, 3GS also holds possibilities for DNA-based antimicrobial resistance detection and strain typing. Even though 3GS might lead to the most comprehensive and most reliable results ever in veterinary microbiology, this technique also holds several risks. First of all, due to the fact that all observed microbial agents that are present in the sample are reported, there is a risk of over-interpretation of obtained results: which of the reported microbial agents are clinically relevant? This is especially important in samples where a microbiome can be expected to be present. In addition, this technique may hold a risk of putting too much focus on the pathogen, while especially in multifactorial diseases also predisposing factors, non-infectious primary problems etc should not be neglected. Considering this technique is still in its infancy, there is still a lot of work to be done on setting up and validating workflows. A possible bottleneck may be the bio-informatics expertise for setting up and validating sequence analysis pipelines. In addition, using DNA-based detection of antimicrobial resistance determinants to predict in vivo susceptibility will also require further research and validation. Both hardware and software-related progress will result in decreasing both price and sample-to-result time of 3GS, provided that general laboratory equipment and 3GS specific consumable prices would not increase significantly. Automated and species-specific bioinformatics protocols will be able to increase quality of (deep) sequencing data and shorten processing and interpretation time (Vereecke et al., 2020). In addition, in near future, it might become possible for diagnostic labs and maybe even veterinary clinics to have their own sequencing station without the need for unrealistic investments. While sample preparation and sequencing can be performed on-site immediately after sampling, online access to the bioinformatics protocols of specialized providers with appropriate computational power may allow high quality and even real-time processing and interpretation of the results. Such

a work flow might result in a sequencing report, possibly within hours after sampling (Marcolungo et al., 2022). Validation of such point-of-care set-ups and clinically relevant interpretation will however be of major importance.

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Filip Boyen

Filip Boyen graduated at the Faculty of Veterinary Medicine, Ghent University, Belgium in 2002. In that year, he started his PhD research on Salmonella pathogenesis in pigs at the Laboratory of Veterinary Bacteriology and Mycology at the same Faculty. He finalized his PhD in 2007, and worked at the Division of Poultry, Exotic Companion Animals, Wildlife and Experimental Animals for 1 year as assistant. In 2008, he joined the Laboratory of Veterinary Bacteriology and Mycology again as laboratory coordinator, a position with diagnostic, teaching and research responsibilities he still holds today.

His research addresses different bacterial diseases in various animals species, ranging from an occasional clinical case report, over pathogenesis research and susceptibility testing, to experimental trials. He considers himself to be a “generalist”, but his main topics of interest are respiratory and intestinal infections and antimicrobial resistance in bacterial pathogens of veterinary and zoonotic importance. The last few years his research focusses also on the use of MALDI-TOF in diagnostics and innovative applications of this technique.

Invited Speaker

EVOLUTION OF ZOONOTIC SARS-COV-2 DATABASE AND ITS INFLUENCE IN ASSIGNING THE PHYLOGENETIC VALUE OF MUTATIONS FOR LINEAGE-SPECIFIC DETECTION

A. Martínez-Murcia

Department of Microbiology, University Miguel Hernández, and [2]Genetic PCR Solutions™, 03300-Orihuela, Alicante, Spain

One of the pending tasks in the research of SARS-CoV-2 is the determination of a possible intermediate host in transmission from the natural reservoir. The development of genetic detection methods is undoubtedly essential to achieve this goal. However, qPCR designs are based only on the sequences that we are describing and depositing in databases that do not necessarily represent all natural diversity. Early on this pandemic, our laboratory has contributed with qPCR kits for SARS-CoV-2 detection and kits specific for some of its variants of concern (VOC). Post-marketing surveillance plan included a rigorous check of the new sequences determined and available at the GISAID database (Global Initiative on Sharing All Influenza Data). In our view, due to the criteria recommended to select samples for sequencing (i.e., the Spike Gene Target Failure methodology) and the geographically uneven sequencing capacity, sequences described so far may have been biased to enrich only a homogenous part of diversity, particularly within the Alpha, Delta and, lately, the Omicron variants. Phenotypic relevance of mutations, or their possible clinical impact, cannot always being taken as phylogenetic markers of SARS-CoV-2 variants. Considerable number of mutations should be determined to properly ascertain the phylogenetic identification of lineages. A simple sequencing strategy, directed to a 952 bp S-gene fragment comprising 29 informative mutations, was developed in our laboratory for affordable determination of most variants of concern.

Antonio Martinez-Murcia

Dr Antonio Martinez-Murcia (6th March 1964) Professor of Microbiology, Miguel Hernández University, Alicante, since 1993), B.Sc. in Biochemistry (Univ. Valencia 1987). Ph.D. in Microbiology (Univ. Reading, UK, 1993). He is an active member of the "Aeromonas Working-group" of the "International Sub-Committee on Vibrionaceae, Aeromonadaceae, and related genera" (International Committee on Systematics of Prokaryotes). Executive member of Association of Veterinary Laboratory Diagnosis Specialists (AVEDILA) and board member of The European Association of Veterinary Laboratory Diagnosticians (EAVLD). Secretary of the board of the Association of Biotech Companies of Alicante (AEBA). Member of Spanish Society of Microbiology since 1989 (SEM). He is co-author of > 90 papers in peer-reviewed journals and book chapters, a patented PCR method (1994), co-director of 8 doctoral theses, and has described 16 new bacterial species. Counts with pioneering experience in sequencing techniques, bacterial phylogeny/evolution/taxonomy, genetic identification/detection/typing. He has participated in >30 R&D projects, including several from the European Commission. Founder and Director of GPS™ laboratory, GENETIC ANALYSIS STRATEGIES SL, awarded in 2002, 2012 and 2015.

Invited Speaker

EUROPEAN NETWORK FOR OPTIMIZATION OF VETERINARY ANTIMICROBIAL TREATMENT

P. Damborg
Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark

The European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT) is a COST Action launched in 2019 and ending in 2023. The aim of ENOVAT is to optimize veterinary antimicrobial use with special emphasis on the development of antimicrobial treatment guidelines and refinement of microbiological diagnostic procedures. In this talk, the structure of ENOVAT, including its five working groups (WGs), will be briefly introduced followed by a presentation of selected results. Focus will primarily be on a WG1 survey from 2021 on practices in veterinary microbiological diagnostic laboratories. Almost 300 respondents from 34 countries took the survey. Results showed large differences between the practices and standards used. For example, the reported average turnaround time for antimicrobial susceptibility testing (AST) of fast-growing organisms ranged from 1-2 days to 6-8 days. Also, quality assurance, use of breakpoints for AST, the level of guidance provided to veterinary clinicians, and other practices varied substantially between laboratories. These results indicate a need to heighten standardization of diagnostic microbiology practices, for example by development of consensus guidelines describing recommended approaches from sample arrival until diagnostic answer.

Peter Damborg

Peter Damborg received his veterinary degree in 2004 from the Royal Veterinary and Agricultural College in Copenhagen, Denmark. Between 2005 and 2008, he completed a PhD on zoonotic enteric bacteria in dogs, and from 2008 to 2013 he was a postdoctoral researcher working on various aspects of antimicrobial resistance and diagnostics. Since 2013, he has been Associate Professor at the Department of Veterinary and Animal Sciences at the University of Copenhagen. His current research focus is primarily on surveillance of AMR in animals and rationalization of veterinary antimicrobial treatment through development of antibiotic treatment guidelines and research into novel treatment regimes, alternative antimicrobial agents (e.g. peptides), and optimization of diagnostic tests. He is head of a local veterinary diagnostic laboratory forming the basis of the ECVM Training Centre at the University of Copenhagen. Since 2018, he has been Chair of the EUCAST subcommittee VetCAST, and since December 2019 he has been Chair of the COST Action ENOVAT (European Network for Optimization of Veterinary Antimicrobial Treatment). Over his career, he has published more than 70 articles in international scientific journals with peer-review (https://orcid.org/0000-0001-9932-5996).

Invited Speaker

ANTIMICROBIAL STEWARDSHIP AND PETS

N.E.M. Hopman[1], I.M. van Geijlswijk[2], L. Portengen, J.A. Wagenaar, D.J.J. Heederik, E.M. Broens

[1]Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

[2]Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

To curb increasing resistance rates, responsible antimicrobial use (AMU) is needed, both in human and veterinary medicine. In human healthcare, antimicrobial stewardship programs (ASPs) have been implemented worldwide to improve appropriate AMU. No ASPs have been developed for and implemented in companion animal clinics yet. The objective of the present study was to implement and evaluate the effectiveness of an ASP in 44 Dutch companion animal clinics. The objectives of the ASP were to increase awareness on AMU, to decrease total AMU whenever possible and to shift AMU towards 1st choice antimicrobials, according to Dutch guidelines on veterinary AMU. The study was designed as a prospective, stepped-wedge, intervention study, which was performed from March 2016 until March 2018. The multifaceted intervention was developed using previous qualitative and quantitative research on current prescribing behavior in Dutch companion animal clinics. The number of Defined Daily Doses for Animal (DDDAs) per clinic (total, 1st, 2nd and 3rd choice AMU) was used to quantify systemic AMU. A statistically significant decrease of 15% (7%-22%) in total AMU, 15% (5%-24%) in 1st choice AMU and 26% (17%-34%) in 2nd choice AMU was attributed to participation in the ASP, on top of the already ongoing time trends. Use of 3rd choice AMs did not significantly decrease by participation in the ASP. This study shows that, although AMU in Dutch companion animal clinics was already decreasing and changing, AMU could be further optimized by participation in an antimicrobial stewardship program.

Els Broens

Els Broens was trained as veterinarian at the Faculty of Veterinary Medicine, Utrecht University. After a few years work in farm animal practice, she had several positions as lecturer and researcher at the Faculty of Veterinary Medicine and the Central Veterinary Institute of Wageningen University and Research. In 2011, she finished a PhD on livestock-associated MRSA in pigs at Wageningen University and the National Institute for Public Health and the Environment. In the same year she became Dutch specialist in Veterinary Microbiology. After the completion of her PhD and residency, she returned to the Faculty of Veterinary Medicine in her current position as Director of the Veterinary Microbiological Diagnostic Centre (VMDC) that processes over 20.000 samples per year. Besides directing the VMDC, she is deputy chair and principal investigator within the Division Clinical Infectiology. She co-authored multiple grant proposals and is involved in several research projects on zoonoses and antimicrobial resistance in companion animals. She is co-promotor of two PhD-students and supervisor of residents in Veterinary Microbiology. First for the Dutch specialization, but in 2019 she became Diplomate for the recently established European College for Veterinary Microbiology (ECVM) and supervisor of residents for this college. Els is member of several (inter)national committees and working groups on veterinary microbiology, public health, zoonoses, antimicrobial resistance and veterinary antibiotic policies. She is co-chair of the COST Action 18217: European Network for Optimization of Veterinary Antimicrobial Therapy (ENOVAT) and chair of the ESCMID Study Group for Veterinary Microbiology (ESGVM).

Invited Speaker

ANTIMICROBIAL AND BIOCIDE RESISTANCE AMONG FELINE AND CANINE PATHOGENS FROM DIAGNOSTIC SUBMISSIONS

A.R. Schug [1,2], A.D. Scholtzek [1,2], A.T. Feßler [1,2], B. Kohn [2,3], C. Weingart [2,3], A.-K. Schink [1,2], A. Bethe [1,2], A. Lübke-Becker [1,2], D. Hanke [1,2], S. Schwarz [1,2]

[1]Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

[2]Veterinary Centre for Resistance Research (TZR), Freie Universität Berlin, Berlin, Germany

[4]Small Animal Clinic, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Objectives: Bacterial resistance to antimicrobials and biocides is an increasing threat. Therefore, the aim of this study was to investigate the resistance properties of canine and feline bacterial pathogens. **Methods:** A collection of 62 *Staphylococcus aureus*, 52 *Staphylococcus pseudintermedius*, 49 *Enterococcus faecalis*, 37 *Enterococcus faecium*, 59 *Escherichia coli*, 56 *Pseudomonas aeruginosa*, and 14 *Acinetobacter baumannii*, was tested for their susceptibility to antimicrobial agents according to the Clinical and Laboratory Standards Institute (CLSI) and to biocides (benzalkonium chloride, chlorhexidine, polyhexanide, and octenidine) using a recently developed broth microdilution method. **Results:** Among staphylococci, penicillin resistance was the dominant resistance property (including 29 methicillin-resistant isolates), followed by macrolide, fluoroquinolone and tetracycline resistance. *E. faecalis* showed no expanded resistance properties, in contrast to *E. faecium* showing resistance to penicillins, macrolides, tetracyclines, and fluoroquinolones. A single vancomycin-resistant isolate carrying *vanA* was detected. *E. coli* displayed expanded multiresistance phenotypes, including a single carbapenem-resistant, bla_{OXA-48}-positive isolate. In addition, three multiresistant *A. baumannii* isolates were identified. The minimal inhibitory concentrations (MICs) of the biocides were unimodally distributed, but differed with respect to the biocide and the bacterial species investigated. No signs of resistance development were observed for the bacterial species-biocide combinations tested, but due to solubility limits, some *P. aeruginosa* isolates exhibited benzalkonium MICs higher than the highest test concentration. **Conclusions:** During this study (multi)resistant isolates were seen among most bacterial species investigated. Therefore, antimicrobial susceptibility testing is highly recommended before starting antimicrobial chemotherapy. However, a development of biocide resistance was not detected among the bacterial species investigated.

Andrea T. Feßler

Dr. med vet. Andrea T. Feßler, PhD
2003 to 2009 study of veterinary medicine at the der Ludwig-Maximilins-Universität in Munich
2012 PhD degree Title of thesis: "Comparative molecular analysis of methicillin-resistant isolates of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* ssp. from cases of mastitis among dairy cattle" 2016 doctors' degree (Dr. med. vet.) "Studien zu Qualitätskontrollbereichen und klinischen Grenzwerten für Cefoperazon zur Bekämpfung boviner Mastitiden" („Studies on quality control ranges and clinical breakpoints for cefoperazone for the treatment of bovine mastitis ") 2009-2016 scientist in the working group „Molecular microbiology and antimicrobial resistance" from Prof. Dr. Stefan Schwarz at the Institute for farm Animal Genetics of the Friedrich-Loeffler-Institute, Mariensee since 2017 scientist at the Institute for Microbiology and Epizootics at the Freie Universität Berlin in the working group of Prof. Dr. Stefan Schwarz
Main research topics

- Antimicrobial Resistance from staphylococci and other bacteria
- Development of quality control ranges and clinical breakpoints for antimicrobial susceptibility testing
- Development of testing methods and quality control ranges for biocide susceptibility testing

Invited Speaker

DEVELOPMENT OF RAPID DIAGNOSTICS FOR THE DETECTION OF PATHOGENS AND AMR

R. M. La Ragione
School of Biosciences and Medicine and School of Veterinary Medicine, University of Surrey, UK

Culture remains the preferred method for the diagnosis of many human and animal pathogens. However, it is labour intensive and expensive. Thus, there is an urgent requirement for rapid, sensitive, specific and economic tests that can be used to diagnose infections in animals and humans. Moreover, the rapid diagnosis of infections can assist with the pragmatic and targeted treatment of infections, thus reducing the emergence of antimicrobial resistance (AMR).
With the advent of Next Generation Sequencing (NGS) technology, and the public availability of many full pathogen genome sequences, it is now possible to use comparative genomics to develop robust molecular tests for the rapid and sensitive diagnosis of many pathogens and AMR genes. One such test, is Loop Mediated Isothermal Amplification (LAMP). LAMP can be used to identify pathogens and AMR in less than 15 minutes, directly from a swab, with high specificity and sensitivity. LAMP can be performed with minimal expertise, simple equipment and the cost is significantly less than culture or PCR. LAMP can be utilised as both a laboratory based test or in Point of Care (PoC) applications, and the advent of Artificial Intelligence (AI) algorithms has revolutionised how the test results can be read and interpreted. This presentation will provide an overview of the currently available rapid diagnostic tests for pathogen and AMR detection, with a focus on the development and validation of LAMP assays for use as PoC tests for livestock, poultry, companion animals and environmental samples.

Roberto La Ragione

Professor Roberto La Ragione – Professor of Veterinary Microbiology and Pathology, School of Veterinary Medicine and Head of the School of Biosciences and Medicine, University of Surrey
Roberto graduated in 1995 and then went on to study for a post graduate degree in veterinary microbiology at the RVC. In 1996 he moved to the government Veterinary Laboratories Agency to undertake a PhD on the pathogenesis of *E. coli* in poultry. In 2005 Roberto was appointed head of pathogenesis and control at the APHA and in 2010 he was appointed Professor of Veterinary Microbiology and Pathology at the University of Surrey.
Roberto gained the FRCPath in 2010 and in 2012 was appointed the Associate Dean for Veterinary Strategy in the School of Veterinary Medicine. In 2014 he was appointed Head of the Department of Pathology and Infectious Diseases and 2019 Deputy Head of School. In 2021 he was appointed Head of the School of Biosciences and Medicine. Roberto is the Chair of the Royal College of Pathologists Veterinary Pathology Specialty Advisory Committee, Chair of the Humanimal Trust, a Trustee of the Houghton Trust a member of the APHA Science Advisory Board, and the past president of the Med-Vet-Net Association and the Veterinary Research Club. Roberto is an Associate member of the European College of Veterinary Microbiology (AECVM). In 2020 he was awarded Honorary Associateship of the Royal College of Veterinary Surgeons (HonAssocRCVS).
Roberto's current research interests focus on AMR and understanding the pathogenesis of zoonotic bacterial pathogens. Roberto has a particular interest in the development of control and intervention strategies, including rapid diagnostics, vaccination, and probiotics for the control of pathogens such as *Salmonella, Brachyspira* and *E. coli* in food producing animals. Roberto has published over 180 peer reviewed papers in the area of microbiology.

ORAL, AULA A

OA1

DIFFERENT GENOTYPES OF *MALASSEZIA PACHYDERMATIS* ISOLATED FROM DOGS

I. Brodard, A. Thomann, V. Perreten, S. Kittl
Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland

Malassezia pachydermatis is a basidiomycotic yeast frequently found in the ears of dogs, where a high load is associated with otitis externa and atopic dermatitis. *M. pachydermatis* is culturable on Sabouraud dextrose agar containing Tween 80 and species identification is usually possible using MALDI-TOF MS. However, we found a number of isolates that could not be identified using the standard Bruker library.
Eight of these isolates were used to establish MALDI-TOF reference spectra and subsequently subjected to whole genome sequencing. In addition, minimal inhibitory concentrations of antifungals were determined using Sensititre™ YeastOne YO10 system with small broth modifications. Wilcoxon rank sum test was applied to compare MIC values. Phylogenetic analysis of whole genome sequences revealed three clusters, two for the here described isolates (designated cluster-15KM2158 and cluster-21KM0316), and one containing the reference strain (NR 126114.1). A similar clustering was observed based on the MALDI-TOF reference spectra. Isolates of a same cluster generated MALDI-TOF scores above 2.0 (the threshold for species identification), whereas the score was below 2.0 when the reference spectra of another cluster were used. MALDI-TOF identification can therefore be difficult if one of the references is missing in the database. Interestingly, isolates belonging to cluster-15KM2158 (n=7) had significantly (p=0.002) higher MICs (8 -64 mg/L) for fluconazole compared to isolates of cluster-21KM0316 (n=7) (2-8 mg/L).Our results show that there is considerable variation among *M. pachydermatis* isolates from dogs, which can complicate diagnostics. Further research is necessary to determine if strains of cluster-15KM2158 consistently show higher fluconazole MICs.

OA2

NOVEL POTENTIAL ANTIFUNGAL COMPOUNDS WITH DUAL MECHANISM OF ACTION SELECTIVELY ACTING AGAINST *MALASSEZIA* SPP.

C. Spadini[1], C. S. Cabassi[1], F. Carta[2], A. Angeli[2], S. Selleri[2], C. T. Supuran[2]
[1]Department of Veterinary Science, University of Parma, Parma, Italy
[2]NEUROFARBA Department, University of Florence, Florence, Italy

Malassezia spp. infections and azole drug resistance phenomena are of great concern in both human and veterinary medicine. Malassezia spp. cause severe human and animal skin disorders, with a zoonotic potential for *M. pachydermatis*, which could include it on WHO fungal pathogens priority list. Inorganic SeS_2 is used as topical treatment, but its mechanism of action on fungal sterol pathways has not been fully revealed, due to the great variability of lipidome among Malasseziomycetes. In this work we evaluated antifungal activity by microdilution broth assay of novel compounds with acyl/selenoureido moieties and primary/secondary sulfonamide groups with a dual mechanism of action: (i) a selective organic selenium fungal toxicity and (ii) the inhibition of a new antifungal target metalloenzyme, the Carbonic Anhydrases (CAs, EC 4.2.1.1). Minimal Inhibitory Concentration (MIC) values of selenium-containing compounds showed very high activity on *M. pachydermatis* (0,5-3,33 µg/ml) instead of *C. albicans* and *C. glabrata* (3,33-256 µg/ml). Suppression of antifungal activity was noted when selenium was replaced with either chalcogen isosteric elements oxygen and sulfur. Compounds library was also tested on *M. furfur* and *M. globosa* showing preferential activity on *M. pachydermatis*, with only a few candidates more active on *M. furfur*. Cytotoxicity properties of selected compounds against MDBK and HaCat cells were assessed, which showed safety profile at MIC values, better than SeS_2. K_I values on *Malassezia* spp. CAs of compounds bearing primary or secondary sulfonamide moiety was in the low-medium nanomolar range, demonstrating a multitarget selective activity on *Malassezia* spp., probably depending on lipidome constitution.

OA3

VULTURES' ORAL MICROBIOME: KEYSTONE YEAST COMMENSALS AND CROSS-INTERKINGDOM INTERACTIONS

M. P. Couto[1,2], F. Lopes[3], M. Liede[3], M. Casero[4], M. Grilo[1,2], E. Cunha[1,2], M. Pereira[5], R. Dias[5], M. Oliveira[1,2]
[1]CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa,Portugal
[2]Laboratório Associado para Ciência Animal e Veterinária (Al4AnimalS),Portugal
[3]CERAS - Centro de Estudos e Recuperação de Animais Selvagens, Castelo Branco, Portugal
[4]RIAS- Centro de Recuperação e Investigação de Animais Selvagens, Olhão,Portugal
[5]BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa,Portugal

Vultures' oral microbiome may encode valuable data for assessing environmental stress-related unbalances, host-associated microbiota's diversity changes, and the efficacy of habitat restorations. The settings at rehabilitation centers may trigger polymicrobial stress-related reorganizations in vultures' microbiome. It is important to characterize, under these specific settings, whether these oral cross-kingdom interactions are symbiotic or pathogenic for the host. As so, this project aimed at describing the fungal constituents and the bacterial microbiome of the oral cavity of recovering vultures (n=23) – namely, Eurasian Griffon Vultures and Eurasian Black Vultures – sampled at wildlife rehabilitation centers in Portugal. The study was divided in three main topics: 1) characterization of oral yeast-like species present in vulture's host-associated microbiota by performing morphological and biochemical identification (using API20CAUX) of oral yeast-like colonies isolated from vultures oral samples, and evaluating their biofilm forming capacity (using Red Congo Agar); 2) evaluation of the bacterial microbiome (16S rRNA profiling by 4th NGS) of animals with and without gross signs of oral yeast-like infections; and 3) evaluation of the potential cross-kingdom interactions between yeasts and other microorganisms present in the oral cavity of the sampled animals.Besides *Candida* and *Trichosporon,* other emergent microorganisms exhibited high relative abundance in vultures' oral cavity, including *Clostridium perfringens a*nd *Paeniclostridium sordelli,* whose toxins have been associated with myonecrosis and gangrene in humans and animals. The detected fungal-bacterial oral interactions linked strictly anaerobic bacteria growth, in aerobic conditions, to polymicrobial complex biofilms, which may influence the dynamics and functions of the oral microbiome. Ackowledgements: This work was supported by CIISA–Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Project UIDB/00276/2020 (Funded by FCT); and by Laboratório Associado para Ciência Animal e Veterinária (LA/P/0059/2020 - AL4AnimalS).
keywords: oral commensals, Vultures, *Candida* sp., *Paeniclostridium sordelli., Clostridium perfringens,* polymicrobial biofilm

OA4

DE NOVO SEQUENCING OF *PROVIDENCIA ALCALIFACIENS* TO STUDY ITS ROLE IN CANINE ACUTE HEMORRHAGIC DIARRHEA

S. Rodriguez-Campos[1], L. Aguilar-Bultet[2], A.H. Haaland[3], T. L'Abée-Lund[4], A.K. Fauske[1], E.M. Soltvedt[1], H. Jørgensen[5], C. Sekse[5], E. Skancke[3], S.F. Nørstebø[1]
[1]Bacteriology and Mycology Unit, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
[2]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland
[3]University Animal Hospital, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
[4]Food Safety Unit, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
[5]Norwegian Veterinary Institute, Ås, Norway

In 2019, an outbreak of canine acute hemorrhagic diarrhea (cAHD) was observed in Southeastern Norway affecting dogs of all ages and breeds, and without known predisposing factors. Recognized causes including infectious agents and intoxication were ruled out; the only major finding was the high prevalence of samples that were culture-positive for *Providencia alcalifaciens*, occasionally in co-occurrence with *Clostridium perfringens*. *P. alcalifaciens* has been associated with diarrhea in humans and animals and was previously isolated from cAHD cases in Norway in 2005 and 2006. Twenty-one *P. alcalifaciens* isolates (dogs from 2019 outbreak, n=16; 2019 healthy control, n=1; 2006, n=2, 2005, n=2) were sequenced by Illumina NovaSeq 6000. Genomes were *de novo*

assembled using the Shovill pipeline and an *ad hoc* core genome Multi Locus Sequence Typing (cgMLST) was developed using the Ridom SeqSphere+ Software and the FDAARGOS_408 strain as seed genome. The sequences revealed virulence factors encoding cytolethal distending protein, flagellar filament structural and motor switch proteins. Thirteen of the 2019 isolates formed a distinct cluster, and a second related cluster grouped three isolates from 2006 (n=2) and 2005 (n=1). Five isolates did not cluster. All isolates from cAHD cases with lethal outcome grouped in the two clusters; no pattern was observed for mild or moderate cases of cAHD. The control strain from the healthy dog was more distantly related to all other isolates. Our results support a possible role for *P. alcalifaciens* as a primary pathogen of cAHD. Unknown virulence factors leading to differences in pathogenicity remain to be disclosed.

OA5

APPLICATION OF MOLECULAR METHODS AND MASS SPECTROMETRY FOR THE DETECTION AND IDENTIFICATION OF MASTIDOGENIC MICROORGANISMS IN RAW MILK PRODUCED IN PUGLIA

L. Del Sambro[1], L. Capozzi[1], C. Quarato[1], E. Catalano[1], G. Schino[1], D. Ridolfi[1], A. Giannico[1], M. Galgano[2], V. Manzulli[1], L. Pace[1], D. Galante[1], A. Bianco[1].
[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italia
[2]Università degli Studi di Bari - Dipartimento di Medicina Veterinaria, Bari, Italia

Mastitis is one of the most common problems on dairy farms.
The aim of the present work was to detect the bacteria present in bovine milk samples, using multiplex qPCR assay and MALDI-TOF MS after bacteriological growth.In February 2022, we collected 83 samples of bovine raw milk which showed somatic cells count higher than 300.000 cell/ml. The mutiplex qPCR assay was performed with the VetMAX™ MastiType Multi Kit. In addition, a loop of milk was streaked on selective growth media; colonies were identified by MALDI-TOF MS. Overall, *in silico* analysis showed a partial agreement with *in vitro* assays. The presence of at least one of the pathogens that may be involved in the pathogenesis of the mastitis has been detected in silico in 98,8% (82/83) of the samples tested, but only 49% (n=41) grew on the isolation media and were identified by mass spectrometry. *In silico* investigations found 63 % (n=52) positive samples for Staphylococcus aureus and 43% (n=36) for Streptococcus agalactiae, confirmed respectively in 8 and 5 samples analyzed phenotypically; these species are recognized worldwide as the most important etiological agents causing mastitis. Our results highlights the importance of using molecular methods for species identification for higher sensitivity than phenotypic methods, which may be affected by the microbial count in the milk sample if less than the sensitivity of the culture. In addition, the study can support veterinarians and breeders by providing valuable tools for managing bovine mastitis in terms of prevention and control.

OA6

ISOLATION AND CHARACTERIZATION OF STREPTOCOCCUS AGALACTIAE FROM BULK TANK MILK IN THE NETHERLANDS

A.E. Heuvelink[1], M.M.C. Holstege[1], M.J. Swarts[1], R. Peerboom[1], G.J. Buter[1], T.J.G.M. Lam[1,2]
[1]Royal GD, Deventer, the Netherlands
[2]Utrecht University, Utrecht, the Netherlands

Streptococcus agalactiae (SAG) is one of the first pathogens described as having harmful consequences for udder health in dairy cows. The recent increase in prevalence of SAG published in literature and of anecdotal cases in the Netherlands prompted us to initiate more research on this pathogen.The objective of the current study was to evaluate the performance of Brilliance™ GBS Agar (GBS) (Thermo Scientific), a commercial chromogenic medium selective for SAG, for isolation of SAG from bulk tank milk (BTM) in comparison with modified Edward's medium (Oxoid Ltd) (mEDW).
BTM samples collected during 10 sampling rounds (August 2020-July 2021) were in addition to the routinely used agars, also inoculated onto GBS. SAG-isolates of a subset of herds were subjected to strain-level typing by Fourier Transform - Infra-Red spectroscopy and Whole Genome Sequencing.
The prevalence of SAG per sampling round (herd prevalence) based on mEDW+GBS varied from 2.3% to 2.8% of samples. Adding GBS agar as a second agar, yielded 24.6% up to 72.2% more

SAG-positive samples per sampling round, indicating GBS has a significant added value in the culture of BTM for the presence of SAG. Overall, sample prevalence increased from 1.5% by using mEDW only to 2.4% (P<0.001) and 2.6% (P<0.001) by using GBS only and both mEDW and GBS, respectively. First typing results showed that isolates from herds found SAG-positive on GBS only do not belong to clearly distinct types than isolates from herds SAG-positive on mEDW only or the combination of mEDW and GBS.

