5th INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

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ABSTRACT BOOK

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Matjaz Ocepek is a Senior Research Fellow and the Head of the National Veterinary Institute at the Veterinary Faculty. He is engaged in development and implementation of the methods for diagnostics of zoonoses. He is also a Leader of several scientific projects and a Principal Investigator of one of the main research groups at the Veterinary Faculty. He has published more than 100 original scientific papers. He is a Member of the European Society of Mycobacteriology, International Association for Paratuberculosis, International Society for Infectious Diseases and Slovenian Microbiological Society (past-President) and a Board Member of the European College of Veterinary Microbiology. Sr. Res. Fell. Matjaz Ocepek is the Head of the National Veterinary Institute. He is engaged in development and implementation of the methods for diagnostics of zoonoses. He is also a Leader of several scientific projects and one of the main research groups at the Veterinary Faculty.

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Dr. Jana Avberšek is a research associate at the Institute of Microbiology and Parasitology at the Veterinary Faculty, University of Ljubljana, and head of the Laboratory for Molecular Bacteriology at the same institute. Her main research interests include molecular methods for detection of various bacterial animal and zoonotic pathogens, genotyping of bacteria by whole-genome sequencing (e.g. Salmonella sp., STEC, Clostridioides difficile, Staphylococcus pseudintermedius) and genetic background of antimicrobial resistance.

Filip Boyen

DVM, PhD, Diplm. ECPHM (non-certified), EBVS® European Specialist in Veterinary Microbiology. Filip Boyen obtained his DVM and PhD at the Faculty of Veterinary Medicine, Ghent University, Belgium. His research addresses different bacterial diseases in various animals species, with a recent focus on the use of MALDI-TOF in diagnostics.

Darja Kušar

Research fellow, BSc Biology, PhD Microbiology, Darja Kušar holds a PhD in microbiology and is a researcher at the Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana. She works in the Laboratory for Molecular Bacteriology. She is experienced in molecular diagnostics and typing of bacterial pathogens, including dPCR and NGS.

Rachel Marschang

PD Dr. med. vet. Diplomate, European College of Veterinary Microbiology and of the European College of Zoological Medicine in herpetological medicine. Consultant at Laboklin in Bad Kissingen, Germany. Adjunct professor at the University of Hohenheim in Stuttgart. Editor-in-chief of the Journal of Herpetological Medicine and Surgery.

Tina Pirš

Dr. Tina Pirš, DVM, is a specialist at National Veterinary Institute, Veterinary Faculty, University of Ljubljana. She is the head of Institute of Microbiology and Parasitology and quality assurance manager. Her main interest is general bacteriological diagnostics in various animals species and she participates in several scientific projects.

Damien Thiry

Professor, DVM, PhD, Dipl ECVM Damien Thiry is Professor of Veterinary Bacteriology at Liège University, Belgium. He obtained a PhD thesis on the interactions between suids and hepatitis E virus in the Virology unit of the FARAH Research Center (Faculty of Veterinary Medicine, ULiège) in collaboration with the Scientific Institute of Public Health in Brussels (Sciensano). Simultaneously, he performed a master degree in Veterinary Public Health – Emerging Diseases. He is working since 2014 in the Bacteriology team of the veterinary faculty. He performed two post-doctoral research stays: one in Pasteur Institute (Paris) and a second one at KULeuven where he developed a Galleria mellonella model of phage therapy against Klebsiella pneumoniae. His main research topics are the study of antimicrobial resistance, especially in Staphylococcus aureus and Enterobacteriacae and the study of bacteriophages as potential treatment against bacterial infections.

Urška Zajc

Urška Zajc is a veterinarian and works in the field of veterinary microbiology at the Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana. She conducts the diagnostics in the Laboratory for especially dangerous bacterial diseases (BSL III). She is experienced in conventional and molecular diagnostics of bacterial pathogens.

SCIENTIFIC COMMITTEE

Prof. Bryan Markey (Chair)

Professor Bryan Markey MVB, PhD, Dipl. ECVM, MRCVS. Bryan Markey is Professor of Veterinary Microbiology at the Dublin School of Veterinary Medicine in Ireland, where he heads up the Section of Veterinary Pathobiology. He is a founding member and de facto diplomate of the European College of Veterinary Microbiology, currently serving as President of ECVM. His research interests include enzootic abortion of ewes, Johne's disease and MRSA infections in small animals. He has co-authored a number of international books on veterinary microbiology, including Clinical Veterinary Microbiology and Veterinary Microbiology and Microbial Disease.

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Matjaz Ocepek is a Senior Research Fellow and the Head of the National Veterinary Institute at the Veterinary Faculty. He is engaged in development and implementation of the methods for diagnostics of zoonoses. He is also a Leader of several scientific projects and a Principal Investigator of one of the main research groups at the Veterinary Faculty. He has published more than 100 original scientific papers. He is a Member of the European Society of Mycobacteriology, International Association for Paratuberculosis, International Society for Infectious Diseases and Slovenian Microbiological Society (past-President) and a Board Member of the European College of Veterinary Microbiology. Sr. Res. Fell. Matjaz Ocepek is the Head of the National Veterinary Institute. He is engaged in development and implementation of the methods for diagnostics of zoonoses. He is also a Leader of several scientific projects and one of the main research groups at the Veterinary Faculty.

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Urška Kuhar

Dr. Urška Kuhar is a Research Fellow with a DVM diploma and a PhD in veterinary medicine at the Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana. She is the head of the Virology Department, head of the National Reference Laboratory for Capripoxviruses and head of the working group of NRLs at the National Veterinary Institute. She is an expert in laboratory diagnostics of viral diseases in animals and works with cell culture techniques, serology, and molecular virological techniques, with a focus on next-generation sequencing and bioinformatics analysis.

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KEYNOTE SPEAKER

Anette Loeffler

Graduated from Munich, Germany, veterinary school in 1994 and subsequently worked in mixed practice in Cumbria. She completed a residency in veterinary dermatology and a PhD on MRSA in companion animals at the Royal Veterinary College. She is Professor in Veterinary Dermatology and Cutaneous Bacteriology at the RVC and divides her time between dermatology referral clinics at the RVC, teaching and research. She has an active role in the referral hospital infection control and antimicrobial guideline activities, co-authored the recent WAVD clinical consensus guidelines on methicillin-resistant staphylococci in small animal practice and is currently Editor-in-Chief of Veterinary Dermatology.

THE IMPORTANCE OF STAPHYLOCOCCI AND THE 'VETERINARY BURDEN OF MRSP'

Anette Loeffler
Royal Veterinary College, University of London, UK

Staphylococci have long been amongst the best known and most troublesome bacterial pathogens in human and veterinary medicine, particularly due to their ability to become multidrug-resistant and adhere to implants. Three recently published systematic analyses show the burden of antimicrobial resistance (AMR) in bacterial pathogens. They identified *S. aureus* as one of the three leading bacterial pathogens for human deaths attributable to and associated with drug resistance globally, and in the WHO European region during 2019 and *S. aureus* was also found to be the leading bacterial cause of death in 135 countries and globally in individuals over 15 years of age also during 2019 (Global Burden of Disease 2019, Antimicrobial Resistance Collaborators).

Although such data are not currently available for veterinary medicine, staphylococcal infections in animals are consistently amongst the most common bacterial infectious diseases in companion animal and livestock medicine and thus lead to considerable morbidity and antimicrobial prescribing. Multidrug-resistance as e.g in *S. pseudintermedius* (MRSP) and biofilm formation amongst the coagulase-negative staphylococci in intensive veterinary care settings have become problematic and mirror many of the medical challenges in human medicine. Molecular techniques have provided new insights into the niche adaptations of staphylococci, resistance genes and virulence factors, but management of staphylococcal diseases remains difficult.

The success of MRSP in small animal settings is largely based on the ubiquity and survival capacity of staphylococci on body surfaces and in the environment and its ability to cause opportunistic infections. And while most staphylococcal infections, including MRSP infections, are not life-threatening to the host, many are associated with chronic underlying diseases and therefore require repeated or ongoing treatment. For skin, ear and eye infections, advances on treatment recommendations in recent years have focused largely on topical therapy and with good success. However, for deeper infections that require systemic therapy, treatment options are often substantially limited due to extensive AMR. A antimicrobials associated with higher toxicity and with ethical concerns regarding their use in animals (rifampicin, amikacin, chloramphenicol).

Significant knowledge gaps remain for drug dosages and laboratory breakpoints. And further insights into host-factors of staphylococcal infections and into the potential of harvesting competitive bacterial flora for disease management are needed. However, positive developments of a reduction in MRSP from dogs following prescribing changes have been seen, supporting the value of collaboration between clinicians, microbiologists and other stakeholders in limiting the threat from multidrug-resistant staphylococci while protecting them as valuable surface colonisers.

INVITED SPEAKER

Irena Zdovc

Professor of Veterinary Microbiology at the Veterinary Faculty, University of Ljubljana. She is employed at the Institute of Microbiology and Parasitology where she works as a clinical bacteriologist. Her research interests include methods for isolation of various fungal and bacterial pathogens with an emphasis on the research of multidrug-resistant bacteria (especially MRSA, MRSP and ESBL). At the National veterinary institut she heads up Section on Bacterial Animal Diseases and she is responsible for two National reference laboratories, NRL for Listeria monocytogenes and NRL for antimicrobial resistance.

DERMATOPHYTOSES IN SMALL ANIMALS

I.Zdovc, M. Golob
University of Ljubljana, Veterinary Faculty, Institut of Microbiology and Parasitology, Ljubljana, Slovenia

OBJECTIVES: Dermatophytoses are the most common fungal diseases of domestic pets, including exotic companion mammals (rodents, rabbit, ferrets, hedgehogs). Dermatophytes are belonging to three genera, *Microsporum, Trichophyton and Epidermophyton*, but only the first two are important in veterinary medicine. Infections in dogs usually occur in a typical form, while cats and rabbits often remain only asymptomatic carriers and pose the greatest risk of infecting other animals and humans.
METHODS: Most of the samples were taken from clinically ill animals but many samples were also examined for the occurrence or suspicion of disease in people who live together with domestic animals and may represent a potential source of infection.
Samples were examined on Sabouraud dextose agar with the chloramphenicol and actidion. Cultures were incubated at 26°C until the appearance of the first suspicious colonies, and in the case of a negative result, up to two weeks. The identification of grown cultures was done on the basis of macroscopic and microscopic characteristics of the colonies.
RESULTS: Among the examined samples, an average of 20% were positive for dematophytes, and the isolates belonged to 10 different species. Three different species are most often found from genus, *Microsporum* (*M. canis, M. gypseum, M. persicolor*) and also from the genus *Trichophyton* (*T. mentagrophytes, T. erinacei, T. verrucosum)*.
CONCLUSIONS: *M. canis* is still the most frequently isolated dermatophyte species, but no longer as dominant as in the past. The results also show some peculiarities that we did not detect in the past. Certain dermatophytes occur more often in animals that are not their primary hosts, and the appearance of unusual types of dermatophytes (eg T. erinaceid) which were not known in Slovenia in the past, is probably the result of importing animals without proper veterinary control.

INVITED SPEAKER

Alessio Lorusso

Italian DVM (*University of Bari*) with a PhD (2005-2009) on animal coronaviruses obtained after a two-years training period (2006-2008) at the Virology Division of the *Utrecht University*, The Netherlands. Next, he moved (2009-2011) to the *National Animal Disease Center* (USDA) of Ames-Iowa (USA) for a postdoc position on swine influenza viruses during the H1N1 pandemic. Dr Lorusso currently works as Research Veterinary Medical Officer, at the Virology Unit of the *Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise* of Teramo, Italy. His current research activities include **i)** viral diagnostics, virus discovery and genome characterization by innovative diagnostic tools including next generation sequencing; **ii)** genetic and antigenic evolution of orbiviruses, flaviviruses, morbilliviruses, and coronaviruses; **iii)** genome manipulation of RNA viruses by reverse genetics and pathogenesis studies. Deputy Director of the FAO Reference Laboratory for zoonotic coronaviruses. Author and co-author of more than 130 peer-reviewed publications in the field of virology.

EMERGING ORBIVIRUSES IN EUROPE

Alessio Lorusso
Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, Teramo, Italy

The World Organisation for Animal Health (WOAH) lists epizootic hemorrhagic disease (EHD) as a disease of wild and domestic ruminants caused by EHD virus (EHDV). EHDV is related to bluetongue virus (BTV), the etiologic agent of the bluetongue disease of ruminants. Both viruses belong to the genus *Orbivirus* and circulate in multiple serotypes. Both viruses cause similar clinical signs in cattle and are transmitted by *Culicoides* biting midges. BT primarily affects sheep and in recent decades has been described multiple times in the European Union (EU), causing devastating repercussions on animal trade. Most European BT outbreaks had a direct origin in North Africa because of wind-driven dissemination of BTV-infected midges from this region. In regards to recent years, BTV-4w strains collected in 2021 in Italy (mainland and Sardinia), France (Corsica) and Spain (Balearic island) were remarkably close (>99.56 % of nucleotide identity in all genome segments) to homologous strains collected in Tunisia in 2019, 2020 and 2021. These novel BTV-4w, along with a different genome constellation, were slightly divergent in Seg-2 (97.85% of nt identity) with respect to Balkanic BTV-4w strains isolated from 2014 onward in Europe including also recent French (Corsica, 2020) and Italian (Sicily, 2021) BTV-4w strains. The novel BTV-4w differed also from Spanish BTV-4w strains which have circulated in mainland Spain since 2010 as these latter were related to BTV-4w strains collected in Morocco and Tunisia in 2012 and 2013, respectively. Moreover, the recent BTV-3w identified in Sardinia shows a novel genome constellation with respect to those identified in previous years but identical to BTV-3w strains recently identified in Tunisia. At the end of October and beginning of November 2022, respiratory distress, fever, erosions of the muzzle and oral mucosa, and drooling were reported in cattle farms in the south-western part of Sardinia and western Sicily. WGS demonstrated that the Sardinian and Sicilian EHDV-8 strains shares high nucleotide sequence identity (>99.9%) with multiple EHDV-8 strains identified in Tunisia in 2022. Predicting future scenarios for the EU cattle production system is difficult, but EHD will probably pose new challenges to EU veterinary authorities. The lessons learned with BT should be a reference for choosing proper control and prevention strategies for EHD.

INVITED SPEAKER

Pavel Kvapil

MVDr. Pavel Kvapil graduated from Faculty of Veterinary Medicine in Brno, Czech republic, in 2005. He started to work in ZOO Ljubljana, Slovenia as a veterinarian in 2008 and became a head veterinarian in 2014. Three years later he has received his ECZM diploma in Zoo Health Management specialty. He has finished his Ph.D. thesis , with focus on Infectious Diseases in ZOO and Wildlife Animals at Department of Biology and Wildlife diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno.

RED PANDA AMDOPARVOVIRUS INFECTION IN EUROPEAN ZOOS

Pavel Kvapil[1], Oldřich Tomášek[2], Endre Sos[3], Marjan Kastelic[1], Urška Jamnikar-Ciglenečki[4], Urška Kuhar[5]
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[5]*Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia*

Red panda amdoparvovirus (RPAV) was first described in captive red pandas at a zoo in the United States in 2018. The prevalence of infection in zoos in the United States was reported to be 50%. That report led us to test for a suspected RPAV infection in a red panda from Ljubljana ZOO, which died in 2019. The results of PCR performed on tissue samples and sequencing of the PCR product confirmed infection with RPAV in the deceased animal. This was the first case of RPAV infection in a European zoo collection. The cause of death of the red panda from Ljubljana ZOO, which was attributed to RPAV infection, was further investigated using ISH, and acute myocarditis was confirmed as the primary cause of death in the infected animal. Later, a study was conducted to investigate the prevalence of RPAV in red pandas from zoos in Europe. Overall, RPAV was detected in 21 of 63 zoos (33.3%) and in 32 of 113 samples (28.3%). The study also showed that the virus is excreted sporadically in faeces, with the virus detected at only 8 of 14 sampling times. We also found that adult females tested virus positive more frequently compared to adult males.

INVITED SPEAKER

Félix Benjamin

European Union Reference Laboratory for Listeria monocytogenes (EURL Lm), French Agency for food, environmental and Occupational health and safety (ANSES), Laboratory for food safety, Salmonella and Listeria unit, Maisons-Alfort, France

IDENTIFICATION AND DETECTION BY REAL-TIME PCR OF 30 MAJOR CIRCULATING *LISTERIA MONOCYTOGENES CLONAL* COMPLEXES IN EUROPE

Félix, B.[1], Capitaine K.[1], Papić B.[2], Kušar D.[2], Kavalič M., Roussel S.[1]

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Listeria monocytogenes (Lm) is a foodborne pathogenic bacterium primarily affecting humans and ruminants. Multilocus sequence typing (MLST)-based studies revealed that the majority of strains belong to few major clonal complexes (CCs) that account for most human and animal cases worldwide. The CCs provide crucial information for risk evaluation and source tracking. Today it is obtained in 5 days by whole genome sequencing. The method GenoListeria developed here provide the CC information in 1 day and cover the 30 major CCs predominant in food, human and animal contamination in Europe. The method was already validated on pure strain according to ISI16140. The method is now, going to be adapted for direct detection in the contaminated matrix, without strain cultivation. We describe here the step involved in this development, made in partnership with the Slovenian national reference laboratory for Lm.

INVITED SPEAKER

Jade Bokma

Dr. Jade Bokma is a veterinarian (2017, Ghent University) who finished her PhD thesis in June 2021 ("Innovations in rapid *Mycoplasma bovis* diagnostics with MALDI-TOF MS and nanopore sequencing"). She is currently working as a postdoctoral researcher at the Department of Internal medicine, Reproduction, and Population medicine (Faculty of Veterinary Medicine, Ghent University). Here she is part of the RESPI-group, and the lead-responsible for all *M. bovis* related matters, both for research, routine diagnostics, and herd health visits. Next to this, she is collaborating in different (inter)national projects, mainly concerning diagnostics, prevention and treatment of pneumonia in cattle.

DIAGNOSTIC TOOLS FOR (RAPID) IDENTIFICATION, STRAIN TYPING, AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *MYCOPLASMA BOVIS*

Jade Bokma

Ghent University

In the last decade, *Mycoplasmopsis bovis* (previously *Mycoplasma bovis*) has emerged as a significant player in the realm of bovine health, antimicrobial use and economics. While traditional control measures and vaccines are often insufficient, a paradigm shift towards early detection and prevention has gained momentum. This emphasises the importance of (rapid) microbiological diagnostics and genomics. An overview of both conventional (including culture and PCR) and innovative diagnostics methods (including MALDI-TOF MS and nanopore sequencing) tailored for *M. bovis* and examples of their direct applicability in practice will be given.

INVITED SPEAKER

Bojan Papić

Dr. Bojan Papić is currently a postdoctoral researcher at the Institute of Microbiology and Parasitology at the Veterinary Faculty, University of Ljubljana. His main research topic is the use of whole-genome sequencing in the genomic surveillance of microbial pathogens, including foodborne bacterial pathogens and *Paenibacillus larvae*, a honeybee pathogen.