OA7

THE CERVICAL MICROBIOME OF SHEEP BREEDS WITH DIFFERENT FERTILITY RATES

S.F. Nørstebø[1], S. Rodriguez-Campos[1], Ö.C.O. Umu[1], L. Abril-Parreño[2], M. Dalland[3], G.D. Gilfillan[3], S. Fair[2], A. Krogenaes[4]

[1]Bacteriology and Mycology Unit, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

[2] Laboratory of Animal Reproduction, Department of Biological Sciences, School of Natural Sciences, Biomaterials Research Cluster, Bernal Institute, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland

[3] Department of Medical Genetics, Oslo University Hospital and University of Oslo, Oslo, Norway

[4]University Animal Hospital, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, PB 5003, 1432 Ås, Norway

In sheep, the use of cervical artificial insemination (AI) using frozen-thawed semen is hampered by low pregnancy rates worldwide, except in Norway where high pregnancy rates are achieved. Previous studies have shown that differences in pregnancy rates following cervical AI are related to the inability of frozen-thawed sperm to traverse the cervix of some ewe breeds but not others. To study the possible effect of the cervical microbiome on sperm transport, we compared the microbiomes of ewe breeds with known differences in pregnancy rates following cervical AI using frozen-thawed semen. These were Suffolk and Belclare (low and medium fertility, respectively) in Ireland and Norwegian White Sheep (NWS) and Fur (both high fertility) in Norway. Cervical swabs from 10 animals of each breed were collected at the follicular phase of both a natural and synchronised oestrus in compliance with ARRIVE Guidelines. DNA was extracted with the IndiSpin Pathogen Kit, and 16S rRNA gene sequencing was performed on an Illumina MiSeq. The R packages DADA2, decontam, phyloseq and ggplot2 were used for downstream analyses. The Irish breeds showed higher microbial diversity compared to the Norwegian breeds. Likewise, the samples taken at a synchronized oestrous showed higher microbial diversity. The top features leading to microbial differences between breeds and between natural and synchronized oestrous corrected for breed, belonged to the genera: *Histophilus*, *Ruminococcus*, *Sphingobium* and *Treponema*. In conclusion, the higher cervical microbial load in low fertility ewe breeds and in the follicular phase of a synchronized oestrous may contribute to reduced sperm transport.

OA8

NEXT GENERATION SEQUENCING ON THE VAGINAL MICROFLORA OF MARES

A. Mataragka[1], A. Symeonidou[1], P. Katsarou[1], G. Diakoudi[2], F. Pellegrini[2], V. Vasinioti[2], G. Lanave[2], N. Decaro[2], D. Vlachakis[3,4,5,6], E. Papakonstantinou[3], John Ikonomopoulos[1].

[1]Laboratory of Anatomy and Physiology of Farm Animals, Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

[2]Department of Veterinary Medicine, University of Bari Aldo Moro, Sp Casamassima Km3, 70010 Valenzano, Bari, Italy

[3]Laboratory of Genetics, Department of Biotechnology, School of Applied Biology and Biotechnology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

[4]University Research Institute of Maternal and Child Health & Precision Medicine, School of Medicine, National and Kapodistrian University of Athens, 11527 Athens, Greece

[5]Division of Endocrinology and Metabolism, Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece

[6]Algorithms and Bioinformatics Group, Informatics Department, Faculty of Natural, Mathematical & Engineering Sciences, Strand Campus,WC2R 2LS, King's College London,UK

Objective: this study was focused to the assessment of the constitution of the vaginal microbial flora of mares. **Materials and Methods:** Vaginal swabs were collected between May to September 2020, from 30 mares, 4 to 26 years old, maintained in different parts of the Attika Prefecture, in central Greece. Before sample collection, the test animals were submitted to clinical examination to determine the absence of pathologic evidence.Samples were processed for PCR amplification of 16srDNA gene and Nanopore sequencing. **Results:** Successful reads were retrieved from 28 of the 30 samples (93.5%), which resulted to the presumptive identification of 192 bacterial species belonging to 87 genera, with diverse genus and species distribution among samples. *Staphylococcus* and *Acinetobacter* spp. were detected in 89% (25 of 28) and 54% (15 of 28) of the test samples, respectively, of which the former referred primarily to *Staphylococcus equorum*, *Staphylococcus saprophyticus*, *Staphylococcus succinus, Staphylococcus cohnii* and *Staphylococcus hominis*, whereas the latter, *to Acinetobacter equi, Acinetobacter bouvetii, Acinetobacter lwoffii* and *Acinetobacter variabilis.* The following genera were detected in at least 25% of the test samples: *Klebsiella* (43%), *Pseudomonas* (36%), *Psychrobacter* (36%), *Bacillus* (32%), *Enterococcus* (25%), *Providencia* (25%), and *Sphingomonas* (25%). Results were compared with those of similar studies, though to the best of our knowledge there is no previous report of an NGS analysis on vaginal microflora of mares.Within the context of the microbiomics analysis, the individuals of the tested population were assigned into groups, based on certain characteristics, including race, reproduction, vaccination. This analysis produced in many cases similarity and discriminating traits, primarily at the genus level.**Discussion/Conclusion:** The NGS analysis of the vaginal microflora of mares provides an abundance of information that can be used to identify diagnostic or prognostic indicators and improve health monitoring and protection in female horses.

OA9

DEVELOPMENT OF A *MYCOPLASMA HYORHINIS* CHALLENGE MODEL IN FOUR WEEK OLD PIGS

D. Földi[1], E. Zsófia Nagy[1], N. Belecz[1], L. Szeredi[2], J.Földi[3], Z. Kreizinger [1,4], M. Tenk[2], A. Kollár[2], M. Gyuranecz [1,4]

[1]Veterinary Medical Research Institute, Budapest, Hungary
[2]University of Veterinary Medicine, Budapest, Hungary
[3]Euvet Bt., Gödöllő, Hungary
[4]MolliScience Kft., Biatorbágy, Hungary

Objective: *Mycoplasma hyorhinis* causes polyserositis, arthritis, and in some cases conjunctivitis and otitis media in piglets. Our aim was to develop a challenge model for the triggering of both polyserositis and arthritis in four-week-old animals.Method: The pigs were challenged two consecutive days with freshly prepared *M. hyorhinis* broth cultures either by intravenous route (10 ml) on both days (group A), or by intravenous route (10 ml) on the first day and by intraperitoneal route (20 ml) the next day (group B). Results: Differences were detected in the weight gain of the challenged groups and the control group. Average weight gains were 11.7, 7.4 and 5.7 kg for the control group, group A and group B, respectively. Swollen joints in the two infected groups were detected. The most affected articulations were stifles, one or both stifles were swollen in 5/6 animals in both infected groups. Pericarditis and pleuritis were also detected in the challenged animals. In the histopathological samples lympho-histiocytic inflammation was detected in the joints, while thickening of the connective tissue was observed in the serosa. After necropsy, *M. hyorhinis* was isolated from two samples of two different animals in group A and from four samples of two different animals in group B. After genotyping the isolates, we were able to confirm that the challenge strain was re-isolated.Conclusions: A *M. hyorhinis* challenge model was established in four-week-old piglets suitable for evaluation of future vaccine candidates.

OA10

PREVALENCE OF PARATUBERCULOSIS IN DAIRY CATTLE HERDS AND APPROCHES TO CONTROL THE DISEASE IN SLOVENIA

U. Zajc, T. Knific, J. J. Hodnik, D. Kušar, J. Starič, M. Kavalič, T. Pirš, J. Ježek, M. Pate, M. Ocepek

University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia

Paratuberculosis (paraTB), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a contagious, incurable and chronic disease that primarily affects the small intestine of ruminants but also a number of other domestic and wild animal species. It is endemic in cattle populations worldwide and has a significant negative economic impact on the dairy industry. The aim of this study was to (i) assess the prevalence of paraTB in large dairy cattle herds using quantitative real-time PCR (qPCR), (ii) evaluate the impact of livestock trade on the spread of paraTB in herds using static and temporal network analysis, and (iii) assess the risk of consumer exposure to MAP from milk and dairy products using stochastic modelling. In 2019 and 2020, pooled fecal samples from 207 Holstein-Friesian cattle herds were analysed by qPCR. In total, 4.35% of herds tested positive for MAP. In the positive herds, faecal and blood samples were collected from all animals older than two years (n=1015). The average prevalence of MAP within herds was 13.6%. The prevalence of paraTB at the herd level was relatively low. Data on animal trade allowed identification of high-risk farms with intensive trade and may therefore help to implement targeted control measures. The risk of consumer exposure to MAP via pasteurized milk and dairy products was generally low, with the exception of individuals consuming raw milk and dairy products from paraTB-infected herds.

OA11

PHENOTYPIC SURVEILLANCE FOR ANTIMICROBIAL SUSCEPTIBILITY IN VETERINARY MASTITIS PATHOGENS FROM IRELAND

A. Naranjo-Lucena [1], G. Madigan [1], A. Johnson [2], C. Sánchez-Miguel [2], S. McGettrick [2], S. Fagan [2], M. Sheehan [2], M. Casey [2], M. Wilson [1], M. McElroy [1], R. Slowey [1]

[1]Department of Agriculture, Food and the Marine (DAFM) Laboratories Backweston, Ireland

[2]DAFM Regional Veterinary Laboratories, Ireland

Surveillance for antimicrobial resistance in veterinary clinical pathogens is not as well developed as for zoonotic and indicator bacteria. Mastitis-causing pathogens isolated from milk submitted to DAFM laboratories in Ireland between 2019 and 2021 were screened for antimicrobial susceptibility. A single isolate per herd of each bacterial species was subject to antimicrobial susceptibility testing as per CLSI guidelines. *Streptococcus uberis* was the most common pathogen isolated from mastitic milk during 2021 (n=151), closely followed by *Staphylococccus aureus* (n=150), *Eschericha coli* (n=128) and finally *Streptococcus dysgalactiae* (n=28). *S. uberis* showed 93.38% susceptibility to β-lactam antimicrobials. *S. dysgalactiae,* however, were 100% susceptible. *S. uberis* had lower levels of susceptibility to erythromycin and pirlimycin (78.7% and 66.2%, respectively) than *S. dysgalactiae* (89.3% and 96.4%). No MRSA was detected. Susceptibility of *S. aureus* to penicillin was greater in 2021 (56.9%) than in 2019 (46.2%), while susceptibility to erythromycin and pirlimycin was above 96% all three years. Comparing the results obtained for *E.coli* isolates in 2021 with 2020, some trends were evident - susceptibility to amoxicillin-clavulanic was 5.3% lower and susceptibility to tetracycline was 6.7% lower, whereas susceptibility to enrofloxacin was slightly higher (1.4%) in 2021. There are preliminary indications that the susceptibility of *S. aureus* to penicillin and erythromycin and that of *E. coli* to enrofloxacin may be increasing. The susceptibility of *S. dysgalactiae* to erythromycin and of *S. uberis* to all anitmicrobials declined in 2021. These findings highlight the importance of establishing a robust surveillance programme to monitor trends and emerging resistance.

OA12

PREVALENCE AND RISK FACTORS ASSOCIATED WITH FAECAL CARRIAGE OF EXTENDED-SPECTRUM B-LACTAMASE-, AMPC- AND CARBAPENEMASE-PRODUCING *ESCHERICHIA COLI* IN STRAY CATS

G. Ratti, A. Facchin, A.Stranieri, A. Giordano, S. Paltrinieri, P. Scarpa, D. Maragno, L. Villa, M. Penati, S. Lauzi

Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Milan, Italy

Antimicrobial resistance in pets is gaining attention worldwide. The aim of this study was to determine the prevalence and risk factors associated with faecal carriage of ESBL-, AmpC-, and Carbapenemase-producing *E. coli* in stray cats from Northern Italy. Rectal swabs were collected between 2020 and 2021 from stray cats admitted to the Veterinary Teaching Hospital of Lodi.

Samples were microbiologically and molecularly analyzed to investigate the presence of ESBL-, AmpC-, and Carbapenemase-producing *E. coli*. Anamnestic data were analyzed to identify risk factors. In total 20/121 (16.5%) animals were positive for the presence of ESBL-/AmpC-/Carbapenemase-producing *E. coli*. Phenotypic test showed 11 (9.1%) ESBL-, 3 (2.4%) ESBL-/AmpC- and 2 (1.7%) AmpC- producing *E. coli*. ESBL genetic determinants were *bla*$_{CTX-M}$ (14, 100%), *bla*$_{TEM}$ (12, 86.7%) and *bla*$_{SHV}$ (2, 14.3%) whereas *bla*$_{CMY-2}$ gene was the most frequently detected gene in AmpC-producing *E. coli* (4, 80%). Moreover, 4 (3.3%) isolates showed Carbapenemase-producing phenotype supported by the detection of *bla*$_{NDM}$ gene in all these isolates. *E. coli* phylogroups F (6/20, 30%) and B2 (5/20, 25%) were the most frequently detected. Nineteen (95%) isolates showed multidrug resistance. Risk factors associated with ESBL-/AmpC-/ Carbapenemase-producing *E.coli* faecal carriage were hospitalization (*P* <0.0001), previous antibiotic treatment (*P* <0.0001) and presence of clinical disease (*P* <0.0001). Our findings highlight the need of surveillance programs and antimicrobial stewardship to reduce the emergence and spread of resistant bacteria. Further studies are needed to define the risk of transmission to humans and other animals.

OA13

ASSESSMENT OF RELIABILITY AND REPRESENTATIVENESS OF ANTIMICROBIAL SENSITIVITY TESTING RESULTS FOR DIFFERENT LIVESTOCK SPECIES AND DIFFERENT BACTERIA IN THE NETHERLANDS FROM 2016 - 2020

J. van Hout[1], M. Augustijn[1], E. Broens[2], T. Derkman[1], M. Gonggrijp[1], A. Heuvelink[1], J. Wiegel[1]
[1]Royal GD
[2]Utrecht University

To further promote prudent use of antimicrobials and to provide solid evidence for policies aimed at reducing antimicrobial resistance levels in bacteria from animals, it's extremely important to have access to validated antimicrobial susceptibility testing (AST) data, representative for specific animal species/bacterial species and with known bias levels (if present). Therefore this study (financed by ZonMw) aimed to assess reliability and representativeness of currently at Royal GD for livestock available AST results of selected antimicrobial/bacterial species combinations. AST results and additional data were collected over the time period 2016-2020 for: *Actinobacillus pleuropneumoniae*/*Escherichia coli*/*Streptococcus suis* (pigs), *Salmonella* Dublin/*Salmonella* Typhimurium (veal calves) and *Escherichia coli* (broilers). Aggregated Minimal Inhibitory Concentration data were statistically evaluated per animal species/bacterial species combination.Overall, AST results are fairly representative for the animal and/or farm density per province (pigs/veal calves) or the number of farms per veterinary practice (poultry). However, the low number of isolates in the dataset of veal calves led to less representative and less reliable AST results. Effort should be made to increase the number of isolates from veal calves. In general, different associations between susceptibility levels and specific factors (i.e., additional information like farm of origin or age) were demonstrated. These factors should be taken into account when showing the final susceptibility patterns for use in antimicrobial treatment guidelines and in veterinary practice. Evaluation of MIC data was hampered by the lack of veterinary clinical breakpoints, clearly emphasising the need for such breakpoints to further improve prudent use of antibiotics in the field.

OA15

ROLE OF COMPANION ANIMAL-HUMAN HOUSEHOLDS IN THE DISSEMINATION OF COLISTIN AND EXTENDED-SPECTRUM BETA-LACTAM RESISTANCE

J. Menezes[1,2,3], A. Belas[1,2,3], C. Marques[1,2,3], J. Moreira da Silva[1,2,3], A. Amaral[1,2], C. Pomba[1,2,3,4]
[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2]AL4Animals, Associate Laboratory for Animal and Veterinary Sciences
[3]ENOVAT—European Network for Optimization of Veterinary Antimicrobial Treatment (COST ACTION CA18217).
[4]Molecular Veterinary Diagnostic Laboratory - Genevet, Rua Quinta da Nora Loja3B, 2790-140 Carnaxide, Portugal

Objectives: To determine the colonization and sharing of colistin resistant, extended-spectrum beta-lactamases (ESBL)- and carbapenem-producing Enterobacterales (CPE) between healthy companion animals and its co-habiting humans. **Methods**: Forty-one Portuguese households (58 humans, 40 dogs, 18 cats) with at least one human–animal pair were studied. Informed consent was obtained. Fecal samples were inoculated on MacConkey agar plates supplemented with 1.5 µg/mL cefotaxime, 1.0 µg/mL meropenem and SuperPolymyxin medium plates. Microdilution susceptibility testing was performed according to EUCAST 2021 guidelines. Beta-lactam and plasmid-mediated colistin resistance (*mcr*-type) genes were screened by PCR and sequenced. Species identification was performed by PCR. Animal-owner pair strains genetic relatedness was estimated by WGS (Illumina NovaSeq), producing paired-end libraries with 150 bp. **Results**: Three dogs (7.5%) presented multidrug-resistant *Escherichia coli* strains harbouring the *mcr*-1 gene, with colistin MICs of 4 mg/L. No CPE was found. Twenty-five ESBL/AmpC-producing Enterobacterales (1 *Citrobacter freundi*, 2 *Klebsiella pneumoniae,* 22 *E. coli*) were detected in 15 households (20.7% from humans and 13.8% from animals); 10 strains presented a multidrug-resistant profile. WGS SNPs analysis suggests human–dog pair shared *E. coli* strains in 2 households: i) ST93 strains harbouring *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{TEM-1}}$ genes isolated from a cat and its owner; ii) **ST457 strains,** harbouring *bla*$_{\text{CTX-M-55}}$ and *bla*$_{\text{CMY-2}}$ genes, and **ST410 strains, harboring** *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{OXA-1}}$ genes **isolated from a dog** and its owner. **Conclusions**: Our findings point toward the role of CA in the dissemination of clinically important genes to humans since they live closely together. Therefore, household hygiene and antimicrobial surveillance is recommended in this community setting.

OA16

HETERORESISTANCE IN A MULTI-RESISTANT CLINICAL *ENTEROBACTER CLOACAE* COMPLEX STRAIN DUE TO AMPLIFICATION OF AN AMPC B-LACTAMASE

J. Kupke[1], J. Brombach[1], F. Ghazisaeedi[1], D. Hanke[1], T. Semmler[2], K. Tedin[1], N. Nordholt[3], F. Schreiber[3], A. Lübke-Becker[1], M. Fulde[1]

[1]Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

[2]Robert Koch Institute (RKI), MF2-Genome Sequencing and Genomic Epidemiology, Berlin, Germany

[3]Federal Institute for Materials Research and Testing (BAM), Department of Materials and the Environment (Dpt. 4), Berlin, Germany

Introduction: Heteroresistance (HR) describes the phenomenon of a subpopulation that grow in the presence of inhibitory antibiotic concentrations. We found HR to ceftazidime in an *Enterobacter cloacae* complex (ECC) strain (IMT49658) isolated from the wound of a horse. **Materials & Methods:** For phenotypic characterisation we conducted population analysis profiles and stability analysis of resistance. Quantification of β-lactamase genes was done with qRT-PCR. Whole genome sequencing (WGS) shed light on the genome of resistant and susceptible population entities. Furthermore, we exposed IMT 49658 to long-term heat stress. Competition assays and ScanLag assessed growth traits of this strain. **Results**: PAPs confirmed HR with growth up to 16-fold the breakpoint concentration. The resistant subpopulation was reversible after subcultivation on non-selective media and comprised a gene-amplification region with the AmpC β-lactamase *bla*$_{\text{DHA-1}}$, detected with WGS. No HR could be detected after heat stress led to gene loss of *bla*$_{\text{DHA-1}}$. Competition assays revealed a loss of biological fitness due to the gene-amplification. Heterogeneous lag times for resistant subpopulations growing on double breakpoint concentration of CAZ in ScanLag revealed that CFUs appearing early maintained higher copy numbers of *bla*$_{\text{DHA-1}}$ than late occurring CFUs, defining the amplifications as the reason for the plasticity of this strain. **Conclusion:** In this ECC strain AmpC β-lactamase *bla*$_{\text{DHA-1}}$ and its amplification lead to a heteroresistant phenotype. In order to combat HR infections in veterinary medicine, strong effort for the investigation of HR is necessary.

OA17

THE ENOVAT SURVEY ON CURRENT METHODOLOGIES USED FOR BACTERIAL IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING IN EUROPEAN VETERINARY DIAGNOSTIC LABORATORIES

D. Timofte[1], I. Cvetkovikj[2], F. Zendri[1], S. Blum[3], S.C. Chaintoutis[4], P.A. Kopp[5], C. Hare[6], Z. Štritof[7], S. Kittl[8], J. Goncalves[9], I. Zdovc[10], E. Paulshus[11], T. Koritnik[12], A. Laconi[13], E. Broens[14],
P. Damborg[15]
[1]Institute of Infection, Veterinary and Ecological Sciences, Leahurst Campus, University of Liverpool, Neston, United Kingdom
[3]Department of Bacteriology and Mycology, Kimron Veterinary Institute, Bet Dagan, Israel
[4]Diagnostic Laboratory, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[5]IDEXX Vet Med Labor GmbH, Kornwestheim, Germany
[6]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK
[7]Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[8]Department of Infectious Diseases and Pathobiology, Institute of Veterinary Bacteriology, University of Bern
[9]Institute of sustainable processes, University of Valladolid, Spain
[10]Institute of Microbiology and Parasitology, Veterinary Faculty of Ljubljana, Croatia
[11]Department of Analysis and Diagnostics, Microbiology, Norwegian Veterinary Institute, Ås, Norway
[12]Department for Public Health Microbiology Ljubljana, Centre for Medical Microbiology, National Laboratory of Health, Environment and Food, Ljubljana, Slovenia
[13]Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro (PD), Italy
[14]Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[15]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark.

Background: Veterinary diagnostic laboratories (VDLs) play a key role in the identification of infectious agents and antimicrobial stewardship. However, there is still a lack of harmonisation of methodologies and procedures used in European VDLs (1,2). **Methods**: The European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT) designed a survey, which was distributed via a online platform to VDLs in 34 European countries. The survey focused on practices and interpretive criteria used for culture and identification (C&ID), and antimicrobial susceptibility testing (AST) of veterinary bacterial pathogens. **Results:** Two hundred and ninety laboratories responded, representing a mixture of academic (39%), government (33%), and private (28%) laboratories. Average C&ID turnaround varied from 1-2 days (78%) to 3-5 days (20%), and 6-8 days (0.5%). For AST, similar time frames were achieved by 63%, 60%, and 0.5% of VDLs, respectively. Only 57% of laboratories attempted bacterial ID to species level. Biochemical ID systems (e.g., API kits) were the most used (56%) followed by MALDI-TOF MS (46%). For AST, Kirby-Bauer disc-diffusion (DD) and MIC determination were conducted by 44% and 33% of laboratories, respectively. A combination of EUCAST and CLSI clinical breakpoints was most commonly used for interpretation of both DD (41%) and MIC (47%). Only 48% and 46% of VDLs routinely screened isolates for methicillin resistance and ESBL production, respectively. **Conclusion:** A variety of methodologies were identified for C&ID and AST in European VDLs. Our results emphasize the need to harmonise diagnostic methodologies to benefit rational antibiotic use and ultimately improve animal and public health.

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OA18

THE PERSISTENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN PETS WITH SKIN AND SOFT TISSUE INFECTIONS: WHICH IS THE ROLE OF THE COHABITANTS AND THEIR LIVING ENVIRONMENT?

E. Spagnolo[1], M. Berlanda[2], L. Ceglie[1], I. Drigo[1], K. Trevisiol[1], M. Cocchi[1], R. Perin[1], C. Losasso[1], K. Capello[1], L. Decastelli[3], D. Pasotto[2], M. Corrò[1].

[1] Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy

[2]Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute, Padua, Italy

[3]Laboratorio Nazionale di Riferimento per gli Stafilococchi Coagulasi Positivi, Turin, Italy

Staphylococcus pseudintermedius (SP), usually belonging to the human and animal saprophytic bacterial flora, could be associated with skin and soft tissue infections (SSTIs) in pets, as well. The presence and the spread of methicillin-resistant SP (MRSP) strains are a matter of concern due to the difficulty of handling SSTIs in pets. In order to understand the cross-contamination between patients and their animal cohabitants, as well as the potential persistence of MRSP in the environment, the sampling was scheduled from armpit, mouth and genitals in all animals, from lesions in pets with SSTIs, and from the environment (bowl, kennel and sofa or bed if shared with the owners) periodically. Three hundred twenty-seven swabs were collected from 24 patients, their 10 cohabitants and the environment. SP and MRSP colonies were identified by phenotypic methods; methicillin resistance gene (*mec*A) was confirmed by PCR. Species identification was performed by MALDI-TOF mass spectrometry. *SP* was isolated in 221/327 samples (67%) and 124 of these strains were *mec*A positive (56%).Interestingly, 26/124 (21%) were strains isolated from the same cohabitants or from the same environment repeatedly.This study reports the persistence of MRSP strains in healthy cohabitants and in the environment. Such persistence could represent an important source of recontamination for the animals and therefore its investigation should be considered in patient management. Moreover, the close sharing of the living environment with humans may represent a health concern due to the risk of human-animal cross-contamination. Work financed by the Ministry of Health (IZSVE 16/18 RC).

OA19

RECOMMENDATIONS FOR THE THERAPY OF LOWER URINARY TRACT INFECTIONS IN CATS AND DOGS

A. Bethe[1,2], C. Weingart[2,3], A.-K. Schink[1,2], J. Brombach[1,2], B. Walther[4], R. Köck[5], R. Merle[6], W. Bäumer[7], B. Kohn[2,3], A. Lübke-Becker[1,2]

[1]Institute of Microbiology and Epizootics, FU Berlin, Berlin, Germany

[2]Veterinary Centre for Resistance Research (TZR), FU Berlin, Berlin, Germany

[3]Small Animal Clinic, FU Berlin, Berlin, Germany

[4]Robert Koch-Institute, Berlin, Germany

[5]DRK Kliniken Berlin, Berlin, Germany

[6]Institute for Veterinary Epidemiology and Biostatistics, FU Berlin, Berlin, Germany

[7]Institute of Pharmacology and Toxicology, FU Berlin, Berlin, Germany

Objective: Lower urinary tract infections (UTIs) are a common reason for antimicrobial therapy in small animal practice. To minimize and improve the use of antimicrobial agents, we aimed to develop treatment recommendations based on detection rates and antimicrobial susceptibility testing (AST) of the respective causative pathogens in clinical samples of cats and dogs.**Material/Methods:** Data on 470 urine samples were collected from dogs (n=258) and cats (n=212), mainly suffering from sporadic (dogs: n=117, cats: n=96) or recurrent cystitis (dogs: n=108, cats: n=105). Minimal inhibitory concentrations (MIC) for eleven antimicrobial agents licensed to treat UTIs in Germany were determined for 308 isolates of clinical relevance (dogs: n=191; cats: n=117) following CLSI guidelines.**Results:** In total, 214 samples were culture-negative or revealed nonspecific bacterial growth. *Escherichia coli* (dog: n=89, cat: n=55) dominated the sample collection. While additionally, *Staphylococcus pseudintermedius* (n=31) and *Streptococcus canis* (n=16) were frequently associated with canine samples, *Enterococcus faecalis* (n=14) and *Staphylococcus felis* (n=14) were common in feline samples. Evaluation of the AST results of the relevant pathogens revealed that potentiated aminopenicillins and 1st generation cephalosporins seem suitable as first line therapeutic choices in sporadic cases of canine and feline lower

UTI.**Discussion:** The high proportion of culture-negative urine samples and the cumulative susceptibility patterns of uropathogens highlight the importance of a sound bacteriological examination including urine sediment analysis to prevent unnecessary treatment as well as treatment failure.

OA20

PROFILING OF THE MICROBIOTA FROM APULIAN COW MILK MOZZARELLA PRODUCED WITH WHEY

D. Simone, C. Trisolini, V. Laera, G. Caldarola, A. Miccolupo, E. Catalano, A. Caffò, A. M. Derobertis, V. Nardelli, M. Iammarino, A. Parisi
Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Via Manfredonia, 20, 71121 Foggia (Italy)

The Mozzarella di Gioia del Colle PDO is a production excellence of Apulia and Italy and its production is based on whey. Microbial communities play a pivotal role in the quality and features of the mozzarella production chain, but to date they are poorly investigated. In this study, we have profiled microbial communities from mozzarella cheese samples together with the wheys used for their production with targeted metagenomics (V3V4 16S). Our survey revealed the presence of taxa commonly associated with mozzarella production (lactobacilli, staphylococci). Additionally, we detected other taxa commonly related to other dairy production chains (e.g. *Thermus*). Overall, within the same production lot, the same non-lactobacilli taxa were more abundant in the mozzarella compared to the whey. We were also able to detect possible contaminants of the production chain with very low abundance. We showed that targeted metagenomics is a valuable tool to profile microbial communities in mozzarella and whey and to identify pathogens that can enter the production line.