MOLECULAR AND GENOMIC SURVEILLANCE OF *Paenibacillus larvae*, THE CAUSATIVE AGENT OF AMERICAN FOULBROOD

B. Papić, M. P. Ocepek, L. Žvokelj, B. Hočevar, M. Kozar, R. Rus, U. Zajc, D. Kušar
University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia

American foulbrood (AFB) is a serious disease of honeybee (*Apis mellifera*) brood caused by *Paenibacillus larvae*. In the last decade, whole-genome sequencing (WGS) has become a method of choice for genomic surveillance of microbial pathogens but is rarely used for *P. larvae.* In this presentation, we will discuss how qPCR and WGS have improved the surveillance of *P. larvae* in Slovenia. P. larvae is divided into five ERIC types (I–V), of which only ERIC I and ERIC II are epidemiologically relevant. While ERIC I is prevalent in most European countries, ERIC II is the predominant *P. larvae* type in Slovenia. The advantages of WGS include outbreak investigation, population structure analysis and virulence gene analysis. WGS highlighted the importance of beekeepers' activities in the spread of *P. larvae* strains over large (> 3 km) geographic distances. The diagnostic value of qPCR quantification of *P. larvae* in hive debris and adult bees for predicting the clinical onset of AFB and the association between disease severity and spore counts will be discussed. qPCR opens new possibilities for a rapid and cost-effective screening of *P. larvae* in hive-related samples that is independent of the occurrence of AFB clinical symptoms and the geographic location of the affected apiary.

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ARNOLD HALL I

L1 SELENOUREIDO MOLECULES ARE EFFECTIVE INHIBITORS OF MALASSEZIA PACHYDERMATIS FIELD STRAINS FROM DOGS

Spadini C.[1], Montanaro S. L.[1], Mezzasalma N.[1], Gandolfo E.[2], Carta F.[3], Angeli A.[3], Selleri S.[3], Supuran C.T.[3], Cabassi C.S.[1]
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[3]NEUROFARBA Department, University of Florence, Florence, Italy

Malassezia pachydermatis (MP) is responsible of severe cutaneous infections in companion animals - particularly in dogs - causing dermatitis and external otitis, which recently gained attention for its increasing azole resistance. For this reason, reaching novel therapeutic strategies is of great interest. In previous work, compounds bearing acyl/selenoureido moieties and primary/secondary sulfonamide groups acting through organic selenium and Carbonic Anhydrases inhibition were evaluated. Eight of these compounds (5g, 7a, 7c, 7k, 8c, 10c, 11b, 11f) were tested *in vitro* by MIC assay against 32 field MP. These strains were isolated from dogs affected by dermatitis and/or external otitis in which a yeast etiology was suspected after cytological examination. Each sample was firstly cultured onto Sabouraud agar then isolates were identified by CHROM-agar® Malassezia. Confirmation of the ID was performed with a nested PCR for internal transcribed spacer region of rRNA gene. In comparison each isolate was tested with MIC assay for its susceptibility to ketoconazole (KTZ). In general, the MIC of tested compounds on field MPs were higher than the reference MP (DSMZ 6172). Despite this, compounds 11b, 8c and 7a showed a lower MIC in 8,1,1/32 field strains, respectively. The MIC of KTZ on field strains showed an average value of 0.94 ± 0.14, including three strains whose MIC values were 16, 8 and 5 µg/ml. On these three strains, compounds 7a, 8c and 10c showed lower MIC values, suggesting greater efficacy of selenoureas than KTZ in phenotypically azole-resistant MP. Confirmation of genotypic resistance to azoles will be needed.

L2 PREVALENCE OF SAPROPHYTIC MYCOBIOTA IN ROAD-KILLED AND HUNTED WILD MAMMALS IN PORTUGAL

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[1]Animal and Veterinary Research Centre (CECAV), UTAD, Vila Real, Portugal; Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
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[3]Centre Research for Natural Resources, Environment and Society (CERNAS-IPCB), Castelo Branco, Portugal
[4]Researcher at Q-RURAL – Quality of Life in the Rural World, IPCB, Castelo Branco, Portugal
[5]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal

Wildlife populations have long been considered a link in the chain of pathogen emergence. An unprecedented number of fungal and fungal-like diseases have been triggering some of the most severe die-offs and extinctions ever witnessed in wild species. The aim of this study was to describe the saprophytic mycobiota in the fur of road-killed and hunted wild mammals in Portugal. Samples were collected with the Mackenzie technique and sent to the Medical Microbiology Laboratory of the University of Trás-os-Montes and Alto Douro, Portugal. Fungal isolation was performed from the fur and scales of 101 wild animals (51 red foxes (*Vulpes vulpes*), 5 beech martens (*Martes foina*), 6 eurasian otters (*Lutra lutra*), 3 European badger (*Meles meles*), genet *(Genetta genetta*), 19 Egyptian mongooses (*Herpestes ichneumon*), 2 hedgehog (*Erinaceus europaeus*), 7 wild boar (*Sus scrofa*), 4 rabbits (*Oryctolagus cuniculus*) and 1 brown hare (*Lepus europaeus*). A total of 426 fungal isolates were obtained. In this study, 84 animals (83.2%; CI 95%: 75.9%- 90.5%) presented saprophytic fungi in their hair or scales, from which 19 genera were identified. The most prevalent genera were *Mucor* (37.6%; CI 95%: 28.2-47.1%), *Penicillium* (20.8%; CI 95%: 12.9-28.7%) and *Aspergillus* (14.9%; CI 95%: 7.9-21.8%). The results obtained suggest the importance of conducting surveys, to increase in knowledge about the mycobiota present in the fur of wild animals and to allocate their importance in a One Health approach.

L3 BACTERIAL AND PARASITIC ZOONOTIC PATHOGENS CARRIED IN THE GUTS OF EAST GERMAN FOXES, RACCOONS AND OTHER PREDATORS, 2021-2022

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Shrinking natural habitats foster the presence of wild predators, including invasive species, in urban areas and interactions with humans. Wild animals may harbor various pathogens as well as resistance genes that can be shed into the environment.
We investigated 77 fecal specimens of legally hunted and road-killed predators from eastern Germany (2021-2022). These included 40 red foxes (*Vulpes vulpes*), 22 raccoons (*Procyon lotor*), eight badgers (*Meles meles*), three raccoon dogs (*Nyctereutes procyonoides*) and four others. The samples were screened for bacteria of the genera *Yersinia*, *Salmonella*, *Listeria,* and *Clostridium* by standard culture and for parasites by the combined sedimentation-flotation method. Three raccoon samples were positive for *Baylisascaris procyonis,* a zoonotic parasite that had so far not been reported around Berlin. *Yersinia enterocolitica* was found in four foxes, seven raccoons, and one badger, and one strain contained a virulence plasmid. A *Salmonella* Choleraesuis strain encoding an aminoglycoside 6′-N-acetyltransferase which also had a *parC* point mutation conferring ciprofloxacin resistance was isolated from a raccoon dog. Three foxes, two raccoons, and one badger were positive for *Listeria monocytogenes*. *Clostridium baratii* was isolated from three foxes, three raccoons, and one badger, where one raccoon isolate encoded a *tetA*(P) tetracycline resistance gene. *Clostridium sordellii* was found in four foxes, one raccoon, and one badger, with one fox isolate containing the tetracycline resistance genes *tetA*(P) and *tetB*(P). In conclusion, predators in urban environments pose a risk of spreading zoonotic pathogens as well as resistance genes and should therefore be monitored.

L4 RAPID TRANSMISSION OF BRUCELLA CANIS IN A CANINE KENNEL IN THE NETHERLANDS FOLLOWING INTRODUCTION OF AN INFECTED DOG

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Introduction: *Brucella canis* is a zoonotic pathogen in dogs and the main causative agent of canine brucellosis. In the Netherlands, *B. canis* had only been detected in individual cases of imported dogs. However, in 2019 an outbreak of *B. canis* occurred for the first time in a cohort of autochthonous dogs in a breeding kennel. **Case Report:** The outbreak began with a positive serological test result of an imported intact male dog showing clinical symptoms of brucellosis. Consequently, urine and blood samples were collected and tested positive for *B. canis* by culture followed by whole genome sequencing (WGS). Screening of the contact dogs in the kennel where the index case was kept, revealed that antibodies against *B. canis* could be detected in 23 out of 69 dogs (34%) by serum agglutination test (SAT). Of the 23 seropositive dogs, *B. canis* could be cultured from the urine and/or heparin samples of 19 dogs (83%). **Discussion:** We describe a case where rapid transmission of *B. canis* in a breeding kennel occurred following the introduction of an infected dog. This is also the first documented case of transmission of *B. canis* to autochthonous contact dogs in the Netherlands. WGS revealed all *B. canis* isolates belonged to the same cluster pointing in the direction of one introduction. **Conclusion:** This outbreak showed that the international movement of dogs from endemic countries poses a threat to the canine population, while serological screening and WGS proved to be valuable tools for respectively screening and the epidemiological investigation.

L5 MOLECULAR TYPING OF MANNHEIMIA HAEMOLYTICA ISOLATES FROM CALVES, COWS, GOATS AND SHEEP.

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Mannheimia haemolytica (*M. haemolytica*) is endemic in The Netherlands. The bacterium is present in non-clinical animals and in pneumonia in cattle, polyserositis in veal calves, and sepsis, arthritis, pleuropneumonia and mastitis in small ruminants. Recently, with whole genome sequencing (WGS) it was shown, that polyserositis in veal calves was caused by *M. haemolytica* serotype 2 strains and that pneumonia in cows was caused by serotype 1 and 6 strains. This raised the question about the genetic relatedness of *M. haemolytica* strains found in sheep and goats compared to those found in cows and calves. To evaluate this, we sequenced 42 *M. haemolytica* isolates. Eight from adult cattle, 12 from calves, 12 from goats and 16 from sheep. Preliminary data showed six different sequence types (ST) and four isolates that couldn't be placed in an ST. Cow-isolates were mainly ST1, calf-isolates were mainly ST4, goat-isolates were mainly ST8 and sheep-isolates were either ST2, ST8 or ST28. The cow-isolates were serotype A1/A6 or A2 (one), calf-isolates were A2, sheep-isolates were serotype A1/A6, A2, 8 and 9 and goat-isolates were serotype A2 and 9. Although both calf, goat and some sheep-isolates were serotype A2, they belonged to different ST. The results suggest that genetically different clades circulate in different animal species. The relative high diversity in sheep-isolates, might be explained, by the fact that these animals traditionally are frequently transported between farms and countries and that they, due to extensive farming have contact with other species like cows and wild ruminants. However, since all these isolates were derived from clinical cases, another possibility is that this pattern of host species-based ST distribution is biased due to different clinical expressions of the *M. haemolytica* STs.

ARNOLD HALL II

L6 NOVEL STOAT CALICIVIRUS IDENTIFIED FROM NGS SEQUENCING DATA

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This study reports a novel vesivirus (family *Caliciviridae*) identified in faecal samples from a stoat (*Mustela erminea*) with two full length sequences, a partial sequence and another partial isolate discovered. Viral detection was performed through metagenomic analysis of sequences obtained through Illumina sequencing. The full-length sequences are 8471 and 8322 nucleotides in length respectively and contained three identified open reading frames (ORF), consistent with a vesivirus. Subsequent phylogenetic analysis has identified the virus as a vesivirus, clustered most closely with the MF677852 mink calicivirus strain (China/2/2016) in all three open reading frames. The full-length sequences shared a nucleotide similarity with MF677852 of 70-72% in ORF1, 61-62% in ORF2 and 71% in ORF3, indicating significant differences despite their close phylogenetic relationship. Further study of the pathology and epidemiology of this virus is necessary to understand the impact on the host species and the potential cross species transmission potential

L7 MOLECULAR DETECTION AND WHOLE GENOME SEQUENCING OF ROTAVIRUS A IN WILD RUMINANTS FROM SLOVENIA

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Rotaviruses A (RVA) are a major cause of acute viral gastroenteritis in humans worldwide and are responsible for about two million hospitalisations per year. They can also infect other mammals such as pigs, calves, goats, lambs and horses, in which they are also considered a major cause of viral diarrhoea. While RVA is well studied in humans and domestic animals, its occurrence in wild ruminants is not well known. The RVA genome is a double-stranded RNA consisting of 11 segments, and genotyping is based on the VP7 (G) and VP4 (P) segments. Currently, there are 42G genotypes and 58P genotypes. RVA has a high mutation rate and some combinations of G and P genotypes can infect different animal species, leading to speculation about the potential for zoonotic transmission. Approximately 430 faecal samples were collected from roe deer, red deer, chamois, mouflon and Alpine ibex in Slovenia between 2018 and 2021. To investigate the presence of RVA in wild ruminants, real-time RT-PCR was used. Positive samples were subjected to NGS using RIP -seq sequencing. In total, 7 samples were RVA positive. Complete genomes were determined and phylogenetically analysed for all 7 RVAs. Genotypes G6P[14] and G10P[15] were found in both roe deer and red deer, representing the first confirmed occurrence of RVA in red deer. In addition, genotype G6P[14] was found in chamois, representing the first known case of positive RVA in this species. Some of these genotypes have also been found in humans, indicating the potential for zoonotic transmission.

L8 PORCINE CIRCOVIRUSES: PECULIAR GENETIC FEATURES AND UNEXPECTED CIRCULATION IN WILD AND RURAL PIG POPULATIONS IN ITALY.

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Porcine circovirus type 2 (PCV-2) is among the most burdensome viruses of the swine industry globally. Several genotypes have emerged, but just three of them (PCV-2a, PCV-2b, and PCV-2d) seem to circulate at worldwide level. Recently, a new porcine circovirus, PCV-3 has been identified and associated with clinical syndromes. Although less marked than PCV-2, a certain genetic variability has been described for PCV-3 as well. The current knowledge of the role of small-scale, backyard pig production on PCVs epidemiology is still obscure. The present study investigated PCVs occurrence in samples collected from commercial and rural pigs, as well as in wild boars located in the same area of Northern Italy. PCVs presence was tested by qPCR and sequencing was performed for strain characterization. Data were analyzed with a combination of statistical, phylogenetic and phylodynamic approaches. A surprising circulation of the minor PCV-2e genotype and peculiar PCV-3 strains was detected in rural farms and wild boars. The phylodynamic analysis highlighted a larger-than-expected viral population size in non-commercial populations and a directional viral flow from these populations to commercial pigs was estimated for PCV-3. A significant flow from wild to rural animals was also proven. Overall, the present study demonstrates the relevant role of non-commercial pig populations in PCVs maintenance and evolution. Although the reason for these preferential ecological niches remains elusive, wild/rural host populations, featured by lower or no control measures and likely posing lower selective pressures, could interact at different levels with viral biology leading to hardly predictable epidemiological outcomes.

L9 FELINE CALICIVIRUS INFECTION IN CAPTIVE CHEETAHS (ACINONYX JUBATUS)

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Feline Calicivirus (FCV) is a significant viral pathogen and ubiquitous in the feline population. FCV infection may be associated with upper respiratory tract disease (URTD) signs, ulcerative lesions in the oral cavity, and, less frequently, cases of lameness and chronic gingivostomatitis. Virulent-systemic (VS)-FCV strains associated with high mortality have also been described. In nondomestic felids has been documented sporadically but repeatedly, mostly as URTD forms and FCV-specific antibodies have been detected in several large felids.
In this study, we describe FCV infection in two cheetahs (Acinonyx jubatus) housed in the Zoo Safari of Fasano (BR) in Puglia, Italy. FCV infection was suspected on the basis of the presence of typical virus manifestations in the two animals and was confirmed by real-time reverse transcription polymerase chain reaction. Interestingly, one of the animals displayed an unusual ulcerative lesion on the nose. The virus was isolated on feline kidney cells and the genome of the virus was amplified and sequenced by MinION Mk1C (Oxford Nanopore Technologies). Mapping of reads generated a sequence of 7583 bp equal to 98.7% of the genome. Analysis by BLAST nucleotide online tool showed the highest identity nt (80.7%) with FCV 15D022 strain identified in 2015 from a cat in South Korea. This study reiterates the increased susceptibility of captive wild felids to FCV infection. The presence of a feline colony in the zoo facilities or indirect transmission through contact with the zoo staff could be associated with the infection of the two animals. Adopting strict bio-safety measures for captive animals is crucial for the management of endangered populations.

L10 CARNIVORE BOCAPARVOVIRUSES – CHALLENGES IN DIAGNOSTICS AND INTERPRETATION OF POSITIVE PCR RESULTS

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Bocaviruses are members of the genus *Bocaparvovirus* within the family *Parvoviridae*. Currently (2023) ICTV classifies 31 different species and although infections are often asymptomatic, bocaviruses are associated with clinical symptoms in a variety of animals as well as humans. There are some studies linking canine bocaviruses to clinical signs, but evidence for their actual pathogenicity is lacking. Since 2019, we have documented individual and connected clinical cases with different clinical pictures and without conclusive diagnosis in which canine bocavirus-specific nucleic acids could be detected by PCR by using several different primer pairs. Obtained PCR products were sequenced and compared to published bocavirus sequences. Canine bocaviruses were detected in a recurring outbreak of severe gastrointestinal disease in an Austrian utility dog team. Several puppies were affected clinically, and viral nucleic acids could also be detected in fecal and respiratory samples of healthy adults. Likewise, bocavirus DNA was detected in association with acute and repeated respiratory symptoms, some severe, over many months in two different dog kennels. We also found positive CBoV PCR results in a fatal case showing liver necrosis, a dog with reproductive disorders and in organ samples of a dog with neurologic signs. Sequenced PCR products showed highest identities to viruses of the *carnivore bocaparvovirus 2* species but are highly divergent between the cases and sometimes also within one outbreak. No other pathogen has been identified that could explain the clinical signs of these cases. Canine bocaviruses are widespread in Austria and can be associated with a wide variety of medical conditions, some of them described in the literature. However, these viruses are not well known by veterinarians and hardly detected diagnostically by commercial laboratories. The generated partial sequences are very heterogeneous, the generation of full genome sequences and the establishment of further diagnostic methods is desirable. The proof that the detected bocaviruses are responsible for the observed symptomatology is still missing.

L11 SPECIES-SPECIFIC REACTION TO INFECTION WITH A HIGHLY VIRULENT FERLAVIRUS: COMPARISON IN CORN SNAKES (*Pantherophis guttatus*) AND BALL PYTHONS (*Python regius*)

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Paramyxoviruses in the genus *Ferlavirus* are an important cause of respiratory disease in snakes. Four genogroups (A, B, C, and tortoise) have been described. There is evidence of transmission of individual strains between genera and families of reptiles. Disease development is believed to depend on virus, host, and environment specific factors. A genogroup B virus was used in transmission studies with corn snakes (*Pantherophis guttatus*) and ball pythons (*Python regius*). The same virus strain and passage was used to

inoculate snakes intratracheally in consecutive studies using the same protocol in both species. Both corn snakes and ball pythons became infected, but both clinical signs and immune responses differed significantly between the species. Corn snakes developed much more severe disease, although systemic infection was present in both species, with virus detected in multiple organs over the course of the study. In the ball pythons, antibodies against the inoculated virus were detected beginning on day 16 and increased steadily to the end of the study, while almost no antibodies were detectable in the inoculated corn snakes. The hematological reaction was dominated by lymphocytosis in the ball pythons and by heterophilia in the corn snakes. Virus was shed mostly through the trachea but also through the cloaca in both species. The reported differences in host reaction to infection are important to understand ferlavirus epidemiology as well as for clinical medicine and for diagnostic testing. Ethics statement: The animal trials were approved by the national authority (Landesdirektion Sachsen, application number TVV 61/13)

L12 AUREAL PLAQUE ASSOCIATE WITH A NOVEL PAPILLOMAVIRUS IN A HORSE

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A papillomavirus (PV) surveillance investigation was conducted on horses with aural plaques. A 4-year-old mare was enrolled in this study after developing multiple lesions (papules and plaques) on the left pinna. A skin tissue sample obtained by biopsy was examined for the presence of PV DNA using three distinct pan-PV consensus PCR assays. The sample was positive in all three PCR assays, and direct Sanger sequencing revealed two types of PV sequences. The complete genomes of the two viruses were obtained after enriching the circular DNA template using rolling circle amplification. The enriched DNA was sequenced using Oxford Nanopore Technologies platform. The complete genome sequence of an EcPV-4 strain was assembled (99.9% identity to the reference sequence). Furthermore, the entire genome sequence of an unclassified PV measuring 7.5 kb was assembled. In the L1 locus, the novel PV was most similar to Equus caballus papillomavirus (EcPV) -1 (64% nt identity), a member of the genus Zetapapillomavirus. The virus was classified as a distinct species of PV based on ICTV classification criteria. The early structural genes E1, E2, E4, E6, and E7, as well as the late structural genes L2, were also mapped. At least 15 types of PVs have been identified in equids, including three bovine PVs (BPV1, BPV2, and BPV13), two donkey PVs (EaPV1 and EaPV2), and ten horse PVs (EcPV1–10). Genotyping studies with specific primers/probes are required to assess the epidemiological significance of this novel EcPV. Genotyping studies with specific primers/probes are needed.