OA21

EVIDENCE OF THE WIDE CIRCULATION OF MULTI-DRUG RESISTANT ENTERIC STRAINS IN LESSER KESTREL (*FALCO NAUMANNI)*

R. Samarelli, N. Pugliese, A. Schiavone, E. Circella, I. Siddique, M. Saleh, C. Zizzadoro, G. Crescenzo, A. Camarda
Università degli Studi di Bari "Aldo Moro", Bari, Italy

This study was aimed to assess the circulation of antimicrobial-resistant, Gram-Negative enteric bacteria in wild birds. Specifically, cloacal swabs were collected from 32 lesser kestrels (*Falco naumanni*) hosted in the apulian wildlife rescue centre in July 2021. All samples were collected upon admittance as part of the routine diagnostic procedures. Swabs were incubated in tryptic soy broth (TSB) with enrofloxacin (enr), selected on McConkey agar with enr, and identified biochemically or by MALDI-TOF. Susceptibility of isolates to the most common antibiotic classes was ascertained by the disc diffusion method. Only one out of 32 samples (3,12%) showed no bacteria growth. From the remaining 31 birds, 41 enr-resistant strains were isolated, specifically 23 *Escherichia coli*, 10 *Proteus mirabilis*, 6 *Klebsiella pneumoniae*, 1 *Citrobacter freundii,* and 1 *Enterobacter cloacae*. Out of them, 39 (95.12%) were resistant to three or more antibiotics.In detail, 35 strains were resistant to tet, 32 to amp, and 25 to sxt or streptomycin (str). No cst-resistant strain was isolated except for *P. mirabilis*, naturally resistant. No strain was resistant to ipm, but 7 were resistant to the fourth-generation cephalosporin cefepime (fep). Those data suggest a wide circulation of multi-drug resistant (MDR) strains, some of them with clinical relevance, in birds that were never treated with antibiotics. Considering its migratory habits, and its position at the top of the food chain, *F. naumanni* should be considered an important indicator of the flow of MDR bacteria between anthropic and wild environments, providing useful information in a One Health view. "The antibiotic names have been abbreviated according to the indications of the British Society for Antimicrobials Chemotherapy, unless otherwise specified"

ORAL- AULA B

OB1

PATTERNS AND DETERMINANTS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN EUROPE: A PHYLODYNAMIC AND PHYLOGEOGRAPHIC APPROACH

G. Franzo, G. Faustini, M. Legnardi, M. Cecchinato, M. Drigo, CM. Tucciarone
Department of Animal Medicine, Production and Health (MAPS), University of Padua, Legnaro (PD), Italy

Porcine reproductive and respiratory syndrome (PRRS) is among the most devastating diseases affecting the pig industry. The remarkable genetic variability of this virus, leading to poor cross-protection, has limited the vaccine efficacy and other measures must be adopted to effectively control the viral circulation. Some recent studies have investigated the factors involved in viral spreading and persistence, at least at the local level. However, despite the topic's relevance, the variables involved in PRRSV epidemiological success at a broader scale, like the European one, have never been investigated using a robust statistical approach, linking classical and molecular epidemiology. In the present study, more than one thousand ORF5 sequences were analyzed through a phylodynamic and phylogeographic approach to investigate the history, dynamics and spreading patterns of PRRSV within European borders. Moreover, several potential predictors representative of swine population features and trade, human population, economy and geographic characteristics, were evaluated through a specifically designed generalized linear model (GLM) to quantify their relevance on viral migration rate between countries over time. Although pig stock density, mean PRRSV strains genetic diversity, investments in agriculture (including a likely role of vaccination) and farmer education were involved to a certain extent, the major determinant was proven to be by far the live pig trade. The present study, which provides a robust depiction of PRRSV European molecular epidemiology patterns and determinants, could contribute to a more rational allocation of resources based on effective prioritization of control measures.

OB2

CANCELLED

OB3

ENTERIC VIRAL CO-INFECTIONS IN ACUTE GASTROENTERITIS OF CATS

A. Palombieri[1], V. Sarchese[1], F. Di Profio[1], P. Fruci[1], V. Martella[2], B. Di Martino[1]
[1]Faculty of Veterinary Medicine, Università degli Studi di Teramo, Teramo, Italy
[2]Department of Veterinary Medicine, Università Aldo Moro di Bari, Valenzano, Italy

Acute gastroenteritis (AGE) is a common clinical problem of cats living in densely housed environments that may recognize a multi-agent aetiology, with more than one virus sequentially or synergistically being involved. The aim of this study was to perform a molecular survey on 62 cats with diarrhoea admitted during 2021 to the veterinary hospital of the Faculty of Veterinary Medicine of Teramo (Italy). Feline enteric samples were tested for selected viral pathogens including feline panleukopenia parvovirus (FPV), feline coronavirus (FCoV), feline kobuvirus (FeKoV), feline chaphamaparvovirus (FeChPV), feline picornavirus (FePV), feline calicivirus (FCV) and norovirus (NoV) using specific or broadly reactive consensus PCR assays at the family, sub-family and/or genus level. The virome composition of eight specimens were further assessed by combining a sequence-independent single-primer amplification (SISPA) approach with Oxford Nanopore Technologies (ONT) sequencing platform. At least one viral pathogen was detected in 67.7% samples with FPV being the most frequently detected virus (35.4%), followed by FCoV (33.8%). FeChPV and FCV were found with rates of 12.9% and 6.4%, respectively. FeKoV, FePV and FeNoV were all detected with a rate of 1.61%. Single infection was identified in 26 samples (41.9%), mainly related to FPV (84.6%). Co-infections were present in 25.8% samples and mainly accounted for by FPV and FCoV. By ONT analysis a picornavirus genetically close to a Feline sakobuvirus A (FeSA) was sequenced. FeSA was first detected in 2012 during a metagenomic study in a healthy cat in Portugal but, since then, it has not been reported again in cats.

OB4

GENOMIC AND TRANSCRIPTOMIC OF RANID HERPESVIRUS 3 AND BUFONID HERPESVIRUS 1 IN NATURALLY INFECTED HOSTS: LINKING MOLECULAR SIGNATURE AND PATHOLOGY PHENOTYPE

F.C. Origgi
Institute of Animal Pathology (ITPA) Vetsuisse Faculty, University of Bern, Bern, Switzerland

Two novel herpesviruses have been recently discovered in free-ranging amphibian populations. Ranid herpesvirus 3 (RaHV3) and Bufonid herpesvirus 1 (BfHV1) have been shown to be associated with a remarkably similar, but distinct proliferative skin diseases in their respective hosts, the common frog (*Rana temporaria*) and common toad (*Bufo bufo*). Interestingly, the disease in both frogs and toads is associated with an inconspicuous or absent detectable, cellular immune response. We have completed the full sequencing of the genome of both viruses and we have analyzed the transcriptomic data obtained from naturally infected frogs and toads. The genome of RaHV3 is 207,914nt long and encodes for 186 predicted protein. The genome of BfHV1 is 158250nt long and encodes for 152 predicted proteins. Both genomes encode for predicted proteins with putative immunomodulatory activity including those known to interfere with macrophage and T-cell activity, all immune cells of critical relevance for antiviral activity. At the same time, an immune modulation appears to occur in the infected host as well. Paralleling this, the transcription of the genes involved in signaling and cell remodeling is the mainly impacted in the infected hosts, suggesting a relevant role in the disease. Furthermore, the genome of RaHV3 contains a predicted gene whose putative encoded protein shares similarities with FOXM1, known to be a critical transcription factor involved in the development of carcinomas in humans. Remarkably, the observed disease phenotype in the infected hosts appears to be the product of a tight interaction between the host and pathogen gene armories

OB5

DO DEFECTIVE KOALA RETROVIRUS SEQUENCE OFFER SELECTIVE ADVANTAGE TO KOALAS?

S. Dudeja, R.E. Tarlinton
School of Veterinary Medicine and Science, University of Nottingham, United Kingdom

Koala Retrovirus (KoRV) is known to be the youngest endogenous retrovirus still settling to its genomic parasitic lifestyle. KoRV has both infectious and endogenous (integrated into the host genome) forms. 100 % of the Koalas in northern Australia are positive for the virus. A higher proviral copy number per cell has been observed in Northern koalas due to endogenized KoRV compared to that in the south. The Koalas in southern Australia show a variability in the prevalence rate of the exogenous virus and a lower rate of KoRV induced disease. Southern Australian koalas earlier considered to be disease free or only having the exogenous counterparts of the virus, demonstrate a defective variant of KoRV known as RecKoRV. RecKoRV is formed due to the recombination between Phascolarctid Endogenous Retroelement (PhER) and KoRV. The presence of RecKoRV variants particularly in the founder population in the French island calls into question the existence of KoRV free animals. The difference in the KoRV profiles between the northern and the southern animals, heighten the chances that these replication defective transcripts may be interfering with full length transcripts of the replication competent KoRV. The research objectives of this project involve screening samples from southern animals for polymorphism of RecKoRV loci using integration site specific PCRs to explore whether these are fixed or variable in the population. Follow on work will then focus on whether there are any links between polymorphic loci and infectious KoRV prevalence and clinical disease incidence.

OB6

A COLD CASE OF EQUINE INFLUENZA DISENTANGLED WITH NANOPORE SEQUENCING

F. Pellegrini[1], G. Lanave[1], A. Hassan Omar[1], G. Diakoudi[1], R. Augelli[2], M. Camero[1], N. Decaro[1], G. Elia[1], C. Buonavoglia[1], V. Martella[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari Aldo Moro, Italy
[2]Ministero della Salute, Ufficio UVAC Bari, Italy

Influenza A viruses (IAVs), family *Orthomyxoviridae*, are enveloped viruses with a ssRNA(-) genome of 13.5Kb, made up of 8 segments. IAVs are classified in subtypes based on the surface proteins hemagglutinin (HA) and neuraminidase (NA), for a total of 18 H and 11 N subtypes. IAVs can infect various avian and mammalian species, causing major acute febrile respiratory disease outbreaks. Equine IAVs (EIAVs) are either H7N7 or H8N3, although H7N7 seems to have disappeared. We determined the whole genome sequence of a H3N8 equine influenza virus (strain Bari/05) identified from a 2005 outbreak in Apulia, Italy, seemingly linked to a 2003 outbreak in a horse racetrack in Rome. After reverse transcription and PCR-based enrichment, a library was generated with the Ligation sequencing kit 1D SQK-LSK110 (ONT) and used for sequencing on MinION Mk1C. The fastq data were assembled to generate contigs of the 8 genome segments and the sequences were analyzed with BLASTn, unveiling a tight correlation of strain Bari/05 to strain A/equine/Pulawy/1/2005(H3N8), identified in Poland in 2005. On interrogation of the national register for animal trading (NSIS), we observed that in 2005 nearly half of the meat (DPA) horses imported in Apulia were from Poland. A small flux of non-DPA horses from Poland was also documented, supporting the hypothesis of introduction of EIAV with horse trading, and ruling out a correlation with the 2003 outbreak. Generating a large database of EIAV genome sequences, including archival viruses, will help understand better the molecular evolution and phylodynamic of EIAV.

OB7

THE EPIDEMIOLOGY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN AN INTEGRATED PIG COMPANY OF NORTHERN ITALY: A MULTIPLE THREATS REQUIRING MULTILEVEL INTERVENTIONS

G. Franzo[1], G. Barbierato[1], P. Pesente[2], M. Legnardi[1], C. M. Tucciarone[1], G. Sandri[2], M. Drigo[1]
[1]Department of Animal Medicine, Production and Health (MAPS), University of Padua, Legnaro (PD), Italy
[2]Laboratorio Tre Valli, 37132 Verona, Italy

Despite the remarkable efforts paid in terms of vaccination administration and biosecurity, eradication and long-term control of Porcine reproductive and respiratory syndrome virus (PRRSV) have often been frustrating. Unfortunately, few studies are currently available that objectively link, using a formal statistical approach, viral molecular epidemiology to the risk factors determining the observed scenario. The present study takes advantage of a remarkable dataset of approximately one-thousand ORF7 sequences, obtained from strains collected between 2004 and 2021 from the largest Italian pig company, which implements strict compartmentalization among independent three-sites (i.e., sow herds, nurseries and finishing units) pig flows. The history and dynamics of the viral population and its evolution over time were reconstructed and linked to managerial choices using a phylodynamic approach. The viral fluxes within and among independent pig flows were evaluated, and the contribution of other integrated pig companies and rurally risen pigs in mediating such spreading was investigated. Finally, viral circulation in Northern Italy was reconstructed using a continuous phylogeographic approach, and the impact of several environmental features on PRRSV strain persistence and spreading velocity was assessed. PRRSV epidemiology was shaped by a multitude of factors, including pig herd management (e.g., immunization strategy), implementation of strict-independent pig flows, and environmental features (e.g., climate, altitude, pig density, road density, etc.). Small farms and rurally raised animals also emerged as a potential threat for larger, integrated companies. Therefore, a multidimensional approach, ranging from individual herd management to collaboration and information sharing among different companies, is mandatory for effective infection control.

OB8

EPIDEMIOLOGICAL INVESTIGATION OF DOMESTIC CAT HEPADNAVIRUS IN HONG KONG

P. Capozza[1], M. Carrai[2], G. Lanave[1], V. Martella[1], J. A. Beatty[2], V. R. Barrs[2]
[1]Department of Veterinary Medicine, University of Bari, Valenzano (Ba), Italy.
[2]Dept Veterinary Clinical Sciences & Centre for Animal Health and Welfare, Jockey Club College of Veterinary Medicine and Life Sciences (JCC), City University of Hong Kong ,Hong Kong

Domestic cat hepadnavirus (DCH) is an emerging virus related to hepatitis-B virus (HBV) of humans. On molecular inestigation from Australia, Italy, Malaysia, Thailand, Japan and UK, DCH

prevalence in feline blood/serum ranges from 0.78 to 12.3%. The aim of this study was to assess the prevalence of DCH in Hong Kong (HK) and to gather information on sequence diversity of HK DCH strains. DCH DNA was detected with virus-specific real-time PCR in 81 (15.8%) of 513 feline blood samples tested, a prevalence higher than reported elsewhere. The mean and median values of DCH in the feline blood were $2.43x10^5$ DNA copies/10 µl and $1.95x10^1$ DNA copies/10µl (range $2.55x10^0$-$1.96x10^7$ DNA copies/10µl), apparently without any significant correlation with the main risk factors considered, i.e. age, sex, breed, neuter status and alanine amino-transferase (ALT). The complete genome sequence of 12 DCH strains was obtained, using a primer walking strategy. On phylogenetic analysis, all the HK DCH strains clustered together with viruses from Australia and Asia (clade A), separate from viruses from Europe (clade B) and from a divergent virus from Japan (clade C). Based on our findings, the DCH strains circulating in HK appear as a continuum of the Asiatic strains.

OB9

CORONAVIRUS DIVERSITY IN UK WILDLIFE

T. Apaa[1], R. Tarlinton[1], C. Staley[1], A. Withers [1], E. Chadwick[2], F. Hailer[2], L. Mckenzie[5], X. Lambin[5], F. Mathews[3], S. Harrison[4], S. Bremner-Harrison[4], M. Loose[1], A. Blanchard[1], M. Bennett[1]

[1]University of Nottingham, Nottingham, UK.
[2]Cardiff University, Cardiff, UK.
[3]University of Sussex, Brighton, UK.
[4]Nottingham Trent University, Nottingham, UK.
[5]University of Aberdeen, UK.

Horseshoe bats (*Rhinolophidae*) are known to be the natural reservoir hosts for *Sarbecoviruses,* and SARS-CoV-2 is likely to have originated from these bat species. Computational analysis and animal studies have demonstrated the presence of high binding affinity of SARS-CoV-2 to ACE-2 receptors in multiple potential animal host species. Continuous field detection of SARS-CoV-2 in a wide range of animal species is reported. Large numbers of cross species transmission events have been recorded in White tailed deer, cats, and mink. The emergence of further reservoir hosts and SAR-CoV-2 variants with potential spill-back to humans is likely. The United Kingdom is home to several wild mustelid, bat and cricetid rodent species that are potentially susceptible to SARS-CoV-2 however, insufficient data on SARS-CoV-2 spill-over/circulation in wild animals is available. The aim of this project was to determine Coronaviruses (CoVs) circulating in UK wildlife and SARS-CoV-2 spill-over from humans into wildlife. A total of 960 samples (faecal, oronasal or rectal swabs, head and neck lymph nodes, and lung samples) collected from UK wildlife (water voles, stoat, otter, pine marten, weasel, mink, badgers, fox, and four UK bat species) have been screened using published generic CoVs *pol* and Sarbecovirus *E* gene PCR protocols. Meta-transcriptomics sequencing and bioinformatic analysis shows the presence of ferret and bat coronaviruses.

OB10

CROSS-TRANSMISSION OF FELINE LEUKEMIA VIRUS (FELV) BETWEEN CHILEAN DOMESTIC AND WILD FELIDS

C. Castillo[1], E. Hidalgo[2], C. Napolitano[3], A. Blanchard[1], R. Tarlinton[1]

[1]University of Nottingham, Nottingham, UK
[2] Fundación Buin Zoo, Santiago, Chile
[3]Universidad de Los Lagos, Osorno, Chile

Feline Leukaemia Virus (FeLV) is a gammaretrovirus of cats. FeLV is widespread among domestic cat populations and can infect many other felids and can produce a serious disease threat to several species of endangered felids. The main consequences of FeLV infection are hematopoietic disorders and neoplasia. Domestic cats have both endogenous (copies of virus in the cat genome) and exogenous variants of FeLV, these frequently recombine producing variants with recombinant

envelope genes and alternate receptor usage. Until recently inter-cat transmission was presumed to be solely the exogenous (FeLV-A) variant but recent studies in domestic cats and in wild pumas in North America have demonstrated that the recombinant FeLV-B variants are also transmitted horizontally. This study applied envelope gene PCR and NGS (Illumina) to determine the envelope gene diversity and transmission dynamics of FeLV variants in Chilean domestic cats obtaining sequences from FeLV-A, B and endogenous FeLV. FeLV-A sequences obtained, are clustered in a Chilean group closer to sequences from the United States and Brazil. The next step will be to apply the same methodology to Chilean wild felids including: free-range wild felids (*Leopardus guigna)*, and felids held in zoological parks (*Caracal caracal*, *Leopardus guigna*, *Panthera leo*, *Panthera uncia*, *Leopardus pardalis*, *Panthera onca*, *Puma concolor* and *Panthera tigris*). The primary aims of the study are to determine if FeLV B (or other envelope variants of FeLV) are being disseminated between wild and domestic felids.

OB11

EVIDENCE OF CIRCULATION OF NEWLAVIRUS IN FOXES IN SOUTHERN ITALY

G. Lanave[1], L.A. Ndiana[1], F. Pellegrini[1], B. Di Martino[2], G. Sgroi[1], V. Vasinioti[1], M. Camero[1], D. Otranto[1], N. Decaro[1], C. Buonavoglia[1], V. Martella[1]
[1]Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy.
[2]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

In 2021, a novel protoparvovirus, provisionally named as Newlavirus (NLV), was reported with a prevalence as high as 44% in foxes in Canada. The virus exhibited a high degree of genetic diversity with low sequence identities in the VP1 gene (70.5–87.8%) and variable detection rates in different fox tissues. Since NLV were detected in other animal species tested, foxes were supposed to be the natural reservoir of the virus.
We assessed the prevalence and genetic diversity of NLV in a collection of fox samples in Italy.
One hundred lymph node samples were collected from necropsied foxes (*Vulpes vulpes*) in Southern Italy over the 2013-2016 period. NLV DNA was detected by real time PCR in 71/100 (71%) samples. NLV load ranged between $2.3x10^1$ and $4.0x10^6$ DNA genome copies/10µl (mean = $2.2x10^5$ DNA genome copies/10µl, median=$4.9x10^3$ DNA genome copies/10µl). The complete genome sequence of 7 NLV strains and the full-length VP2 sequence of other 14 NLV strains were obtained. Whilst in the NS1 region the 7 Italian strain clearly segregated apart from the Canadian viruses, in the partial (663 bp) VP1/VP2, the 21 Italian NLV strains were intermingled with the Canadian strains throughout three distinct genetic clusters, a pattern consistent with recombination. Overall, the NLV prevalence in foxes in Italy was higher than expected. The high genetic diversity observed in the VP1/VP2 gene (70.2-99.7%) of NLVs collides with the high genetic conservation of carnivore protoparvovirus species 1 (CPV/FPV), suggesting complex evolutionary dynamics of this novel protoparvovirus in wildlife.

OB12

MOLECULAR PREVALENCE OF EQUINE PARVOVIRUS-HEPATITIS IN ITALIAN HORSES

R. Cardone, G. Diakoudi, G. Lanave, V.I. Vasinioti, V. Martella, C. Buonavoglia, G. Elia
Department of Veterinary Medicine, University of Bari "Aldo Moro", Italy

In 2018 a novel equine parvovirus (EqPV-H) was identified in horses with hepatitis and tentatively associated with Theiler's disease. Since then, surveillance studies have demonstrated a global distribution of EqPV-H in horses with subclinical to severe hepatitis. To assess the distribution of EqPV-H infection in equid population in Italy, a collection of 1090 serum samples was screened for the presence of viral DNA using a specific real time PCR assay. Viral DNA was extracted using a commercial Kit (Qiagen S.p.A., Milan, Italy). Pooled serum samples were initially screened and EqPV-H positive pools were separated and re-screened in order to identify the virus-positive samples. Overall, 61/1090 (5.6%) sera tested positive for EqPV-H. EqPV-H transmission still remains unclear, even if iatrogenic transmission via contaminated biological products of equine-origin has been demonstrated experimentally. Interestingly, a cluster of infections was identified in the same stable, in Apulia, South Italy, with 7/14 contact horses being positive to EqPV-H. This finding hints to a contagious nature of EqPV-H and highlights the need for further investigation on the modalities of virus transmission.

OB13

STANDARDISATION OF MOLECULAR AND MICROBIOLOGICAL METHODS FOR THE DETECTION AND IDENTIFICATION OF PATHOGENS RESPONSIBLE FOR BOVINE RESPIRATORY DISEASE (BRD)

L. Capozzi[1], A. Bianco[1], D. Simone[1], C. Quarato[1], C. Bulzachelli[1], M. Solito[1], G. Schino[1], L. pace[1], M. Galgano[2], A. Pratelli[2], L. Del Sambro[1]
[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia , Italy
[2]Department of Veterinary Medicine, University Aldo Moro of Bari, Valenzano (BA), Italy

Bovine Respiratory Disease (BRD) is one of the most common causes of morbidity and mortality in cattle. It affects the lower respiratory tract, is characterized by severe clinical conditions and high contagiousness. BRD is sustained by several pathogens, both viral and bacterial, and mixed infections are also frequently identified in pneumonia cases.
The aim of the present study was to identify, using microbiological and molecular methods, the pathogens responsible for pulmonary lesions detected in cattle at the abattoir, related to BRD. Sampling was carried out during the slaughter phase, exclusively on carcasses presenting pulmonary lesions referable to BRD, at two slaughterhouses located in the province of Bari. All the samples taken (n.44) were subjected to culture examinations by sowing on selective media, in order to search for the micro-organisms responsible for the disease. Isolated colonies were identified by biochemical and molecular methods (Table 1). Next generation sequencing methods allowed the investigation of virulence and antibiotic resistance characteristics. Nucleic acids extracted from tissue fragments taken from lesions were tested by Real-Time PCR for specific targets of the main viral and bacterial pathogens responsible for BRD (Table 2).
Microbiological and molecular tests revealed in 88.63% of samples tested (39/44) the presence of at least one of the pathogens that can be involved to the pathogenesis of BRD.
The outcomes obtained from this surveillance study may lay the foundations for promoting therapeutic and prophylactic strategies, aimed at reducing the spread of BRD as well as assessing

the economic impact on regional animal husbandry.

Table 1. Results of species identification tests, carried out on colonies isolated from each sample tested (ID: BRD_001-BRD_044).

ID Sample	Species identification confirmed by Sanger sequencing			
	Peptone Phosphate Broth	Blood agar	MacConkey	TSB
BRD_001	-	-	-	-
BRD_002	*M. bovigenitalium*	*Trueperella pyogenes*	-	-
BRD_003	-	-	*Pasteurella multocida*	-
BRD_004	-	-	*Microvirgula aerodenitrificans*	-
BRD_005	-	*Trueperella pyogenes*	-	-
BRD_006	-	*Aeromonas hydrophila*	-	-
BRD_007	-	*Hafnia alvei*	-	-
BRD_008	*M. bovis*	-	-	-
BRD_009	-	-	*Pasteurella multocida*	-
BRD_010	-	-	-	-
BRD_011	-	*Serratia liquefaciens*	-	-
BRD_012	-	*Aeromonas hydrophila*	-	-
BRD_013	-	*Macrococcus caseolyticus*	-	-
BRD_014	-	*Hafnia alvei - Macrococcus caseolyticus*	-	-
BRD_015	-	*Escherichia coli*	-	-
BRD_016	*M. arginini*	*T. pyogenes - Enterococcus hirae*	*Escherichia coli*	-
BRD_017	-	*Enterobacter*	-	-
BRD_018	*M. arginini*	*Enterococcus*	-	-
BRD_019	*M. arginini*	*Advenella kashmirensis*	-	-
BRD_020	-		*Pseudomonas koreensis*	-
BRD_021	*M. arginini*	-	-	-
BRD_022	*M. arginini*	*Staphylococcus equorum*	-	-
BRD_023	*M. bovis*	*Pseudomonas putida*	-	-
BRD_024	*M. bovirhinis*	*Advenella kashmirensis*	-	-
BRD_025	-	*Aeromonas*	*Escherichia coli*	-
BRD_026	*Ureaplasma*	*Aeromonas - S. chromogenes*	-	-
BRD_027	*M. bovis*	*Trueperella pyogenes*	-	-
BRD_028	*M. bovis*	*Pasteurella multocida + Trueperella*	-	-
BRD_029	-	-	-	*Hafnia alvei*
BRD_030	*M. bovis*	-	-	*Streptococcus-Serratia*
BRD_031	-	*Staph. vitulinus*	*P. multocida - Pantoea agglomerans*	-
BRD_032	-	*Streptococcus - Staph. vitulinus*	-	-
BRD_033	-	-	-	*Escherichia coli*
BRD_034	*M. bovigenitalium*	*Pasteurella trehalosi - T. pyogenes*	-	-
BRD_035	-	*Pasteurella multocida*	-	-
BRD_036	-	*Pasteurella multocida*	-	-
BRD_037	-	-	-	-
BRD_038	-	*Pasteurella multocida*	-	-
BRD_039	*M. arginini*	*Trueperella pyogenes*	-	-
BRD_040	-	*Staph. epidermidis*	-	-
BRD_041	*M. bovigenitalium*	*Aeromonas*	-	-
BRD_042	-	-	*Aeromonas*	-
BRD_043	-	*Proteus mirabilis*	*Acinetobacter baumannii*	-
BRD_044	-	*Mannheimia haemolytica*	-	-

Table 2. Results of Real-Time PCR tests, performed on the DNA/RNA extracts of each sample tested (ID: BRD_001-BRD_044).

ID Sample	Real Time PCR										
	M.bovis	*P.multocida*	*H. somni*	*M. haemolytica*	*T.pyog*	*BCoV*	*BVDV*	*BRSV*	*BAdV*	*BoHV1*	*BPiV3*
BRD_001	-	+	-	-	-	-	-	-	-	-	-
BRD_002	+	-	+	-	+	+	-	-	-	-	-
BRD_003	-	+	-	-	-	-	-	-	-	-	-
BRD_004	-	-	-	-	-	-	-	-	-	-	-
BRD_005	-	-	-	-	-	-	-	-	-	-	-
BRD_006	-	-	-	-	+	-	-	-	-	-	-
BRD_007	-	+	+	-	+	-	-	-	-	-	-
BRD_008	+	-	-	-	-	-	-	-	-	-	-
BRD_009	-	+	-	-	-	-	-	-	-	-	-
BRD_010	-	-	+	-	-	-	-	-	-	-	-
BRD_011	-	-	-	-	-	-	-	-	-	-	-
BRD_012	-	-	-	-	-	-	-	-	-	-	-
BRD_013	-	+	-	-	-	+	-	-	-	-	+
BRD_014	-	-	-	-	-	-	-	-	-	-	-
BRD_015	-	-	-	-	-	+	-	-	-	-	-
BRD_016	+	+	+	-	+	-	-	-	-	-	-
BRD_017	-	+	+	-	-	-	-	-	-	-	-
BRD_018	+	+	+	-	+	-	-	-	-	+	-
BRD_019	-	-	-	-	-	-	-	-	-	-	-
BRD_020	-	-	-	-	+	-	-	-	-	-	-
BRD_021	-	+	-	-	+	+	-	-	-	-	-
BRD_022	+	+	-	-	+	+	-	-	-	-	+
BRD_023	+	+	+	-	+	+	-	-	-	-	-
BRD_024	-	+	-	+	+	+	-	-	-	-	-
BRD_025	+	+	+	-	+	-	+	-	-	-	-
BRD_026	+	+	+	-	-	-	+	-	-	-	-
BRD_027	+	+	+	-	+	+	-	-	-	-	-
BRD_028	+	+	+	-	-	-	-	-	-	-	-
BRD_029	+	-	-	-	-	-	-	-	-	-	-
BRD_030	+	-	-	-	+	-	-	-	-	-	-
BRD_031	-	-	+	-	-	+	+	-	-	-	-
BRD_032	-	+	+	-	-	-	+	-	-	-	-
BRD_033	-	-	-	-	-	-	-	-	-	-	-
BRD_034	-	-	+	-	+	-	-	-	-	-	-
BRD_035	-	+	-	-	+	+	-	-	+	-	-
BRD_036	-	+	-	-	+	-	-	-	+	-	-
BRD_037	-	-	-	-	+	+	-	-	-	-	-
BRD_038	-	-	-	-	+	+	-	-	-	-	-
BRD_039	+	+	+	-	+	-	-	-	-	-	-
BRD_040	-	-	-	-	-	+	-	-	-	-	-
BRD_041	+	+	+	-	-	+	-	-	-	-	-
BRD_042	-	-	-	-	-	+	-	-	+	-	-
BRD_043	-	+	+	-	+	-	-	-	-	-	-
BRD_044	-	+	+	+	-	-	-	-	-	-	-

OB14

GENETIC DIVERSITY OF *SALMONELLA* INFANTIS ISOLATES FROM BROILERS, HUMANS AND FOOD, 2007–2021

B. Papić[1], D. Kušar[1], J. Mićunović[1], M. Pirš[2], M. Biasizzo[1], M. Kavalič[1], M. Ocepek[1], J. Avberšek[1]

[1]*University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia*

Salmonella Infantis is the most common *Salmonella* serotype in broilers and is among top five serotypes isolated from human patients. A decade ago, *S.* Infantis clone harboring a mosaic megaplasmid named pESI was first reported, associated with increased virulence, biofilm formation and multidrug resistance. Since then, *S.* Infantis clones with pESI-like plasmids have been reported worldwide, replacing pESI-free clones. Here, 212 *S.* Infantis isolates from Slovenia, 2007–2021, underwent whole-genome sequencing to compare the population structure of *S*. Infantis from different sources: broilers (*n* = 150), humans (*n* = 30) and meat (*n* = 32). Core genome multilocus sequence typing (cgMLST) revealed that all isolates were highly genetically homogeneous and belonged to sequence type (ST) 32, except for one human isolate, which was assigned to a novel ST. Out of 30 human isolates, five were closely related (≤7 allele differences) to broiler and/or food isolates, indicating possible zoonotic transmission. Several additional clusters of non-human isolates were observed and were mostly supported by the underlying epidemiological data. On farms where multiple broiler isolates were analyzed, persistence of a single clone was observed; however, reintroduction of additional clones was also noted. pESI-positive clones predominated in all three groups. Because the spread of *S*. Infantis clones was observed across all three analyzed sources, the Slovenian broilers and meat are the source of infection for humans in some cases. Nevertheless, the source of infection for most human cases remains unknown.

OB15

METAGENOMICS INVESTIGATION IN A MULTI-PATHOGEN FOODBORNE OUTBREAK OF ACUTE GASTROENTERITIS USING NANOPORE TECHNOLOGY.

G. Diakoudi[1], G. Lanave[1], F. Pellegrini[1], F. Beikpour[1], E. Suffredini[2], C. Catella[1], D. Loconsole[3], M. Chironna[3], V. Martella[1]

[1]*Dipartimento di Medicina Veterinaria, Università degli Studi di Bari Aldo Moro, Italy*
[2]*Istituto Superiore di Sanità, Rome, Italy*
[3]*Dipartimento di Scienze Biomediche, Università degli Studi di Bari Aldo Moro, Italy*

Syndromic diagnostics have unveiled that the aetiology of acute gastroenteritis (AGE), in either sporadic cases or in outbreaks, can be multifactorial, thus posing a challenge for the current diagnostic approaches. In April 2018, an AGE outbreak related to consumption of raw seafood in Bari, Italy, was identified. Up to twelve different viral strains of norovirus, astrovirus and aichivirus were detected from both symptomatic and asymptomatic persons, suggesting massive contamination of the food source. Accordingly, these samples were considered an excellent target for metagenomics investigation using Nanopore sequencing technology. The fecal sample of a symptomatic patient was used to generate a library with SISPA protocol, producing ~5.8 GB of data (> four million reads) in MinION Mk1C flow cell. About 12% of the reads were recognized as viral on Genome Detective virus tool. We generated the complete genome sequence of a human mamastrovirus (family *Astroviridae*), the nearly complete genome sequence of a human aichivirus A (family *Picornaviridae*) and reads of norovirus genogroup I (family *Calicivirdae*), all associated with AGE in humans. Also, we detected reads of picobirnaviruses (family *Picobirnaviridae*) and of salivirus A (family *Picornaviridae*), whose role in AGE is unknown/unclear. In addition, reads of 4 plant viruses and of 138 phages were identified. Overall, the experiment unveiled the complexity of fecal virome in the patient and confirmed the importance of metagenomics approach in investigation of foodborne outbreaks.

OB16

GENOME SEQUENCING OF A RABBIT ROTAVIRUS A STRAIN WITH NANOPORE PLATFORM

A. Hassan Omar, F. Pellegrini, G. Lanave, C. Catella, G. Diakoudi, A. Cannarozzi, G. Casalino, E. Circella, F. D'Amico, C. Buonavoglia, V. Martella
Dipartimento di Medicina Veterinaria, Università degli Studi di Bari Aldo Moro, Italy

Group A Rotaviruses (RVAs), family *Reoviridae* genus, are important gastroenteric pathogens associated with acute gastroenteritis in humans and animals. RVA genome is composed of 11 segments of double-stranded RNA (dsRNA) for a total of 18.5kb. A genome-based classification was developed in 2008 to compare effectively different RVA strains and explore their genome make-up. RVA are associated with enteric diseases in rabbit, mainly in post-weaning animals.
We identified the RNA of RVA by a quantitative TaqMan real time RT-PCR (qRT-PCR) in a group of young rabbits (9 out of 11 stool samples, mean CT= 25.88, range CT=15.33-31.34) with enteritis complex from Santeramo in Colle (Bari, Italy).
By using a PCR-based enrichment protocol, the complete genome of the rabbit RVA strain was reconstructed. The PCR product was used for library preparation with a Ligation sequencing kit SQK-LSK110 (Oxford Nanopore Tech.) and sequenced on a MinION MK1C device. The fastq files were analysed with Genome Detective. The 96.8% of the genome was assembled, using 62,1268 reads, with a 69,739.5-depth coverage. The virus showed the genotype constellation G3-P[14]-I2-R2-C2-M3-A9-N2-T6-E5-H3, with the highest nucleotide identity in BLAST to either RVA/Human-wt/BEL/B4106/2000/G3P[14] or RVA/Human-wt/BEL/BE5028/2012/G3P[14], in all the genome segments. These human viruses were detected in children in Belgium in 2000 and 2012 and were regarded as zoonotic viruses of rabbit origin. Gathering sequence data on animal RVA is pivotal to reconstruct the origin of unusual human RVAs of zoonotic origin.

OB17

CANCELLED

PSA 23 – OB18

A SUSPECTED CASE OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN THE NETHERLANDS – A RARE DISEASE REVISITED

H. Graham[1], M. van der Most[1], R. Dijkman[2], L. Harkema[2], L. Labout[3], P.H.M.M. Jacobs[3], M.A.H. Spierenburg[3], M. Heijne[1]
[1]Wageningen Bioveterinary Research, Lelystad, The Netherlands
[2]Royal GD, Deventer, The Netherlands
[3] Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, The Netherlands

The histologic features of lung tissue of a bull that was euthanized due to severe respiratory symptoms shortly after import in the Netherlands, were indicative of contagious bovine pleuropneumonia (CBPP), a notifiable disease caused by *Mycoplasma mycoides* subspecies *mycoides* (Mmm). The Netherlands had been free of this rare disease since 1886. The suspicion prompted an investigation, which involved the veterinary authorities and the national reference laboratory (NRL). A real-time PCR was performed on tissue samples of the lung and bronchial tree, yielding a negative result. Furthermore, a farm visit was made to inspect the health of the herd and to collect serum samples from the co-housed animals. One out of twenty serum samples tested positive in an in-house CBPP complement fixation test (CFT), which led to a second farm visit to repeat the sampling. In addition, tissue samples of the bull and the collected serum samples were forwarded to an OIE reference laboratory for confirmation. The OIE reference laboratory confirmed the absence of Mmm DNA in the samples of the bull and sequencing revealed the presence of *Mycoplasma bovis*. Because of this and by performing the cELISA for CBPP on the serum samples, the initially positive CFT result was considered to have been caused by cross-reactivity. This case may be closed, but is a valuable reminder for laboratories and microbiologists not to forget this disease and be prepared with the right set of specific diagnostics tests: not only to diagnose CBPP, but more importantly, to be able to exclude it.

OB19

NOVEL *CORYNEBACTERIUM* SPECIES ISOLATED FROM A CORNEAL ULCER AND URINE IN DOGS

M. Tresch[1], C. Watté[2], M. Stengard[2], S. Feyer[1], E. Akdesir[1], C. Ritter[3], S. Kittl[1]
[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland
[2]Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland
[3]Veterinary Office Villereuse, Geneva, Switzerland

Background: Corynebacteria are Gram-positive club-shaped bacteria, which cause various diseases in animals. Though corynebacteria can occur in conjunctival sacs of healthy dogs, species such as *C. pseudodiphtheriticum* and *C. renale* have also been isolated from dogs with corneal ulceration. *C. urealyticum* is described as a rare cause of cystitis in dogs. Methods: A swab from a corneal ulcer and a urine sample from two canine patients were routinely cultured in our laboratory. Coryneform bacteria were isolated which could not be identified using MALDI-TOF MS. Therefore, 16s rRNA gene sequencing was performed. Antimicrobial susceptibility was determined by broth microdilution (Sensititre®) following CLSI guidelines. The isolates were subjected to whole genome sequencing (Illumina®) followed by digital DNA-DNA hybridization (dDDH) and Genome Blast Distance Phylogeny analysis (Type Strain Genome Server). Furthermore, average nucleotide identity (ANI) was determined using OAT v0.93.1. Results: While 16s rRNA sequencing preliminarily identified the isolates as *Corynebacterium oculi*, first isolated from ocular specimens in humans, analysis of the whole genome sequencing data indicated that these isolates cluster together as a potentially new, closely related species. ANI with the most closely related species *C. oculi* was only 87%, confirming this finding. While the ocular isolate showed low MIC ranges for all tested antibiotics, the isolate from urine showed high MIC values for fluoroquinolones and a mutation in the *gyrA* gene was detected. Conclusion: As a versatile and potentially fluoroquinolone-resistant pathogen in dogs, this novel species should be further investigated. More isolates are needed for further characterization.

OB20

ARYL HYDROCARBON RECEPTOR IS ACTIVATED BY INFECTION WITH CANINE CORONAVIRUS

C. Cerracchio[1], M.G. Polverino[2], V. Iovane[3], A.R. Attili[4], M.G. Amoroso[2], F. Fiorito[1,4]
[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy
[2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055, Portici, Portici (Naples), Italy
[3]Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy
[4]BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples Federico II, Portici, Naples,Italy

Objectives: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that interacts with endogenous and exogenous substrates including bilirubin, biliverdin, tryptophan metabolites, environmental pollutants, and microbial metabolites. The activation of AhR by these substances induces the control of the expression of target genes such as AhR repressor, detoxifying monooxygenases, and cytokines. Recent advances reveal that AhR signaling regulates aspects of the intrinsic, innate and adaptive immune response to diverse microorganisms. AhR is involved in the host response to Coronaviruses (CoVs) (i.e. MCoV, SARS-CoV-2, HCoV 229E) infection. Particularly, AhR agonists decrease the expression of ACE2 via AhR activation, resulting in suppression of SARS-CoV-2 infection in mammalian cells. Here, we report that AhR is activated by infection with genotype II of canine coronavirus (CCoV-II), an alphacoronavirus. Moreover, pharmacological inhibition of AhR suppresses in vitro replication of CCoV.
Methods used: Infection of CCoV (378/strain) in canine fibrosarcoma (A72) cell line was performed in the presence of CH223191, an AhR antagonist. Bioscreen, immunofluorescence, and virus yield

analyses were carried out.
Results: Following CCoV infection, we found a considerable stimulation of AhR, a receptor expressed in A72 cells. At non-toxic concentration, CH223191 noticeably reduced cell death signs and increased cell viability. Furthermore, the AhR antagonist induced a meaningful decline in virus yield, accompanied by the inhibition of the expression of viral nuclear protein.
Conclusions: Taken together, our findings show that infection with CCoV activates AhR. Furthermore, pharmacologic AhR inhibition reduces CoVs replication in vitro, identifying AhR as a possible candidate target for antiviral therapy..

POSTER SESSION A

PSA01

ANTIBODIES AGAINST TESTUDINID HERPESVIRUSES 1 AND 3 IN PET TORTOISES IN EUROPE

R. E. Marschang, E. Müller, C. Leineweber
LABOKLIN GmbH & CO KG, 97688 Bad Kissingen, Germany

Herpesviruses can cause serious disease in turtles and tortoises. Four genetically distinct herpesviruses have been described in tortoises, testudinid herpesvirus (TeHV) 1-4. Types 1 and 3 are by far most commonly described. Both have been isolated in cell culture and virus neutralization tests (VNT) can be used to detect antibodies against each in plasma or serum from infected tortoises. In a retrospective study, antibody detection by VNT in samples from 1728 captive tortoises submitted to our laboratory between 2016 and 2020 were evaluated. Antibodies against at least one of the viruses were found in 122 animals (7.06%; 95% CI 5.95-8.37%), including 41 (2.37%; 95% CI 1.75-3.20%) with antibodies against TeHV1 only, 62 (3.59%; 95% CI 2.81-4.58%) against TeHV3 only and 19 (1.1%; 95% CI 0.71-1.71%) against both viruses. Detection rates differed significantly between different species ($P < 0.001$) with the highest rates against TeHV1 found in Russian tortoises (*Testudo horsfieldii*) (22.92%) and the highest rates against TeHV3 found in marginated (*T. marginata*) (22.47%) and spur-thighed tortoises (*T. graeca*) (12.23%). TeHV3 detection rates also differed significantly depending on the country from which the samples were submitted ($P = 0.0014$), while those for TeHV1 did not ($P = 0.5567$). Compared to results of virus detection studies in pet tortoises in Europe, antibodies were detected in a significantly ($P = 0.0059$) lower percentage of Hermann's tortoises (*T. hermanni*) compared to virus detection rates. This supports the hypothesis of previous authors that immune response to herpesvirus infection is dependent on host species in tortoises. **Key words**: Russian tortoise, Hermann's tortoise, spur-thighed tortoise, testudinid herpesvirus, *Testudinid alphaherpesvirus 3*, *Testudo*, serology

PSA02

DETECTION OF PORCINE CIRCOVIRUS IN AN ITALIAN FOX

F. Alfano[1], M. G. Lucibelli[1], N. D'Alessio[1], C. Auriemma[1], A. Gallo[1], E. De Carlo[1], N. Decaro[2], V. Martella,[2] G. Fusco[1]
[1]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy
[2] Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano (BA), Italy

The aim of our work was to study the circulation of porcine circovirus (PCV-2) in domestic and wild carnivores in Italy.Out of 206 carcasses (100 dogs, 77 cats, 14 wolves, 15 foxes) analysed in our investigation, 5 tested positive for PCV-2 (2 dogs**,** 1 cat, 1 fox, 1 wolf). A single PCV-2 infection was found in the brain of a fox (#119493), whereas in the other PCV-2 positive animals, co-infections with other pathogens were observed. The localization of circoviruses in the brain is not unusual in animals and has recently been reported in humans with paraplegia and acute central nervous system infections (Smits et al. 2013; Tan et al. 2013). In 2016 in southern Italy a canine circovirus was found in the brain of dogs and wolves, but not in foxes and it was associated to neurological and enteric signs (Zaccaria et al. 2016). According to our knowledge, this is the first report of porcine circovirus in the brain of a fox.
The results of our study highlight the need to monitor the PCV-2 circulation in carnivores, as well as the clinical forms associated to this viral agent in animals different from swine.

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PS03

EFFECTS OF FARMACOLOGICAL INHIBITION ON THE AUTOPHAGIC MACHINERY DURING FELINE HERPESVIRUS (FHV-1) INFECTION IN VITRO.

G. Ferrara[1], V. Iovane[2], F. P. Nocera[1], C. Longobardi[3], U. Pagnini[1], S. Montagnaro[1]
[1]Department of Veterinary Medicine and Animal Productions, University of Naples "Federico II", Naples, Italy
[2]Department of Agricultural Sciences, University of Naples "Federico II", Naples, Italy.

[3]Department of Mental, Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy.

Feline herpesvirus type 1 (FeHV-1) is a common virus that causes rhinotracheitis and conjunctivitis in cats. This virus, like other members of the alphaherpesviridae subfamily, interacts with autophagic machinery to complete its biological cycle. We investigated the effects of pharmacological autophagy inhibition on viral progeny production in this study.
Permissive CRFK cells were infected with FeHV-1, in the presence of different autophagy inhibitors (3-Methyladenine, LY294002, bafilomycin A1, and chloroquine diphosphate). Following viability evaluation (MTT assay), we investigated autophagic flux using autophagy markers (LC3 and p62/SQSTM1) by western blot analysis. Furthermore, we used viral titration (TCID50) and real-time PCR to investigate viral production in the absence and presence of inhibitors. Bafilomycin and chloroquine treatment reduced the conversion of LC3I in II as well as the degradation of p62/SQSTM1. Autophagy inhibition was accompanied by lower viral titers and viral gene expression. Using other inhibitors, no effects were observed.
These results were confirmed when siRNA was used to interfere with the late stages autophagy related gene ATG5. On the other hand, the use of rapamycin (an autophagy inducer) had the opposite effect on the viral titers as well as on the viral gene expression. These results suggest the importance of basal autophagy for the accomplishment of the FeHV-1 cycle, as well as the reduction of viral spread caused by the use of late-stage autophagy inhibitors. These compounds require additional experiments to determine their potential therapeutic use, considering the in vivo cytotoxicity of bafilomycin and the wide application of chloroquine in humans.

PS04

THE IMPORTANCE OF INDIRECT ELISA TESTS IN THE DETECTION OF SMALL RUMINANTS LENTIVIRUS INFECTION IN PORTUGAL

J. Jacob-Ferreira[1], A.C. Coelho[1], A. Grau Vila[2], O. Mínguez González[2], D. Lacasta[3], R. Valentim[4], H. Quintas[4]
[1]CECAV – Animal and Veterinary Research Centre; Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
[2]Servicio de Sanidad Animal, Dirección General de Producción Agropecuaria e Infraestructuras Agrarias, Consejería de Agricultura y Ganadería, Junta de Castilla y León, Valladolid, Spain,
[3]Animal Pathology Department, Instituto Agroalimentario de Aragón-IA2, Universidad de Zaragoza-CITA, 50013 Zaragoza, Spain
[4]Mountain Research Centre (CIMO), School of Agriculture, Polytechnic Institute of Bragança (IPB), Campus de Santa Apolónia, Bragança, Portugal

OBJECTIVES: Small ruminant lentiviruses (SRLVSs) are the group of viruses responsible for Maedi-Visna in ovine and for Caprine Arthritis Encephalitis in caprine species. Theses diseases result of progressive and chronic infections as well as are one of the major causes of severe economic loss. On current days, in Portugal, there is few information about SRLV infection. The main aim of this research is to quantify the seroprevalence associated to SRLV in Portugal. **MATERIAL AND METHODS:** Seroprevalence of SRLV research was done in small ruminant herds of the Portugal. Between 14 to 19 blood samples of different ages, based on the total number of animals in each herd were collected. The infection by SRLV in each sample was made using a commercial test of indirect ELISA (ID Screen® MVV/CAEV Indirect). The herd was considered positive if at least one animal was seropositive. **RESULTS:** We collected samples in a total of 102 herds, having 75 of ovine, 18 of caprine and 9 of mixed species. We verified that 91(89.22%) herds were positive to SRLV, of which 66 (88%) of ovine, 16 (88.89%) of caprine and 9 (100%) of mixed species. Of 1774 blood samples, 745 (42%) were positive, with 519 (39.74%) positive animals in ovine herds, 160 (51.78%) in caprine herds and 66 (41.51%) in mixed species herds. **CONCLUSION:** There is a highly seroprevalence of SRLV infection in Portugal. These results highlight the importance of using laboratorial methods such as ELISA tests for the early detection of diseases in small ruminant herds.

PS05

ANTIVIRAL ACTIVITY OF FUNGAL SECONDARY METABOLITE 6-PENTYL-a-PYRONE AGAINST CANINE CORONAVIRUS INFECTION

C. Cerracchio[1], M.G. Polverino[2], A. Staropoli[3,4], F.P. Nocera[1], L. De Martino[1], MG Amoroso[2], F. Vinale[1,5], F. Fiorito[1,5]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy

[2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055, Portici, Portici (Naples), Italy

[3]Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy

[4]Institute for Sustainable Plant Protection, National Research Council, Portici, Naples, Italy

[5]BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples Federico II, Portici, Naples, Italy

Objectives: Genotype II of canine coronavirus (CCoV-II), an alphacoronavirus, can cause self-limiting enteric disease in dogs. The remarkable plasticity of CoVs occurs through mutation and recombination processes. The current SARS-CoV-2 pandemic as well as the recent detection of a novel canine-feline recombinant alphacoronavirus isolated from a human patient highlights the cross-species transmission ability of CoVs. In this scenario, antiviral compounds are considered necessary to fight CoVs infections. Fungi produce secondary metabolites (SMs), often developed as antibiotics, fungicides, hormones, and plant growth regulators. Screening performed on benzo-γ-pyrone 3-*O*-methylfunicone, a SM produced by *Talaromyces pinophilus*, showed that it reduces infectivity of hepatitis C virus and bovine herpesvirus 1. 6-pentyl-α-pyrone (6PP), a SM produced by *Trichoderma* genus, displays activity against plant pathogens, and anti-biofilm-producing bacteria. Up to now, no studies about the potential antiviral activity of 6PP are known. So, here antiviral ability of 6PP was assessed against CCoV-II infection. **Methods used:** During CCoV (378/strain) infection in canine fibrosarcoma (A72) cell line, bioscreen, immunofluorescence staining, cytomorphological and virus yield analyses were performed. **Results:** Following CCoV infection, the non-toxic concentration of 0.1 µg/mL of 6PP markedly increased cell viability and cell proliferation. These results were accompanied by reduced cell death signs. Moreover, 6PP significantly reduced virus yield and the expression of the nucleocapsid protein NP. **Conclusions:** Overall, our findings suggest that nontoxic concentration of 6PP shows potential activity against CCoV infection. Noteworthy, in the screening of potential antivirals, *in vitro* animal model of CoVs avoids the manipulation of extremely hazardous CoVs (SARS-CoVs, MERS-CoV).

PS06

DETECTION OF SAPOVIRUSES IN ITALIAN CARNIVORES

F. Alfano[1], M. G. Lucibelli[1], G. Miletti[1], N. D'Alessio[1], A. Gallo[1], C. Auriemma[1], R. Brunetti[1], M. P. Valentino[1], L. Baldi[1], E. De Carlo[1], N. Decaro[2], V. Martella,[2] G. Fusco[1]

[1]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy

[2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano (BA), Italy

The aim of our work was to study the circulation of sapoviruses (SaVs) in carnivores in Italy. Out of 206 carcasses (100 dogs, 77 cats, 14 wolves, 15 foxes) analysed in our investigation, 9 (5 dogs, 3 cats, 1 fox) tested positive for SaV RNA. Single SaV infection was found in only 2 dogs (#3354 and #23021), whereas in the other SaV-positive animals, co-infections with other pathogens were observed. Dog #23021 displayed gastroenteric lesions, as commonly observed in SaV infections in humans (4) and dogs (1). In contrast, in dog #3354 there was no gastroenteric involvement and the observed lesions were referable to a uremic syndrome (US). Whether the SaV infection in the dog was associated with US or it was a mere coincidence could not be assessed. Also, since we did not use an open diagnostic system, we cannot rule out the presence of other undetected pathogens. In humans, US has been reported in patients with bacterial gastroenteritis (3). However, there is also a limited number of cases not related to gastroenteritis. These "atypical" forms of US recognize various causes, including viral infections (2). The results of our study highlight the need to monitor the SaV circulation in dogs as well as the clinical forms associated to this viral agent.

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PS07

MOLECULAR SURVEILLANCE FOR NOROVIRUS AND ROTAVIRUS INFECTIONS IN DOMESTIC CARNIVORES IN ITALY

F. Mira[1], F. Gucciardi[1], G. Schirò[1], G. Chiaramonte[1], G. Purpari[1], D. Vicari[1], F. Antoci[1], V. Martella[2], A. Guercio[1], S. Di Bella[1]
[1]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy
[2]Department of Veterinary Medicine, University of Bari, Valenzano, Italy

A One Health approach is strongly suggested for management of infectious diseases in humans and animals. Companion animals, dogs and cats, have a close interaction with humans, sharing the same environment. This generates occasional exposure of humans to potential zoonotic enteric viruses, such as norovirus (NoV) and rotavirus (RoV) and viceversa of domestic carnivores to human enteric viruses. We investigated the epidemiology of NoVs and RoVs in dogs and cats in Italy. Intestines, faeces or rectal swab were collected from n=170 dogs and n=47 cats in the period 2020-22. NoV was detected in 4/170 (2.3%) dogs and 3/47 (6.4%) cats, whilst RoV was detected in 2/170 (1.1%) dogs and 3/47 (6.4%) cats, either alone or in mixed infections with other viral pathogens. None of the NoV- and RoV-positive animals lived in a household. All but two (2 and 3 years old) were young animals (10 days - 3 months old). The amplicon generated from the diagnostic region B (ORF1) of NoVs was sequenced. The canine NoVs displayed the highest nucleotide (nt) identities to 2018/19 GVI.2 strains from China (95.72%), to 2007 GIV.2 strains from Italy (93.90%) and 2008 NoVs from Greece (93.89-94.86%). Feline NoVs displayed the highest identities to 2013 GVI.2 strain (91.95%), to 2010 GIV.2 strains from USA (90.10-94.79%), Italy 2006 (91.75-92.33%), and Japan 2017 (91-91.95%). Gathering sequence data on enteric viruses of domestic carnivores is useful to define better the plasticity of the enteric virome at the human/animal interface.

PS08

HEPATITIS E VIRUS IN TUSCANY: EVIDENCE OF PRESENCE IN CO-HABITING WILD AND DOMESTIC ANIMALS

M.I. Pacini, M. Forzan, N. Fonti, M. Mazzei
Department of Veterinary Sciences, University of Pisa, Italy

Hepatitis E virus (HEV) is a non-enveloped ssRNA+ virus belonging to the *Hepeviridae* family responsible for sporadic and epidemic outbreaks in industrialized countries. Although suids are recognized as the main reservoirs of HEV, other species may act as HEV hosts, including domestic and wild animals. In central Italy, Tuscany represent a model for studying HEV epidemiology in complex ecosystems since it is one of the Italian regions with the largest number of wild animals and hunting activity is largely diffused.The aim of our research is to investigate and analyse the circulation of HEV in several wild and domestic animals sharing the same ecosystem in Tuscany.
From 2014 to 2021, serological and molecular investigations were carried out in wild species mostly diffused and hunted in Tuscany (wild boar, deer, brown hares, and wild rabbits), and in domestic animals living in close contact with wildlife (hunting dogs and semi free-range cattle). Serological analysis recorded variable seroprevalences with values ranging from 50% (wild boar, 186/374) to 5% (rod deer, 2/43); no serological positivity was found in cattle (0/10), fallow deer (0/13) and brown hares (0/103). Moreover, HEV RNA was detected in liver samples collected from wild boar, wild rabbit, roe deer and fallow deer and in swabs sampled from wild boar carcasses; no molecular positivity was found from hunting dogs' rectal swab. In conclusion our data indicate a diffuse circulation of HEV in a wide range of animals living in an area characterised by a wide interface between wildlife and domestic animals.

PS09

DETECTION OF INFLUENZA A VIRUS-SPECIFIC ANTIBODIES IN HOUSE CATS AND STRAY CATS IN THE NETHERLANDS, 2020-2022

M.B.H.M. Duijvestijn[1*], N. Schuurman[1], T.F. Verweij[1], J.M.A. van den Brand[1], J.A. Wagenaar[1,2], F.J.M. van Kuppeveld[1], C.A.M.de Haan[1], H.F. Egberink[1] , J.H.Verhagen[1].

[1]*Utrecht University, Faculty of Veterinary Medicine, Department of Biomolecular Health Sciences, Utrecht, The Netherlands.*
[2]*Wageningen Bioveterinary Research (WBVR), Lelystad, The Netherlands.*

Objective: Cats have been shown to be susceptible to infection and disease with highly pathogenic avian influenza (HPAI) H5 viruses, yet limited knowledge is available about cats' susceptibility to different influenza A virus (IAV) subtypes. Haemagglutinin of IAV is a major target for protective antibodies. Here we used sera from house cats and stray cats sampled in the Netherlands (2020-2022) to investigate pre-exposure to H1, H3, H5, H7 and H9 IAVs. **Materials and Methods:** A total of 296 house cats (95 municipalities) and 476 stray cats (52 locations) were tested for presence of antibodies against human origin ($H1N1_{pdm2009}$, $H1N1_{2007,}$ H3N2), canine origin (H3N8), and avian origin (H5N8, H7N9, H9N2) IAV using an in house developed ELISA based on recombinant haemagglutinin proteins. **Results:** The seroprevalence of antibodies against $H1_{pdm2009}$ in house cats was 7.1% (21/296) and 3.2% (15/476) in stray cats. The H5 seroprevalence was 0.7% (2/296) in house cats and 6.9% (33/476) in stray cats. Seroprevalence for $H1_{2007}$ and H3 in both groups was <1%. Less than 1% (2/296, 1/296) of house cats had H7- or H9-specific antibodies, while stray cats had 1,5% (7/476) H7- and 1,7% (8/476) H9-specific antibodies. The H5 seroprevalence in stray cats varied by location, ranging from 0% to 100% (8/8). Three cats <1 year had H5N8-specific antibodies, reflecting recent exposure.**Conclusion**: House cats showed higher seroprevalence to human origin $H1_{pdm2009}$ IAV and stray cats showed higher seroprevalence to avian origin H5N8 IAV. A role of cats as mixing vessel for IAVs needs further research.

PSA10

VAGINAL ADMINISTRATION OF A BOHV-4-BASED VECTOR EXPRESSING CpHV-1 gD PROTECTS GOATS AGAINST CpHV-1 INDUCED PATHOLOGY

A.E. Odigie[1], M.S. Lucente[1], C. Catella[1], D. Mrenoshki[1], I.C. Ugochukwu[1], F. Pellegrini[1], P. Capozza[1],A.A.K Zarea[1], V. Franceschi[2], G. Greco[1], A. Pratelli[1], G. Donofrio[2], M. Tempesta[1],
[1]*Department of Veterinary Medicine, University of Bari "Aldo Moro", SP 62 Casamassima km 3, Valenzano, Italy*
[2]*Department of Veterinary Medical Sciences, University of Parma, Via del Taglio 10, Parma, Italy*

Objectives: Caprine herpesvirus 1 (CpHV-1), an *Alphaherpesvirus* of the *Herpesviridae* family and *Varicellovirus* genus, is a globally occurring non-vaccine preventable genital infection linked with major economic losses due to returns in service, abortions and stillbirths. We evaluated the efficacy of a recombinant BoHV-4 virus vector expressing CpHV1 gD (BoHV-4-A-gD(cp)gD(106)ΔTK), already validated when inoculated parenterally, vaginally administered as a potential vaccine candidate against CpHV-1 infection in goats. **Methods:** Four CpHV1-seronegative goats received via vaginal mucosa three dose of the BoHV-4-A-gD(cp)gD(106)ΔTK at intervals of three weeks while three other goats remained unvaccinated as controls. Two weeks after the third vaccination, all goats were intravaginally challenged with a highly pathogenic dose of BA.1 strain of CpHV-1 and underwent a 20-day observation for clinical signs. Vaginal swabs, vaginal washes and sera were collected for serology and virological tests. **Results:** All vaccinated goats were clinically protected against CpHV-1 induced genital disease with short duration of mild hyperemia, minimal viral shedding, and very low to absent humoral response. In contrast, the unvaccinated controls developed severely graded hyperemic and oedematous vaginal lesions with prolonged high titer viral shedding. **Conclusion:** This study validated BoHV-4-based vector administered mucosally as a safe and effective vaccine candidate against CpHV-1 induced genital disease in goats with potentially diverse applications.