L13 SPATIOTEMPORAL PATTERNS, FARM CONTACTS AND VIRUS TRAITS AS DETERMINANTS OF SARS-COV-2 TRANSMISSION BETWEEN MINK FARMS, THE NETHERLANDS 2020

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The epidemic spread of SARS-CoV-2 between Dutch mink farms between April and November 2020, led to 69 farms becoming infected and being culled, and to the decision by the Dutch government to forward the termination mink farming to January 2021. To elucidate which factors determined the transmission dynamics, we analyzed the spatiotemporal outbreak pattern, virus genetic sequencing data, variation in clinical outcome and information on the between-farm contact structures. In particular, we used the spatiotemporal epidemic pattern to estimate the between-farm transmission kernel for different genetic clusters of outbreaks. This kernel is defined as the transmission hazard between an infected and a susceptible mink farm as a function of the distance between the two farms, and its distance dependence can be used as a signature to assess different potential transmission routes. Subsequently, the analyses of data on clinical outcome and on the between-farm contact structure were used to elucidate the role of contact patterns versus virus strain properties as determinants of transmission that could explain between-cluster differences in the transmission kernel. Phylogenetic analysis of the sequences found in samples from 66 outbreak farms suggested six distinct genetic clusters, with five clusters corresponding to separate introductions of SARS-CoV-2 into mink. Virus evolutionary rates were used to assess virus reproduction rates within the clusters Between four of these clusters, that together comprise the majority of outbreaks, we identified significant differences in the spatial transmission characteristics. One cluster in particular, referred to as A2, showed a higher transmission potential, with expected larger spatial range and higher number of farms expected to become infected in comparison to the other virus clusters. Analysis of the between-farm contact structure showed a significant correlation between clusters and the frequency distribution of different types of contact. Different AA mutations in the spike protein were identified among these clusters with some specific mutations being dominant in Cluster A2. Infection characteristics such as shedding and clinical presentation were also assessed and compared among

clusters. Combining genetic and epidemiological models led to assessing transmission in higher detail and identifying potential differences in transmission between variants of SARS-CoV-2 in minks.

ARNOLD HALL I

L14 HOST-SPECIFICITY MECHANISMS IN *SALMONELLA ENTERICA* SEROVAR GALLINARUM

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A few *Salmonella* serovars are host-restricted and causes systemic infection rather than self-limiting gastroenteritis. The reason for this is unknown. We used the avian specific serovar *S.* Gallinarum, the cause of Fowl Typhoid and Pullorum disease, to study host specificity mechanisms and to elucidate the reasons why they cause systemic disease. Putative host specificity/systemic infection genes were identified by genomic and transcriptomic approaches. Genomic comparisons identified 161 genes, which where unique to *S.* Gallinarum compared to the closely related, broad host range serovar, *S.* Enteritidis. Further, the core genome of the two serovars contained, 1288 genes with conserved Single Nucleotide Polymorphisms (SNPs) in the coding region (1080) or in the up-stream (promoter) region (208). A method to study gene expression inside the hen was developed. Based on this, expression of genes in pathogenicity islands SPI-2, SPI-13 and SPI-14 were shown to differ significantly during infection between *S.* Gallinarum and *S.* Enteritidis/*S.* Dublin, used for comparison, just as *S.* Gallinarum uniquely expressed genes of the ascorbate utilization pathway during infection, making such genes candidate genes for further studies. Mutation by gene knock-out or single nucleotide mutation to change the SNP sequence type to that of *S.* Enteritidis was performed in selected candidate genes from above, and challenge of HD11 macrophage cells and measurement of cytokine expression with such mutants were used to investigate the importance of the genes/ SNPs in host pathogen interaction. Results will be reported at the meeting.

L15 PHENOTYPIC AND PHYLOGENETIC CHARACTERIZATION OF STREPTOCOCCUS GALLOLYTICUS STRAINS ISOLATED ON POULTRY FARMS

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Streptococcosis in poultry usually occurs in young flocks, where it affects their performance and often causes subclinical infections. In this research, 23 *Streptococcus gallolyticus* strains originating from five laying hen and broiler farms were investigated to determine their phenotypic and phylogenetic characteristics. Phenotypic analyses included disk diffusion assays, biofilm-forming ability assays and proteomic typing. Antibiotic susceptibility was tested to seven most commonly used antibiotics in this region. Biofilm-forming ability assays were performed twice in triplicates using quantitative adherence test on microtiter plates, while the proteomic phenotypes of the strains were analysed using MALDI-TOF MS Biotyper. Phylogenetic analyses were performed based on the 16S and *soda* gene sequences. The highest resistance rates were detected against norfloxacin (91.3%), doxycycline (60.87%) and enrofloxacin (47.83%). Two and seven strains showed high and moderate biofilm formation ability, respectively. The results of the phylogenetic analyses confirmed that 16S gene cannot be used as an indicator of diversity for *S. gallolyticus* due to the high level of homogeneity between the strains. However, the analysis based on *sodA* gene sequences was able to differentiate between the similar strains and group them according to the farm of origin on the phylogenetic tree. Proteomic phenotyping showed more detailed differentiation between the strains, although the results did not fully correlate with the phylogenetic tree based on *sodA* gene. The overall results showed relatively high resistance rates and biofilm formation ability, while phylogenetic analysis indicated the investigated strains have a zoonotic potential based on their high similarity to the strains isolated from people with *S. gallolyticus* infections.
Key words: *St*reptococcus gallolyticus, poultry, MALDI-TOF MS, disk diffusion, biofilm, phylogeny, 16S, *sodA*

ARNOLD HALL II

L16 DEVELOPMENT OF TWO NOVEL SEQUENCE TYPING SCHEMES FOR *MYCOPLASMA HYOSYNOVIAE*

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Background: *Mycoplasma hyosynoviae* (Mhs) is a commensal of the upper respiratory tract in swine. After systemic spread, Mhs exhibits an affinity to joint tissue and causes polyarthritis. Although clinical cases are recently emerging, its epidemiology is largely unknown and no genome-based typing tools are

available yet. **Materials and Methods**:A core genome MLST (cgMLST) scheme for Mhs containing 390 target genes was developed using Ridom© SeqSphere+ based on nine published genomes and whole genome sequences of 64 Mhs strains from Austria, Germany and Norway, including isolates from clinically affected pigs, commensal isolates, and strains originating from wild boars. Allelic profiles were compared *in silico* to identify suitable target genes for a conventional MLST scheme. Primer pairs for the chosen target genes were designed and tested by amplification of target genes followed by sequencing. **Results**: Seven target genes (*dnaA, ftsY, gyrB, recA, rpoB, uvrA, fusA*) were selected for MLST based on their discriminatory power *in silico*. cgMLST and MLST resulted in corresponding clusters and sequence types for isolates from one epidemiological unit, but not for isolates from different origins. The Adjusted Rand coefficient of 0.893 indicates high concordance and the discriminatory power was very similar for both typing methods, displayed by Simpson's IDs of 0.992 (cgMLST) and 0.990 (MLST). **Conclusion**:The selection of seven target genes based on 73 Mhs strains *in silico* resulted in an MLST scheme with sufficient discriminatory power for diagnostic approaches while being practicable for most labs. cgMLST and MLSt facilitate epidemiological studies on a pathogen that has been recently emerged in Europe but is still not given the appropriate attention both in diagnostics and research.

L17 INACTIVATION OF AIRBORNE CORONAVIRUS BY UVC RADIATION

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Introduction: The global COVID-19 pandemic has underscored the need for innovative technologies to mitigate indoor transmission of viruses through aerosols. This plays a role in both human and veterinary medicine. One potential solution gaining significant attention is the utilization of air purification systems with various filter mechanisms, known for their ability to effectively remove aerosols. Additionally, the application of specific ultraviolet (UV) wavelengths has demonstrated promising results in inactivating viruses. In light of these advancements, our study aimed to investigate the efficacy of 270-nm UVC-LEDs against coronaviruses in aerosols. In order to assess the effectiveness of an air purification unit featuring a prototype UV-LED chamber, an experiment was conducted to evaluate its ability to reduce a predetermined viral load from stationary aerosols in an experimental chamber. **Materials and Methods:** The Feline Coronavirus (FCoV) was selected as the model virus. An ultrasonic nozzle nebulizer was utilized to generate FCoV aerosols within an aerosol chamber with a volume of 7m³.This chamber facilitates the controlled production of bioaerosols, while simultaneously monitoring climatic conditions such as temperature and relative humidity. The air purification unit was positioned precisely in the center of the chamber. To facilitate comparison, the air purification unit was tested in two configurations: one with a HEPA filter included and the other without, but with the UV-LED chamber activated. After the aerosolization process and subsequent decontamination by the air purification unit, air samples were collected using a Coriolis µ cyclone air sampler. The concentration of FCoV in the collected air samples was determined in cell culture by using tissue culture infectious dosis 50 per m³ ($TCID_{50}/m^3$). **Result:**The initial level of infectious airborne FCoV in the experimental setup was 5.3 $\log_{10}$ $TCID_{50}/m^3$ after 10 minutes of aerosolization and 4.8 $\log_{10}$ $TCID_{50}/m^3$ after 20 minutes. In the absence of any measures (UVC or HEPA), when only the fan of the air purification unit was active and there was also no fresh air flow, the levels of infectious viruses were measured as 3.9 $\log_{10}$ $TCID_{50}/m^3$ after 10 minutes and 3.2 $\log_{10}$ $TCID_{50}/m^3$ after 20 minutes. These measurements served as the control. Based on these values, the decrease of airborne viruses was calculated after 10 or 20 minutes with the activated UVC module or the presence of the HEPA filter. Specifically, after 10 minutes, there was a 1.5-log reduction of airborne FcoV compared to the control when the UVC unit or HEPA filter was activated. After 20 minutes, a 2-log reduction with both variants was observed compared to the control. **Discussion/Conclusion:** In conclusion, our study showed the effectiveness of the air purification unit in reducing the concentration of the infectious FCoV virus within a predefined aerosol. Both measures we implemented, such as the inclusion of the UVC module and the utilization of the HEPA filter, resulted in a reduction in the concentration of airborne FCoV when compared to the control group. These findings emphasize the efficiency of the air purification unit in reducing viral contamination in experimental settings. Moreover, this study's results contribute to our understanding of UVC technologies in air purification units and their applicability in the context of infectious viruses in aerosols of indoor environments.

L18 PERMISSIVENESS OF DIFFERENT TMEM154 GENOTYPE CELL LINES TO DIFFERENT SRLV GENOTYPES/SUBTYPES

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Abstract: Recently, an association between the polymorphism in the ovine transmembrane protein 154 (TMEM 154) gene and resistance against different small ruminant lentivirus (SRLV) subtypes infection was demonstrated, representing a possible control tool in sheep.
Thus, 10 skin fibroblastic cell lines belonging from the three genotypes of the TMEM154 gene were infected with 8 SRLV viral strains and tested for retrotranscriptase (RT) activity and cytopathic effect. Moreover, viral pseudotypes were used to evaluate SRLV pattern.

Two out of eight viral strains showed a statistically significant difference among the three cell lines. Within genotype A, the It-561 strain produced fewer syncytia, a reduced degree of cell fusion, and a lower RT-activity in the 4 homozygous K/K cell lines, suggesting a resistant pattern for these cells. Interestingly, within genotype B, only subtype B1 showed a similar pattern of resistance. The entry assay using the *env* gene confirmed restriction in resistant lines in contrast to the permissive lines, suggesting an entry blockade as the main restriction factor. The B1 subtype is considered one of the most virulent for goats, so much so that it is the target of specific eradication programs in different Countries. In such programs, usually sheep population is not tested. The possibility to control the infection in sheep through genetic selection simplifies the management of sheep, reducing the risk of being a B1 *reservoir*. Moreover, these results suggest that the use of *ex-vivo* approach is a valid tool for the study of resistance patterns against different viral strains in several countries.

Keywords: Small ruminant lentivirus, gene TMEM154, genetic resistance.

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L19 PLANT-BASED VACCINES FOR AVIAN VIRAL DISEASES-CHALLENGES AND OPPORTUNITIES

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Viral diseases are an important cause of morbidity and mortality in poultry, resulting in significant economic losses. The ongoing outbreak of H5N1 avian influenza in Europe has called into question our ability to control the disease through biosecurity and culling, with increasing recognition that vaccination may be needed. More widely, despite the availability of commercial vaccines for the major viral diseases of poultry, these diseases continue to pose significant risk to global food production. There are multiple factors for this: vaccines may be too expensive in low-middle income countries, cold chain storage, if required, may not be readily achievable, and currently available vaccines may not protect well against local emerging strains. Plant derived vaccines offer several advantages, including low cost, ability to rapidly produce large quantities of protein and reduced risk of contamination from infectious agents. Plant based systems can be used to produce subunit protein vaccines or virus-like particles (VLPs). VLPs comprise a “virus-like” structure which assembles following the expression of a limited number of viral proteins within the plant cell. For example, for avian influenza vaccines, HA (haemagglutinin) expression is sufficient for VLPs to form, while Newcastle disease virus requires expression of multiple virus proteins. The use of additional “chaperone proteins” can also enhance VLP assembly and yields. VLP are able to induce both antibody and cell mediated responses in vaccinated animals. We will discuss our experiences with the production of plant-based vaccines for avian influenza and Newcastle disease virus and consider the possible pathways to their use in vaccination of poultry.

ORAL

SATURAY, SEPTEMBER 23rd 2023

5th INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

ARNOLD HALL I

L20 EPIDEMIOLOGICAL CUT OFF VALUES (ECOFFs) AND CLINICAL BREAKPOINTS FOR VETERINARY PATHOGENS

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** on behalf of the VetCAST steering committee (veterinary subcommittee of EUCAST)*
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Antimicrobial susceptibility testing (AST) plays a key role as a rational basis for targeted antimicrobial therapy. Correct interpretation of AST in veterinary medicine is hampered by the lack of clinical breakpoints (CBPs) for relevant antimicrobials and bacterial species. According to the requirements of the European committee on antimicrobial susceptibility testing (EUCAST) an important prerequisite for establishing a CBP is the availability of an epidemiological cut-off (ECOFF). Moreover, ECOFFs are important as a basis for harmonized antimicrobial resistance monitoring and, under certain conditions, they may serve as surrogate CPB. Therefore, members of VetCAST and the COST Action ENOVAT conducted a joint project for generating minimum inhibitory concentration (MIC) distributions for setting new ECOFFs of six veterinary pathogens (*Staphylococcus* (*S.*) *aureus, S. pseudintermedius, Streptococcus equi, Actinobacillus pleuropneumoniae, Pasteurella multocida, Mannheimia haemolytica*) and eight first-line antimicrobials, including penicillin, aminopenicillins and tetracyclines as well as of reference strains for quality control. Based on the MIC distributions created, the EUCAST steering committee successfully set new quality control ranges for *S. aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 reference strains as well as several new ECOFFs were determined for the above mentioned pathogens. The newly generated ECOFFs will be used to support the setting of CBPs for bacteria/antimicrobial combinations, for which PK/PD cutoffs are available. To get VetCAST CPBs published on the EUCAST homepage, disk diffusion cut off values are required. Therefore, volunteers are needed to assist VetCAST in order to perform disk diffusion studies.

L21 ANTIMICROBIAL RESISTANCE, VIRULENCE AND BIOFILM FORMATION IN COAGULASE-NEGATIVE STAPHYLOCOCCI FROM ANIMALS

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Coagulase-negative staphylococci (CoNS) are commensal organisms that colonize the skin and mucosal membranes of humans and several animal species. Nevertheless, CoNS are emergent pathogens often associated with antimicrobial resistance that can cause severe infections. Therefore, we aimed to

characterize the antimicrobial resistance and biofilm formation of CoNS isolated from different animal species. A total of 180 CoNS were recovered from hares, owls, chickens, quails, donkeys, and camels. The staphylococci species were identified by MALDI-TOF. The presence of antibiotic resistance genes was investigated by PCR. Biofilm formation was evaluated by the microplate assay. Among the 180 CoNS, 73 *S. sciuri*, 63 *S. lentus*, 22 *S. urealyticus*, 5 *S. haemolyticus*, 5 *S. vitulinus*, 3 S. *xylosus*, 3 *S. epidermidis*, one *S. succinus* and one *S. saprophyticus* were identified. *S. lentus* and *S. sciuri* were isolated from all animals and *S. urealyticus* was mostly detected in quails. CoNS isolates from wild hares, owls and camels were generally susceptible to most antibiotics tested. In contrast, most CoNS isolates from donkeys and all isolates from chickens and quails were multidrug-resistant being mostly resistant to erythromycin, clindamycin, penicillin, tetracycline, ciprofloxacin encoded by the *erm*A, *erm*B, *erm*C, *mph*C *tet*K, *tet*L, *tet*M and *tet*O genes. Regarding the biofilm formation, all isolates had the capacity to form biofilm. *S. urealyticus* isolates produced significantly more biofilm biomass than the other species. In our study, some staphylococci strains with zoonotic potential were multidrug-resistant and all had the capacity to form biofilms which may pose a threat to human health. **Acknowledgements**: This work was supported by the projects UIDB/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT) and was also supported by LAQV-REQUIMTE, which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020).

L22 RESISTANCE GENES IN MRSP ISOLATED FROM PETS WITH SOFT TISSUE INFECTIONS

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Staphylococcus pseudintermedius (SP) belongs to the saprophytic bacterial flora of animals, but it is also associated with skin and mucosal infections. The increasing levels of methicillin resistant SP (MRSP) isolated in animals has become a concern in terms of public health, especially for multi-resistant clones, as both resistant strains and resistance genes may be transferred from animals to humans. In fact, MRSP are usually multi-resistant. In this scenario, we investigated MRSP isolates from pets to identify resistance genes, other than the beta-lactamase resistance genes, using the microarray. Moreover, a survey was performed in order to collect anamnestic data. 58/81 SP isolated from pet suffering from soft tissue infections were *mecA* positive and its presence was statistically related to the administration of antibiotics in the animals in the previous 24 months (Chi Square Test, p=0.002). In addition to *mecA,* these strains harbour genes encoding for kanamycin (*aaacA-aphD; 51/58*), tetracycline (*tetK, tetM; 29/58*), erythromycin (*ermB; 55/58*). These resistance genes are located on mobile portion of the genome (transposon or plasmid) meaning that they could be easily transferred among bacterial species of animals and humans. In conclusion, most of our MRSP are potentially resistant to aminoglycosides, tetracycline and macrolides, so they are difficult to treat. The knowledge of the resistance pattern would be useful in the clinical and in the epidemiological setting in order to reduce the number of treatment attempts and to monitor the antimicrobial resistance. The Italian Ministry of Health [IZSVE 16/18 RC] supported this work.

ARNOLD HALL II

L23 EVALUATION OF THE BRUKER® RAPID SEPSITYPER PROTOCOL FOR THE RAPID DIAGNOSIS OF BACTERAEMIA, JOINT AND URINARY TRACT INFECTIONS IN COMPANION ANIMALS

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Background: The Rapid Sepsityper Kit (Bruker Daltonics) allows pathogen detection directly from samples through matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS). The method considerably accelerates results timeframe while accurately identifying microorganisms in human flagged blood culture bottles. We evaluated the usefulness of the MBT Sepsityper IVD module for rapid diagnosis of blood, joint and urinary tract infections in companion animals. **Methods**: Three-hundred (*n*=300) veterinary clinical specimens collected from 255 dogs, cats and horses altogether underwent Rapid Sepsityper direct on-plate detection. Samples consisted of *n*=10 blood and *n*=19 synovial fluid in signal culture bottles and *n*=271 cystocentesis urine in sterile universals. Results were compared to bacterial culture and identification attained the next day. **Results**: Synovial fluid and blood samples showed superior diagnostic sensitivity (92% and 63%, respectively), whilst only 33% of culture positive urine were identified via Sepsityper. Good correlation was observed between Sepsityper direct detection and positive culture results for both blood and synovial specimens, with positive predictive values (PPV) of 100% and 86%. Ninety-five percent of culture negative urine were accordingly identified by Sepsityper. Gram-negative bacteria displayed greater detection rate than Gram-positives across all sample types. **Conclusions**: Rapid Sepsityper results appear reliable for blood and

synovial veterinary specimens, although a larger sample size is warranted. Reports could be issued shortly after signal positive culture, supporting accurate antimicrobial selection for treatment of bacteraemia and joint infections in companion animals. For urine samples, antibiotic use may be excluded based on negative Sepsityper results, achievable in 20 minutes from sample receipt.

L24 SEROGOUPS OF *DICHELOBACTER NODOSUS* IN DUTCH SHEEP FARMS DETECTED USING A MULTIPLEX REAL-TIME PCR

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Objective: The efficacy of commercially available multivalent *Dichelobacter nodosus* footrot vaccines in control and management of the disease in sheep has been proven to be less than ideal. Australian studies have proven that flock specific mono- or bivalent vaccines are more effective. To test the feasibility of the application of flock specific vaccines under European conditions, we investigated serogroup diversity in Dutch sheep farms. Methods: Twenty-four sheep farms with suspected footrot were visited in Spring of 2021, footrot lesions were scored and 10 lesion swab samples were collected from each farm. *D. nodosus* was detected using a qPCR targeting the *pnpA* gene. *PnpA*-positive samples were tested with a multiplex qPCR targeting the *fimA* gene for identification of serogroups A-I and M.
Results: Sixty-five percent (157/240) of swabs and 88% (21/24) of farms tested were *D. nodosus*-positive. At least one serogroup was identified in 94% (148/157) of positive samples and in 95% (20/21) of positive farms. On 13 farms only one or two serogroup(s), and on the remaining 6 farms 3 to 6 serogroups were detected. All serogroups were found, except M. Serogroups A and B were the most prevalent ones. Conclusions: Results of this study indicate that a considerable diversity of *D. nodosus* serogroups is present on Dutch sheep farms. As majority of farms have only one or two serogroups, a farm specific vaccination approach with mono- or bivalent vaccine is likely to be a promising option for more effective control and management of the disease.

L25 THE FIRST CASE OF LACTOCOCCOSIS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FROM SLOVENIA

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OBJECTIVES: The objective is to present a case of lactococcosis in farmed rainbow trout from Slovenia caused by *Lactococcus garvieae*. *L. garvieae* is the aetiological agent of an emerging fish disease that affects various fish species, causing acute and haemorrhagic septicaemia. It affects both marine and freshwater aquaculture and can lead to substantial economic losses. METHODS: In December 2022, a Slovene fish farm reported an outbreak of acute to subacute mortality. Water quality parameters were within normal limits, water temperature was 12.8°C. Necropsy was performed, and fish were sampled for bacteriological and histopathological investigations. Bacteriology was performed on blood agar incubated at 21°C and 37°C for 48 hours. Isolates were identified using MALDI-TOF MS. Susceptibility test was performed by Kirby-Bauer disc diffusion method. RESULTS: The *L. garvieae* isolate was susceptible to amoxicillin with clavulanic acid, enrofloxacin, gentamicin, oxytetracycline, and florfenicol and resistant to flumequine and trimethoprim/sulfamethoxazole. The diseased fish showed lethargy, anorexia, hyperpigmentation, and bilateral exophthalmus with haemorrhages. Necropsy revealed gill anaemia, severe congestion and petechiae in the internal organs, and enlargement of the spleen. Histopathological investigation demonstrated congestion and haemorrhages in most organs, panophthalmitis, meningitis and encephalitis with necrosis, proliferative branchitis with necrosis, pericarditis with necrosis, periaerocystitis, hepatitis and hepatocellular lipid depletion, spleen congestion with lymphopaenia, and hyaline droplet degeneration of kidney tubulocytes. CONCLUSIONS: Despite regular bacteriological diagnostics in Slovenia and reports of *L. garvieae* isolation in neighbouring countries and worldwide, lactococcosis has never been confirmed in Slovene fish until now.

ARNOLD HALL I

L25A LEVOFLOXACIN ACTIVITY AGAINST *P. MULTOCIDA* AND *E. COLI* ISOLATED FROM RABBITS

WITHDRAWN

L25A COMPLICATE SITUATION OF FELV IN DOMESTIC CAT POPULATION IN CHILE. HIGH PREVALENCE AND MULTIPLE-VARIANT INFECTING CATS

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Feline Leukemia Virus is a gamma-retrovirus and has world-wide distribution among domestic cat populations. The virus infection causes a broad range of clinical signs, principally associated with

hematopoietic disorders and neoplasia. Its main transmission is horizontally, with close social activities (grooming, sharing food and water, nursing, or fights). It can also be vertically transmitted (trans-placentally and during parturition). The clinical signs are caused by infection with exogenous variants (mainly FeLV-A), but the virus can also recombine with endogenous FeLV (carried in the cat genome) or with itself, producing other variants with worse clinical outcomes (FeLV-B, C, T, E). FeLV infection has been notably reduced in developed countries due to high vaccination rates and cat population management, however, it is still a big concern in other countries. Here we show a high prevalence of FeLV and multiple variants infecting Chilean domestic cats. The study applied PCR to diagnose FeLV in Chilean cats and modelled risk factors (such as test reason, husbandry, age, vaccine status, etc.) for infection. Additionally, NGS (Next-generation sequencing) illumine and nanopore was applied to determine the envelope gene diversity in FeLV. Sequences were obtained from FeLV-A, B and endogenous FeLV. A total of 354 cats were tested by PCR: 51% of these were positive for FeLV, results of this modelling will be presented. The NGS analysis demonstrated quasispecies of FeLV-B and FeLV-A variants co-infecting cats. These Chilean isolates demonstrate clear regional clustering compared with other FeLV sequences. SNP detection and phylogenetic clustering within these Chilean isolates was very dependent on the sequencing technology used. These results confirm the importance of FeLV as one of the main threats to cat's health in countries that have not implemented adequate preventive measures.

L25B ANTIMICROBIAL RESISTANT E. COLI IN IRISH RAW MILK

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Food-producing animals can act as potential reservoirs of resistant bacteria and therefore pose a risk to public health if these enter the food chain. Resistant *Escherichia coli* (*E. coli)* have been detected in milk from animals with mastitis. However, there is limited information on the prevalence of antimicrobial resistant (AMR) *E. coli* in raw milk and raw milk products in Ireland. This study aimed to assess the AMR patterns present in *E. coli* isolated from raw milk and raw milk products. Isolates were phenotypically characterised by selective media culture and broth microdilution to obtain minimum inhibitory concentrations (MIC). Whole genome sequencing (WGS) was performed on resistant isolates to gain a better understanding of the genetic determinants and resistance mechanisms present. Overall, one hundred and thirty-three samples were tested, with *E. coli* recovered from fifty-seven (42.85%). Twenty isolates (35.08%) showed resistance, with eight presenting multi drug resistance (MDR). Among those resistant to critically important antimicrobials, only one isolate was phenotypically resistant to cefotaxime and ceftazidime (presumptive AmpC ß-lactamase producer). This was confirmed by WGS as chromosomal mediated (mutation in AmpC promoter). Two isolates were phenotypically confirmed as Extended Spectrum β-Lactamase producers (ESBL) with one of them harbouring the *blaCTX-M-3* gene indicating plasmid mediated resistance. No carbapenemase producing *E. coli* were isolated. Overall we observed a low prevalence of AMR, however we show that raw milk may be a vehicle for transmission of MDR bacteria and ESBL or AmpC producing *E. coli* into the food chain.

L26 SURVEILLANCE OF MDR "ESKAPE" ORGANISMS AND *ESCHERICHIA COLI* IN EQUINE HOSPITAL ENVIRONMENTS INFORMING INFECTION CONTROL

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Background: Bacteria from the ESKAPE group (i.e. ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa and* ***E****nterobacter species*) and *Escherichia coli* are frequently multidrug resistant (MDR) and can be associated with nosocomial infections, an increasing concern for veterinary settings. **Objective**: To determine the environmental prevalence of ESKAPE+MDR_*E. coli* on surfaces which can act as reservoirs of infection in equine hospital clinical areas. **Methods:** Routine environmental sampling was performed with Swiffer-wipes to screen surfaces from high-risk areas (e.g., surgical theatres, intensive care and isolation units), equipment (e.g., endotracheal tubes) and high-touch areas (e.g., computer keyboards) following species-specific protocols for each target pathogen (ESKAPE+MDR_*E. coli*). Monitoring hand-hygiene of hospital staff and students was conducted by hand-plate sampling. **Results**. One thousand environmental and hand-plate samples were collected between April 2010-Feb 2023. The overall ESKAPE+MDR_*E. coli* prevalence was 35.6%, where MDR_*E. coli* was the most (and *K. pneumoniae* the least) common target pathogen identified (18% and 1.7% respectively). MDR_*E. coli* was most prevalent in stables (40.7%), stocks (21.1%) and high-touch areas (14.3%). *E. cloacae* was the main ESKAPE pathogen in the environment from ICU (20.9%), isolation (21.4%) and from high-touch areas (10.5%). Concerningly, hand-plate sampling showed that MRSA was the most common ESKAPE isolate detected on hands (8.4%), followed by *A. baumannii* (5.2%) and *E. cloacae* (3.9%); however, *E. coli, P. aeruginosa* and *K. pneumoniae* were not found in these samples. **Conclusion:** We demonstrate high ESKAPE+MDR_*E. coli* hospital surface and hand contamination, findings which inform infection control actions to prevent environment-to-patient cross-transmission.

L27 STAPHYLOCOCCUS AUREUS ST612 OUTBREAK IN AN EQUINE REFERRAL CLINIC IN THE NETHERLANDS

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Objective: Carriage of methicillin resistant *Staphylococcus aureus* (MRSA) in horses is a zoonotic risk as well as a risk for infection and transmission in equine. The aim of this study was to investigate relatedness of two suspected MRSA outbreaks (2020 and 2022) in an equine referral clinic. **Methods:** In 2020 and 2022 eight cases of surgical site infections (SSI) in horses were MRSA positive. In total 38 samples from eight MRSA positive SSI cases were cultured. Retrospectively 14 MRSA isolates (horses, n=8; environment, n=3; and staff members, n=3), were analysed using Nanopore whole genome sequencing. **Results:** The MRSA isolates were identified as ST398 (n=9) and ST612 (n=5). The ST398 isolates from 2020 (n=7) had an average SNP distances of 97 SNPs. MRSA ST612 (all 2022) were from SSI (n=2) and environmental screening (n=3); stable, operation-preparation and recovery. All ST612 show SNP distances <10. ST398 isolates from 2022 from one person and a horse show a SNP distance of 47 (Figure 1). **Conclusions:** MRSA ST398 isolates from 2020 are related but there is no evidence for an outbreak. Transmission of ST398 between staff member and horse in 2022 is suspected. The MRSA ST612 isolates from the two horses and the environmental samples show SNP distances <10, indicating transmission of MRSA ST612 causing an outbreak in this clinic in 2022. Presence of MRSA ST612 has been described in Australia and New South Wales in horses and is increasingly detected in humans patients in Western Australia and Africa, indicating the importance of surveillance.

L28 SURVEILLANCE AND INFECTION CONTROL IN EQUINE HOSPITALS (SENTINEL): A PILOT STUDY IN THE EQUINE ICU

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Objective: Hospital associated infections (HAIs) caused by multidrug resistant (MDR) bacteria are a challenge in both human and veterinary medicine. However, data on HAIs in equine hospitals are scarce. Objectives of this pilot study are 1) to identify potential reservoirs and transmission routes of MDR bacteria in the Intensive Care Unit (ICU) of the Utrecht University Equine Hospital (UUEH) and 2) to improve and evaluate the existing infection prevention protocol (IPC). **Methods:** A first sampling round was performed, in which patient related samples of horses in the ICU (e.g., faeces, nasal swabs and clinical samples) and environmental samples (e.g., twitches and stable door samples) were collected and tested for the presence of MDR bacteria. Relevant isolates were sequenced to evaluate the relatedness. Results of the first sampling round and literature research were used to improve the IPC. A second sampling round is currently performed to evaluate the IPC.
Results: During the first sampling round, 14 horses and their environment were sampled. Two clusters of *Acinetobacter baumannii* (11 isolates, e.g., from faeces, a stable door and a drinking bucket) and two clusters of MRSA (18 isolates, e.g., from nasal swabs and twitches) were found. For both bacteria, isolates were found in multiple horses and the environment suggesting transmission within the ICU.
Conclusion: Several MDR bacteria were found in the ICU. Therefore, the importance of hygiene measures was discussed and the awareness of MDR bacteria was underlined. Usage of gloves when handling horses was made obligatory and an extra trash bin was placed. The second sampling round is currently performed, these results are expected in June 2023.

ARNOLD HALL II

L29 CANINE STAPHYLOCOCCACEAE CIRCULATING IN A KENYAN ANIMAL SHELTER

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Abstract: Animal shelters, especially in resource-poor countries, bring together pets from different regions and history. This study aimed at the characterization of the nasal *Staphylococcaceae* of dogs gathered in an animal shelter in Kenya, to determine their genetic relatedness, the presence of dominant

clones and antibiotic resistance profile. Nasal swabs were collected from >100 crossbreed dogs in 2015 and screened for *Staphylococcaceae* using standard cultivation techniques. Ninety-two strains were isolated and their genetic characteristics as well as phylogenetic relationships were determined using complete genomes obtained by PacBio long-read sequencing. Strains encompassed nine validated species, with *S. aureus* (*n*=47), *S. pseudintermedius* (*n*=21) and *Mammaliicoccus (M.) sciuri* (*n*=16) being the most dominant species. Two *S. aureus* clones of ST15 (CC15) and ST1292 (CC1) were isolated from 9 and 37 dogs, respectively. Strains were tested for their antimicrobial susceptibility by determining the minimum inhibitory concentrations (MICs). Eighty six strains had non-wildtype minimal inhibitory concentrations to at least one of the following antimicrobials: tetracycline, benzylpenicillin, oxacillin, erythromycin, clindamycin, trimethoprim, gentamicin or streptomycin, encoded by *tet*(K)/*tet*(M)/*tet*(L), *blaZ*, *mecA*/*mecA1*, *msrA*/*mphC*/*erm*(A)/*erm*(B), *salA*/*lnu*(A)/*lnu*(B)/*lsa*(E), *dfrG*/*dfrK*, *aac(6')-aph(2'')* and *str*, respectively. Many virulence-encoding genes were detected in the *S. aureus* strains, other *Staphylococcaceae* contained a lower number of such genes. Plasmids and prophage sequences were linked to distinct resistance and virulence-encoding genes. The unsuspected high presence of *S. aureus* clones in many dogs suggests dissemination within the shelter and a human source.

L30 EPIDEMIOLOGICAL SURVEILLANCE AND STRAIN TYPING OF *ENTEROBACTER CLOACAE* ISOLATES RETRIEVED FROM CLINICAL CASES AND ENVIRONMENTAL SOURCES IN VETERINARY SETTINGS (2019-2023)

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Objectives: Surveillance of hospital-acquired infections associated with ESKAPE pathogens is key to limit their intra-hospital spread. The objectives of this study are to determine the strain diversity, as well as transmission of *Enterobacter cloacae* isolated from clinical cases, environmental samples and hand-plates retrieved from the Small Animal and the Equine Hospitals of the University of Liverpool. **Methods:** A total of 112 *E. cloacae* isolates from clinical (CL, *n*= 21), environmental (ENV, *n*= 84) and hand-plate sampling (HP, *n*=7) identified by MALDI-TOF MS were selected for typing using Fourier Transform Infrared (FTIR) spectroscopy. The FTIR is a rapid and user-friendly metabolic fingerprinting method for establishing clonal relatedness of microbial strains which is important for outbreak investigation. **Results:** FTIR analysis revealed that isolates were distributed across eight main clusters, where three of these were exclusively composed of ENV isolates obtained between 2020-2023. Five clusters comprised a mix of CL and ENV including nine CL isolates obtained in a close timeframe with the ENV isolates suggesting cross-transmission. In addition, four clusters also included closely related hand-plate and environmental isolates, also suggesting hand cross-transmission. **Conclusions:** Clonally related *E. cloacae* isolates have been identified across HP, CL and ENV veterinary hospitals' sources, indicating possible cross-transmission events. Interestingly, some clusters included *E. cloacae* isolates obtained at broad timeline, indicating possible persistence of the bacteria in the hospital environment or re-introduction in certain hospital areas. Our findings suggest that FTIR is useful for detecting intra-hospital transmission events, therefore informing infection control and preventative measures within veterinary settings.