PSA11

SARS-CoV-2 INFECTION IN DOGS AND CATS IS ASSOCIATED WITH CONTACT TO COVID-19 POSITIVE HOUSEHOLD MEMBERS

M.M. Kannekens-Jager[1], M.M.T. de Rooij[2], H.S. Kooistra[3], H.F. Egberink[1], J.A. Wagenaar[1], E.M. Broens[1]
[1]*Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[2]*Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

[3]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

The aim of this research was to assess the SARS-CoV-2 prevalence amongst cats and dogs (PCR- and/or antibody positive) and potential risk factors in two different populations in the Netherlands during the period July 2020 – April 2021. Samples were collected from dogs and cats living in a household with at least one confirmed COVID-19 positive person (household study; 156 dogs and 152 cats) and dogs and cats visiting a veterinary clinic (veterinary clinic study; 183 dogs and 140 cats). Collected oropharyngeal and rectal swabs were tested for presence of virus (PCR) and blood samples were tested for antibodies (ELISA). PCR-positive animals were followed over time and retested. In the household study, 18.8% (27 dogs, 31 cats) tested SARS-CoV-2 positive, whereas in the veterinary clinic study SARS-CoV-2 prevalence was 4.6% (6 dogs, 9 cats). SARS-CoV-2 prevalence amongst dogs and cats was significantly higher in multi-person households with two or more COVID-19 positive persons compared to multi-person households with only one COVID-19 positive person. In both study populations, no associations could be identified between SARS-CoV-2 status of the animal and health status, age or sex of the animal. During follow-up of PCR-positive animals, no other pets in the household were tested positive despite long-lasting SARS-CoV-2 RNA positivity in cats (up to 35 days). In conclusion, SARS-CoV-2 infection in dogs and cats suggests no severe clinical signs and SARS-CoV-2 infection in dogs and cats appeared to be clearly associated with confirmed COVID-19 positive status of the household.

PSA12

VIRULENCE PROFILE OF ORAL ENTEROCOCCI OBTAINED FROM DOGS

E. Cunha[1,2], L.M. Carreira[1,2], M. Videira[3], A.F. Ferreira[1,2], R. Abreu[1,2], I. Chambel[4], L. Tavares[1,2], M. Oliveira[1,2]

[1]*CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal*

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

[3]*Casa dos Animais de Lisboa, Câmara Municipal de Lisboa, Lisboa, Portugal* [4]*BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal*

Background: Enterococci are ubiquitous bacteria with high genome plasticity. As opportunistic bacteria and according to their virulence signatures, enterococci are a leading cause of difficult to treat healthcare infections in both humans and animals. This work aimed to isolate, identify and characterize the virulence profile of enterococci obtained from the oral cavity of healthy dogs. **Materials/methods:** Twenty dogs were selected from an official animal's rescue institution. All procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Lisbon. Oral swabs were obtained from each animal and conventional bacteriological techniques were used for enterococci isolation, followed by PCR identification as described by Jackson et al 2004. The phenotypic virulence profile of each isolate was assessed by evaluating lipase, gelatinase, lecithinase, protease and DNase activity, haemolysin's' production and biofilm-forming ability. **Results:** A total of 68 enterococci were recovered from 17 animals. PCR identification revealed that 35.29% of the isolates were identified as *E. faecalis,* 13.24% as *E. faecium,* 5.88% as *E. hirae,* and 45.59% as *Enterococcus* spp. Virulence evaluation revealed that 55.88% of the isolates were able to produce lipase, 27.94% gelatinase, 27.94% lecithinase, 26.47% protease, 0% DNase, 51.47% haemolysins, and 92.65% biofilm. Isolate´s virulence index ranged from 0.14 to 0.86. **Conclusions:** Enterococci with high virulence index are present in the oral cavity of most dogs, having potential to became opportunistic pathogens. Our results reinforce the establishment of enterococci as an important reservoir of virulence determinants, being a relevant bacterial model in veterinary bacteriology. **Ackowledgements:** This work was supported by CIISA–Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Project UIDB/00276/2020 (Funded by FCT); and by the Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020 - AL4AnimalS).

PSA13

GENOMIC ANALYSIS OF *ESCHERICHIA COLI* ISOLATES FROM A CHICKEN HATCHERY

A. Papoušková[1,2], M. Masaříková[1,2], J. Palkovičová[2], A. Čížek[1,2]

[1]*Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, Veterinary University Brno, Brno, Czech Republic*
[2]*Central European Institute of Technology, Veterinary University Brno, Brno, Czech Republic*

E. coli is one of the most important bacterial pathogens in commercial poultry. Recent research shows that pathogenic poultry strains (APEC) form a dynamic variable population in which "risk" genotypes and clonal lines play an important role. These can also spread vertically from parent farms to offspring, potentially from poultry products to consumers. Hatcheries appear to be a key site of cross-contamination and primary colonization of newly hatched chickens. Samples of shells and dust were taken from hatchery boxes in a commercial hatchery in the years 2020-21. Based on preliminary characterization, 74 *E. coli* isolates were selected for whole genome sequencing by Illumina. The acquired data were analyzed by available tools to obtain a complex genetic characterization (VirulenceFinder, ResFinder, SeroTypeFinder, PlasmidFinder) and phylogenetic analysis (MLST, pMLST). In contrast to previously analyzed clinical isolates, the B1 phylogenetic group dominated among the hatchery population, with a high prevalence of ST155 and ST162. Typical APEC genotypes, especially ST23 and ST117, were detected sporadically, which proves their occurrence in the hatchery environment, where they readily colonize day-old chicks. Repeated detection could indicate their persistence in parent flocks or their spread between farms, presumably through hatcheries. Whole genome sequencing makes a fundamental tool for monitoring the occurrence of risk lines in the production chain of commercial poultry. Only long-term sampling at different levels of the pyramid with detailed genetic analysis will provide sufficient data to assess the significance of *E. coli* risk clonal lines in poultry.

PSA14

ISOLATION AND CHARACTERIZATION OF FOUR NEW BACTERIOPHAGES AGAINST *AEROMONAS SALMONICIDA*, THE CAUSATIVE AGENT OF FURUNCULOSIS

S. Desmecht[1], C. Antoine[1], F. Laforêt[1], J.-N. Duprez[1], A. Schonbrodt[2], F. Lieffrig[3], D. Thiry[1]
[1]*Bacteriology, Departement of parasitic and infectious diseases, FARAH, ULiége, Liége, Belgium.*
[2]*Independent fish farm veterinarian, Hargimont, Belgium.*
[3]*Fish Pathology Laboratory, CER groupe, Marche-en-Famenne, Belgium.*

Aeromonas (A.) salmonicida, a Gram-negative bacteria belonging to the *Aeromonadaceae* family, is a primary fish pathogen that causes furunculosis in salmonids, carp and perch, as well as septicemia in a variety of fish. This species is considered as one of the main bacterial pathogens responsible for important economic losses in aquaculture industry. Large amounts of antibiotics such as oxytetracycline, quinolones and sulfonamides are used to treat this infection, which highly contributes to the emergence of antibiotic-resistant strains. The application of bacteriophages (phages) in aquaculture seems to be a promising solution to control pathogenic bacteria in this field because these organisms are well adapted to aquatic environments. The aim of this work was to isolate and characterize new lytic phages against *A. salmonicida.* The enrichment method used to isolate phages consists in mixing a centrifuged and filtered water sample with a bacterial culture in exponential phase. When clarification of the medium was observed, the supernatant of this mixture was spread on the surface of LB agar and covered with a bacterial overlay in exponential phase. Phages present in distinct clear lysis plaques were then purified three times by subculturing. For this purpose, a sampling campaign of water from fish farming ponds and natural aquatic environments in the south of Belgium was carried out in early 2022. Out of 48 water samples, four new lytic phages were isolated. A preliminary host spectrum test showed that three of these four phages were active against other wild *A. salmonicida* strains while the fourth one showed a narrower host spectrum. These four phages were not active against any of the *A. hydrophila* strains tested. After having determined temperature and pH stabilities, adsorption times and kinetics of these four new phages, further studies are needed to analyse their genomes and to assess the *in vivo* safety and efficacy of these phages.

PSA15

DISSEMINATED NOCARDIOSIS CAUSED BY NOCARDIA FARCINICA IN TWO PUPPY SIBLINGS

F. Zendri, P. Richards-Rios, I. E. Maciuca, E. Ricci, D. Timofte

Department of Veterinary Anatomy, Physiology and Pathology, Institute of Infection, Veterinary and Ecological Sciences, Leahurst Campus, University of Liverpool, Neston, United Kingdom.

Background: Systemic nocardiosis due to *Nocardia farcinica* has not been reported in canine outbreaks. **Case presentation**: Two 14-week-old female Dogue de Bordeaux siblings presented with fever and severe, acute onset limb lameness; traumatic lesions with evidence of infection were identified over the lame limbs of both dogs. The patients had to be euthanised due to poor response to therapy and rapid escalation to systemic infection with severe central nervous system manifestations. The post-mortem changes consisted of multiple disseminated abscesses, mainly affecting the skin and subcutis of the wounded skin, lung, kidney and brain, with regional suppurative lymphadenitis. Bacterial culture isolated *Nocardia farcinica* from several of these sites in both dogs, whose identification was achieved via MALDI-TOF MS and confirmed by 16sDNA sequencing. Clinical significance of the isolate was supported by cytology of the post-mortem organs' impression smears showing numerous branching filamentous bacteria associated with purulent inflammation. The organism displayed marked multidrug-resistance including to doxycycline and imipenem. No history of immunosuppression was available neither histopathological signs of lymphopenia were detected, although further testing would be required to rule out viral pathogens as canine distemper and parvovirus. **Conclusion**: *N. farcinica* should be considered as a potential differential cause of sudden lameness and systemic infection in dogs with traumatic skin lesions. This is the first reported small-scale outbreak of systemic nocardiosis in dogs due to *N. farcinica*.

PSA16

CHARACTERIZATION OF FLAVOBACTERIUM PSYCHROPHILUM ISOLATES FROM CZECH AQUACULTURE

V. Nováková, M. Palíková, A. Čížek
University of Veterinary Sciences Brno, Brno, Czech Republic

Flavobacterium psychrophilum is an important fish pathogen causing economical losses mainly in salmonids all over the world, including the Czech Republic. Several studies have characterized *F. psychrophilum* isolates from various countries, but none from the Czech Republic. Aim of this study was to characterize isolates gained in 2012-2019 from aquaculture in the Czech Republic. Seventy isolates from 11 farms were included in this study. Analysis of antimicrobial susceptibility to most frequently used antibiotics in aquaculture using broth microdilution method, presence of selected virulence factors using culture methods and serotyping using mPCR protocol was performed. Antimicrobial susceptibility testing showed high MIC for oxolinic acid, oxytetracycline, flumequine, enrofloxacin and increasing resistance to florfenicol, which is currently drug of choice for flavobacteriosis treatment in many countries. By mPCR-based serotyping type 1, corresponding to serotype Fd, was most frequently identified, less often type 2, corresponding to serotype Th, and only sporadically types 3 and 4, corresponding to serotype Fp^T. No type 0 was detected. All isolates were able to create visible biofilm, high proportion created sediment and hydrolyzed gelatin, but only one third of isolates was positive for gliding motility. Characterization of isolates is essential for revealing ways of introduction of the pathogen in the farms and enables to create effective protective measures in the aquaculture. This study showed increasing resistance to antibiotics similarly as in other countries, meaning that alternative therapy methods are necessary to be studied.

PSA17

ANTIMICROBIAL SUSCEPTIBILITY AND MULTILOCUS SEQUENCE TYPING OF *CAMPYLOBACTER JEJUNI* ISOLATES FROM DOGS WITH DIARRHEA

M. Cvetnić, V. Mojčec Perko, S. Pintarić, S. Hađina, J. Habuš, I. Benvin, Z. Štritof
Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia

Campylobacteriosis is one of the most common zoonoses worldwide, with dogs being one of the possible sources of infection. The aim of this study was to investigate the antimicrobial susceptibility and sequence types of Campylobacter jejuni isolated from the feces of dogs with diarrhea. Campylobacter spp. were isolated on modified charcoal-cefoperazone-deoxycholate agar (mCCDA). Species was identified by MALDI TOF MS. Antimicrobial susceptibility of isolates was

tested using the disk diffusion method according to the EUCAST. Multi-locus sequence typing (MLST) was performed according to Dingle et al (2001).
From 248 dogs, 46 (18.5%) Campylobacter spp. were isolated. Of these, 26 (56.5%) were C. jejuni. Resistance to ciprofloxacin was 57.7%, to tetracycline 23.1%, and to erythromycin 3.8%. Five (19.2%) isolates were resistant to two antimicrobials; four to ciprofloxacin and tetracycline and one to ciprofloxacin and erythromycin. Fifteen isolates were randomly selected for MLST and classified into six clonal complexes (CC-21, CC-206, CC-403, CC-45, CC-443, and CC-48) and 10 sequence types; three each from ST-1943 and ST-10039, two from ST-538, and one each from ST-3156, ST-19, ST-2086, ST-572, ST-3335, ST-475, and ST-51. Six of these STs have not been previously detected in dogs. Six STs have not been confirmed in Croatia to date. High genetic diversity was found among the typed isolates. Given the increasing contact of dogs and humans, continuous epidemiological studies of campylobacteriosis and antimicrobial susceptibility of isolates from all sources are needed.
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PSA18

***SEROPOSITIVITY OF COXIELLA BURNETII* IN WILD BOAR (*SUS SCROFA*) AND RED DEER (*CERVUS ELAPHUS*) IN PORTUGAL**

H. Pires[1], L. Cardoso[2,3], A.P. Lopes[2,3], M.C. Fontes[2,3], M. Matos[4], C. Pintado[1], L. Figueira[5,6], J.R. Mesquita[7,8,9], A.C. Matos[5,6], A.C. Coelho[2,3]
[1]Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal
[2]Animal and Veterinary Research Centre, Vila Real, Portugal, Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Portugal
[3]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
[4]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), UTAD, Portugal
[5]Centre Research for Natural Resources, Environment and Society, Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal,
[6]Researcher at Q-RURAL – Quality of Life in the Rural World, Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal
[7]ICBAS – School of Medicine and Biomedical Sciences, Porto University, Porto, Portugal
[8]Epidemiology Research Unit (EPIUnit), Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal
[9]Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal

OBJECTIVES: *Coxiella burnetii* is a zoonotic microorganism that infects a wide range of wild and domestic species, causing the disease Q fever, frequently involving ticks as vectors. To better understand the occurrence of *C. burnetii* infection in wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*), an epidemiological study was conducted in the Centre region of Portugal. **MATERIAL AND METHODS:** A serological survey was performed on samples from 377 wild boar and 240 red deer, totalizing 617 animals from the Centre of Portugal. Only adult animals were sampled in this study. Antibodies specific to *C. burnetii* were detected with a commercial enzyme-linked immunosorbent assay (ELISA; IDVet®, Montpellier, France) according to the manufacturer instructions.**RESULTS:** A total of 9/617 samples (1.5%, 95% confidence interval [CI]: 1.1-1.9%)) were reactive to *C. burnetti* and regarded as positive. The seropositivity for wild boar was found to be 1.1% (4/377, 95% CI: 0.6-1.6%), and for red deer 2.1% (5/240, 95% CI: 1.5-2.7%). **CONCLUSION:** Results indicate that wild boar and red deer from the Centre of Portugal are exposed to *C. burnetii*. This study demonstrates that wild boar and red deer can be reservoirs of infection for both livestock and humans.

PSA19

USEFULNESS OF MALDI-TOF MASS SPECTROMETRY IN THE DIAGNOSIS OF BOVINE MASTITIS

A. Bellato, P. Robino, M. Furlan, F. Traverso, C. Pegorin, A. Mannelli and P. Nebbia
Department of Veterinary Sciences, University of Turin, Italy

In udder microbiology, MALDI-TOF MS method could become the reference method due to its ability to identify bacteria at the species level faster and at a lower cost than classical methods. Accurate and fast diagnosis can result in a timely and better treatment plan. Information about the strains involved is of crucial importance due to the difficulty in treating the disease, whether clinical or subclinical. The aim of this work was to explore the use of the MALDI-TOF for the diagnosis of subclinical mastitis and providing more information regarding the differences among the bacterial strains circulating in the herd. The study involved 256 clinically healthy cows; milk samples were cultured on conventional media and a colony representing each population was analysed, obtaining a high-quality mass spectrum. Peak-intensity matrices were produced from mass spectra analysis, thus dendrograms were obtained using R coding language to measure the dissimilarities among strains of the same species. Our findings confirmed that the MALDI-TOF MS method easily identifies the pathogens usually involved in subclinical mastitis. The cluster analysis, despite having limitations in generalizability due to the small sample size and the lack of standardisation, can be useful in differentiating bacterial strains between different herds. Differences in the reliability of cluster analysis are likely linked to the bacterial species. Future developments concerning the diagnosis of mastitis with the MALDI-TOF method should be the implementation of the mass-spectra database in order to improve the accuracy of the method, and the possible identification of additional species. Key words: *subclinical mastitis, MALDI-TOF, cluster analysis*

PSA20

LOAD OF *CAMPYLOBACTER* SPP. IN THE ENVIRONMENT OF POULTRY FARMS IN GERMANY

B. Reichelt, V. Szott, U. Roesler, A. Friese
Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Berlin, Germany

Introduction:*Campylobacter* (C.) *jejuni* is the most common cause of campylobacteriosis in humans, and broiler meat is considered one of the major sources. The poultry house environment may be a reservoir for *Campylobacter* spp. to some extent. However, to date, there are limited data on the spillover of *Campylobacter* from houses with *Campylobacter*-positive flocks and the buildup of environmental reservoirs. In addition, *Campylobacter* spp. are capable to transit into a viable but non-culturable (VBNC) state as a result of various extrinsic stress factors. In order to identify possible reservoirs of persistent and VBNC-Campylobacter and thus uncover relevant transmission pathways, a longitudinal study was conducted. Material/Methods: Three Broiler farms and their environment close to the barn were intensively investigated at the end of two consecutive fattening cycles in summer and winter over three years. The selected farms were also examined after cleaning and disinfection. All samples were processed according to the semi-quantitative method for the detection and enumeration of *Campylobacter* spp. (ISO/TS 10272-3). A systematic selection of isolates from all sampling collections was examined by whole genome analyses. Moreover, environmental and selected broiler house samples were treated with propidium monoazide (PMA) and analyzed by live/dead discrimination using real-time PCR (qPCR). Results: In two out of three farms, *Campylobacter* was frequently detected in high amounts in the chicken barns, especially in summer. However, the pathogen was only occasionally detectable in the environment, particularly in water-associated matrices, especially in winter. However, *Campylobacter* could not be isolated in broiler houses after cleaning and disinfection. The emission source of culturable *Campylobacter* was found to be primarily contaminated chicken manure. *C. jejuni* proved to be the dominant species of the isolates examined. PMA-qPCR revealed no detection of VBNC-*Campylobacter* in selected barn and environmental samples. In contrast, *Campylobacter* DNA was more frequent detected in environmental samples. Discussion: The present study provides insight into the significance of *Campylobacter* in the environment in relation to prevalence in the broiler farms investigated in Germany. The results established indicate sporadic environmental findings in the immediate vicinity, suggesting spread, persistence and possible reintroduction. *C. jejuni* was found in nearby water bodies, indicating that the pathogen is ubiquitous by spread and circulation. Although the findings were sporadic and no significant source of transmission has yet been identified, it should be kept in mind that even very low levels of *Campylobacter* may initiate the colonization of whole poultry flocks.

PSA21

VIRULENCE CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA* FROM CANINE OTITIS

R. Abreu[1,2], F. Monteiro, E. Cunha[1,2], M. Lourenço, L. Tavares[1,2], M. Oliveira[1,2]
[1]CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal
[2]Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS), Portugal

Background: Otitis externa is a recurrent problem in small animal general practice. Despite of being a multifactorial disorder, secondary infections are common. *Pseudomonas aeruginosa* is one of the most frequent gram-negative pathogens involved in infectious otitis. These ubiquitous bacteria are found in water and decaying matter, but when present in the ear canal they can cause disease. It presents multiple intrinsic resistances and produces multiple virulence factors; thus, its treatment can be challenging. This work aimed to identify and characterize the virulence profile of *P. aeruginosa* obtained from the ear canal of dogs with otitis externa. **Materials/methods:** A total of 48 *P. aeruginosa* isolates from the collection of clinical isolates from the Laboratory of Bacteriology, Faculty of Veterinary Medicine, University of Lisbon, Portugal (2016-2021) were analyzed, by evaluating various phenotypic virulence traits (biolfim and haemolysins production, lipase, lecithinase, DNAse protease and gelatinase activity) using specific media. The virulence index (V. Index) values were determined for all isolates. **Results:** All isolates produced lipase and haemolysins (100%), 20 isolates (41.67%) produced gelatinase, 26 isolates produced lecithinase (54.17%), 46 isolates produced protease (95.83%), none of the isolates produced DNAse (0%) and 6 were able to produce biofilm (7.69%). *P. aeruginosa* V. Index ranged between 0.28 to 0.86, with a 0.58 media and a 0.57 median. **Conclusions:** *P. aeruginosa* isolates tested presented a high virulent index, demonstrating the importance of adequate treatment for related infections, avoiding their recurrence and reducing the harmful effects on the health and welfare of the patient. **Ackowledgements:** This work was supported by CIISA–Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Project UIDB/00276/2020 (Funded by FCT) and by Laboratório Associado para Ciência Animal e Veterinária (LA/P/0059/2020 - AL4AnimalS).

PSA22

***DIETZIA* SP. B32 INFECTION IN A HORSE: FIRST GENETIC CHARACTERIZATION**

A.-R. Attili[1], R. Gonçalves dos Santos[2], R. Hurtado Castillo[2], D.L. Neres Rodrigues[2], A. Lima[2], W. Ferreira dos Anjos[3], C. Rifici[4], S. Tiwari[2], A. Kumar Jaiswal[2], T.L. de Paula Castro[2,5], N. Seyffert[2,6], A. Santos[3], A. Góes-Neto[7], T. de Jesus Sousa[2], V. Azevedo[2]
[1]School of Biosciences and Veterinary Medicine, University of Camerino, Matelica Italy
[2]Cellular and Molecular Genetics Laboratory, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[3]Department of Computer Science, Federal University of Uberlandia, Uberlandia, Brazil
[4]Department of Veterinary Science, University of Messina, Messina, Italy
[5]Institute of Health Sciences, Federal University of Bahia, Salvador, BA, Brazil
[6]Institute of Biology, Federal University of Bahia, Salvador, BA, Brazil
[7]Molecular and Computational Biology of Fungi Laboratory Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil

Environmental and opportunistic *Dietzia* species belong to the *Dietziaceae* family, order *Mycobacteriales*, class *Actinobacteria*. In the absence of accurate techniques, *Dietzia* spp. could be misidentified as *Rhodococcus hoagii*, formerly *Rhodococcus equi*. **Objectives:** In order to study the complete genome sequence of *Dietzia* strain isolated from diffuse subcutaneous nodules of a mare in Reggio Calabria (Italy), comparative and phylogenetic analyses, searches for antibiotic resistance genes, virulence factors, and genomic islands were performed. **Methods:** Analyses were performed for 49 genomes of *Dietzia* species (NCBI), and one genome was sequenced using Hiseq (Illumina), *Dietzia* sp. isolate. All genomes were homogenized by Prokka software. Phylogenomic trees, presence of antibiotic resistance and virulence genes were predicted by MEGAX software, CARD and VFDB databases, respectively. Images were created using iTOL. Islands of pathogenicity (PAIs), resistance (RIs), metabolic (MIs) and symbiotic islands (SIs) were predicted using GIPSy software.

Results: *Dietzia* sp. B32 resulted as a new species not yet described because of similarity values below 90.1% and 41 unique genes involved in "cellular component" and "molecular function. Four genes (*rpo*B, *mtr*A, *rbp*A, and APH(3')-Iia) related to the antibiotic resistance, and six genes associated with virulence factors (*pho*P, *pho*R, *icl*, *tuf*A, *rel*A, and *cap8E*) were described. The mutation in the *rpo*B gene confers resistance to rifampicin. Five pathogenicity (PAIs), five resistance (RIs), two metabolic (MIs), and one symbiotic island (SI), were found. **Conclusions:** As the true pathogenic potential of *Dietzia* species is not known, the comparative analysis of *Dietzia* B32 contributes to better understand this increasingly emerging genus.

PSA24

RESULTS OF URINARY BACTERIAL CULTURE AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF DOGS AND CATS FROM A VETERINARY LABORATORY IN PORTUGAL IN 2021

V. Campos da Silva, H. Carvalho, N. Carvalho
Cedivet – Centro Diagnóstico Veterinário, Oporto, Portugal

Objectives: The aim of this study was to investigate the aetiology and antimicrobial resistance of pathogens isolated from urinary samples of dogs and cats submitted to analysis in a private veterinary clinical laboratory in Portugal. Materials and methods: Data from urinary samples, submitted as part of a urinary profile investigation, between January and December of 2021 were used (n=4001). Results: A total of 601 samples showed significant bacteraemia (>100,000 UFC/mL) and 61,1% of urine samples from dogs and 38,9% from cats had significant cultural growth. *E. coli* was the most frequently isolated microorganism in both dogs (49,7%) and cats (49,3%), followed by *Proteus mirabilis* in dogs (23,3%) and coagulase-negative staphylococci in cats (11,3%). Amoxicilin-clavulanic acid was the drug with the lowest percentage of resistance (11,4%), fluoroquinolones showed levels of resistance above 10% (enrofloxacin 16,3%, marbofloxacin 16,1%, pradofloxacin 15,0%) and trimethoprim-sulfamethoxazole showed a total level of resistance of 20,8%. Conclusions: Our work confirms *E. coli* as the most common cause of bacterial urinary tract infections in both dogs and cats, provides evidence of high levels of resistance to fluoroquinoles and enhances the need of surveillance of antimicrobial resistance in the establishment of guidelines at a national level.

PSA25

PRELIMINARY EVALUATION OF THE EFFICACY OF A NEW INACTIVATED ORAL VACCINE AGAINST ENTEROTOXIGENIC *E. COLI* INFECTIONS IN POST-WEANING PIGLETS

A. Inglesi[1], G.A. Clemente[2], S. Draghi[1], J. Filipe[1], A. Maisano[3], F. Riva[1], M. Amadori[2], F. Guarneri[2], V. Lorenzi[2], C. Montagnin[2], L. Bertocchi[2], F. Fusi[2]
[1]Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy
[2]Reparto produzione e controllo materiale biologico, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy
[3]Animal Health and Welfare, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna 'Bruno Ubertini', Lodi, Italy

Weaning is a highly stressful period for piglets, which become more susceptible to diverse diseases. At the intestinal level, weaned piglets show severe inflammatory responses that impair the gut epithelial barrier and predispose them to pathogen colonization and invasion. In post-weaning piglets several *E. coli* strains cause diarrhea leading to the reduction of production performances and the increased use of drugs. In order to limit and mitigate the impact of *E. coli* infections in post-weaning piglets, we developed a new oral vaccine to be easily administrated to the animals via drinking water. The vaccine is based on heat-inactivated enterotoxigenic *E. coli* strains expressing F4 and F18 fimbriae for 26 days. The efficacy of our vaccine has been preliminarily evaluated in terms of mucosal anti-F4 and anti-F18 IgA production in the saliva (IgAF4sal and IgAF18sal) by ELISA analysis and the number of IgA-secreting cells in mesenteric lymph nodes (IgAcell) by ELISPOT assay. The level of IgAF4sal was significantly higher in vaccinated piglets compared to the unvaccinated control ones, whereas no differences were observed for IgAF18sal. Based on the increment of IgAF4sal production the vaccinated animals were classified as low, medium and high responders. Interestingly, only high responders showed a significant increase in the number of IgAcells. These encouraging preliminary results suggest a possible efficacy of our new vaccine and

highlight diverging immune responses to different *E. coli* fimbrial antigens. This work was supported by the Italian Ministry of Health: Grant n. PRC2019001.

PSA26

VIABLE MICROBIAL COMMUNITY COMPOSITION OF HEALTHY AND DAMAGED SKIN IN STRAY DOGS AND CATS.

F. Paola Nocera[1], C. Di Palma[1], S. Cappiello[2], A. Masullo[1], M. Pompameo[2], B. Lamagna[1], Luisa De Martino[1,3]

[1]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Via F. Delpino 1, 80137 Naples, Italy

[2]Veterinary Hospital, ASL Napoli 1 Centro, via M. Rocco di Torrepadula 13, 80145 Naples, Italy

[3]Task Force on Microbiome Studies, University of Naples "Federico II", Naples, Italy

Objectives: The purpose of this report was to describe the microbial community composition regarding three sites of healthy skin and the site of traumatic wounds in stray dogs and cats, and to verify the correlation between the bacteria resident on healthy skin and those of the damage skin of the same animal **Materials and methods:** Skin swabs were collected from stray dogs and cats presenting traumatic wounds. Precisely, swabs were performed on injured skin; furthermore, a collection of nasal and ear swabs as samples of skin cavities, and nasal spine swab as sample of external skin, representing samples of healthy skin sites, were performed for each animal. Samples were plated onto different types of solid culture agar media and incubated aerobically at 37°C for 24-48 h. Once bacterial growth was detected, the identification was performed by MALDI-TOF-MS **Results:** Stray cats presented a larger bacterial diversity in skin samples than stray dogs. *Staphylococcus pseudintermedius* was the most frequently identified bacterium in stray dogs both from damaged and healthy skin samples, whereas in stray cats *S. pseudintermedius* represented the third most frequently isolated bacterial species. However, in both animals *S. pseudintermedius* isolation was significantly higher in damaged skin samples than healthy ones ($P < 0.05$). In stray cats the Gram-negative *E. coli* resulted to be the most common identified bacterium, and its presence was significantly higher in damaged skin samples than healthy ones ($P < 0.05$) **Conclusions:** According to this study, traumatic wounds provide an opportunity for microorganisms from the skin microbiota, to enter the underlying tissues and find the optimal conditions for their overgrowth

PSA27

DITHIOTHREITOL PROTECTION ON MICROBICIDAL ACTIVITY OF INNOVATIVE LIGHTS: AN *IN VITRO* STUDY

M. Ambrosio[1,2], F. P. Nocera[1], C. Cerracchio[1], F. Fiorito[1], M. Hohenegger[2], L. De Martino[1,3]

[1]Department of Veterinary Medicine and Animal Production, Infectious Diseases Unit, University of Naples "Federico II", Naples, Italy

[2]Institute of Pharmacology, University of Medicine, Vienna, Austria

[3]Task Force on Microbiome Studies, University of Naples "Federico II", Naples, Italy

Objectives: Innovative mechanisms using the microbicidal photodynamic effect of light energy are used in bacterial eradication strategies. The light is emitted in the visible light spectrum (VIS) and is safe for mammalian cells. When microorganisms are exposed to light, excitation of porphyrins, photosensitizing chromophores endogenous to cells and sensitive to VIS light, is created. The resulting excitation leads to the production of reactive oxygen species (ROS), which completely damage the cell. We tested the Biovitae ® light, which emits white light from a light-emitting diode (LED) and investigated the effect of a sulfhydryl compound, dithiothreitol (DTT), a protective reagent against ROS, on lytic activity of the lights. **Materials and Methods:** The lights (by company *Nextense*) have a special combination of frequencies and intensities: 400-420 nm, 400-450 nm, 400-700 nm. 96-well plates containing *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* were exposed for six hours, and bacterial growth was monitored over 24 hours by optical density (OD) measurement. Bacterial strains were also exposed to light in combination with DTT. **Results:** During the six hours light exposure, a slight increase in growth was observed for both bacteria strains. Bacterial growth was significantly ($P \leq 0.005$) decreased 18 hours after the end of exposure. DTT significantly protected ($P \leq 0.05$) both *E. coli* and *S. aureus,* when exposed to light.