L31 DETECTION OF HONEYBEE PATHOGENIC VIRUSES IN BUTTERFLIES IN SLOVENIA

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Abstract: A decline in the number and diversity of pollinators has been observed worldwide. Various butterfly species are also threatened by extinction. The objective of our study was to determine whether pathogenic honeybee viruses can be transmitted to butterflies. A total 120 butterfly and honeybee samples from four locations in Slovenia were analysed using the quantitative RT-PCR methods. Of six viruses examined, we found low levels of the bee viruses: Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Lake Sinai virus 3 (LSV3), and Sacbrood bee virus (SBV) in butterflies compared to bee samples, although these viruses were present in large amounts in honeybees from the same locations. The viral load in the positive butterfly samples was much lower than in the positive bee samples, which could indicate that they are passive carriers of bee viruses. The percentage of positive samples in butterflies was higher when collected at sampling sites with higher density of apiaries. We assume, that the possible sites of virus transmission are flowers of nectar plants visited by both honeybees and butterflies. This is the first study that pathogenic bee viruses have been detected in butterflies.
Keywords: butterflies; honeybees; viruses; pathogens transmission

ARNOLD HALL II

L32 HIGH CAPACITY OF BIOFILM FORMATION IN MULTIDRUG-RESISTANT *SALMONELLA* INFANTIS STRAINS AND MORPHOTYPE ASSOCIATION

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The ability of Salmonella species to adhere to surfaces and form biofilms is a direct link between environmental contamination and food processing/contamination. The purpose of this study was to investigate the ability of biofilm-formation of 80 MDR and ESBL *S.* Infantis isolated from broiler food chain production and to characterize biofilm production through WGS and morphotype association characteristics. Biofilm formation was quantified using the 96-well polystyrene microtiter plates test. The rdar morphotype was judged visually on CRA plates. Our results demonstrated that all tested strains were biofilm producers, although with varying degrees of production with a rdar morphotype. Six/80 (7,5%) strains resulted moderated biofilm producers and 74/80 (92,5%) strong biofilm producers. The WGS analysis was performed on 6 *S.* Infantis strains resulted strong biofilm-producers. This assay showed the presence of the fim cluster (fimADF) also described in *S.* Typhimurium, which is necessary for fimbrial production. Biofilm presence has an impact also on antimicrobial therapy. The population of bacterial cells harnessed in the biofilm is in a state defined as "dormant," or metabolically inactive, and this may contribute to the mechanism of antibiotic resistance. It is important to emphasize that within the broiler farm, the environmental temperature is between 18-22°C which is the optimum temperature for in vitro biofilm formation by Salmonella spp. *S.* Infantis persistence in broiler flocks may be related precisely to its ability to form biofilms, making disinfection protocols on the farm and in the production chain more difficult, thus posing serious Public Health concerns

L33 ANTIBIOTIC SUSCEPTIBILITY AND BIOFILM FORMATION IN ESBL-/AmpC-/CARBAPENEMASE-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM FECAL SAMPLES IN DOGS AND CATS

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Escherichia coli able to produce extended-spectrum β-lactamases (ESBL), AmpC β-lactamases and carbapenemases represents a serious public health threat. Biofilm-producing *E. coli* are highly resistant to sanitation procedures and contribute to the transfer of antimicrobial resistance genes between bacteria. The aims of this study were to investigate the presence of biofilm-forming ability and antibiotic resistance rates in 38 ESBL-/AmpC-/carbapenemase-producing *E. coli* isolated from dog and cat faeces harboring bla_{CTX-M} (84.2%), bla_{TEM} (76.3%), bla_{SHV} (7.89%), bla_{CMY-2} (21.1%), cAmpC (5.36%), bla_{NDM} (13.2%) and bla_{OXA-48} (2.63%) genes. The phenotypic detection of biofilm production was performed with the microtiter plate method and biofilm-associated genes (*csgA*, *sdiA*, *rcsA*, *rpoS*) were studied with PCR. The minimum inhibitory concentration (MIC) was determined by the broth microdilution method. All the ESBL-/AmpC-/carbapenemase-producing *E. coli* investigated were able to form biofilm. Based on the results of the mean optical densities, all strains were classified as weak biofilm producers, regardless of the frequency of ESBL/AmpC/carbapenemase-encoding genes. All strains possessed the *csgA*, *sdiA* and *rpoS* biofilm-associated genes. Multidrug resistance was observed in 36 (94.7%) isolates. Results confirmed the presence of biofilm formation in ESBL-producing strains but did not confirm the association with higher biofilm production previously reported in *E. coli* harboring bla_{CTX-M} gene compared to those carrying bla_{CMY-2} and bla_{SHV} genes. The extent of multidrug resistance and biofilm-producing *E. coli* carrying ESBL/AmpC/carbapenemase genes isolated from fecal samples of pets highlights the need to consider biofilm production in future studies aimed to improve knowledge on factors to reduce the global spread of resistant bacteria.

L34 ANTIMICROBIAL USE AND ANTIMICROBIAL RESISTANCE IN THE LIVESTOCK SECTOR OF EASTERN EUROPE, CAUCASUS, AND CENTRAL ASIA

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Antimicrobial resistance (AMR) poses a worldwide health threat to humans, animals, plants, and the environment. The use of antimicrobials triggers the development of AMR; hence, understanding antimicrobial use (AMU) practices and supporting prudent use of antimicrobial drugs are crucial across all sectors. The Food and Agriculture Organization of the United Nations (FAO) carried out Knowledge, Attitude, and Practices (KAP) surveys to evaluate AMU in the livestock sector in several countries of Eastern Europe, Caucasus, and Central Asia from 2020 to 2023.
In-depth face-to-face interviews with farmers of priority livestock production systems, field veterinarians, veterinary pharmacists, and feed mill operators were conducted in 12 countries (500-700 participants/country). Data were collected using tablets, recorded in KoboCollect platform, and analysed using Microsoft Excel and R software. When possible, faecal samples from various animal species and milk samples from cows with mastitis were also collected from the surveyed livestock farms for AMR detection. KAP surveys provided a comprehensive overview of AMU in the livestock sector of targeted countries, and revealed important gaps in the knowledge and practices of participants, such as the purchase of

antimicrobials without prescriptions, the use of antimicrobials for prophylaxis and growth promotion, and the misunderstanding around the difference between AMR and antibiotic residues. AMR was detected in commensal indicator and pathogenic bacteria. The findings of KAP surveys are highly important for guiding awareness-raising efforts, developing targeted trainings, and informing revision or development of policies, to promote responsible AMU and to reduce the development and circulation of AMR.

ARNOLD HALL II

L35 *PSEUDOMONAS AESTUS* ISOLATION FROM THE NASAL CAVITY OF A CAT WITH CHRONIC RHINITIS

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Background: In companion animals, *Pseudomonas* species are frequently associated with the development of skin, urinary, respiratory and systemic infections and oral disease. Because of their multiple intrinsic resistance to several antimicrobials and capacity to acquire new resistance mechanisms and virulence determinants, they are challenging opportunistic pathogens to manage. **Materials/methods:** A 9-year-old cat was presented to consultation due to chronic respiratory signs. A rhinoscopy was performed and samples for histopathological evaluation and mycological and bacterial analysis were collected. Bacterial culture allowed to obtain an isolate, which was identified by API20NE and PCR using primers BCR1 and BCR2, followed by sequencing of the amplicon fragment. Isolate susceptibility profile was determined using the disk diffusion method. **Results:** Histopathology revealed chronic lymphoplasmacytic rhinitis. Bacterial culture allowed to obtain an isolate in pure culture, first identified as *Burkholderia cepacia* by API20NE, but later identified as *Pseudomonas aestus* CMR5c by sequencing of a 1750 bp PCR amplicon, followed by homologous sequences analysis using the NCBI database. Considering antimicrobial susceptibility testing, it was observed that the isolate was susceptible to gentamicin, ceftazidime and piperacillin, intermediate to tobramycin, amikacin, piperacillin-tazobactam, ciprofloxacin, enrofloxacin, marbofloxacin and ofloxacin, and resistant to imipenem, meropenem, cefepime and aztreonam, being classified as multidrug-resistant (MDR). **Conclusions:** This case corresponds to the first description of *Pseudomonas aestus* isolation from the upper airways of a cat with chronic rhinitis, pointing out for the importance of establishing monitorization protocols aiming at the isolation, identification and characterization of non-traditional, multidrug-resistant *Pseudomonas* strains in the veterinary setting. **Ackowledgements:** This work was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon; by AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences; by Institute for Bioengineering and Biosciences and by Institute for Health and Bioeconomy at Instituto Superior Técnico.

L36 A MENTABOLOMICS APPROACH FOR THE EARLY DETECTION OF CAMPYLOBACTER SPP. IN BROILER HOUSES BY VOLATILE ORGANIC COMPOUNDS.

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Campylobacter is the most reported foodborne pathogen in the European Union and chickens are the main reservoir for human campylobacteriosis[1]. Rapid on-site detection methods could be a powerful tool in the control of *Campylobacter* in the primary production sector. It would provide farmers real-time information on the *Campylobacter* status of flocks, leading to better insight into relevant introduction routes of *Campylobacter* into broiler houses. Such knowledge will allow implementation of more adequate control strategies. The potential of measuring volatile organic compounds (VOCs) to detect the presence of *Campylobacter* in broiler flocks was investigated. VOCs were captured from the air of 27 different broiler houses in the second half of the production round through dynamic headspace sampling on sorbent tubes. Thermodesorption was used to recover the trapped VOCs before analyzing them by gas chromatography mass spectrometry (TD-GC-MS). Simultaneously with the air samples, fecal droppings were collected to determine the *Campylobacter* status of the flocks by PCR. Using a metabolomics approach, a total of 162 VOCs were detected in the air of the broiler houses and out of the investigated flocks almost 30 percent (8/27) was *Campylobacter* positive. Differences in VOC profiles were detected between *Campylobacter* positive and negative flocks. Remarkably, many VOCs, including the most differential ones, were more abundant in the samples from negative flocks. In conclusion, the study resulted in a couple of candidate VOCs but more field trials are needed to validate the robustness of VOC profiles as markers for the presence of *Campylobacter* in broiler houses.

References: EFSA, 2020. Update and review of control options for Campylobacter in broilers at primary production. EFSA Journal 18, e06090.

L37 DETECTION AND MOLECULAR CHARACTERISATION OF *ACTINOMYCES DENTICOLENS* CAUSING LYMPH NODE ABSCESSATION IN HORSES

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We describe the identification and molecular characterization of *Actinomyces denticolens* that caused abscessation of the submandibular lymph node in three unrelated horses. PCR, culture and serology of samples of the cases specific for *Streptococcus equi* subsp*. equi* yielded a negative result. Bacterial culture and subsequent identification with MALDI-TOF resulted in decisive identification of *A. denticolens* in two of the three isolates. Final confirmation that *A. denticolens* was found to be present in all three isolates was achieved using whole genome sequencing, supported by genotyping using multi locus sequence typing (MLST). The three isolates showed 95% nucleotide sequence identity. The number of single nucleotide polymorphisms (10 170 to 36 058) indicated that the strains were polyclonal, suggesting that these cases were epidemiologically unrelated. Four known virulence genes were detected. The absence of known antibiotic resistance genes was in line with the high susceptibility as indicated through the susceptibility patterns obtained for two of the three isolates. We conclude that *A. denticolens* should be included in the differential diagnosis of (submandibular) lymph node abscessation in horses, especially if strangles due to *S. equi* subsp*. equi* cannot be confirmed with laboratory diagnostics. Furthermore we report the first draft genome of *A. denticolens* isolated from horses.

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PSA01 EVIDENCE FOR SPORADIC *COXIELLA BURNETII* EXCRETION IN SHEEP AND GOAT MILK, CENTRAL PORTUGAL

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Sheep and goat are the primary reservoirs for Q fever, a widespread zoonotic infectious disease caused by *Coxiella burnetii*. Limited information on the epidemiology of *C. burnetii* is available from Portugal, particularly concerning *C. burnetii* excretion in ruminant milk. In this study, the presence of *C. burnetii* in bulk tank milk of sheep and goat flocks was tested to assess *C. burnetii* circulation in sheep and goat products that are consumed by the human population in Central Portugal. A total of 78 sheep milk farms from 46 parishes of five municipalities the Centre region of Portugal were collected in all farms providing a 2 ml bulk milk sample both in January 2015 and in January 2016 resulting in a total of 156 bulk milk samples. DNA was extracted and *C. burnetii* IS1111 region was targeted using a SYBR® Green qPCR. Positivity was assessed by analysis of the amplicon melting curves followed by confirmation by further bidirectional SANGER sequencing and nBLAST analysis. One sample (1.3%; 95%CI: 0.03-6.94) collected in 2015 was positive for *C. burnetti*. Bidirectional sequencing followed by nBLAST analysis confirmed the presence of *C. burnetii* DNA. Further surveillance efforts should be made as milk from small ruminant species is widely consumed and used in the region.

PSA02 DETECTION OF *CANDIDATUS* MYCOPLASMA HAEMOLAMAE IN SOUTH AMERICAN CAMELIDS FARMED IN ITALY

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Candidatus Mycoplasma haemolamae (CMhl) is a hemotropic bacterium that can infect several South American camelids such as alpacas (*Vicugna pacos*) and llamas (*Lama glama*). To date the presence of CMhl has been reported in different countries but studies in Italy are lacking. In 2021, blood smear examination of an alpaca cria admitted to the Veterinary Teaching Hospital (VTH) of Lodi, Italy, revealed the presence of numerous small basophilic coccoid structures attached to the surface of erythrocytes compatible with CMhl infection. Following this finding, the aim of this study was to investigate the presence of CMhl in alpacas and llamas in Italy in 2021-2022. Animals from seven herds were included in this study and up to 90% of the animals for each herd were tested. Blood samples were collected and analyzed by a CMhl species-specific qPCR. Overall, CMhl was detected in 37/91 (40.7%) animals, occurring in both alpacas (32/85; 37.6%) and llamas (5/6; 83.3%). CMhl was detected in at least one animal in all the tested herds. Positivity in the tested animals/herd ranged from 8.3% to 71.4%. The results of this study showed the presence of CMhl in Italy. Further investigations are needed to assess the transmission dynamics and the clinical relevance of CMhl in South American camelids farmed in Italy.

PSA03 BACTERIA AND ANTIMICROBIAL SUSCEPTIBILITY FROM CANINE LIQUOR SAMPLES OVER A 3 YEAR PERIOD

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In this retrospective study, results of bacteriological culture and antibiotic sensitivity testing on cerebrospinal fluid from 169 dogs submitted to a commercial diagnostic laboratory between 2020 and 2022 were evaluated. Aerobic bacterial culture was carried out according to standard protocols and anaerobic culture was also carried out on 22 of the samples. Aerobic bacteria were cultured from 41 samples (24.3%), no growth was observed in 128 samples (75.7%). An obligate anaerobe was only cultured from one of 22 samples (4.5%). Cultured gram-positive bacteria included *Staphylococcus pseudintermedius* (8.5%), coagulase-negative staphylococci (21.3%), beta-haemolytic streptococci (4.3%), enterococci (2.1%), micrococcacea (6.4%), aerobic spore-forming bacteria (4.3%), and *Exiguobacterium* spp. (2.1%). *Escherichia coli* was the most commonly cultured gram-negative bacterium (29.8%), followed by other enterobacteria (4.3%), nonfermenters (8.5%), and *Acinetobacter* spp. (8.5%). All *S. pseudintermedius* isolates were susceptible to cefovecin, enrofloxacin, pradofloxacin, chloramphenicol, and trimethoprim/sulfonamide. Doxycyclin, chloramphenicol and trimethoprim/sulfonamide were most often effective against the coagulase negative staphylococci isolated. The isolated *E. coli* had the highest susceptibility rate to chloramphenicol. This study provides an overview of bacteriological findings in canine cerebrospinal fluids in a larger number of clinical samples. The isolated bacteria are similar to those reported in similar studies previously. Although information on the clinical status of the dogs included in this study was not available, evaluation of such tests including antibiotic sensitivity testing is still rare in the literature and should continue to be monitored to optimize understanding of causes of bacterial meningoencephalitis in dogs and help guide treatment choices.

PSA04 A NOVEL SPECIES OF THE *PASTEURELLACEAE* CAUSING PNEUMONIA IN A BEARDED DRAGON (POGONA VITTICEPS)

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Background: *Pasteurellaceae* are Gram-negative coccobacilli with a worldwide distribution. They are commensals on the mucous membranes of the upper respiratory and intestinal tracts of many animals but may also act as opportunistic pathogens causing pneumonia and septicemia. In reptiles, the knowledge of the pathogenicity of *Pasteurellaceae* is still limited. Snipes *et al.* described *Pasteurella testudinis* associated with respiratory disease in the Californian desert tortoise *Gopherus agassizii*. However, *Mycoplasma* was concurrently isolated, and the clinical importance of *P. testudines* as a pathogen remains unclear. **Methods and Results**: An 8.5-year-old male bearded dragon (*Pogona vitticeps*) was submitted for post-mortem examination. The reptile was found dead, shortly after showing signs of dyspnea and lethargy. Macroscopically, both lungs were markedly filled with purulent material, and bilateral suppurative pneumonia was diagnosed after histological assessment. To identify the etiological agent, lung samples were submitted to bacteriology and cultured on Trypticase Soy agar (TSA) at 37°C, aerobically, and MacConkey agar (MC) at 37°C, aerobically. Microscopically the bacteria appeared as Gram-negative rods but could not be identified using MALDI-TOF MS (Bruker). Therefore, 16S rRNA gene sequencing was performed. The sequence was compared to the EzBioCloud 16S database where the closest match was *Avibacterium endocarditidis* (DQ465412), with 94.24% identity. This result is clearly below the 98-99% cutoff threshold for species delineation. **Conclusion:** Here we present a case of suppurative pneumonia in a bearded dragon likely caused by a previously undescribed species of the *Pasteurellaceae* family. Further research is necessary to determine the distribution and pathogenic potential of this species.

PSA05 PERFORMANCE OF FOURIER-TRANSFORM INFRARED SPECTROSCOPY FOR TYPING OF *Mannheimia haemolytica*

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Fourier-Transform InfraRed Spectroscopy by using the IR-Biotyper (Bruker) is a promising tool to group bacterial isolates that belong to the same species based on their IR absorption spectrum. In the Netherlands, *Mannheimia haemolytica (M. haemolytica)* is an endemic pathogen in cattle, sheep and goats. We used the IR-Biotyper for two different purposes. Firstly, we used it to evaluate if *M. haemolytica* that were isolated from infected sites of necropsied lambs, submitted at different episodes of severe pneumonia in the same herd in the same season, were caused by the same or by different bacteria. In this case, we showed that the *M. haemolytica* generated different IR spectra. This implicates that several different *M. haemolytica* bacteria are present at the same time or that there is a shift in presence of different *M. haemolytica*. Secondly, we used the IR-Biotyper to investigate if *M. haemolytica* that were isolated from clinical cases of pneumonia in cattle or polyserositis in veal calves belonged to serotype 1 or 6 versus serotype 2. In this latter case, we compared results of IR-biotyping with results of whole genome sequencing. We were able to build a model for classification of the generated IR spectra in two groups corresponding with these two serotype groups of bovine *M. haemolytica*. These results indicate that IR-biotyping seems to be a valuable and relatively cheap tool to distinguish different *M. haemolytica* phenotypes and serotypes.