Discussion: These results confirmed the microbicidal photodynamic effect of Biovitae ® light against bacteria. Since DTT protected bacterial strains from antimicrobial light effects, these results are indicative for a redox-sensitive mechanism of action.

PSA28

***LEPTOSPIRA INTERROGANS* SEROGROUP SEJROE SEROVAR HARDJO IN A DAIRY CATTLE FARM IN SOUTH OF ITALY (SICILY)**

G. Macaluso[1], V. Blanda[1], R. D'Agostino[1], C. Sciacca[1], F. Arcuri[1], I. Giacchino[1], A. Stancanelli[2], A. Torina[1], F. Grippi[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy*

[2]*Istituto Zooprofilattico Sperimentale della Sicilia, Caltanissetta, Italy*

Bovine leptospirosis is a zoonoses causing economic losses in livestock. This work reports an outbreak of *Leptospira interrogans* serogroup Sejroe serovar Hardjo in a officially free from Brucellosis, Tuberculosis and Enzootic Bovine Leucosis cattle farm in province of Enna (Sicily). In February 2022, 2 cows showing full-term abortion from three months without placental retention, were sampled at three times: on day 0 (T0), eight days after the first abortion signs, on day 30 (T1) and on day 60 (T2), after antibiotic treatment. At T1 and T2 42 other animals were sampled. Blood, milk and stool samples were also collected at T2. Research of antibodies against pathogenic *Leptospira* species was carried out by Micro Agglutination Test (MAT); ELISA against the main abortion agents (*Chlamydia abortus*, *Coxiella burnetii*, *Neospora caninum* were also performed. Blood, milk and drinking water samples were subjected to Real Time-PCR to detect *Leptospira* pathogenic species and the main abortion agents (*Chlamydia abortus*, *Coxiella burnetii*, *Neospora caninum, Coronavirus, Cryptosporidium spp.*, *Escherichia coli* K99, *Rotavirus*). No animals resulted positive at differential diagnosis except for two ones seropositive for *N. canimun* and *C. burnetii*, without showing signs of abortion. At T0 one of the two sera resulted positive at MAT. Thirty and twenty-five sera resulted MAT positive at T1 and T2 respectively. This study describes clinical manifestations, diagnostic implications and epidemiological characteristics of an outbreak in cattle due to *L. interrogans* serogroup Sejroe serovar Hardjo, confirming that *L. interrogans* plays a role in determining leptospirosis infection in cattle reared in Sicily

PSA29

BOVINE TUBERCULOSIS *(MYCOBACTERIUM BOVIS/MYCOBACTERIUM CAPRAE)* IN SLOVENIA: A REVIEW 2015-2021

T. Pirš, U. Zajc, M. Ocepek

University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Gerbičeva 60, SI-1115 Ljubljana, Slovenia

Slovenia has been officially free of bovine tuberculosis (bTB) since 2009. However, the country achieved less than 0.1% infected cattle herds for six consecutive years as early as the mid-1970s. Since 2009, routine tuberculin testing in line with the relevant EU legislation for maintaining officially free status was conducted. In the last 20 years, only four outbreaks of bTB in cattle have been confirmed in Slovenia. Most of the animals were traded from other EU member states, only in one case we couldn't determine the origin of the animal involved. From 2015 to 2021, a total of 1098 samples from cattle were submitted for bacteriological testing. In 2015, 14 cases caused by *M. caprae* were detected in a single herd of fattening bulls. *M. caprae* was isolated from suspicious lesions of the lungs and adjacent lymph nodes, detected at regular slaughter. In 2018, another case was confirmed following the postmortem examination of an intradermal tuberculin test reactor. In addition to regular surveillance of cattle, comprehensive disease control also requires surveillance of wildlife species. From 2015 to 2021, 1860 samples of wildlife were analysed as part of annual state programmes, most of which were from wild boar (1300 samples from 625 animals) and deer (411 samples from 211 animals). The presence of *M. caprae* was comfirmed in one red deer (*Cervus elaphus*) in 2018 and the presence of *M. bovis* was confirmed in one wild boar (*Sus scrofa*) in 2019. Data were obtained from regular disease surveillance programme financed by the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection.

PSA30

SUBCUTANEOUS NOCARDIOSIS IN A 3-YEAR-OLD FEMALE CAT IN ITALY: A CASE REPORT

M. Medardo[1], F. Clemente[2], M. Marino[1], C. Lisi[1], G. Cocciolo[1], P. A. Martino[3], N. Decaro[4]
[1]Laboratorio di Analisi Veterinarie MYLAV, Passirana di Rho, Italy
[2]Ambulatorio Veterinario San Luca, Bologna, Italy.
[3]One Health Unit - Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy.
[4]Department of Veterinary Medicine, University of Bari, Valenzano, Italy.

Introduction: *Nocardia* spp. includes worldwide, ubiquitous zoonotic bacteria that can cause suppurative to pyogranulomatous infections, with localized or disseminated lesions. *Nocardia* spp. is considered an opportunistic pathogen, with the majority of clinical cases occurring in immunocompromised hosts. **Case report information:** A 3-year-old European cat presented with severe pododermatitis with fistulas in the left limb. **Intervention/Treatment:** An immunosuppressive therapy was administered after two tentative histopathological diagnoses of *pemphigus foliaceus*. After initial regression, lesions became more severe in the following 15 days, so that surgical curettage and other biopsies for histology and culture were carried out. **Results:** Histopathology was suggestive of severe pyogranulomatous dermatitis; microbiological culture showed positive results with presumptive diagnosis of *Nocardia* spp. infection, which was confirmed by modified Ziehl-Neelsen staining and *Nocardia* genus-specific PCR. Drug sensitivity was determined by the Kirby-Bauer disk-diffusion method and the isolate was susceptible to amikacin, doxycycline, trimethoprim-sulfamethoxazole and chinolones. Immunosuppressive therapy was discontinued and antibiotic therapy with doxycycline and enrofloxacin was administered. Follow up in July 2022 showed a remarkable improvement of the clinical conditions.**Discussion and conclusion:** This case report aims to highlight the importance of a multi-level steps for correct diagnosis and tempestive treatment of feline nocardiosis, which should be regarded as a potential zoonosis.

PSA31

PHAGE-MEDIATED SHIGA-TOXIN (STX2D) GENE TRANSDUCTION FROM O80:H2 SHIGA TOXIGENIC *ESCHERICHIA COLI* (STEC) TO NON-STEC STRAINS AND *IN VIVO* VIRULENCE ASSESSMENT

A. Habets[1], C. Antoine[1], J. Wagemans[2], M. Vermeersch[3], F. Laforêt[1], R. Lavigne[2], J. Mainil[1], D. Thiry[1]
[1]Bacteriology, Department of Parasitic and Infectious Diseases, FARAH, ULiège, Liège, 4000, Belgium
[2]Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, 3000, Belgium
[3]Center for Microscopy and Molecular Imaging, Electron microscopy laboratory, Gosselies, ULB, 6041, Belgium

Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne pathogens that cause human diseases ranging from diarrhea to life-threatening complications including hemolytic–uremic syndrome (HUS). Virulence of STEC strains and their ability to cause severe diseases are associated with the activity of prophage-encoded Shiga toxins (Stxs). The first objective of this work was to isolate and characterize the Stx2d phage from STEC O80:H2 and study the transfer of this phage in non-STEC strains. The second objective was to assess the survival of *Galleria mellonella* larvae inoculated with these transduced trains. One bacteriophage isolated from a STEC O80:H2 strain was used to infect five non-STEC strains. Three strains (K12-MG1655, K12-DH5α and O80:H26) were successfully converted. The genome of the phage was analyzed. A stability study was performed and showed that this phage was stable in the new STEC strains after three successive subculturing steps. This phage (vB_EcoS_ULI-O80_Stx2d) presents resistance to high temperature (60°C) and low pH (2-8). The phage belongs to the *Caudoviricetes* class (unclassified genus and family) and to the siphovirus morphology. It encodes several proteins involved in the lysogenic cycle. *Galleria mellonella* experiments showed that mutant strains caused significantly higher mortality rates than the corresponding non-STEC strains. In conclusion, this study showed that *stx2d* gene from O80:H2 *E. coli* can be transferred to non-STEC strains and contributes to their virulence. This research was funded by the University of Liège (project HYBRID_COLI_O80)

PSA32

IN VITRO CHARACTERIZATION AND ASSESSMENT IN *GALLERIA MELLONELLA* OF NEWLY ISOLATED PHAGES AGAINST *ESCHERICHIA COLI* K1

C. Antoine[1,2], F. Laforêt[1,2], B. Blasdel[3], A. Fall[4], J.N. Duprez[1], J. Mainil[1], V. Delcenserie[2], D. Thiry[1]

[1]Department of Infectious and Parasitic Diseases, Bacteriology and Pathology of Bacterial Diseases, FARAH, ULiège.Belgium

[2]Department of Food Science, Quality management in the food chain, FARAH, ULiège.Belgium

[3]Vésale Bioscience, 5310 Noville sur Mehaigne, Belgium

[4]Genalyse Partner SA, En Hayeneux 62, 4040 Herstal, Belgium

Extra-intestinal *Escherichia coli* express several virulence factors that increase their ability to colonize and survive in different localizations. The K1 capsular type is involved in several infections, including meningitis, urinary tract, and bloodstream infections. The aims of this work were to isolate, characterize, and assess the in vivo efficacy of phages targeting avian pathogenic *E. coli* (APEC) O18:K1, which shares many similarities with the human strains responsible for neonatal meningitis. Eleven phages were isolated against APEC O18:K1, and four of them presenting a narrow spectrum targeting *E. coli* K1 strains were further studied. The newly isolated phages vB_EcoS_K1-ULINTec2 were similar to the *Guernseyvirinae* subfamily, and vB_EcoP_K1-ULINTec4, vB_EcoP_K1-ULINTec6, and vB_EcoP_K1-ULINTec7 to the *Autographiviridae* family. They are capsular type (K1) dependent and present several advantages characteristic of lytic phages, such as a short adsorption time and latent period. vB_EcoP_K1-ULINTec7 is able to target both K1 and K5 strains. This study shows that these phages replicate efficiently, both in vitro and in vivo in the *Galleria mellonella* model. Phage treatment increases the larvae survival rates, even though none of the phages were able to eliminate the bacterial load.This research was funded by the Walloon Public Service, BIOWIN project: Inteliphages

PSA33

THE EFFECTIVENESS OF THYME VULGARIS ESSENTIAL OIL ON ANTIBIOTIC RESISTANCE BACTERIA ISOLATED FROM POULTRY LITTER

M. Galgano[1], F. Pellegrini[1], G. Fracchiolla[2], D. Mrenoshki[1], A. Attia Koraney Zarea[1,3], A. Bianco [4], L. Del Sambro[4], L. Pozzi[4], A. Schiavone[1], A. Assan Omar[1], A. Camarda[1], F. Cirone[1], M. Tempesta[1], D. Buonavoglia[1], A. Pratelli[1]

[1]Department of Veterinary Medicine, University Aldo Moro of Bari, Sp Casamassima Km 3, 70010 Valenzano (Ba), Italy

[2]Department of Pharmacy-Drug Sciences, University Aldo Moro of Bari, Via Orabona 4, 70125, Bari, Italy.

[3]Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre (NRC), Egypt

[4]Istituto Zooprofilattico della Puglia e della Basilicata, Contrada San Pietro Piturno, 70017, Putignano (Ba), Italy

The development and spread of antibiotic-resistanceamong bacteria is a major cause for concern worldwide. The indiscriminate use of antimicrobial agents in poultry farms is linked to the increase of multi-resistant bacteria and their spread in the environment. Furthermore, the presence of antimicrobial residues in poultry products is also documented. Accordingly, this study aims to identify bacteria in chicken litter, test their resistance to antibiotics and evaluate the antimicrobial efficacy of Thyme Essential Oil (TEO). Litter sample bulk was collected from a broiler farm and was subjected to qualitative and quantitative investigations of microorganisms. The litter was treated with aqueous solutions of TEO at different concentrations (5% to 1.25%). The pathogens detected, Lactose positive *Enterobacteriaceae* and Mannitol salt-positive *Staphylococcaceae*, was identified as *Escherichia Coli* and *Mammaliicoccus lentus*, respectively. These pathogens showed phenotypic and genotipic resistance to different class of antibiotics (b-lactams, macrolids, glycopeptides, lincosamids, polimixine).The results showed that TEO is able to reduce the total number of bacteria, expressed as % reduction of $\log_{10}$CFU/g of litter, from 76.63% to 54.85% based on the concentration of use. In particular, the strongest antibacterial action was observed against *Enterobacteriaceae* with a reduction, at the lowest concentration, of 73.3% compared to the positive control, while *Staphylococcaceae* showed a reduction of 50%. In the One Health perspective, the antibacterial

action of TEO against multidrug-resistant bacteria and the absence of any oil residues, unlike what happens with antibiotics and disinfectants, represent fundamental advantages for encouraging and supporting the use of oils as antibacterialin animal husbandry.

PSA34

ANTIMICROBIAL RESISTANCE ANALYSIS OF *ACINETOBACTER* SPP. STRAINS ISOLATED FROM A DOMESTIC CAT DURING ROUTENELY VETERINARY ACTIVITY ON ANIMAL CARCASSES

D. Vicari, A. Carrozzo, M. La Giglia, F. Mira, V. Randazzo, G. Schirò, F. Antoci, M. Vitale
Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy

During animal carcasses inspection to establish the cause of death, microbiology analyses are routinely performed from different organs in search of pathogenic bacteria. During this activity, strains of *Acinetobacter* spp. were isolated from different organs of the carcass of a domestic female Bengala cat. Before death, the cat had been treated with antibiotic and prostaglandine following an abortion. After the treatment however, a symptomatology of restlessness and convulsions began which within 6 days determine the death. Necroscopic examination revealed the presence of necrotized material inside the uterine horns and pulmonary oedema. The microbiological analysis showed the isolation of *Acinetobacter spp.* from the lung, liver, brain, intestine, heart. The identification of strains was performed through Api 20 Gallery (Biomerieux) and antibiogram by Kirby Bauer method was performed to establish antimicrobial resistance and multidrug resistance was detected toward ampicillin, cephuroxime, aztreonam, cephixime and trimethoprim. The presence of a multidrug resistant strain of *Acinetobacter* spp. in a pet is a serious concern for human health also and highlight the importance of one health approach to manage antimicrobial resistance (AMR) avoiding its spreading. The cat was hospitalized in a veterinary clinic, with the risk of MDR strains diffusion for health operator or other animals. The strain was resistant also to third generation cephalosporin. The bacteria has been identified as *Acinetobacter baumanni* but since API gallery is not valid for the correct identification at specie level, further molecular analysis will be performed for the fully characterization.

PSA35

COMBATING CAMPYLOBACTER IN THE DUTCH BROILER MEAT PRODUCTION – A JOINT VENTURE

M. van der Most[1], E. Pacholewicz[1], M.A. Dame-Korevaar[1], J. Schreuder[2], M.L. den Hartog[3], J. Visser[4], M.G.J. Koene[1]
[1]Wageningen Bioveterinary Research, Lelystad, The Netherlands
[2]Wageningen Livestock Research, Wageningen, The Netherlands
[3]NEPLUVI, Veenendaal, The Netherlands
[4]AVINED, Nieuwegein, The Netherlands

Campylobacter is the most reported foodborne pathogen in the European Union and chickens are the main reservoir for human campylobacteriosis[1]. This emphasizes the need for enhanced *Campylobacter* control at various stages of poultry meat production. In the Netherlands, shared responsibility of managing food safety led to a public-private partnership (PPP) between government, private stakeholders and research institutes to reduce *Campylobacter* prevalence in broiler meat production. In a PPP all parties show commitment to an applied research project, either financially or by an in-kind contribution. Since 2015, the Ministry of Agriculture, Nature and Food Quality, the Ministry of Health, Welfare and Sport, the Dutch Association of Poultry Processing Industries and representatives of the primary broiler production sector are working together with research institutes towards reducing *Campylobacter* at farm level and in slaughterhouses. After almost 8 years filled with a wide variety of studies, it's time to make up the balance. Valuable achievements of the PPP are research projects on intervention strategies like vaccination and biosecurity measures, maternal immunity and early detection methods. Leads for further research, for example towards differences between conventionally and alternative production systems, were provided by a multi-year monitoring study into *Campylobacter* presence on Dutch broiler farms and associated risk factors. Whether it's attributable to the PPP or not, with 43,1% in 2015 and 27,9% in 2021, monitoring on Dutch slaughterhouses shows a decreasing trend in the percentage of

Campylobacter positive flocks[2]. Although consistent effort is needed, slow and steady might win the race!

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PSA36

VIRULENCE PROFILES OF *KLEBSIELLA* SPP. FROM DIFFERENT SMALL ANIMAL INFECTION SITES

L. Fernandes[1,2,3], J. M. da Silva[1,2], J. Menezes[1,2], C. Marques[1,2,5], C. Caneiras[6,7] and C. Pomba[1,2,3]
[1]CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2]AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal
[3]Molecular Veterinary Diagnostic Laboratory - Genevet, Rua Quinta da Nora Loja 3B, Carnaxide, Portugal
[5]Faculty of Veterinary Medicine, Lusófona University, Lisbon, Portugal
[6]Laboratory of Microbiology Research in Environmental Health (EnviHealthMicro Lab), Institute of Environmental Health (ISAMB), Faculty of Medicine, University of Lisbon, Lisbon, Portugal
[7]Institute of Preventive Medicine and Public Health, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Objectives: To characterize antimicrobial-resistance and virulence of *Klebsiella* spp. isolated from different small animal infection sites. **Materials and Methods**: During 2020, sixty-two *Klebsiella* spp. isolates were obtained from clinical samples from small animals and categorized according to infection site. Antimicrobial susceptibility testing followed the EUCAST guidelines. Beta-lactamase encoding genes were characterized by PCR and sequencing. Virulence phenotyping included the string test and microplate crystal violet biofilm quantitation. Virulence genotyping screening by PCR included 15 virulence encoding genes. **Results**: Sixty-one isolates were multidrug-resistant, and most strains were from urinary tract infection (UTI)(n=31/62). Forty-five isolates carried the $bla_{CTX\text{-}M\text{-}15}$ gene (n=45/62), three the $bla_{KPC\text{-}3}$ and one the $bla_{OXA\text{-}181}$. Two *Klebsiella pneumoniae* $bla_{CTX\text{-}M\text{-}15}$ carriers were positive on the string test but *rmpA* negative. The more frequent virulence genes were *fimH-1*, *entB* and *mrkD* (85.5%, 88.7% and 79.0%, respectively), followed by *kpn* (53.2%), *ycfM* (41.9%), *kfu* (40.3%) and *traT* (38.7%). *allS* was absent. The Yersiniabactin High-Pathogenicity Island (YHPI) was present in 22.6% of the isolates. No apparent relationship between infection site and biofilm production was found. Nevertheless, 64.5% of the isolates were at least moderate biofilm producers, of which a slightly higher proportion of non-UTI isolates were moderate-to-strong producers compared to UTI isolates (35.5% versus 29%). **Conclusions**: *Klebsiella* spp. from different types of small animal infections can harbor virulence genes associated with increased invasiveness, such as YHPI, and be multidrug-resistant, constituting an emerging problem in small animal practice. The two *K. pneumoniae* showing a hypervirulent suggestive phenotype (positive string test) require further studies, highlighting the need for continuous surveilling of this serious phenotype.

PSA37

PREDATORY ATTEMPTS ON BATS: INJURY FREQUENCIES, BACTERIOLOGICAL PATTERNS OF WOUNDS AND ESCAPE ABILITY OF BATS

Ciocanau M.A., Danes D.
University of Agronomic Sciences and Veterinary Medicine, Bucharest Romania

The interactions between bats and their predators are difficult to observe, one of the factors that are restricting bats to their nocturnal activity being exactly the predation risk exposure. However, predator-inflicted wounds can offer insights into the predation risk faced by a range of bat taxa. During 4 years of observation, we investigated the predatory attempts on active bats and hibernating bats. Furthermore, we investigated the ecological connectivity and habitat use of adults and juvenile

bats, to estimate their escape ability. Bite-related wounds during the hibernation period were more frequent on bats nesting in wild areas, in caves with large entrances and less frequent in those inhabiting urban areas. In contrast, during the active period, predatory bite-injuries were more frequently observed in bats inhabiting in urban areas rather than those from wild areas. The escape ability is a factor related with ecological connectivity, with the habitat and roosting activity during the hibernation and during the active period. The bite injuries were contaminated with specific oral microbiota members of the predator or with unusual polymicrobial association of oral flora, skin/fur microbiom and environmental microorganisms. The bacteria present were quite diverse, counting from 4 to 13 genra isolated. The most common cultural isolates reveal: *Staphylococcus sp., Pasteurella sp., Escherichia sp., Streptococcus sp.,* inconsistant isolates were *Enterococcus avium, Bacillus subtilis, Enterococcus faecalis, Proteus vulgaris, Pseudomonas sp., Lactobacillus sp., Klebsiella sp., Campylobacter sp.* In urban areas the species most frequently responsable for predation attempts on bats were represented by *Felidae* during the active period of bats: the wild bats face a higher risk of predation from *Accipitriformes, Falconiformes* and *Mustelidae* more frequently during the hibernation period. Our results show that the bacterial species identified in the bite inflicted injuries on bats might give an insight upon the predators. The pathogenic significance of these isolates request further investigations.

PSA38

OCCURRENCE OF *ALTERNARIA ALTERNATA* IN THE FUR OF DOGS AND CATS – AN IMPORTANT FUNGAL ALLERGEN IN A ONE HEALTH APPROACH

P. Afonso[1,2,3], H. Quintas[3,4], A.F. Vieira[5], E. Pinto[5], M. Matos[6,7], A.S. Soares[1,2], L. Cardoso[1,2,5], A.C. Coelho[1,2,5]

[1]CECAV – Animal and Veterinary Research Centre, Vila Real, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
[3]Agrarian School, Instituto Politécnico de Bragança, Bragança, Portugal
[4]Mountain Research Center (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal
[5]Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[6]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal [7]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

OBJECTIVES: *Alternaria alternata* is an important human allergen that can be found in the fur of animals. Inhalation of *A. alternata* spores is associated with respiratory hypersensitivity, asthma, and allergic fungal rhinosinusitis. The aim of this work was to study the occurrence of *A. alternata* in the fur of dogs and cats from 13 clinic and three shelters in northeast Portugal, to understand their significance for public health under a One Health approach. **MATERIAL AND METHODS:** Fur samples were collected using the toothbrush technique. A convenience sample of 412 animals belonging to a shelter was examined for the presence of *A. alternata* in the fur. Samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25ºC and 37ºC, for 3-7 days. Identification of *A. alternata* was based on the morphology of their colonies and micromorphology of spore and hyphae. **RESULTS:** *Alternaria alternata* was identified in culture in 34 out of the 412 sampled animals (8.3%; 95% confidence interval [CI]: 7.8–8.7%). *Alternaria alternata* was identified in 28 animals from the shelters out of 156 ones (17.8%; 95% CI: 17.0-18.6%) and in six animals from clinics out of 256 (2.3%; 95% CI: 5.4–6.6%). **CONCLUSION:** Occurrence of *A. alternata* was high in this study. Since this agent is an important allergen, more studies are required to better understanding the relevance of the isolation of this fungus in the fur and its significance for public health.

PSA39

MICROSPORUM CANIS CARRIAGE IN STRAY CATS AND DOGS FROM SHELTERS AND CLINICS IN THE NORTH OF PORTUGAL

P. Afonso[1,2,3], H. Quintas[3,4], A.F. Vieira[5], E. Pinto[5], M. Matos[6,7], A.S. Soares[1,2], L. Cardoso[1,2,5], A.C. Coelho[1,2,5]

[1]CECAV – Animal and Veterinary Research Centre, Vila Real, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
[3]Agrarian School, Polytechnic Institute of Bragança, Bragança, Portugal
[4]Mountain Research Center (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal

[5]*Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*
[6]*Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal* [7]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

OBJECTIVES: Dermatophyte carriage data are crucial for assessing epidemiology and designing potential control strategies. To study the occurrence of dermatophytosis, a survey was carried out in dogs and cats, from October 2021 to June 2022 in shelters and clinics of northeast Portugal. **MATERIAL AND METHODS:** Fur samples (n = 412) were collected from three shelters and 13 pet clinics using the toothbrush technique. Dermatophyte culture was performed using Dermatophyte test medium®. Petri dishes were handled under sterile conditions and incubated at 28°C for up to 21 days. **RESULTS:** *Microsporum canis* was identified in seven animals (5 dogs and 2 cats) out of the 412 animals (1.7%; 95% confidence interval [CI]: 1.2–2.2%). Dermatophytes were identified in two animals from the shelters (1 dog and 1 cat) out of the 156 animals (1.3%; 95% CI: 0.5-2.1) and in five animals from clinics (4 dogs and 1 cat) out of 256 (1.9%; 95% CI: 1.3-2.5%). **CONCLUSION:** These results suggest that dermatophyte shedding is rare in animals admitted to the shelters and clinics under study. Considering the scarcity of epidemiological reports in Portuguese shelters and clinics, these results could be a useful contribution towards diagnosis and prevention.

PSA40

CUTANEOUS MYCOBIOTA OF PET RABBIT (*ORYCTOLAGUS CUNICULUS*) AND GUINEA PIG (*CAVIA PORCELLUS*)

R. Abreu[1,2], A. Ramos[1], A. T. Reisinho[1], E. Cunha[1,2], L. Tavares[1,2], M. Oliveira[1,2]
[1]*CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal*
[2]*Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS),Portugal*

Background: Rabbits and guinea pigs are frequently adopted as companion animals. We aimed to characterize the cutaneous mycobiota of these species and to evaluate their potential role as dermatophyte carriers. **Materials/methods:** Samples of hair and scales (n=102) were obtained from 32 rabbits and 19 guinea pigs using two different methods: by pulling hairs surrounding lesions and collecting scales (in case of dermatological localized lesions) or along the body of the animal (in case of absence of dermatological signs) (n=51); and using the Mackenzie's method (brushing the animal with a toothbrush) (n=51). Samples were inoculated in Sabouraud Chloramphenicol Agar and Dermatophyte Test Media and observed daily during the incubation period. Finally, isolated fungal species were identified through their microscopic and macroscopic morphology. **Results:** It was possible to obtain 168 isolates, the majority being saprophytic moulds (83.96%), belonging to the genera *Aspergillus* (26.8%), *Scopulariopsis* (15.5%), *Penicillium* (14.9%), *Mucor* (7.1%), *Rhizopus* (7.1%), *Cladosporium* (4.8%), *Alternaria* (4.2%), *Phoma* (1.2%) and *Chaetomium* (0.6%). Yeast growth was observed in some samples (5.89%), corresponding to *Candida* spp. (5.4%) and *Rhodotorula* spp. (1.2%). It was not possible to identify 19 fungal colonies (11.3%), or isolate any dermatophytes. **Conclusions:** The three fungal genera *Chaetomium*, *Phoma* and *Rhodotorula* were identified for the first time in pet rabbits and guinea pigs. The majority have already been reported in the hair and skin of rabbits, guinea pigs and other domestic animals, such dogs and cats. Some species have already been reported to cause infection in human and animals, especially in immunocompromised individuals. **Ackowledgements:** This work was supported by CIISA–Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Project UIDB/00276/2020 (Funded by FCT) and by Laboratório Associado para Ciência Animal e Veterinária (LA/P/0059/2020 - AL4AnimalS)

PSA41

HYPERBETAGLOBULINEMIA IN 3 DOGS WITH SYSTEMIC MYCOSIS

A.Grassi[1], S. Rossi[2], M. Pantoli[1], R. Ferriani[2], M. Gambini[1], C. Cafarchia[3]
[1]*I-Vet laboratorio analisi, Flero- Brescia- Italy*
[2]*Ospedale Veterinario San Francesco, Milan- Italy*
[3]*Dipartimento di Medicina Veterinaria, Università degli Studi Aldo Moro, Bari- Italy*

Introduction: Serum protein electrophoresis (SPE) is a technique used to determine circulating proteins composition, to identify typical pathological patterns and, and to evaluate the patient's health status when combined with haematological and biochemical profiles There are no published data about SPE changes in dogs with systemic mycosis (SM), which are difficult to diagnose and require specific laboratory tests. The aim of this study is to analyse SPE of 3 dogs with MS and their follow-up after antifungal therapy. Material and Methods: The study included a Giant Schnauzer (P1), Russian Terrier (P2) and Corso dog (P3) ranging from 3 to 4 years-old, suffering from SM by *Scytalidium* spp, *Aspergillus deflectus* and *Cladosporium* spp. respectively. Diagnosis was made by means of imaging, cytological examination, culture examination and fungal typing. Patients underwent antifungal therapy and complete haematological and biochemical analysis at the time of diagnosis and after clinical improvement as follow-up. Results: All dogs initially showed hyperproteinaemia with hyperbetaglobulinemia (1- 10.2 g/dl, 46%; 2- 8.8 g/dl, 44%; 3- 8.2 g/dl, 33%); after antifungal therapy and improvement of clinical conditions SPE values decreased (1- 7.2 g/dl, 24%; 2- 7.10 g/dl, 33%; 3- 7.3, 30%). Subsequently dog1 died due to owner's, dog2 following heart complications while dog3 survived and was healthy. Conclusion: SPE is already useful technique in veterinary medicine to support clinical suspicion, diagnosis and monitoring of some canine diseases caused by etiologic agents (e.g. Leishmaniosis, Ehrlichiosis, Angiostrongylosis). Hence more studies are needed to correlate SPE changes with SM and use it as a diagnostic and monitoring tool.