PSA06 PATHWAYS- ENLIGHTENING THE ROLE OF *PASTEURELLA MULTOCIDA* IN THE PATHOGENESIS OF BOVINE RESPIRATORY DISEASE

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Introduction: Bovine respiratory disease (BRD) is a common, costly, and multifactorial disease in cattle. Primary viral infections predispose the animal to a secondary infection with bacterial pathogens such as *Pasteurella multocida* (PM). The pathogenicity of PM is largely unknown. However, major surface components of PM, like capsule, lipopolysaccharide (LPS), iron-acquiring proteins, and surface proteins, play important roles in resistance to the host immune response and phagocytosis. **Objectives:** To shed light on the possible link between PM genotype and BRD, we aimed to characterize the genomic population of PM in Norwegian calves with and without BRD using whole genome sequencing (WGS). **Methods:** PM isolates (n=370) from different parts of the respiratory tract (nasal, nasopharynx, and bronchioalveolar) of calves with and without BRD were subjected to DNA extraction using DNeasy Blood and Tissue kit (Qiagen). The extracted DNA was sequenced on the Illumina MiSeq platform. Bioinformatic analyses included trimming, genome assembly with quality check, annotation, virulence factor (VF) analysis, phylogeny, pangenome definition, and genotyping (MLST, *in silico* serotyping). **Results:** Initial MLST analyses for a subset of the samples (n=118) indicate that most of the isolates belong to sequence type 1 (ST1), followed by ST3, and ST68. In addition, the *in-silico* capsular serogrouping identified capsular serogroups A and F in both calves with and without BRD with no clear differences. Also, the BLAST search shows no clear tendency in the distribution of PM-relevant virulence factors between cases with and without BRD. **Conclusions:** Preliminary results have not given any clear indications of the relatedness of ST and serogroup of PM comparing cases with/without BRD, but it remains to analyze samples from 2/3 of the total material. To our knowledge, this is the largest genomic collection of *P. multocida* from calves and the first ever from Norwegian cattle.

PSA07 THE COMPOSITION OF MASTITIS CAUSING MICROORGANISMS AND CYTOKINES IN HEALTHY COW'S MILK: A PILOT STUDY

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Background and objectives. Dairy products are an ideal culture medium for pathogenic microorganisms. Among most common monitoring methods of milk quality and possible pathology in animals, are the evaluation of somatic cell count (SCC) in the milk, clinical signs and bacteriological analysis. The aim was to examine mastitis causing strains in clinically healthy cow's udder milk and presence of cytokines in spring and autumn seasons. Materials and methods. Milk samples taken from the 10 cows were checked for the presence of Gram-positive and Gram-negative bacteria, and the SCC was detected. Immunohistochemistry method was performed for interleukin (IL) -2, IL-4, IL-8, IL-10, IL-12, IL-17a, β-defensin-3, transforming growth factor (TGF)-β1, interferon-γ and nuclear factor (NF)-κB presence in the milk. Results. S. agalactiae, S. uberis, S. aureus, E. coli, and Klebsiella, Enterobacter, Citrobacter spp. were found in healthy cow's milk. In the spring round, the highest prevalence was observed for S. aureus. In the autumn round, the highest mean levels were observed for S. uberis, then followed S. aureus. IL-4, IL-17a and TGF-β1 demonstrated the highest expression in the milk, while NF-κB had the lowest one. Conclusions. The presence of a rich bacterial microbiome (S. aureus, S. uberis) in the milk of healthy animals, as well as changing bacterial species between the collecting rounds (in spring and autumn) occur as a result of both - the immune state of the animal and many external factors, which consequently affects the number of positive cytokine cells to establish a congruently high expression of IL-2, IL-4, IL-17a and TGF-β1 positive cells into the milk.

PSA08 ENTEROBACTERALES DOMINATE IN EGGS AFTER HATCHING FAILURE IN THE TREE SPARROW (PASSER MONTANUS)

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Populations of Tree Sparrows (*Passer montanus*) are declining. Monitoring reproductive successrate of this birds in nest boxes showed that there was a considerable amount of hatching failure. To test the hypothesis that specific bacteria might be involved in the failure of eggs to hatch, 56 eggs from different nests and broods were aseptically sampled and used for general bacterial culture after which the bacteria were identified. Twenty nine different bacterial species could be identified. Bacteria of the order *Enterobacterales* were relatively more present in heavy cultures and in eggs with rotten content and without known other cause of hatching failure. *Enterobacterales* are correlated to egg spoilage in Tree Sparrow nest boxes, however, if they are the prime cause of failure or opportunistic infections afterwards remains to be elucidated.

PSA09 *IN VITRO* CHARACTERIZATION AND PRELIMINARY EFFICACY ASSESSMENT IN *GALLERIA MELLONELLA* LARVAE OF FOUR NEWLY ISOLATED BACTERIOPHAGES ACTIVE AGAINST *AEROMONAS SALMONICIDA*

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The Gram-negative bacteria *Aeromonas (A.) salmonicida* 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 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. The aims of this work were to isolate new phages active against *A. salmonicida*, characterize them *in vitro* and assess their potential use for phage therapy in a preliminary *in vivo* model. Four new phages were isolated from water samples collected in fish farms and natural aquatic environments in southern Belgium. Transmission electron microscopy allowed to classify these four phages in the *Caudoviricetes* class which includes tailed-phages with dsDNA and an icosahedral capsid. Genomic analysis showed that three of these phages, named vB_AsaM_ULASA2 (170,823bp), vB_AsaM_ULASA3 (164,381bp) and vB_AsaM_ULASA4 (171,205bp), belong to the *Straboviridae* family while vB_AsaM_ULASA1 (47,813bp) stay in the unclassified part of the *Caudoviricetes* class. Four strains of *A. salmonicida* were tested for virulence on *Galleria mellonella* larvae and two of them, named ATCC7965 and Asa-CER1, were selected for further experiments. Inoculation doses were determined as 10^2 CFU/10 µl and 10^4 CFU/10 µl, respectively. The safety and efficacy of the four phages at MOI 10 and 100 in this model are currently assessed.

PSA10 PERSISTENCE AND SPREAD OF *SALMONELLA* INFANTIS ON A BROILER FARM

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Salmonella Infantis is the most prevalent *Salmonella* serotype in broilers and one of the top five serotypes causing infections in humans. Here, we investigated the persistence and spread of *S.* Infantis in a broiler farm. Eight broiler houses operated by three farmers were included in the study. From February 2021 to July 2022, different sample types were collected from service rooms, farm surroundings, slaughterhouse and in each broiler house at the end of the production cycle, after house cleaning and before housing a new flock. A total of 69 *S.* Infantis isolates were obtained from all sampled areas and underwent whole-genome sequencing. All isolates belonged to sequence type 32 (ST32), exhibited two multidrug-resistant types and harbored a pESI plasmid. The cgMLST analysis revealed five clusters (≤ 10 allele differences) comprising 3–30 isolates. The clusters mainly comprised isolates from the houses operated by a single farmer, although three clusters also contained individual isolates from neighboring houses operated by other farmers. Isolates obtained during the most recent sampling formed a new cluster and had the additional resistance gene *bla*$_{\text{TEM-1B}}$. The wide distribution of *S.* Infantis in the farm environment indicates complex transmission dynamics of *S.* Infantis in broiler farms. The results obtained suggest that new *S.* Infantis strains are constantly being introduced into the farm, replacing the old strains.

PSA11 CHARACTERIZATION OF FORESKIN MICROBIOTA OF BUCKS WITH MICROLITHIASIS BY USING NGS TECHNOLOGIES

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Testicular microlithiasis (TML) is a condition in which microcalcifications are located within the lumen of spermatic or seminiferous tubules with sonography considered the gold standard in the diagnosis. Fertility disorders are often reported associated with TML although pathogenesis has not yet been elucidated. The present study aimed to evaluate if the foreskin microbiota composition affects the TML occurrence in male goats. Foreskin swabs from 8 healthy and 8 TML affected bucks, from Basilicata region, Italy, were taken. Extracted DNAs were submitted to the 16S rRNA V1-V9 region amplification with the products used for setting libraries by using the 16S barcoding kit SQK-16S024 (ONT, UK). Sequencing was performed by using a Flongle FLO-FLG001, version R9.4.1 on the Mk1C device (ONT, UK) for 24h. FastQ files were uploaded on the online EPI2ME platform and analyzed by the Fastq 16S 2021.09.09 (Metrichor Agent, ONT) workflow. Statistical analyses were performed with R v.4.1.3. After quality control, a total of 420,502 bacterial 16SrRNA region sequence reads (mean 3,162, median 1,004, range 502-59,002) were obtained, with a total of 52 genera identified. In details, the most abundant phyla included: *Campylobacter* spp.

(62.50%), *Corynebacterium* spp. (50.00%), *Fusobacterium* spp. (50.00%), *Streptobacillus* spp. (50.00%), *Porphyromonas* spp. (37.50%) and *Ureaplasma* spp. (31.25%). Diversity indexes analysis did not reveal significant differences between the two groups of bucks (p>0.05) thus not supporting possible links between microbiota composition and lesion occurrence. Of interest, by using NGS the occurrence of zoonotic bacteria, such as *Campylobacter* spp., was detected in both groups, thus suggesting a possible zoonotic risk in the field, although deep investigations are required to obtain the identification at the species level.

PSA12 LONG-TERM FOLLOW-UP OF MYCOPLASMA HYOPNEUMONIAE-SPECIFIC CELL-MEDIATED IMMUNITY IN VACCINATED PIGS

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Vaccination of piglets against *M. hyopneumoniae* is commonly practiced. The long-term *M. hyopneumoniae* vaccine-induced cell-mediated immunity (CMI) has not been investigated thoroughly. Therefore, this study aimed to investigate the persistence of *M. hyopneumoniae* vaccine-induced CMI in fattening pigs under field conditions. On two farrow-to-finish farms, 25 piglets (n=50) were intramuscularly *M. hyopneumoniae* vaccinated with Ingelvac MycoFLEX® (Boehringer Ingelheim Vetmedica GmbH) at 16 days of age and blood was collected monthly till slaughter. Using a recall assay and multicolor flow cytometry, the ability of different T cell subsets to produce cytokines and to proliferate upon stimulation with the vaccine strain was investigated. On both farms, one month old pigs had high percentages of TNF-α, IFN-γ and both TNF-α/IFN-γ-producing $CD4^+CD8^+$ T cells. The percentages significantly decreased from one to two months of age or from two to three months of age. Afterwards, these percentages either decreased further or remained stable over time. On one farm, from one to two months of age, the percentage of IFN-γ^+ $CD4^-CD8^+$ T cells significantly decreased, while the percentage of TNF-α^+ $CD4^+CD8^-$ T cells significantly increased. Furthermore, *M. hyopneumoniae*-specific proliferation of T cells was observed from one month of age till the end of the fattening period. After vaccination and in absence of infection, *M. hyopneumoniae*-specific single cytokine-producing and polyfunctional $CD4^+CD8^+$ T cells are present and proliferating T cells are observed till the end of the fattening period. An *M. hyopneumoniae*-specific vaccine-induced CMI might contribute to a long term protection against *M. hyopneumoniae* infections in fattening pigs.

PSA13 THE ROLE OF THE DEPARTMENT OF VETERINARY SCIENCE OF TURIN UNIVERSITY (ITALY) IN THE PRESERVATION OF MICROORGANISMS OF VETERINARY INTEREST

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The Turin University Culture Collection (TUCC) is a biobank developed since 2016 to address the need for a common approach among research institutes for the collection, characterisation, storage, and use of pathogens from human and non-human sources. The TUCC, that encompasses the Department of Veterinary Science (DSV), has been included as part of the Italian National Recovery and Resilience Plan (PNRR), with a project entitled "Strengthening the MIRRI Italian Research Infrastructure for sustainable Bioscience and Bioeconomy (SUS-MIRRI.IT)". In particular, through an online platform, TUCC is implementing a database that will allow to appraise all the available microorganisms (bacteria, yeasts, filamentous fungi, microalgae); besides, TUCC provides important services for the academic world, industry and institutions. The PNRR funding will allow TUCC to modernize its laboratories and equipment, increase its microbial culture capacity, and develop new tools and services for users, including an online platform for accessing and sharing microbial strains and associated data. These upgrades will enhance TUCC's role as a national and international reference centre for microbial biodiversity and biotechnology, promoting the sustainable use of microbial resources for the benefit of society and the environment. The inclusion of TUCC in the PNRR reflects the Italian government's commitment to supporting research and innovation as key drivers of sustainable development and economic recovery.

PSA14 STABILITY AND LYTIC ACTIVITY ASSESSMENT OF BACTERIOPHAGES TARGETING STAPHYLOCOCCUS AUREUS CAUSING BOVINE MASTITIS IN MILK

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Bovine mastitis is a major cause of culling in dairy cattle and the antimicrobial treatment of the infection contributes to the emergence and the spread of antimicrobial resistances. Phage therapy could be a

promising approach but the biological and physicochemical properties of milk can affect the phages properties. The objective of this study was to compare the stability and lytic activity of phages targeting *Staphylococcus aureus* in raw and pasteurized milk. A total of 28 bacteriophages previously isolated against *S. aureus* were spotted on 44 *S. aureus* strains isolated from bovine mastitis to evaluate the phage host range. The phage stability was assessed by enumerating them in milk prior and after 6h incubation at 37°C. The lytic activity was assessed by inoculating milk with *S. aureus* and phages at a MOI of 1000. Bacterial and phage enumeration at different timepoints allowed to compare the lytic activity of the phages and their replication dynamics. A broad host spectrum was observed for 24 phages and no lysis was observed for the remaining phages. Stability analysis showed that all phages were still active after 6h incubation in both raw and pasteurized milk with an average stability rate of 9%. No significant titer variation was observed between both groups. These results show that components in milk alter the phage properties, but the remaining phage titer (log5-log8 PFU/ml) should be high enough to lyse the bacteria. Further phage stability tests in selective milk components should now be performed to assess the phages inactivation in milk.

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PSA15 EARLY ADMINISTRATION OF GRAMNEGATIVE GUT ANAEROBES REDUCES E. COLI COLONIZATION OF DAY-OLD-CHICKS INCLUDING A VIRULENT APEC STRAIN

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High level of microbial contamination of newly hatched chicken is a risk factor for increased first-week mortality, massive use of antibiotic to reduce the losses a potentially transmission of high-risk virulent APEC clones throughout the production chain. Chicken hatched in commercial hatcheries, lacking a source of beneficial "adult" microflora, are prone to colonization by potential pathogens of the Enterobacteriaceae. Unlike common probiotics, administration of complex mixtures of anaerobic gut microflora proves to be more efficient in competitive exclusion of unwelcome bacteria and it is a promising means of reducing the microbial burden in broiler chicken. An experimental group of one-day-old chicken from a commercial hatchery was inoculated by a mixture of 7 gut anaerobes of known species. The next day the chicks of both experimental and control group were colonized by a ciprofloxacin-resistant APEC strain O78:H4-ST117. On days 7., 14., 21. and 28. 7 chicken of each group were euthanized, autopsied and serial dilutions of cecal contents cultivated on ciprofloxacin-supplemented Levine EMB agar. Specifically, the presence of the experimental strain was detected with an O78 antiserum. Significant reduction of colonization rates in the first two weeks was recorded in the experimental group for the numbers of ciprofloxacin-resistant *E. coli*. While the chicken of the control group showed 100% positivity for the experimental strain in first two weeks, in experimental chicken it remained under the detection level during the whole experiment. No differences in weight gain between groups were noted. The results show that colonization of chicken by defined anaerobic mixtures may provide a decisive protection during the critical period of the chicken intestinal microflora development and may reduce the need for antibiotic use in broiler chicken.

PSA16 WILD BOAR (SUS SCROFA) S A POSSIBLE SOURCE OF CLOSTRIDIOIDES DIFFICILE FOR HUMNAS

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Introduction. *Clostridioides difficile* (*C. difficile*) is one of the most important causative agents of life-threatening infections of the human colon, and until recently clostridial colitis was considered as a disease of mainly nosocomial origin. However, there has been an unusually high increase in the number of community infections in recent years and this has initiated the search for alternative sources of clostridia in people without a history of previous hospitalization (Hernandez et al., 2020). Currently, there is an abnormal increase in the population of wild boars in the Czech Republic, which is why these animals look for food even near human settlements where they can find and feed on sources contaminated with resistant bacteria of human origin. On the contrary, the consumption of boar meat may pose a risk of colonization of the human intestine with *Clostridioides difficile.* The aim of our work was to verify whether the wild boars really represent a possible risk for human colonization by toxin producing strains of *C. difficile*. **Material and methods.** For the *C. difficile* isolation, the examined samples were the diaphragm muscle of hunted wild boars (376 animals), which are being examined by the laboratories of the State Veterinary Institutes for the purpose of screening for infection with the *Trichinella spiralis* parasite. A previously established procedure was used to culture the samples on selective media (Masaříková et al., 2020). Typical colonies were identified by the MALDI – TOF MS method and *C. difficile* isolates were then characterized via PCR for the presence of *tcdA*, *tcdB* and *cdtA/cdtB* encoding genes. Antibiotic susceptibility to eight antimicrobials (amoxicillin, ciprofloxacin, clindamycin, erythromycin, metronidazole, moxifloxacin, tetracycline and vancomycin) was determined using E-tests. **Results.** We

obtained 21 *C. difficile* isolates from 376 diaphragm muscle samples (5%), in thirteen (62%) of them was confirmed presence of genes encoding toxin production. Most of the toxinogenic isolates (10/13) carried the *tcdA* and *tcdB* genes, only three isolates had the *cdtA/cdtB* genes for the binary toxin identified. None of the isolates was equipped with the genes encoding toxins A, B and binary toxin together. All thirteen isolates showed sensitivity to the antibiotic amoxicillin, erythromycin, metronidazole, moxifloxacin, tetracycline and vancomycin. Conversely, one hundred percent of the isolates showed resistance to ciprofloxacin. In addition, three isolates were also resistant to clindamycin. **Conclusion**. The detection of toxinogenic clostridia in wild boars may represent a risk of alimentary infection for humans in case of consumption of insufficiently heat-treated boar meat. The important finding is that none of our isolates showed resistance to the antibiotics of choice in the treatment of human pseudomembranous colitis caused by toxinogenic strains of *C. difficile*, which are metronidazole and vancomycin.

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PSA17 SAMPLING AND INTERPRETING BLOOD CULTURES OBTAINED FROM SEPSIS-SUSPECTED CALVES

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Objectives: Sepsis is a life-threatening condition, gaining awareness in animals due to the vital role of critically important antimicrobials in its treatment. In calves, guidelines are lacking on appropriate blood culture sampling and assessment of isolates as contaminants or relevant pathogens. Therefore, our first objective was to compare the diagnostic performance of two blood culture media (pediatric plus (PP) and plus aerobic (PA)), with a different volume and composition. The second objective was to estimate blood culture contamination in calves. **Methods:** A diagnostic test study was performed on 126 critically ill calves, comparing the performance of PP, PA, and hypoglycemia (<60mg/dL) using a Bayesian latent class model. Survival analysis was used to compare time to positivity (TTP). Contamination was descriptively analyzed. Pathogens were considered relevant when; (1) member of the Enterobacteriaceae family, (2)highly relevant in other bovine pathologies, or (3)isolated from both blood cultures. **Results:** The sensitivities for PP, PA, and hypoglycemia were estimated at 68.7%, 87.5%, and 61.3%, respectively. Specificity was 95.1%, 94.2%, and 72.4%. No significant difference in TTP was identified between PP and PA. An overall contamination rate of 8.3% was assumed, and blood culture positivity reduced nearly 50% when presumed contaminants were excluded. The most frequently isolated assumed true pathogens were *Escherichia coli (*n=14) and *Salmonella* sp. (n=5). **Conclusions:** The highest diagnostic accuracy was observed for PA. The type of culture did not influence TTP or the contamination rate. Therefore, sampling PA culture twice is theoretically the best option to determine sepsis in critically ill calves.