POSTER SESSION B

PSB01

EVALUATION OF ANTIBODIES AGAINST *PANLEUKOPENIA VIRUS (FPV)* IN CATS: COMPARISON OF TWO METHODS

A. Cavalli, C. Desario, G. Lanave, A. Trotta, M. Corrente, V. Martella
Department of Veterinary Medicine, University of Bari, Valenzano, Italy

Feline panleukopenia virus (FPV) causes acute lethal infection with signs of enteritis and bone marrow suppression in domestic cats and wild felids. Due to the severity of the disease in cats, vaccination is strictly recommended in all age groups.
Evaluating the levels of antibodies in the serum of cats to establish the optimal time for vaccination is important and virus neutralization (VN) and inhibition of hemagglutination (HI) may be used. Although VN is regarded as the gold standard in FPV serology for its ability to assess the actual protection status against infection, viral replication in cells must be detected by indirect immunofluorescence (IFI). HI is universally considered a good proxy for VN but the presence of non-specific agglutinins in feline sera and artefactual precipitation of red blood cells (RBC) can cause false negative and false positive results, respectively. In this study, we optimized the HI assay to quantify precisely FPV-specific antibody titres in 30 serum samples of cats of various age groups and vaccine status. Overall, removal of nonspecific agglutinins from the sera by adsorption with RBCs and decreasing the concentration of RBCs from 0.8% to 0.1% improved the performance of the HI assay. Using the VN-IFI test as a standard, the modified HI test showed 100% sensitivity and 83.3% specificity. The negative and positive predictive values were 90% and 100%, respectively. When comparing the VN and the modified HI tests, the statistically significant ($p < 0.001$) AUC was 0.917 (CI95%; 0.81; 1.03), highlighting the reliability of the optimized protocol

PSB02

ASSESSMENT OF THE PREVALANCE OF THE MAJOR MICROBIAL AND PARASITIC PATHOGENS OF BEES IN A CLINICALLY HEALTHY POPULATION MAINTAINED UNDER CLOSE SERVEILLANCE

A. Mataragka[1], P. Katsarou[1], A. Symeonidou[1], F. Hatzina[2], L. Haristos[2], T. Papadopoulos[3], J. Ikonomopoulos[1]
[1]*Agricultural University of Athens, Athens, Greece*
[2]*Hellenic Agriculture Org. "DEMETER", Nea Moudania, Greece*
[3]*Aristotle University of Thessaloniki, Thessaloniki, Greece*

Objective: This study was focused to the application of the polymerase chain reaction (PCR) for the assessment of the prevalence of major microbial and parasitic pathogens in a clinically healthy population of bees and investigate for potential diagnostic indicators based on test-positivity associations.
Materials and Methods: The study was conducted on a swarm of 250 stationary colonies located in Northern Greece, in which varroosis is considered enzootic. The study population had not shown clinical evidence of disease and had not received any antimicrobial or antiparasitic agents for a period of at least six months before sampling. A total of 820 bees were collected from 22 colonies between July and August 2020, and processed for PCR, qPCR, and RT-qPCR for the detection of Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Sacbrood Virus (SBV), Varroa Destructor Virus-1 (VDV-1), European Foulbrood (EFB), *Nosema* spp., Small Hive Beetle (SHB), *Tropilaelaps* spp. and *Varroa destructor.*
Results: All samples (100%) reacted positively to the PCR assays conducted for the detection of ABPV and BQCV, whereas 22.7% were also positive to a third pathogen i.e., VDV-1 (18.2%) and Nosema spp. (4.5%). The prevalence per pathogen was 100% for ABPV and BQCV, 18.2% for VDV-1, and 4.5% for *Nosema* spp. Positive test results were not recorded for CBPV, DWV, SBV, *Tropilaelaps* spp., *Varroa destructor*, EFB, and SHB.
The investigation for diagnostic indicators based on test-positivity association did not produce statistically significant results.
Discussion/Conclusion: In spite close health monitoring and having remained at the same location for many years, positivity of the target population to ABPV and BQCV was 100%. More than 1/5th of the study population was co-infected by three pathogens. This study indicates the need to apply urgently test and removal measures for the control primarily of ABPV and BQCV.

PSB03

PRELIMINARY SEROPREVALENCE STUDY OF PARATUBERCULOSIS IN DAIRY HERDS IN APULIA (SOUTHERN ITALY)

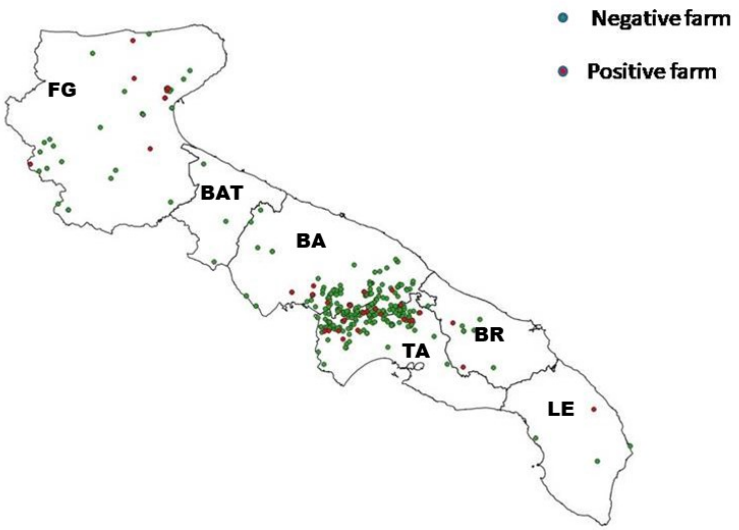
M.G. Galgano[1], A. Pratelli[1], M. Tempesta[1], G. Greco[1], D. Mrenoshki[1], A.A.K. Zarea[1,4], A. Sposato[3], M. Beverelli[3], C. Trisolini[2], V. Laera[2], G. Caldarola[2], D. Buonavoglia[1]
[1]Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano (Ba), Italy
[2]Istituto Zooprofilattico della Puglia e della Basilicata, Putignano (BA), Italy
[3]Istituto Zooprofilattico della Puglia e della Basilicata, Torre Santa Susanna (BR),Italy
[4]Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre (NRC), Egypt

John's disease (JD) is a chronic granulomatous disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) which mainly affects ruminants and whose zoonotic potential has been debated for over a century. This disease results in huge economic losses in the cattle livestock.
In Italy, voluntary programs for the control of MAP in infected dairy cattle were implemented in the Northern and Central part of the country, where many studies have been conducted. The development of an adequate control plan requires knowledge of the epidemiological situation in the other non-investigated areas. There are several diagnostic tests to identify infected animals and/or herds. Currently, ELISA is the most commonly used test for the detection of antibodies against MAP.
A total of 6.056 serum samples were collected from 341 different farms in the Apulia region (Italy) during the screening plans for brucellosis, according to the protocol approved by the Ethics Committee for Animal Experimentation (approved n° 19/21) of the Veterinary Medicine Department. Sera were tested using a commercial ELISA test. Based on the performance of the ELISA test (sensitivity 43%, specificity 99.3%), the prevalence rate of JD detected in Apulia was 1.07% (95%, CI: 0.5 to 1.7%) at animal level and 22.25% (95%, CI: 15.8 to 30.6%) at herd level.
Our results are in agreement with the data observed in literature and highlight the need to design and implement effective JD control program at national level to prevent the spread of the disease.

Table 1: Apparent and true prevalence, with 95% confidence intervals (CI), of *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds of Apulia region.

	Apparent Prevalence	True Prevalence	Number of positive	Number of negative
Animal level	1.17% (95%CI: 0.9% to 1.4%)	1.07% (95%CI: 0.5% to 1.7%)	71	5985
Herds level	10.56% (95%CI: 7.7% to 14.3%)	22.25% (95%CI: 15.8% to 30.6%)	36	305

Figure1: Map showing the study areas with the positive and negative cases of *M. avium* subsp. *paratubercolisis* from dairy cattle serum samples.



PSB04

A POSSIBLE CO-INFECTION RELATIONSHIP BETWEEN CACV AND CPV-2 IN IRANIAN DOGS

R. Faraji[1], M. Sadeghi[1], S.-R. Miraei-Ashtiani[1], V. Vasinioti[2], L. A. Ndiana[2], C. Desario[2], F. Beikpour[2], N. Decaro[2]

[1]Department of Animal Science, College of agriculture & natural resource, University of Tehran, Karaj, Iran.

[2]Department of Veterinary Medicine, University of Bari Aldo Moro, Italy.

Since the first detection of canine circovirus (CanineCV), several reports have been published over the last decade about the worldwide distribution of this emerging virus of dogs. Canine circovirus (CanineCV) is a small, icosahedral, non-enveloped, and spherical virus, with a circular single-stranded DNA genome of nearly 2 kb in size (Kapoor, Dubovi et al. 2012) that infects domestic dogs and wild carnivores (Zaccaria, Malatesta et al. 2016). So far, CanineCV and genetically related circoviruses have only been reported in a few countries, including the USA (Kapoor, Dubovi et al. 2012, Li, McGraw et al. 2013), Argentina (Kotsias, Bucafusco et al. 2019), Brazil (Weber, Cibulski et al. 2018), Italy (Decaro, Martella et al. 2014), Germany (Hsu, Lin et al. 2016), China (Sun, Zhang et al. 2019), Thailand (Piewbang, Jo et al. 2018), Colombia (Giraldo-Ramirez, Rendon-Marin et al. 2020), Norway (Urbani, Tryland et al. 2021), the UK (Bexton, Wiersma et al. 2015) and more recently in Iran (Beikpour, Ndiana et al. 2022). This study aimed to survey the circulation of CanineCV in Iranian dogs and also to characterize the detected strains at the molecular level. In order to investigate the prevalence and genomic features of CanineCV in Iranian dogs, a total of 203 dog faecal samples was collected between February and November 2018 from five different geographical regions and screened by real-time PCR (qPCR). Thirteen samples (6.4%) from a total of 203 screened samples were positive for CanineCV by qPCR, while 49 samples (24.13%) were found to contain the CPV DNA (Table 4). The MGB probe assays showed that all CPV positive samples contained CPV2a strains. All the CanineCV positive samples were also positive for CPV. Three partial replicase nucleotide sequences of the detected CanineCV strains were obtained and compared with the reference sequences deposited in the GenBank depository database. By sequence analysis, the Iranian CanineCV strains displayed a nt identity of 96.4-98.2 % each to other and of 88.3-98.2 to CanineCV reference strains. Through analysis of the partial Rep nt sequences, the Iranian CanineCV strains were more closely related to strains detected in Turkey (GenBank accession no. MK783223), allowing to hypothesize a possible introduction of the virus

from a neighbor country. Due to the limited number of analyzed sequences, however, other studies are needed to better understand the evolutionary pattern of Iranian CanineCVs. Continuous molecular surveillance for CanineCV should be also carried out to better understand the pathogenic potential of this emerging virus of the canine enteric tract. The present study provides new insights into the CanineCV molecular epidemiology and its possible role as a co-infectious pathogen.
Keywords: Dog; Canine circovirus; Genetic analysis; Canine Parvovirus; Iran.

PSB05

DIAGNOSTIC INVESTIGATION FOR THE DETECTION OF MYCOBACTERIA IN SAMPLES OF FISH FEEDS AND TISSUE FROM SEA BREAM AND SEA BASS WITH SEVERE TUBERCULAR LESIONS

A. Mataragka[1], N. Tzimotoudis[2], M. Kolygas[3], E. Karavanis[4], J. Ikonomopoulos[1]
[1]School of Animal Biosciences, Department of Animal Science, Agricultural University of Athens, Athens, Greece
[2]Hellenic Army Biological Research Center / Laboratory of Microbiology, Athens, Greece
[3]Veterinary School, University of Thessaly, Karditsa, Greece
[4]Histology-Histopathology Laboratory, C' Army Veterinary Hospital, Camp Makri, Thessaloniki, Greece

Objective: We report on a case of a sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) aquaculture farm in Greece, with many incidents of severe dermal and visceral tubercular lesions detected during routine inspection and post-mortem examination.
Materials and Methods: The investigation was conducted on samples of fish feeds (n=13) and fish feed ingredients (n=15) available commercially, and tissue samples (n=51) collected from the affected population, and consisted of molecular analysis and histopathology. The former was performed using five PCR assays targeting the main mycobacterial pathogens. Histopathology examination relied on staining of tissue sections of various visceral organs with hematoxylin-eosin and Ziehl-Neelsen.
Results: Presence of mycobacteria was demonstrated in the fish feeds (100% and 46.2% positive for *Mycobacterium* spp. and *Mycobacterium avium*, respectively), in the fish feed ingredients (73.3% and 33.3% positive for Mycobacterium spp. and Mycobacterium avium, respectively) and the tissue sections (76.5% and 3.9% positive for *Mycobacterium marinum* and *Mycobacterium avium*, respectively) that were examined. Sequence analysis of the PCR products was confirmatory of the specificity of the amplification process, at a 98% minimum level of identification. Histopathology revealed in all cases evidence consistent with mycobacterial infection.
Conclusion: The result of the analysis indicates that in addition to contaminated feeds, fish are exposed to mycobacteria through another source, which is mainly associated with *Mycobacterium marinum.* This probably accumulates in large numbers over the years in the organic sediment collected on the seabed of the aquaculture, causing severe infections.

PSB06

POTENTIAL EFFICACY OF A COMBINATION OF DIFFERENT NON-BIOSECURITY BASED INTERVENTION MEASURES TO REDUCE *C. JEJUNI* COLONIZATION OF BROILER CHICKENS

V. Szott[1], E. Peh[2], B. Reichelt[1], A. Friese[1], S. Kittler[2] , U. Roesler[1]
[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Germany
[2]Institute for Food Quality and Food Safety, University of Veterinary Medicine, Hannover, Germany

Objective: The objective of this study was to evaluate the efficacy of combined non-biosafety measures to reduce *C. jejuni* colonization. We combined a CE culture with bacteriophages and an essential oil (carvacrol) with organic acids. **Methods:** To examine the effect of carvacrol and organic acids, broilers were fed daily with 120 mg/kg feed of carvacrol and a mixture of four acids in their drinking water. To evaluate the efficacy of a CE-culture with bacteriophages, broiler chickens were treated with the CE-culture twice and received a phage combination of two phages continuously via drinking water prior to necropsy. **Results:** Cecal count enumeration demonstrated that the *C. jejuni* load was significantly reduced for the group receiving a combination of the CE-culture and bacteriophages log reduction of 1.0 $\log_{10}$ MPN/g**.** Likewise, colon counts were significantly decreased for the group receiving a combination of bacteriophages and the CE-culture (log

reduction of 1.0 log_{10} MPN/g). In contrast, although we observed a log reduction of 1.0 log_{10} MPN/g in *C. jejuni* cecal counts in the group receiving a combination of carvacrol and organic acids, this reduction was nonsignificant. Likewise, there was no significant difference in *C. jejuni* counts in the colon. **Conclusion:** We conclude that a combination of this particular CE-culture and bacteriophages may be a promising practical approach in broiler production to reduce *C. jejuni* colonization in broiler chickens at slaughter age. However, why the combination of carvacrol and organic acids failed to reduce *C. jejuni* intestinal colonization is unclear and remains to be investigated.

PSB07

ZOONOTIC POTENTIAL OF HEPATITIS E VIRUS: THE ROLE OF PIGS IN THE TRASMISSION OF INFECTION TO HUMANS

F. Gucciardi[1], G. Macaluso[1], S. Di Bella[1], F. Mira[1], A. Princiotta[1], G. Chiaramonte[1], C. Castronovo[1], A. Lastra[1], S. Vullo[1], G. La Rosa[2],A. Torina[1], A. Guercio[1], G. Purpari[1]
[1]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" – Palermo – Italy
[2]Department of Environment and Healt, Istituto Superiore di Sanità – Rome - Italy

Hepatitis E virus (HEV) is responsible for acute entero-transmissible hepatitis in humans. In Italy, an increase in autochthonous cases of HEV has recently been documented, and several cases have been epidemiologically linked to the consumption of raw or undercooked wild boar and pork liver or meat. Strains circulating in pigs and humans have a high sequence similarity, suggesting that transmission between pigs and humans is common. This study investigated the occurrence of HEV-RNA in different sample types of pigs intended for human consumption. Faeces, diaphragmatic muscle, liver, and blood samples were collected from 325 pigs in 46 farms in Sicily, Italy. The organs were prepared by the Trizol/chloroform homogenization/purification method. A 10% stool suspension in MEM medium was obtained. HEV RNA was detected via Real Time RT-PCR targenting ORF3, and by nested RT-PCR targeting ORF1 and ORF2 , followed by sequencing and phylogenetic analysis. Overall, 76/325 (23.38%) animals belonging to 35/46 herds (76.1%), tested positive for HEV RNA, detected in faeces (16%), livers (3.9%), muscles (9%), and plasma (1%). Phylogenetic analyses performed on the sequence of ORF1 and ORF2 fragments showed the circulating of genotype 3 and subtypes 3C and 3F. The data obtained confirm the circulation of the virus in the suidae in the Sicilian territory. The presence of the virus in the plasma of animals is important and can contribute to the contamination of meat. As the survival time of the virus in pig products is not known, precaution are important to prevent HEV infection in animal facilities.

PSB08

EARLY-MYCO: AFLATOXIN B1 EFFECTS ON OFFSPRING GUT IMMUNITY AND MICROBIOTA

P. Bastos-Amador[3], E. L. Duarte[1,2*], I. Silva[1], R. Assunção[5] , P. Alvito, A. T.Caldeira [1,6,7], M. Ferreira[3,4]
[1]School of Science and Technology, Universidade de Évora, Évora, Portugal
[2]MED-Mediterranean Institute for Agriculture, Environment and Development, Évora, Portugal
[3]Champalimaud Centre for the Unknown, Lisboa, Portugal
[4] Centre for Neuroscience and Cell Biology, University of Coimbra, UC Biotech, Biocant Park, Cantanhede, Portugal
[5] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal & Food and Nutrition Department, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.
[6] Hercules Laboratory, Universidade de Évora, Portugal
[7]City U Macau Chair in Sustainable Heritage, Universidade de Évora, Portugal

Aflatoxin B1 (AFB1) produces acute or chronic deleterious health effects in humans and animals. Still, long-term effects derived from initial exposure in early life, a critical period for colonization and development of gut microbiota, has not been fully evaluated. Particularly, aflatoxins could impair gut microbiota and immunity settlement, as they have been proven to cross the placental barrier and can be found in breast milk. We investigated the impact of maternal exposure to AFB1 on early-life microbiota in a mouse model. Females were fed jelly pellets containing 400 µg/kg AFB1 diluted in DMSO (treated animals n=6) or DMSO vehicle alone (control group n=6) during pregnancy and

lactation. Faeces from the offspring of both treated and control females were collected immediately after weaning and faecal DNA was extracted and purified. Bacterial taxa diversity and relative abundance were assessed by High-Throughput Sequencing performed in an Illumina Miseq® sequencer, targeting the V3 and V4 regions of the 16S rRNA gene. Operational taxonomic units (OTUs) were determined by clustering reads to 16S reference databases. A hundred and twenty-four (N=124) bacterial genera were found in both groups, 5 were only present in AFB1 treated group and 27 exclusively in control groups. A hundred and fifty-one (N=151) bacterial species were common to both groups, 15 species exclusively found in AFB1 litters and 34 species were exclusively found in control litters. To assess abundance and characterize species diversity and evenness, Shannon diversity index was calculated but no significant differences were found between groups. Although present in both groups, *Akkermansia muciniphila* and *Bacteroides acidifaciens* were significantly higher in controls. *A. muciniphila* colonizes the intestinal tract in childhood and regulates mucus thickness, intestinal barrier integrity and is involved in immune modulation. *B. acidifaciens* participates in the metabolism of lipids and sugars and activates some cytokines and immune cell receptors. Sulfidogenic bacteria recently related to inflammatory bowel disease such as *Desulfovibrio piger* and *Bilophila wadsworthia* were exclusivly found in the treated litters. Early-life gut microbiome is paramount to trigger the gut immune defences, but is far less stable than the adult microbiome. Moreover, previous work identified aflatoxins intake as a potential health hazard in Portuguese children. These preliminary results open an extensive field to further investigate the association between mycotoxins and microbiome, as the latest is increasingly recognized as a major player in a wide spectrum of diseases. This work was funded by FCT/MCTES through national funds, to earlyMYCO (PTDC/MED-TOX/28762/2017), and CESAM (UIDP/50017/2020+UIDB/50017/2020).

PSB09

RICKETTSIA MASSILIAE CIRCULATION IN SHEEP AND ATTACHED *RHIPICEPHALUS SANGUINEUS* IN CENTRAL PORTUGAL

J. R. Mesquita[1,2], S. Santos-Silva[1], A. de Sousa Moreira[1], M. B. Baptista[3], R. Cruz[3,4], F. Esteves[3,4], H. Vala[3,5], P. F. Barradas[1,2,6,7]

[1]Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal
[2]Epidemiology Research Unit (EPIUnit), Instituto de Saúde Pública da Universidade do Porto, Portugal
[3]Agrarian School of Viseu, Polytechnic Institute of Viseu (ESAV), Portugal
[4]Centre for Studies in Education and Health Technologies (CI&DETS), Viseu, Portugal
[5]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB) and Institute for Innovation, Capacity Building and Sustainability of Agri-food Production (Inov4Agro), UTAD
[6]Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde (CESPU), Gandra, Portugal
[7]Agrarian School, Polytechnic Institute of Coimbra (IPC), Portugal Country,

Rickettsiosis poses a serious public health threat among emerging and re-emerging vector-borne diseases. Ticks are reservoirs and vectors of *Rickettsia* having a significant role in the transmission of rickettsiae. In Portugal, little is known about tick-borne *Rickettsia* species in sheep. Therefore, this study aimed to investigate rickettsiae infection in ticks and their sheep host from 27 farms of four districts of central Portugal, to clarify the role of the sheep host in the circulation of this zoonotic agent. Between March and May 2021, EDTA blood samples (n=100) of healthy grazing sheep and their ticks (n=100, one tick per animal) were collected during a herd health program in Central Portugal. Obtained ticks were all identified as *Rhipicephalus sanguineus* sensu lato by PCR targeting a partial sequence of 16S rDNA gene followed by sequence analysis. *Rhipicephalus sanguineus* s.l. and host sheep blood were tested for the presence of *Rickettsia* spp. by conventional PCR targeting a partial sequence of *omp*B and *omp*A genes. From a total of 100 paired *Rhipicephalus sanguineus* s.l. ticks and host sheep, *Rickettsia massiliae* was detected in 62 *Rhipicephalus sanguineus* ticks and 35 grazing sheep blood samples, collected in central Portugal, 2021. These findings suggest that sheep may develop rickettsiemia and probably, could be capable of transmitting and amplifying the infection to uninfected ticks maintaining rickettsiae in circulation in the domestic cycle.

PSB10

STATUS OF THE CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA* ISOLATES AND ANTIMICROBIAL RESISTANCE FROM HUMAN INFECTIONS

T. de Sousa[1,2,3,4], E. Costa[5], O. Alves[5], M. Hébraud[6,7], G. Igrejas[1,2,3], P. Poeta[3,4,8,9]

[1]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[2]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[3]LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Caparica, Portugal;
[4]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal;
[5]Medical Centre of Trás-os-Montes and Alto Douro, Clinical Pathology Department, Vila Real, Portugal;
[6]Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), Metabolomic and Proteomic Exploration Facility (PFEM), 60122 Saint-Genès-Champanelle, France;
[7]Université Clermont Auvergne, INRAE, UMR Microbiologie Environnement Digestif Santé (MEDiS), 60122 Saint-Genès-Champanelle, France;
[8]CECAV – Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal;
[9]Veterinary and Animal Research Centre, Associate Laboratory for Animal and Veterinary Science (AL4AnimalS).

P. aeruginosa is an opportunistic pathogen being one of the major opportunistic pathogens. The general excessive use of antibiotics is increasing their resistance to these substances. The emergence of multi-drug resistant *P. aeruginosa* is a serious threat due to the lack of therapeutical options. This work aims to phenotypically and genotypically determine resistance to different classes of antibiotics. Thirty-tree *P. aeruginosa* strains recovered between September 2021 and February 2022 from human patients infected with this pathogen were used in this study. All strains were isolated using VITEK 2® COMPACT (bioMérieux) in Medical Centre of Trás-os-Montes and Alto Douro. The antibiotic susceptibility tests were performed using the Kirby Bauer diffusion test and the minimum inhibitory concentration (MIC). For the antibiotic colistin, the minimum bactericidal concentration (MBC) method was also performed. All methods were in accordance with EUCAST standards. PCR tests performed with the specific primers sets. Of the thirty-tree clinical isolates of *P. aeruginosa*, 33% presented resistance to several antibiotic classes. Resistance to imipenem (n=28, 85%), meropenem (n=11, 33%) and piperacillin-tazobactam (n=10, 30%) were the most represented. In contrast, strains were very sensitive to the amikacin (n=33, 100%) and tobramycin (n= 32, 97%). The MBCs was 8 µg/mL (56,25%), 16 µg/mL (37,50%) and 32 µg/mL (6,25%) of the 32 samples (range between 0.25 to 32 µg/mL). Our results confirm that multidrug-resistant *P. aeruginosa* is emerging among clinical isolates and, although, colistin remains an effective option, 12% of the isolates are resistant to colistin, which pose a major public health problem.

PSB11

PSEUDOMONAS AERUGINOSA PHENOTYPIC CHARACTERIZATION IN CANIS FAMILIARIS

T. de Sousa[1,2,3,4], A. Fortunado[3,4,5], M. Hébraud[6,7], A. Garcês[8,9,10], M. Ribeiro[1,2], C. Sabença[1,2,3,4], G. Igrejas[1,2,3] P. Poeta[3,4,11]

[1]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[2]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[3]LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Caparica, Portugal;
[4]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal;
[5]First degree biology student, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;

[6]*Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), Metabolomic and Proteomic Exploration Facility (PFEM), 60122 Saint-Genès-Champanelle, France;*
[7]*Université Clermont Auvergne, INRAE, UMR Microbiologie Environnement Digestif Santé (MEDiS), 60122 Saint-Genès-Champanelle, France;*
[8]*INNO - Veterinary Laboratory, R. Cândido de Sousa 15, 4710-503 Braga, Portugal;*
[9]*Cooperativa de Ensino Superior Politécnico e Universitário, CRL -CESPU, R. Central Dada Gandra, 1317, 4585-116 Gandra, Portugal;*
[10]*CITAB - University of Trás-os-Montes and Alto Douro, Quinta de Prados 5000-801, Vila Real, Portugal.*
[11]*Veterinary and Animal Research Centre, Associate Laboratory for Animal and Veterinary Science (AL4AnimalS).*

Pseudomonas aeruginosa is a pathogenic bacterium that has a high natural resistance to several antibiotics. Companion animals are potential transmitters of this bacterium to humans, and the dog (Canis familiaris) has a special prominence among this group. Therefore, from a One Health perspective, it is important to evaluate and to characterize the antimicrobial resistance of P. aeruginosa from dogs. This work aims to determine phenotypically resistance to different classes of antibiotics. Twenty-seven P. aeruginosa strains were recovered between September and December 2021 from dogs infected with this pathogen. All dog samples were collected from the INNO-Veterinary Laboratory and Veterinary Hospital of Trás-os-Montes and Alto Douro and isolated using VITEK 2® COMPACT (bioMérieux) to be used in this study. Their identification was confirmed using the selective medium Pseudomonas CN selective agar. The susceptibility tests were performed to microbial agents using the Kirby Bauer diffusion test and the minimum inhibitory concentration (MIC) (EUCAST standards). Eleven antibiotics disk were tested: Ceftazidime, Cefepime, Amikacin, Gentamicin, Tobramycin, Doripenem, Imipenem, Meropenem, Aztreonam, Ciprofloxacin and Ticarcillin-clavulanic acid. The antibiotics Enrofloxacin, Ceftiofur, Marbofloxacin and Cefovecin were performed by MIC. The phenotypic profile of the twenty-seven isolates revealed a resistance to cefovecin (74%) and ceftiofur (59%), demonstrating a high resistance to the cephalosporins class of antibiotics. The isolates were susceptible to amikacin (100%) and tobramycin (100%). In this preliminary study, most of the strains showed intermediate resistance to the various antibiotics tested, but we confirm that the samples from dogs do not yet show high resistance to antibiotics. However, their use has to be controlled to minimize the acquisition of resistances.

PSB12

DETECTION OF ESBL-PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATED FROM HUMAN URINARY TRACT INFECTIONS IN THE NORTH OF PORTUGAL

C. Sabença[1-4], E. Costa[5], S. Sousa[5], T. de Sousa[1-4], G. Igrejas[2-4], C. Torres[6], P. Poeta[1,4,7,8]
[1]*MicroART-Antibiotic Resistance Team, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[2]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[3]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[4]*Associated Laboratory for Green Chemistry, University NOVA of Lisbon, Caparica, Portugal*
[5]*Hospital Centre of Trás-os-Montes and Alto Douro, Clinical Pathology Department, Vila Real, Portugal*
[6]*Area Biochemistry and Molecular Biology, University of La Rioja, 26006 Logroño, Spain*
[7]*CECAV – Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[8]*Veterinary and Animal Research Centre, Associate Laboratory for Animal and Veterinary Science (AL4AnimalS) - Vila Real (Portugal)*

The most frequent human infections in communities and hospitals are urinary tract infections (UTIs). Extended-spectrum beta-lactamases (ESBL) producing bacteria is a growing concern because this resistance mechanism means that fewer antibiotic options are available to treat common infections. *Klebsiella pneumoniae* samples were isolated from urinary tract infections in the Medical Centre of Trás-os-Montes and Alto Douro between the December 7th of 2021 and the February 3rd of 2022.

K. pneumoniae strains were tested for antimicrobial resistance by the disc diffusion method, and ESBL production was tested according to the Etest ESBL PM/PML. Finally, resistance genes were detected by conventional Polymerase Chain Reaction procedure. A total of 85 *K. pneumoniae* were isolated from the urinary tract infections. From the ESBL production test, 25 ESBL-producing *K. pneumoniae* (29.4%), which were then submitted to the antimicrobial susceptibility test (AST), were identified. AST has shown that all strains were resistant to ampicillin, cefotaxime, cefepime, and aztreonam. A high percentage of resistance to ciprofloxacin (96%), ceftazidime (96%), and trimethoprim-sulfamethoxazole (92%) was verified. Nevertheless, a low percentage of resistance to imipenem (4%), meropenem (12%), ertapenem (24%), cefoxitin (24%), amikacin (28%), and tetracycline (24%) was observed. From the 20 resistance genes tested, we detected the bla_{CTX-M} (92%), and bla_{TEM} (84%) β-lactamase genes. Concerning the other resistance genes, we detected most frequently the *sul2* (88%), *aac(3)-II* (76%) and *aadA1* (64%) genes. In opposition, *tetB*, and *aac(3)-IV* genes were not observed. Results obtained highlighted a worrying percentage of ESBL-producing *K. pneumoniae* strains with multidrug resistance isolated from urinary tract infections. However, the low resistance to some antibiotics gives us hope in fighting this global public health problem. Close contact with animals and their environments allows zoonotic pathogens, such as *K. pneumoniae*, to pass from animals to humans and vice versa. So it is still essential to study these isolates from human infections since, according to the One Health concept, people's health is closely linked to the health of animals and our shared environment.

PSB13

SCREENING OF ENVIRONMENTAL SWABS AT A VETERINARY TEACHING HOSPITAL AFTER ISOLATION OF KLEBSIELLA PNEUMONIAE IN A CAT WITH URINARY TRACT INFECTION.

N. Mezzasalma, C. Spadini, M. Iannarelli, S. L. Montanaro, F. Fidanzio, A. Corsini, E. Cataldo[1], C. Quintavalla[1], C. S. Cabassi

Department of Veterinary Science, University of Parma, Parma, Italy.

Nosocomial infections (NIs) represent an important public health issue of the 21th century, in humans and animals. On May 2022 a case of NI has been documented in a cat presented at the Veterinary Teaching Hospital of University of Parma for lower urinary tract disease (FLUTD). The first urine sample was cultured onto suitable culture solid media and BHI broth, resulting sterile. During the hospitalization the cat underwent catheterization due to urethral obstruction and developed clinical signs suggestive for sepsis. Urine and blood samples were cultured, isolating *Klebsiella pneumoniae* (KP). Antibiogram (AB) was performed by Kirby-Bauer, testing 20 antibiotics selected for the treatment of systemic diseases of cats, resulting not effective *in vitro*. A NI was hypothesized and environmental screening was performed. Seventy swab samples were collected in intensive care unit, examination rooms and ultrasound rooms. KP was confirmed in the cage where the patient was hospitalized and on the keyboard in the ultrasound room, together with other bacteria agents of nosocomial infections: *E.coli, Staphylococcus aureus, Enterobacter cloacae, Pseudomonas fluorescens, Burkholderia cepacia, Stenotrophomonas maltophilia, Acinetobacter Iwoffii*. Each bacterial strain was evaluated by AB, then 13 strains including KP, resistant to at least 14 antimicrobials, were evaluated through MIC assay. According to Magiorakos et al (2012), Sweeney et al (2018), referring to antimicrobials used in companion animals, 11 strains were multidrug-resistant (MDR). These findings highlight the need for active surveillance and improved hygiene standards to decrease the risk of NI transmission in the setting of a veterinary teaching hospital.

PSB14

DETERMINING THE PREVALENCE OF ANTIMICROBIAL DRUG RESISTANCE IN WILD BADGERS IN THE REPUBLIC OF IRELAND

R. Dunne[1], G. Madigan[1], S. Murphy[1], R. Maguire[1], E. Bracken[1], U. Fogarty[2] A. Naranjo Lucena[1], R. Slowey[1] and M. McElroy[1]

[1]Central Veterinary Research Laboratory, Bacteriology and Parasitology division,Co. Kildare, Ireland.

[2]Irish Equine Centre, Co. Kildare, Ireland.

Antimicrobial resistance (AMR) is recognized as a global threat to human and animal health. Under the WHO - One health approach, regular surveillance and reporting of the incidence of AMR in humans and livestock enables trends to be identified and monitored. Wildlife is recognized as a potential vector of AMR to humans and livestock, yet there is little data available in the Republic of Ireland. This study examined the prevalence of AMR from fecal samples (n=95), collected from European badgers (*Meles meles*) throughout the Republic of Ireland. The samples were screened for commensal *Escherichia coli* (E. coli), as well as extended spectrum beta lactamases (ESBLs) using selective agar plates and MALDI-ToF. Commensal E. coli was isolated and identified in 85 samples (80.8%). Anti-microbial susceptibility testing (AST) on the 85 E. coli isolates was then carried out, identifying 5 samples (4.8%) as ESBLs, including one AMP C producing Beta Lactamase. A further 5 samples (4.8%) were identified as having resistance to various antimicrobial agents including ampicillin, tetracycline, sulfamethoxazole, trimethoprim and chloramphenicol. 4 of these samples (3.8%) were multi-drug resistant. This study provides a snapshot of AMR prevalence in wildlife in the Republic of Ireland. With the potential of wildlife to act as a reservoir for zoonotic AMR pathogens, and with the limited amount of data available, further studies are needed to monitor levels of AMR in wildlife. This will help to establish its significance to humans and livestock as well as identify potential drivers of AMR.

PSB15

QUANTIFYING TOPICAL ANTIMICROBIAL USE AND EXPLORING THE EFFECT OF PARTICIPATION IN AN ANTIMICROBIAL STEWARDSHIP PROGRAMME IN DUTCH COMPANION ANIMAL CLINICS

N.E.M. Hopman[1], N. Kardomatea[1], L. Portengen[2], J.A. Wagenaar[1], D.J.J. Heederik[2], I.M. van Geijlswijk[2], E.M. Broens[1]

[1]Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

[2]Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

Objective: Emergence of antimicrobial resistance in both human and veterinary medicine has raised concerns, which applies to systemic and topical antimicrobial use (AMU). However, little information is available on topical AMU. The objective of this study is to quantify topical AMU in 44 Dutch companion animal clinics and to explore the effect of participation in an antimicrobial stewardship programme (ASP). **Methods:** Prescription data were obtained (from 2012-2018) during previous project 'Antimicrobial Stewardship and Pets'. The Defined Daily Dose for Animals (DDDA)-method was used, and a mixed effect times series model was applied to monthly topical AMU data. Clinic characteristics were assessed using a multivariable regression model and the intervention effect was modelled using a step function with a change in (linear) time trend. **Results:** A seasonal topical AMU pattern was observed, with highest AMU in July-August and lowest AMU in February-March. Topical AMU significantly decreased over time and the proportion of dogs was positively associated with topical AMU. No strong effects were seen after the introduction of an ASP, except for second line antimicrobials and for skin products, which both showed a decrease. **Conclusion:** This study demonstrated a seasonal pattern and a decreasing time trend in topical AMU. The effect of an ASP was less clear than for systemic AMU. This might be an effect of the focus on reducing systemic AMU, resulting in a shift towards topical AMU. As topical AMU appeared to be a substantial part of total AMU in these clinics, topical AMU should be taken into account when optimizing AMU.

PSB16

MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* IN CHICKEN STERNAL BURSITIS

V. Silva[1-4]*, A. Igrejas[1], J. Ribeiro[1,2], A. Silva[1-4], P. Pinto[5], M. Vieira-Pinto[5-6], G. Igrejas[2-4], P. Poeta[1,2,6,7]

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Portugal.

[2]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Portugal

[3]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Portugal

[4]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Portugal*
[5]*Department of Veterinary Science, University of Trás-os-Montes and Alto Douro (UTAD), Portugal*
[6]*Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Portugal*
[7]*Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), Portugal*

Infectious bursal disease (IBD) is an infectious disease, very contagious in young birds, that affects the tissues of the animal's immune system, such as Fabricio's bursa. The disease alters the immune response and leaves infected animals immunosuppressed which may lead to the development of secondary infections. Furthermore, it is known that chickens with IBD are more susceptible to develop infections caused *Mycoplasma synoviae*, *Staphylococcus aureus*, and *Pasteurella* spp. Therefore, this study aimed to isolate *S. aureus* from chickens with IBD. Swab samples were collected from 44 chickens with IBD. The swabs were incubated at 37°C for 24 h in BHI broth supplemented with 6.5% of NaCl. Then, the inoculum was seeded on Baird-Parker agar plates and incubated at 37°C for 24 h. *S. aureus* species were identified by MALDI-TOF MS. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method. From the 44 samples, 24 (54.5%) were positive for *S. aureus*. Six (25%) isolates showed resistance to at least three classes of antimicrobials and were classified as multidrug-resistant. All isolates were resistant to aminoglycosides and resistance to penicillin (n=2), macrolides and lincosamides (n=11), tetracycline (n=2), trimethoprim-sulfamethoxazole (n=2), fusidic acid (n=5), chloramphenicol (n=2) and ciprofloxacin (n=2) was also detected. This study demonstrated that *S. aureus* carrying resistance to several different antimicrobials is frequently present chicken with IBD which may can raise a problem since secondary infections can cause significant economic losses. Funding 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) and by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Vanessa Silva and Adriana Silva are grateful to FCT (Fundacão para a Ciência e a Tecnologia) for financial support through the PhD grants SFRH/BD/137947/2018 and SFRH/BD/04576/2020, respectively.

PSB17

ANTIBIOTIC-RESISTANT ENTEROCOCCI ISOLATED FROM POULTRY'S STERNAL BURSA LESIONS

J. Ribeiro[1,2]*, V. Silva[1-4], P. Pinto[5], M. Vieira-Pinto[5-6], G. Igrejas[2-4], P. Poeta[1,2,6,7]
[1]*Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Portugal*
[2]*Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Portugal.* [3]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Portugal*
[4]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Portugal.* [5]*Department of Veterinary Science, University of Trás-os-Montes and Alto Douro (UTAD), Portugal*
[6]*Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Portugal*
[7]*Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), Portugal*

Hundreds of thousands of annual deaths worldwide are caused by antibiotic-resistant bacterial infections, making antibiotic resistance a serious threat to public health. One main contributor to the spread of antimicrobial-resistant bacteria is the poultry industry since it allows contact between humans, live animals, and the environment. Poultry can be affected by infection or traumatic injuries in the sternal bursa that can be responsible for economic losses at the abattoir level due to total or partial condemnations. Therefore, to determine whether the sternal bursa lesions could represent a source of antibiotic-resistant bacteria, forty-eight swabs were collected from the bursal lesions of slaughterhouse chickens. Each sample was seeded onto Slanetz-Bartley agar and Slanetz-Bartley agar supplemented with vancomycin (4 mg/L) for the isolation of *Enterococcus* spp. and vancomycin-resistant enterococci (VRE), respectively. Colonies with typical morphology were identified by the bile-aesculin reaction. The antimicrobial susceptibility of the isolates was determined according to the Kirby-Bauer disc diffusion method against ampicillin, vancomycin,

teicoplanin, erythromycin, tetracycline, ciprofloxacin, chloramphenicol, quinupristin-dalfopristin, linezolid, and gentamycin. Forty-one (85%) samples presented *Enterococcus* spp. and their phenotypic profile revealed that 33% (n=16) were resistant to erythromycin, and 52% (n=25) were resistant to tetracycline. Furthermore, one (2%) sample presented VRE, and its phenotypic profile revealed that it was also resistant to erythromycin. These results demonstrate that sternal bursa lesions in poultry represent a source of antibiotic-resistant bacteria. To prevent the further spread of these bacteria, precautions should be taken when handling carcasses affected by this lesion. Funding: 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) and by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Vanessa Silva is grateful to FCT (Fundacão para a Ciência e a Tecnologia) for financial support through the PhD grant SFRH/BD/137947/2018.

PSB18

GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF *ESCHERICHIA COLI* ISOLATES RECOVERED FROM THE UTERUS OF MARES WITH FERTILITY PROBLEMS

F. Paola Nocera[1], C. Zagaglia[2], L. Maurizi[2], A. Lucia Conte[2], C. Longhi[2], L. De Martino[1,3]

[1]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy

[2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, 00185 Rome, Italy

[3]Task Force on Microbiome Studies, University of Naples "Federico II", Naples, Italy

Introduction: *Escherichia coli* (*E. coli*) is reported worldwide as one of the main causative agents of equine endometritis, and its ability to form biofilm within the uterus is a potential cause of persistent endometrial infections. This study aimed to define the phenotypic antimicrobial resistance profile, the biofilm formation capacity, and the detection of some virulence genes of equine *E. coli* strains isolated from mares suffering from endometritis. **Materials and Methods:** *E. coli* strains were isolated from uterine swabs collected from mares diagnosed with reproductive disorders. Antimicrobial susceptibility testing was performed by disk diffusion method. Biofilm formation ability was established by crystal violet staining. The presence of some virulence genes was assessed by multiplex PCR assay. **Results:** A total of 24 *E. coli* strains were collected. Hemolytic phenotypes were detected in two out of 24 studied strains (8.3%). The antimicrobial resistance profiles showed high resistance to penicillin (95.8%), ampicillin (95.5%), imipenem (91.7%), amoxycillin/clavulanic acid and tetracycline (41.7%), whereas the most efficient antimicrobial was amikacin (100% of cultures), followed by enrofloxacin (95.8%), ceftiofur (86.9%) and gentamicin (75.0%). 54% (13/24) of the isolated *E. coli* resulted to be multidrug-resistant strains. Each tested strain exhibited biofilm-forming capacity. However, most of them were moderate biofilm producers (54.2%), 20.8% strong biofilm producers and 25.0% weak biofilm producers. A wide genetic diversity about tested virulence genes was observed. **Discussion and Conclusions:** In the present study, the worrying prevalence of multidrug-resistant *E. coli* biofilm producing strains represents a serious challenge in the treatment of mares with fertility problems.

PSB19

EPIDEMIOLOGICAL LINK BETWEEN CLINICAL DHA-1-PRODUCING MDR *KLEBSIELLA PNEUMONIAE* ST11 OF FELINE AND HUMAN ORIGIN

C. Marques[1,2,3] J. Perdigão[4], A Spadar[5], J Phelan[5], S Campino[5], T.G. Clark[5], L. Pinheiro[1,2,6], P. Cavaco-Silva[7], A. Duarte[7,8], C. Pomba[1,2,6]

[1]CIISA - Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal

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

[3] Faculty of Veterinary Medicine, Lusófona University, Lisbon, Portugal

[4]iMed.Ulisboa - Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

[5]Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

[6]Molecular Veterinary Diagnostic Laboratory - Genevet, Rua Quinta da Nora Loja 3B, 2790-140 Carnaxide, Portugal

[7]*Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, Portugal*
[8]*Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal*

Objectives: This study aimed to characterize two clinical multidrug-resistant (MDR) *Klebsiella pneumoniae* (KP) ST11 strains obtained from a cat and an unrelated hospitalized human with urinary tract infection using whole genome sequencing (WGS). **Methods:** Disk diffusion antimicrobial susceptibility against 28 antimicrobials was conducted according to CLSI. Both strains were subjected to WGS (Illumina HiSeq 4K,2x151bp). De novo assembly was carried out from raw sequence reads and the genome assemblies characterized for its resistome and virulence gene content. Phylogenetic reconstruction was performed from a set of genome-wide high-quality SNPs. **Results:** Both KP ST11 strains were, wzi75 (KL105), DHA-1 producers and had similar MDR profiles. Other antimicrobial resistance genes detected in both strains included: *bla*OXA-1, *bla*SHV-11, *qnrB4*, *aac(6')-Ib-cr5*, *aadA2*, *sul1, arr-3*, *catB3*, *fosA*, mph(A) and *qac*E. The replicon detected in the cat's strain (IncR1) was also found in the human´s KP strain. According to the phylogenetic analysis, these strains differed in only 13 SNP, suggesting an epidemiologically link between them following the 23 SNP cut-off proposed by Sherry et al. (2019). Noteworthy, the unrelated cat and human were unrelated, but both lived in Lisbon, therefore pointing to a possible scenario of local hospital-to-community dissemination. **Conclusion:** By using NGS, this study brings strong evidence that cats and unrelated hospitalized humans may become infected with closely related MDR *K. pneumoniae* ST11 strains. Thus, dissemination of MDR strains seems to be possible even in the absence of direct contact between humans and pets, highlighting the need for an OneHealth approach. **Financing:** CIISA/FCT project-UIDB/00276/2020; AL4AnimalS-LA/P/0059/2020.

PSB20

ENVIRONMENTAL CONTAMINATION WITH MULTIDRUG RESISTANT BACTERIA AND CARBAPENEMASE-PRODUCING *ACINETOBACTER* SPP. IN PORTUGUESE SMALL ANIMAL VETERINARY PRACTICES

J. M. da Silva[1,2], J. Menezes[1,2], C. Santos[3], L. Fernandes[1,2,3], A. J. Amaral[1,2], C. Pomba[1,2,3]
[1]*CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*
[2]*AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences , Portugal*
[3]*Molecular Veterinary Diagnostic Laboratory - Genevet, Rua Quinta da Nora Loja3B, 2790-140 Carnaxide, Portugal*

Objectives: Little is known about the prevalence and transmission of multidrug resistant (MDR) bacteria in Veterinary Medicine, particularly in small animal veterinary practices (SAVPs). The aim of this study is to determine the prevalence of multidrug resistant bacteria in SAVPs. **Materials and Methods**: Six SAVPs were studied. Environmental samples from critical surfaces were collected. Nasal swabs were voluntarily obtained from Veterinarians and staff. All swabs were plated on specific media selective for resistant bacteria: ESBL- and carbapenemase-producing Enterobacterales; Methicillin-Resistant *Staphylococcus* (MRS) and MDR Acinetobacter. Gram negative isolates were screened by PCR for the presence of major families of beta-lactamases and carbapenemase genes. Staphylococci isolates were screened by PCR for the presence of *mecA* gene. **Results**: At least one resistant isolate was found in 20.8% (n=26/125) of the analysed surfaces: i) 58% (n=15/26) were positive for MDR *Acinetobacter* spp.; ii) 19% (n=5/26) were positive for MRS *Staphylococcus pseudintermedius*; iii) 8% of the surfaces (n=2/26) were positive for MRS *Staphylococcus epidermidis*. In one SAVP, 18.2% of surfaces analysed (n=4/22) tested positive for OXA-23-producing *Acinetobacter* spp. Forty-three percent of human nasal isolates carried the *mecA* gene, of which 20% (n=6/30) were MRS *Staphylococcus aureus*. In one SAVP, one Veterinarian and one nurse were nasally colonized by a CTX_M-15-producing *Klebsiella pneumoniae*. **Conclusions**: This study demonstrates a concerning level of environmental contamination, which highlights the need for harmonization of infection, control and prevention guidelines SAVPs to prevent the dissemination of multidrug resistant bacteria onto the community.

PSB21

ASSOCIATIONS BETWEEN ENTEROCOCCI SPECIES ISOLATED FROM ORNAMENTAL ANIMAL FEED AND GENES OF ANTIBIOTIC RESISTANCE

C. Miranda[1-4,†], R. Soares[1,†], A. Martins[5,6], S. Cunha[1], L. Ferreira[1], G. Igrejas[4,7,8], P. Poeta[1,4,6,9,]

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2]Department of Sciences, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal
[3]Toxicology Research Unit (TOXRUN), IUCS, CESPU, CRL, Gandra, Portugal
[4]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Caparica, Portugal
[5]Department of Zootechnics, UTAD, Vila Real, Portugal
[6]Animal and Veterinary Research Center (CECAV), UTAD, Vila Real, Portugal
[7]Department of Genetics and Biotechnology, UTAD, Vila Real, Portugal
[8]Functional Genomics and Proteomics Unit, UTAD, Vila Real, Portugal
[9]Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), Portugal
[†]These authors have contributed equally to this work.

Ornamental animals can represent an important reservoir of *Enterococcus* carrying resistances to various antibiotics, allowing their transmission to humans by direct or indirect contact through the environment, fomites and contaminated feces. In this line, the aim of this study was to investigate the presence of statistical associations between three obtained enterococci species from ornamental animal feed between February and December 2020 and their antibiotic resistance. Fifty-six enterococci isolates, *E. faecalis* (n=22), *E. faecium* (n=19) and *E. gallinarum* (n=15) were tested by PCR assay for the presence of the resistance genes for 13 antibiotics like vancomycin (*van*A and *van*B), erythromicyn (*erm*A, *erm*B and *erm*C), gentamicin (*aac*(6')-*aph*(2'')-Ia), tetracycline (*tet*L, *tet*M and *tet*K), quinupristin/dalfopristin (*vat*D and *vat*E), chloramphenicol (*cat*A) and streptomycin (*ant*(6)-Ia). The data was analyzed by the chi-square independence test, using the SPSS 15® software. A probability level (p) <0.05 was considered statistically significant in the association of variables. The genotypic characterization showed that the most prevalent resistance genes detected in enterococci species were *erm*B (54%), *tet*L (48%), *van*A (37%), *tet*M (32%), *tet*K (30%), and *ant*(6)-Ia (30%). The statistical analysis showed significant associations for the *erm*B (p=0.0001), *tet*M (p=0.0002), *tet*L (p=0.0014), *van*A (p=0.0205) and *ant*(6)-Ia (p=0.0015) resistance genes. Thus, *E. faecalis* was more likely to be positive for *tet*M, *E. faecium* for *erm*B and *tet*L, and *E. gallinarum* for *erm*B, *tet*L, *van*A and *ant*6-Ia. In conclusion, *E. gallinarium* was the enterococci species that demonstrated a higher probability of carrying a variety of antibiotic resistance genes.

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PSB22

PREVALENCE STUDY OF ANTIBIOTIC RESISTANCE GENES IN *E. faecalis* AND *E. faecium* ISOLATED FROM BOVINE COLOSTRUM

S. Cunha[1], C. Miranda[1-4], A. Martins[5,6], R. Soares[1], M. Maia[1], F. Silva[6,7], G. Igrejas[4,8,9], P. Poeta[1,4,6,7,10,]

[1]Microbiology and Antibiotic Resistance Team (MicroART), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2]Department of Sciences, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal
[3]Toxicology Research Unit (TOXRUN), IUCS, CESPU, CRL, Gandra, Portugal
[4]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Caparica, Portugal
[5]Department of Zootechnics, UTAD, Vila Real, Portugal
[6]Veterinary and Animal Research Centre (CECAV), UTAD, Vila Real, Portugal
[7]Department of Veterinary Sciences, UTAD, Vila Real, Portugal
[8]Department of Genetics and Biotechnology, UTAD, Vila Real, Portugal
[9]Functional Genomics and Proteomics Unit, UTAD, Vila Real, Portugal

[10]*Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), Portugal*

Enterococcus faecalis and *E. faecium*, two of the most prevalent nosocomial agents worldwide, are frequently isolated from animals and animal foods, including bovine colostrum. These bacteria are, in most cases, resistant to a large number of antibiotics, which they are capable of transmitting to others. The aim of this study was to assess whether there is a statistical association between the presence of an antibiotic resistance gene or virulence gene in the enterococci isolate, obtained from bovine colostrum, and its identified species. Forty-four *E. faecalis* isolates and eleven *E. faecium* isolates were tested, by PCR assay, for the presence of antibiotic resistance genes and virulence genes. For the statistical analysis, the chi-square (χ^2) independence test and Fisher's exact test were performed, using the SPSS 15® program. A probability level (p) <0.05 was considered statistically significant in the association of variables. Statistically significant relationships were obtained for the *erm*(C) and *tet*(M) resistance genes (p=0.003 and p=0.048, respectively), in which *E. faecium* was more likely to be positive for both. For virulence genes, significant relationships were observed for *ace* and *gel*(E) genes (p=0.007 and p=0.024, respectively), showing that both are more prevalent in *E. faecium*. According to this study, *E. faecium* isolates from calf colostrum are more likely to act as a reservoir and transmission vehicle for erythromycin and tetracycline resistance genes, and collagen-binding protein and gelatinase genes, than *E. faecalis* isolated from colostrum. **Acknowledgements:** 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) and by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

PSB23

EVALUATION OF THE LOWEST CONCENTRATION/TIME RATIO OF THE OXYGEN/OZONE GAS MIXTURE AGAINST BACTERIA RESPONSIBLE OF METRITIS

A. Trotta, E. Lillo, A. Rizzo, A. Carbonari, M. Cordisco, M. Corrente
Department of Veterinary Medicine, University of Bari "Aldo Moro", S.P. per Casamassima km. 3 70010 Valenzano (BA),

Ozone is a gas with oxidizing power and showing a strong microbicidal activity on bacteria, fungi, viruses and protozoa. The aim of this study was to *in vitro* determine the lowest dose of gaseous oxygen/ozone (O_3/O_2) mixture, and the minimum exposition time, showing the optimal antibacterial effect, on different bacterial species. A pilot study was carried out on reference strains (*E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923) starting from 0.5 McFarland suspension, diluted 1:1000 in 5 ml sterile saline and then plated on Petri dishes, which was exposed to a gaseous constant flow of an O_3/O_2 mixture respectively at 50, 35 and 20 µg Ozone/mL (O_3/mL) for 5, 3 and 1 minutes. Based on preliminary results, a collection of 17 field isolates from cases of cow metritis (belonging to *Klebsiella pneumoniae, Enterobacter agglomerans, E. coli, S. aureus* and *Proteus mirabilis*), was subjected to the minimum concentration of O_3/mL for the lowest exposition time, and bacterial growth was evaluated after 24/48 hrs by counting the UFC/ml in Petri dishes. The experiments highlights the bactericidal activity of the 20 µg O_3/mL for 1 minute against all the field isolates. Ozone appears to have an optimal antibacterial activity on different bacteria and the promising *in vitro* low dose/exposition time, encourages the possible *in vivo* application, as part of the strategy to limit the use of antimicrobials in bacterial diseases such as metritis in cows.

OA14

ANTIMICROBIAL RESISTANCE, VIRULENCE CHARACTERISTICS AND PHYLOGENETIC DIVERSITY OF *ESCHERICHIA COLI* ISOLATED FROM FOOD-PRODUCING ANIMALS

A. Silva.[1-6], V. Silva [1-6], R. Cordeiro[7], G. Igrejas[2,5,6], V. Falco[8], P. Valentão [9] and P.Poeta[1-4]
[1]*Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.*
[2]*LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, Caparica, Portugal.*
[3]*CECAV – Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[4] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal*

[5] *Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.*
[6] *Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.*
[7] *Intergados, SA, Av. de Olivença, S/N 2870-108 Montijo, Portugal*
[8] *Chemistry Research Centre (CQ-VR), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.*
[9]*REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto.*

Antimicrobial resistance has emerged in food-producing animals and the antibiotic therapy induces the selection of resistance bacteria. Not only limited the therapy options but also generate the spread of resistance bacteria from livestock to humans and cause a major public health problem. The main objective of this work was to determine the antibiotic resistance profiles of *Escherichia coli* isolates from pigs' farms in Portugal and undertake the presence of virulence and antibiotic resistance genes, and to carry out a phylogenetic classification of the isolates. Of the 45 *E. coli* strains obtained, 8.8% of them belong to the D phylogenetic group, which is implicated in extraintestinal infection and 57.4% of them belong to the A phylogenetic group and 31.1% belong to the B1 phylogenetic group, which is indicated that are commensal strains. All the isolates presented a multi-resistance profile, with high levels of resistance to β-lactams, aminoglycosides, sulfonamides and tetracycline. Twenty-four resistance genes were detected, the most frequent of which were *amp*C and *bla*TEM conferring resistance to β-lactams and *sul*3 conferring resistance to sulfonamides.The presence of genes involved in the expression of aerolysin (*aer*), necrotizing factor-type 1 (*cnf* 1), type1-fimbriae (*fim*A), haemolysin (*hly*), typeC-fimbriae (*pap*C) and adhesin *PapG* class III (*papG*III) was also investigated. In total, 97.7% of the strains carried virulence factors. This study has revealed a high incidence of resistance to commonly used antimicrobials in animal production and human medicine. Food-producing animal represent an important reservoir of *E. coli* isolates with genes with the potential risk to humans. 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). Vanessa Silva and Adriana Silva are grateful to FCT (Fundação para a Ciência e a Tecnologia) for financial support through the PhD grant SFRH/BD/137947/2018 and SFRH/BD/04576/2020, respectively.

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DETECTION OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* SEROVAR KENTUCKY IN FALCO TINNUNCULUS

A. Bianco[1], L. Capozzi[1], L. Del Sambro[1], D. Simone[1], M. Galgano[2], R. Fraccalvieri[1], L.M. Difato[1], L. Palazzo[1], I. Pietragalla[1], M. Toce[1], A.C. Romano[1], A. Parisi[1]
[1]*Experimental Zooprophylactic Institute of Apulia and Basilicata, Via Manfredonia, 20 - 71121 Foggia,Italy*
[2]*Dipartimento di Medicina Veterinaria (DIMEV) dell'Università degli Studi di Bari Aldo Moro,Italy*

Wild birds are considered potential sources of zoonoses because they can host enteropathogens and can became antimicrobial resistance (AMR) reservoirs. In this study we describe the isolation and the molecular typing of *Salmonella* spp. from Falco tinnunculus. The Whole Genome Sequencing (WGS) was performed using MiSeq platform (Illumina) to the aim to characterize the isolate. Draft genome was assembled using SPAdes v3.15.4 and annotated using Prokka (Version 1.14.6). The serovar and Sequence type (ST) were predicted by Salmonella In Silico Typing Resource (SISTR) and Multi Locus Sequence type (MLST) analysis, respectively. AMR genes were detected using biotool ABRIcate (Version 1.0.1). The isolate was typed as *Salmonella enterica* subsp. *enterica* serovar Kentucky (*S.* Kentucky) belonging to ST152. The *S.* Kentucky ST152 is frequently isolated from asymptomatic cattle and poultry in United States, which represent probable reservoirs, but rarely from wild animals. Generally, in recent years, *S.* Kentucky has been described as cause of infection in food animals in several countries. Thus, to safeguard the welfare of domestic animals and human health, it is therefore important to know the antibiotic resistance of strains isolated in wild animals. The *in silico* prediction identified AMR genes correlated with resistance to beta-lactams, aminoglycosides and fluoroquinolones. The antimicrobial resistance was confirmed

by phenotypic test. Among these, fluoroquinolone is listed as “Critically important antimicrobials” by the World Health Organization. Our results stressed the importance of a continuous bacteria surveillance in wild animals.