PSA18 PASTEURELLA MULTOCIDA OCULAR INFECTION IN A LITTLE OWL (ATHENE NOCTUA): A CASE REPORT

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Pasteurella multocida is a gram-negative bacterium, common in the oropharyngeal microflora of numerous hosts. It is responsible for fowl cholera in birds and hemorrhagic fever in cattle. However, it has been rarely described in ocular infections (endophthalmitis, keratitis, corneal ulcers, and conjunctivitis). A juvenile female little owl (*Athene noctua*) was presented to the Centro Animali Non Convenzionali, the wildlife rescue centre of the Veterinary Teaching Hospital of Turin, with bilateral devastating ocular lesions. The animal showed severe discomfort, poor body conditions (flat pectoral muscles, easily palpable keel), and blindness. Bilateral eyeball enlargement and positive fluorescein eye stain test of the whole corneas were recorded. Pupillary light reflex test could not be performed due to evident corneal opacity. The little owl was treated with topical ofloxacin eyedrops QID, meloxicam 0.5 mg/kg IM SID, and enrofloxacin 10 mg/kg SQ SID with no response. A swab from vitreous fluid by vitreous tap was collected. The animal died within few days and complete necroscopy was performed. Eyes and main organs samples were collected for microbiology and histopathology. Viral and bacterial DNA was extracted. Histopathologically, the only significant lesion detected was a severe and bilateral hemorrhagic-necrotizing panophthalmitis. A viral and bacterial metagenomic protocol was performed using Illumina Miseq platform. The results showed high percentage of sequencing reads, from eyes (0.3% of total) and vitreous swab (60% of total) only, belonging to *Pasteurella multocida* subsp. *multocida*. The full genome

sequence of the strain was obtained. The preliminary results suggest a panophthalmitis due to *Pasteurella multocida* infection.

PSA19 EVALUATION OF ANTIMICROBIAL RESISTACE PATTERN IN GRAM NEGATIVE BACTERIA AFTER A CASE OF HOSPITAL-ASSOCIATED INFECTION IN A CAT

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Hospital-associated infections (HAIs) are a major challenge in human and animals. On May 2022, a case of HAI was documented in a cat referred to the Veterinary Teaching Hospital of University of Parma for signs of lower urinary tract disease (FLUTD) with a sterile urine specimen. During hospitalization, the cat underwent catheterization for urethral obstruction and subsequently developed clinical signs suggestive of sepsis. *Klebsiella pneumoniae* was isolated from cat urine and blood. A HAI was hypothesized, and 65 environmental swabs were collected from different hospital surfaces (cages, fomites, computers). After bacteriological examination a wide environmental spread of Gram-negative bacteria was detected, among which 15 bacterial strains were selected for their zoonotic potential: 7 *Enterobacter cloacae*, 4 *Klebsiella pneumoniae*, *E. coli*, *Acinetobacter lwoffii, Stenotrophomonas maltophilia*, and *Pantoea agglomerans*. Antimicrobial susceptibility of the isolates was evaluated by disk-diffusion and MIC assays and for the presence of specific related resistance genes, namely ESBL, carbapenemases and AmpC. The results showed that most isolates were Multidrug-resistant organisms (MDROs); the presence of ESBL genes was detected in 13/15, AmpC genes in 7/15 and carbapenemase genes in 10/15 bacterial strains. Subsequently, all Klebsiella pneumoniae profiles were compared using Enterobacterial Repetitive Intragenic Consensus Sequences PCR showing close phylogenetic similarities. All *Klebsiella* isolates showed the same resistance profile (*bla*CTXM1, *bla*TEM, *bla*SHV, *bla*IMP) suggesting a possible common origin. This study underlines the importance of HAI control program in veterinary hospitals, aimed at avoiding the spread of zoonotic MDROs, thus reducing the public health associated risk.

PSA20 SELECTION OF AQUATIC-ORIGIN LACTIC ACID BACTERIA AND THEIR SAFETY FOR SUBSEQUENT APPLICATION IN HUMAN AND ANIMAL HEALTH

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This study focuses on the identification and evaluation of lactic acid bacteria (LAB) derived from aquatic organisms for their potential use in human and animal health. Species such as *Lactiplantibacillus plantarum* and *Pediococcus acidilactici* are known to have probiotic properties, while others are considered commensal or opportunistic pathogens. Our objective was to isolate and identify QPS-type LAB species, as defined by the European Food Safety Authority, from healthy fish, and conduct safety assays to evaluate their suitability. Mucus samples were collected from *Oncorhynchus mykiss*, *Salmo trutta* and *Acipenser baerii*, from fish farms in Northeast Spain. LAB isolates were identified using molecular techniques. *In vitro* antagonism assays were performed to evaluate their inhibitory effects against both fish and human pathogens. Antibiotic resistance activity assays were conducted to determine the susceptibility of the QPS-type LAB isolates. Cytopathic effect assays were carried out on a fish cell line, and a toxicity assay was performed on salmonid embryonic eggs. As result, various QPS-type LAB isolates were obtained. They exhibited inhibitory effects against the tested pathogens to varying degrees. Antibiotic resistance assays revealed variation in resistance profiles, some exhibiting resistance, while others displayed no significant resistance. Cytopathic effect and toxicity assays demonstrated differences in the effects of the LAB strains on fish cells and embryonic eggs. We suggest that this methodology holds promise for further refinement. Additional techniques could enhance the selection of safe LAB isolates. This research contributes to the investigation of LAB as potential alternatives to antibiotics following a One Health approach.

PSA21 EUROPEAN VETERINARY BAROMETER FOR BOVINE RESPIRATORY DISEASES: A COMPREHENSIVE TOOL FOR MAPPING DIAGNOSTIC TEST RESULTS AND GEOLOCATION OF RESPIRATORY TRACT SAMPLES FROM CATTLE

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Objectives: Awareness of circulating BRD pathogens could support pathogen-oriented decision making, such as preventive measures. To provide support to veterinarians and farmers in the control of BRD, we aim to develop the European Veterinary Barometer, which integrates, visualizes, and analyzes diagnostic test results from European laboratories. **Methods:** As part of DECIDE (Horizon 2020, No. 101000494), anonymized diagnostic test results from cattle with BRD (2016-2022) were collected from Belgian, Dutch, and Irish laboratories. The datasets included test results for BRD pathogens, date, sample types, diagnostic methods, and geolocation. A prototype website was developed to visualize the results. **Results:** After aggregation to the herd level, 11,838 results remained (BEL: 8,678, NLD: 1,404, IRL: 1,756). So far, 18.1% (1,327/7,355) tested positive for Bovine Respiratory Syncytial Virus, 18.0% (899/4,957) for Bovine Corona Virus, 6.4% (470/7,387) for Parainfluenza Type-3, 22.4% (1,917/8,544) for *Mycoplasma bovis*, 25.7% (2,728/10,602) for *Mannheimia haemolytica*, 46.3% (4,894/10,581) for *Pasteurella multocida*, and 17.2% (1,822/10,596) for *Histophilus somni*. Viruses were more prevalent in winter months, and Dutch samples showed lower rates for *P. multocida* and *H. somni* compared to other countries, probably due to variations in diagnostic methods. **Conclusions:** The European Veterinary Barometer for BRD could be used to provide insight in the prevalence of pathogens that cause BRD. The tool can stimulate pathogen specific implementation of risk mitigating measures in certain areas and periods. We aim to expand the tool with data from additional countries, an early warning component, and the economic impact of the pathogens.

POSTER
PSB

FRIDAY, SEPTEMBER 22nd 2023

5th INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

PSB01 *CAMPYLOBACTER* REDUCTION AND MODE OF ACTION BY THE POTENTIAL PROBIOTIC *BACILLUS SUBTILIS* PS-216 *IN VITRO* AND *IN VIVO* IN THE CHICKEN HOST

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Antibiotic resistance and the spread of *Campylobacter* sp. in poultry and thus in food, are a growing problem worldwide, causing enormous economic losses. Regulations on antibiotic use, health and economic concerns have opened the door for more intensive research into alternative solutions to this problem, such as the use of probiotics. We focus on the use of *Bacillus subtilis* PS-216 as a potential probiotic in poultry for *Campylobacter jejuni* reduction in cecum and increased weight gain of broilers. In co-cultivation experiments *in vitro,* we confirmed a strong inhibition of 20 *C. jejuni* strains (from 1,5 to 6,5 log reduction) and confirmed the mode of action with antimicrobials bacillisine and plipastatin. However, *C. jejuni* strains with an active type 6 secretion system were significantly ($p<0,05$) less sensitive to PS-216. Furthermore, we investigated the efficacy of *B. subtilis* PS-216 to control *C. jejuni in vivo* in broiler chickens (up to 21-days of age) and the influence on weight and welfare (up to 42-days of age). It was confirmed that *B. subtilis* PS-216 significantly reduced the level of *C. jejuni* in the cecum content of broilers when treated for the entire period (21 days) for 1,3 log CFU/g cecum content. Interestingly, the addition of spores resulted in significant increased weight gain of broilers, compared to the untreated group. We conclude that *B. subtilis* PS-216 has the potential to be used as a probiotic in poultry to reduce foodborne pathogens and increase broiler welfare.

PSB02 3M™ MOLECULAR DETECTION ASSAY FOR RAPID DETECTION OF SALMONELLA SPP. AND LISTERIA MONOCYTOGENES IN FOOD SAMPLES

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3M™ Molecular detection assay (MDA) is a molecular method for rapid detection of some bacteria important in the food industry. The method is based on loop-mediated isothermal amplification of specific nucleic acid sequences and bioluminiscence detection of amplicons. The analysis is performed on enriched media, buffered peptone water for *Salmonella* spp. or (Half) Fraser broth for *Listeria monocytogenes* and has been implemented and accredited in our laboratory. MDA was initially validated by AFNOR certification according to ISO 16140-2 compared to ISO 6579 and 11290-1. In our laboratory, we have also validated ISO standard protocols for enrichment so that isolation of the detected pathogens can be performed directly from MDA-positive enriched sample using the ISO standard. MDA *Salmonella* spp. was used to analyze 2421 food samples of animal origin during 2019–2022. 23 (1%) samples were positive for *Salmonella* spp. *Salmonella* spp. (*S.* Infantis, *S.* Saintpaul, *S.* Typhimurium, *S.* Enteritidis) was isolated from all MDA-positive samples using ISO 6579:2017. With MDA *Listeria monocytogenes*, 595 food samples of animal origin were tested during 2021–2022, and 91 (15%) samples were positive for *Listeria monocytogenes*. Isolate was obtained from 80 MDA-positive samples using ISO 11290-1:2017. The method is very useful to obtain negative results quickly, but positive MDA results should be confirmed with the ISO standard methods. During validation, we found that mixing the incubated enriched sample in Fraser broth before lysis is a very important step to obtain reliable results.

PSB03 CHALLENGE TEST FOR ASSESSING THE GROWTH POTENTIAL, MAXIMUM GROWTH RATE AND DURABILITY STUDY OF LISTERIA MONOCYTOGENES IN "ZASEKA" SAMPLE

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Razmnoževanje bakterij *Listeria monocytogenes* (*LM*) v vzorcih živil je odvisno od lastnosti živila, to so fizikalno-kemijski (a_w, pH, % NaCL, druge dodane snovi) in mikrobiološki parametri (vzporedna mikrobiota) ter pogojev hranjenja (temperatura, atmosferski pogoji, vlažnost, čas). Za živila za neposredno uživanje, ki omogočajo rast *LM* (Točka 1.2, Poglavje 1, Uredba Komisije (ES) št. 2073/2005) velja, da število LM pri proizvodih danih v promet med rokom uporabnosti, ne sme preseči 100 cfu/g (mejna vrednost). To merilo se uporablja, v kolikor proizvajalec pristojnemu organu dokaže, da ta vrednost med rokom uporabnosti ne bo presežena. V ta namen se izvajajo študije s katerimi ugotavljamo potencial rasti bakterije, največjo stopnjo rasti in oceno trajnosti. Ta testiranja smo izvedli na treh različnih serijah zasek (različna receptura). Pri izvedbi smo sledili smernicam EURL za LM (*Lm* technical guidance document on challenge tests and durability studies for assessing shelf-life of ready – to – eat foods related to *Listeria monocytogenes,* version 4, 1 July 2021) ter kriterijem v ISO 20976-1:2019. Vzorci vseh treh serij zaseke so bili pozitivni na *Listeria monocytogenes*, v koncentraciji < 10 cfu/g. V laboratorij smo jih dobili na dan priprave in jih periodično testirali do konca roka uporabe. Za namen ugotavljanja potenciala rasti (Δ) in največje stopnje rasti smo vzorce iz vsake serije dodatno kontaminirali do koncentracije 50 – 200 cfu LM

/g. Potencial rasti za *Lm* v vseh treh serijah je bil manjši od 0,5, ugotovili smo, da se *Lm* ni množila niti v eksponentni fazi rasti.

PSB04 *DIETZIA CALABRIAE* SP. NOV.: FIRST CHARACTERIZATION OF AN ANIMAL BACTERIAL SPECIES

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Abstract: *Dietzia* B32T was isolated from subcutaneous nodules of a 12-year-old mare in Reggio Calabria, Italy. Based on previously MALDI-TOF MS identification and studies using 16S rRNA gene sequence similarity, the B32T strain was confirmed as belonging to the genus *Dietzia* and most closely related to *Dietzia lutea* (97.07%).
Objectives: To describe the *Dietzia* B32T strain within the genus, morphological, biochemical, physiological, genomic analyses, and antibiotic susceptibility testing, were performed. **Methods:** For the study, the following tests were conducted: Gram staining, and motility by optical and transmission microscopes; growth temperature (4-50 °C), growth curve at 37 °C using Thermo-Spectrophotometer Multiskan SkyHi-UV-Vis Reader; NaCl tolerance and haemolytic activity using liquid medium and BHI plates. For biochemical tests (catalase, glucose, sucrose, urea, nitrate reduction tests) the kit RapID™ CB-Plus (Remel ThermoFisher) was used. Susceptibility to Gentamicin-CN10, Ampicillin-AMP10, Tetracycline-TE30, Chloramphenicol-C30, Vancomycin-VA30, Clindamycin-CD2, Streptomycin-S10, Erythromycin-E15, was evaluated using Kirby Bauer method. In addition, sequencing (Illumina Hiseq) and complete genome assembly (FastQC-v.0.11.5, Edena-3.0) used as an external assembly for tree inference was performed. Digital values of DNA-DNA hybridization and mean nucleotide identity were calculated using the Genome-to-Genome Distance Calculator-2.1 (Leibniz-Institute DSMZ) and JSpecies Web Server, respectively. **Results:** On the basis of biochemical, morphological, and genotypic data, the *Dietzia* B32T strain formed a monophyletic clade with the known species of this genus and represents a new species for which the name *Dietzia calabriae* sp. nov. has been proposed. **Conclusions:** The genome of *Dietzia calabriae* sp. nov. is available in the GenBank database under accession number CP093845.

PSB05 GENETIC DIVERSITY OF VIRULENCE FACTORS AND SECONDARY METABOLITES IN *Paenibacillus larvae*

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Paenibacillus larvae is the causative agent of American foulbrood, a serious disease of honeybees (*Apis mellifera*). Its key virulence genes include chitin-binding protein Cbp49, AB toxins and secondary metabolites. The aim of this study was to gain insight into the genetic diversity of *P. larvae* virulence genes and biosynthetic gene clusters (BCGs). Virulence genes and BGCs were determined in nine public (ERIC I–V) and 33 genetically diverse Slovenian *P. larvae* genomes (ERIC I–II) using BlastN and antiSMASH. Chitin-binding protein *cbp49* was present in all genomes. An intact *plx1* gene was present in two public ERIC I genomes, whereas the Slovenian ERIC I genomes harbored a truncated *plx1*. Intact *plx2A* and *plx2B* genes were present in most ERIC I genomes and in all ERIC III–V genomes. A functional *C3larvinAB* locus was present only in strain 11-8051 (ERIC III/IV-ST9, public). A functional S-layer protein gene *splA* was present only in ERIC II genomes and in strain SAG 10367 (presumable novel ERIC type, public). Bacillibactin BGCs were classified into a single type, whereas paenilamicin, paenilarvin and sevadicin BGCs were more genetically diverse and could be classified into multiple (2–7) types. All BGCs were generally conserved in ERIC I and ERIC II genomes. The results highlight that the reference strains do not encompass the entire genetic diversity of *P. larvae* virulence genes and BGCs.

PSB06 GENETIC CHARACTERISTICS OF *Paenibacillus larvae* STRAINS CAUSING RECURRENT OUTBREAKS OF AMERICAN FOULBROOD IN EASTERN SLOVENIA, 2022

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Paenibacillus larvae is the causative agent of American foulbrood (AFB), a serious disease of honeybee (*Apis mellifera*) brood that leads to weakening and gradual collapse of honeybee colonies. The aim of the study was to perform a comprehensive genetic characterization of *P. larvae* strains associated with recurrent AFB outbreaks in Eastern Slovenia, 2022. For this purpose, a panel of 31 *P. larvae* isolates was

constructed, which originated from 24 apiaries maintained by 19 beekeepers. Six out of 24 apiaries had recurrent cases of AFB in 2022. Whole-genome sequencing revealed that the predominant genotype was ERIC I-ST2 (*n* = 24), followed by ERIC II-ST 11 (*n* = 7). Five cgMLST clusters were observed, which were geographically restricted to a single AFB zone except for one cluster associated with a single beekeeper. The isolates associated with recurrent AFB cases belonged to three cgMLST clusters, but a single *P. larvae* strain was always responsible for recurrent AFB cases within a given apiary. The present study confirms the importance of beekeepers' activities in the spread of *P. larvae* over large geographic distances and shows that recurrent AFB outbreaks are caused by a single *P. larvae* strain persisting within an apiary.

PSB07 THE MICRO EPIDEMIC ONE HEALTH: A PROJECT OF NARRATIVE MEDICINE INVOLVING STUDENTS, PROFESSIONALS IN TRAINING AND CITIZENS, TO INCREASE KNOWLEDGE AND SKILLS ON ZOONOSES.

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Micro Epidemic One Health (MEOH, https://spvet.it/microepidemic.html) is a research project of the Italian Ministry of Health dedicated to narrative medicine on zoonoses and their control, involving institutional partners. The "Stories of Zoonosis" are derived from interviews with key witnesses, such as veterinarians and health practitioners and operators. These narratives depict real-life situations, capturing the emotions and challenges that occurred and were resolved at that particular moment. Since the stories often revolve around notifiable infections, they also serve as an opportunity to learn how to manage specific situations related to infectious diseases, taking into consideration the legislative context that existed at the time. These narratives are available to readers on a web platform (https://storiedizoonosi.spvet.it/) as free narratives accessible from personal computers or smartphones. The platform serves as an ongoing open anthology of case studies, licensed under Creative Commons Attribution 4.0 International. Each story is accompanied by a brief peer-reviewed scientific literature, annotations/integrations, and biomedical Open Data. The material is intended to facilitate learning on various topics, including self-learning for young students and training for specialists in Human and Veterinary Medicine. Additionally, it aims to enhance citizens' competence in the management and prevention of zoonoses. Moreover, this project fosters intergenerational exchange and coaching among healthcare professionals of different ages, enabling younger practitioners to benefit from the experiences of their older colleagues and gain insights from various geographical and historical contexts.

PSB08 CHARACTERISATION OF *FLAVOBACTERIUM* SPP. FROM DISEASED AND APPARENTLY HEALTHY RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FARMED IN SLOVENIA

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OBJECTIVES: Flavobacteria are one of the main causes of bacterial diseases in freshwater fish worldwide and are responsible for significant economic losses. Main pathogenic species include *F. psychrophilum, F. columnare*, and *F. branchiophilum*. The aim of this study was to characterise *Flavobacterium* spp. isolated from rainbow trout in Slovenia. METHODS: Necropsy and sampling of 324 rainbow trout (six samplings of 54 fish each) from two Slovene fish farms was conducted in October 2022, January, and April 2023. Anacker and Ordal's agar and modified Shieh's agar supplemented with tobramycin were used for isolation and incubated at 15°C and 21°C, respectively. Suspect *Flavobacterium* isolates were identified using MALDI-TOF MS. Additionally, general bacteriological, ectoparasitic, and histopathological investigations were performed. RESULTS: *F. psychrophilum* was isolated from the skin, gills, and/or internal organs of at least 4/54 fish at each sampling. In addition, more than 30 other flavobacterial species, including several described as potentially pathogenic, such as *F. aquatile, F. hydatis, F. johnsoniae, F. araucananum, F. oncorhynchi, F. plurextorum,* and *F. piscis,* were isolated from the skin and/or gills. At least one species was isolated from almost all fish at each sampling. *F. aquatile, F. piscis* and several unidentified *Flavobacterium* spp. were also isolated from internal organs. No other pathogenic bacteria were isolated from internal organs on blood agar. CONCLUSIONS: The study confirms the significance of flavobacteria as pathogens in rainbow trout production in Slovenia. Besides *F. psychrophilum*, several other potentially pathogenic flavobacterial species were isolated and their role in disease will be further investigated.

PSB09 OCCURENCE OF INFECTIOUS AGENTS CAUSING ABORTION IN SLOVENIAN AUTOCHTHONOUS SHEEP BREEDS

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Within the research project, the health status of two flocks of Slovenian autochthonous sheep breeds (Istrian Pramenka and Jezersko-Solčavska) was monitored over a period of two years. The presence and dynamics of the occurrence of antibodies against the causative agent of Q fever, enzootic abortion of ewes, toxoplasmosis, ovine epididymitis and neosporosis were determined using the ELISA tests. In total, the most samples were tested positive for toxoplasmosis and the least were positive for *Chlamydophila abortus*. In addition, samples from three aborted lambs were screened for *Salmonella* spp., *Listeria monocytogenes*, *Anaplasma phagocytophilum*, BHV-4, *Coxiella burnetii*, *Campylobacter fetus*, *Chlamydophila* spp. and *Leptospira* spp. by the qPCR method using the VetMAX Ruminant Abortion Screening Kit (Thermo Fisher Scientific) and for *Brucella* spp. and *N. caninum* by the in-house qPCR assays. All three samples were positive for *Salmonella* spp. and *S. enterica* subsp. *diarizonae* was isolated.

PSB10 FUNGAL PNEUMONIA WITH *PURPUREOCILLIUM LAVENDULUM* IN A PLUMED BASILISK (*BASILISCUS PLUMIFRONS*)

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Introduction: Fungal infections in reptiles are frequently caused by opportunistic saprophytic fungi. Among them, various entomopathogenic fungi have been described to cause pneumonia in lizards. A plumed basilisk kept in a zoological collection was found in reduced general condition with unspecific signs of illness. Despite supportive therapy, the animal's condition worsened. It was euthanized and subjected to pathological and microbiological examination. **Methods and Results:** Necropsy revealed multifocal, round, whitish foci of up to 1mm in diameter in the lung. Lung tissue was cultured on Trypticase™ Soy II agar with 5% Sheep Blood (BD™) and Sabouraud Glucose Chloramphenicol Selective Agar (Thermo Scientific™) at 37°C aerobically. After 48 hours, fungal growth was seen on both growth media. Identification using MALDI-TOF (Bruker, Germany) was not successful. Sequencing of the ITS-2 region using universal primers revealed a 100% identity with *Purpureocillium lavendulum* (NR_166039.1) and a 99% identity with *Purpureocillium lilacinum* (NR_165946.1). Histology of the lung revealed granulomatous inflammation with intralesional fungal hyphae without histochemical evidence of additional infectious agents. **Conclusions:** *P. lavendulum*, an entomopathogenic fungus, has been isolated from cases of fibrinous pneumonia in a green tree python and a panther chameleon. Both were captive animals, just like the here described plumed basilisk. Infections of wild reptiles with *P. lavendulum* have not been reported so far. The first description of the species stated that *P. lavendulum* would not grow at 35°C. However, our isolate grew at 37°C, indicating that the species identification needs to be further verified.

PSB11 SCREENING OF *ASPERGILLUS* SPP. IN PETS AND FOMITES IN PORTUGAL

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Aspergillus is a genus containing several hundred species of molds, which are found in various environments around the world. Species of *Aspergillus* are important medically and commercially. Some species can cause infection in humans and other animals. *Aspergillus* infection ranges over a wide clinical spectrum, including invasive pulmonary aspergillosis and its disseminated extrapulmonary form as invasive aspergillosis. They usually affect severely immunocompromised hosts, but sometimes also affect the immunocompetent population. Understanding *Aspergillus* spp. and its distribution and host diversity is extremely important. The aim of this study was to screen fur of pets and fomites (dog beds, blankets, pillows and rugs) for the presence of *Aspergillus* spp. Samples were collected with the Mackenzie technique and sent to the Medical Microbiology Laboratory of the University of Trás-os-Montes and Alto Douro, Portugal. The samples collected were inoculated in Potato Dextrose Agar medium (PDA), and the colonies were identified microscopically by the Lactophenol with Cotton Blue technique. In total, 341 pets and 21 fomites were analysed. Considering the location of the fomites, *Aspergillus* spp. (100%) and *Aspergillus niger* (75%) were identified in fomites from inside the homes. *Aspergillus fumigatus* was isolated in 0.09% of the fur of pets. After *A. fumigatus* was isolated, it was inoculated in Brain Heart Infusion Blood Agar, for the search for haemolysis. Virulence factors such as haemolysins were not found. These results highlight the relevance of this study, along with the need to use complementary techniques in order to obtain a more global and in-depth view, and establish a possible relationship between the presence of these fungi and their impact on public health in a One health approach.

PSB12 *PENICILLIUM LABRADORUM* DISSEMINATED INFECTION IN A GERMAN SHEPHERD DOG

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On 11 November 2022, a 10-year-old spayed female German shepherd dog with urine incontinence was presented. Physical examination of the right front limb revealed non-painful soft tissue enlargement with a firm consistency. Peripheral lymph nodes were normal. Hematological, serum biochemical, and urinalysis tests revealed no remarkable alterations. X-ray examination unveiled a proliferative lesion of the right radial bone. Abdominal ultrasound examinations did not reveal anatomical lesions. Computer tomography revealed aggressive proliferative and lytic lesions in the radius with soft tissue edema. Cytologic examination of fine-needle aspirates revealed marked neutrophilic and moderate macrophagic inflammation with evidence of fungal hyphae. A biopsy of the lesion was made, and histologic examination revealed severe fibroplasia-fibrosis associated to mixed inflammation. Grocott and PAS staining confirmed the presence of fungal hyphae. Urine sediments were cultured in different media. Fungal cultures were positive and identified macro-microscopically as *Penicillium* spp. The initial identification was confirmed molecularly by amplification and sequencing of three genes (β-tubulin, calmodulin and nuclear ITS region) from urine and from the biopsy. The sequences were characterized as *Penicillium sp* upon interrogation of GenBank™ and Mycobank, displaying 100% nucleotide identity in the β-tubulin to *Penicillium labradorum.* The dog was treated with itraconazole and pantoprazole, and approximately 190 days after the initial diagnosis passed away but necropsy could not be done. Disseminated fungal infections usually have a poor prognosis probably because they are not diagnosed timely. Fungal infections should be always considered in the differential diagnosis of bone lesions.

PSB13 *ALTERNARIA ALTERNATA* IN THE FUR OF OUR DOGS AND CATS – IMPLICATIONS IN PUBLIC HEALTH

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The genus *Alternaria* consists of widely recognized plant pathogens that are responsible for several diseases and are also prevalent allergens for both animals and humans. The aim of this study was to carry out a screening of *Alternaria alternata* in dogs and cats in the North of Portugal. A sample of 341 animals belonging to a shelter and 13 veterinary clinics were examined for the presence of *A. alternata* in the fur. The samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25ºC and 37ºC for 3–7 days. A total of 286 dogs and 55 cats were studied, with 45.0% (n = 157) of samples from shelters and 54.0% (n = 184) from clinics. The prevalence of *A. alternata* was 23.8% (n = 81; 95% confidence interval [CI]: 19.34–28.63%). The prevalence values among males and females were 23.4% (95% CI: 17.06–30.80%), and 24.0% (95% CI: 18.05–30.90%), respectively (p = 0.892). This mold was detected in 64/286 dogs and 17/55 cats. Among the positive species, prevalence in cats (30.91%; 95% CI: 19.15–44.81%) was higher than in dogs (22.38%; 95% CI: 17.68–27.66%), but the difference was not statistically significant (p = 0.184). 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 the fungus in the fur and their significance for public health in a One Health approach.

PSB14 SYSTEMIC CANDIDA INFECTION AND PULMONARY ASPERGILLOSIS IN AN ALPACA (VICUGNA PACOS): A CASE REPORT.

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Systemic fungal infections are rarely reported in veterinary literature and are usually associated with an immunocompromised state. This study reports a peculiar case of systemic candidiasis infection associated with pulmonary aspergillosis in an apparently immunocompetent alpaca. A captive 7-year-old female alpaca sharing the habitat with conspecifics developed respiratory symptoms. The animal had a no history

of previous disease and was treated with benzylpenicillin and dexamethasone. Despite initial improvement, the symptoms worsened and it died 40 days later. Gross post-mortem examination showed subcutaneous emphysema, diffuse pneumonia with multiple suppurative foci, also disseminated in kidney and liver. Cytological examination conducted on renal and pulmonary necrotic lesions, revealed a myriad of yeasts and pseudohyphae. Histological examination of collected tissues, revealed purulent tubular-interstitial nephritis, necrotic-purulent pneumonia with presence of fungal hyphae, liver fibrosis and focal calcifications. Fungal cultures confirmed the infection, demonstrating the growth of yeasts and filamentous fungi form lungs and growth of yeasts from liver, kidney and heart, identified as *Aspergillus fumigatus* and *Candida albicans*, respectively, by MALDI-TOF and ITS sequencing. Prior corticosteroid and antibiotic therapy was the only risk factor for candidiasis and pulmonary aspergillosis identified in this case. Other underlying factors may have remained undetected. Nonetheless, systemic candidosis and aspergillus pulmonary infection must be considered as a possible differential diagnosis in respiratory infection of camelids, especially after long-term glucocorticoids or antibiotics treatments, as they are important immunosuppressive factors.

PSB15 *OPHIDIOMYCES OPHIDIICOLA* FROM LAKE GARDA (ITALY) BELONGS TO CLADE I

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In a previous study we detected *Ophidiomyces ophidiicola* (Oo) and its associated disease in the Italian ophidiofauna, establishing a diagnostic workflow that included molecular detection of fungal DNA combined with histopathology of scale clips. Samples from 9 live snakes and 8 road-killed specimens, including dry swabs, scale clips, and preserved tissues, were collected and investigated with a SYBR Green-based real-time PCR assay targeting Oo ITS2 (rRNA). Further molecular detection targeting Oo nad1 (mitochondrial - real-time PCR) and LSU (genomic - panfungal PCR) was performed if a positive result was obtained. Scale clips from positive individuals underwent histopathology. Swabs or scale clips from 4 dice snakes *Natrix tessellata* found in Lake Garda (Trentino - Northern Italy), 3 of which exhibited gross signs, tested positive for Oo with molecular detection and sequencing. Histopathology of clipped lesioned scales showed ulceration, inflammation and intralesional hyphae and conidia consistent with ophidiomycosis. DNA extracted from a positive swab (NT1.s2) was submitted to amplification of the most polymorphic fragments (≤ 200bp) belonging to ITS2, transcription elongation factor, and actin genes (Origgi et al. 2022). The resulting sequences were concatenated, aligned (MUSCLE) with the corresponding concatenated genes from other Oo and an outgroup, deleting all gaps, to obtain a maximum likelihood phylogenetic tree.
Oo from Lake Garda clearly clustered with Clade I, also known as the European Clade. The findings confirm and localise Oo Clade I in Italy after 60+ years, and the approach offers prospects for phylogenetically characterizing Oo from swab-extracted DNA without available isolates.

PSB16 IN VITRO EVALUATION OF INNOVATIVE ANTIMICROBIAL APPROACHES AIMING AT CONTROLLING OTITIS BY PSEUDOMONAS AERUGINOSA IN DOGS

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Otitis externa (OE) is one of the most common dermatological diseases in dogs, having a multifactorial etiology. Among the most frequent bacterial agents associated with canine OE, Pseudomonas aeruginosa is of special concern, due to its frequent multidrug resistance profile and remarkable ability to form biofilms, structures that make treatments challenging and predispose to infections chronicity and recurrence. The increasing prevalence of resistant bacteria in dogs with OE leads to the need to find new therapeutic agents, including Essential Oils or gels containing antimicrobial peptides, such as Peptivet®. This study aimed to compare the antibiofilm potential of different antimicrobials towards P. aeruginosa from canine OE, using a collection of 12 biofilm-producing clinical isolates and the reference strain P. aeruginosa ATCC27853. A conventional microtiter assay was applied to evaluate the inhibitory potential of gentamicin (1505UI/ml, used as control), Peptivet® and Lavanda Essential Oil (LEO). After a 30min incubation, LEO demonstrated the highest biofilm inhibition and eradication abilities (69.2% and 61.5%), followed by gentamicin (61.5% and 53.9%), and Peptivet® (61.5% and 53.9%). After 24h, LEO was able to inhibit and eradicate 76.69% of the isolates, gentamicin was able to inhibit and eradicate 100% of isolates, and Peptivet® was able to inhibit 84.6% of the isolates and eradicate 69.2%. In conclusion, LEO can be a potential alternative to conventional antibiotics for the treatment and prevention of external otitis caused by P. aeruginosa in dogs. Future studies should be performed to confirm its anti-biofilm potential using conditions better mimicking the ears in vivo environment.

PSB17 THE ROLE OF WILD BOARS IN THE CIRCULATION OF TICK-BORNE PATHOGENS: THE FIRST REPORT OF RICKETTSIA MONACENSIS

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Most wild mammals can serve as hosts both for tick-borne pathogens (TBPs), as well as for the ticks themselves. Among these, wild boars, due to their large body size, habitat and life span show high exposure to ticks and TBPs. The species is now one of the widest-ranging mammals in the world, as well as the most widespread suidae and overabundant in most parts of the world, including Europe. Altogether, long-life expectancy, large home ranges including migration, their feeding and social behaviors, wide distribution, overabundance and increased chances of interactions with livestock or humans make them suitable sentinel species for general health threats, such as: antimicrobial resistant microorganisms, ASF, or pollution, as well as for hard ticks' distribution and abundance, and for certain TBP's, such as *Anaplasma phagocytophilum*. The aims of this study were to evaluate the presence of rickettsial agents in wild boars from two counties in Romania. Among 203 blood samples of wild boars (*Sus scrofa*) collected during three hunting seasons, fifteen were found positive for DNA of TBP's. Six wild boars were positive for *A. phagocytophilum* and nine for *Rickettsia* spp. The identified rickettsial species were *R. monacensis* and *R. helvetica.* No animal was positive either for *Borrelia* spp., *Ehrlichia* spp. or *Babesia* spp. To the best of our knowledge, this is the first report of *R. monacensis* in European wild boars, thus adding the third species from the SFG Rickettsia, in the epidemiology of which this wild species could have a role as a reservoir host.

Keywords: *Anaplasma* spp.; *Rickettsia* spp.; *Sus scrofa*.
Acknowledgement: This research was funded by USAMV CN project 21865/4.10.2021

PSB18 THE SCREENING OF POTENTIALLY PATHOGENIC BACTERIA IN WILD UNGULATES WITH HOME RAGES IN PROXIMITY OF LIVESTOCK FLOCKS

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Wild ruminants such as *Capreolus capreolus* (roe deer) and *Sus scrofa* (wild boar) are widely distributed in Europe, and overabundant in certain environments. This overabundance has multiple negative effects which include an increased contact with the anthropic environment, particularly with free-grazing livestock. From this point of view, this species can be used as sentinel species in One Health approach for the assessment of common risks such as infectious diseases. Considering this, the aim of the present study was to evaluate the bacteria population in feces in individuals living in proximity to livestock. Fecal samples were collected from 30 roe deer, 30 wild boars meeting the criteria. The samples were inoculated on culture media and the obtained isolates were microbiologically examined. The species identification was done using the 16S rRNA amplification and sequencing. In total, 261 obtained sequences allowed the identification. The most prevalent identified genera with the predominant species were: *Escherichia - E. coli, Yersinia - Y. enterocolitica*, *Pseudomonas* sp., and *Bacillus* sp. Among the potentially pathogenic bacteria with importance in human or veterinary health regardless of their prevalences, it is worthily mentioning: *Staphylococcus aureus, S. haemolyticus*, *Y. pestis/Y. pseudotuberculosis, Listeria monocytogenes/L. seeligeri, L. ivanovii, Shigella dysenteriae, Acinetobacter Enterococccus faecium* and *E. faecalis*. The most of identified bacteria were reported as commensals in wildlife species which may serve as reservoir hosts for some of them. Wildlife species may transmit them to domestic animals and humans, spreading the diseases at the wildlife-livestock interface.

Keywords: Bacteria, wild ruminants, 16S rRNA, One health
Acknowledgement: This research was funded by USAMV CN project 21865/4.10.2021

PSB19 DETECTION OF ANAPLASMA PHAGOCYTOPHILUM AND EHRLICHIA CANIS IN DOGS FROM A VETERINARY UNIVERSITY HOSPITAL IN ITALY: A RETROSPECTIVE STUDY 2012-2020

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Anaplasma phagocytophilum and *Ehrlichia canis*, responsible for canine granulocytic anaplasmosis (CGA) and canine monocytic ehrlichiosis (CME) respectively, are tick-borne pathogens with a proven or potential zoonotic role that have shown increasing prevalence worldwide. The aims of this retrospective study were to assess the frequency of *A. phagocytophilum* and *E. canis* infections in dogs tested at the Veterinary University Hospital (VUH) of the University of Bologna (Italy), to evaluate whether infections were related to clinical data, and to genetically analyse the identified bacteria. Dogs referred to the VUH between 2012 and 2020 that had undergone at least one of the rapid immunoenzymatic (SNAP 4Dx Plus Test, IDEXX, USA), indirect immunofluorescent antibody or PCR test for *A. phagocytophilum* and/or *E. canis*, were included. Signalment and clinicopathological data were retrieved from medical records and statistically analysed. The GroEL gene of bacteria identified in PCR positive dogs were sequenced and phylogenetically analysed. During the study period, 1322 dogs were included and 94/1322 (7,1%) tested positive for at least one pathogen: 55 for *A. phagocytophilum* and 63 for *E. canis*. Twenty-six dogs were coinfected. The highest frequency of positivity was in 2014, followed by a progressive decline. The frequency of infection was significantly higher in mixed breed than in purebred dogs and, only for *A. phagocytophilum*, in hunting dogs than in the other breeds. No other significant association was found between infection and clinical data. The results obtained expand knowledge about prevalence of CGA and CME in Italy and possible risk factors in dogs.

PSB20 HIGH SEROPREVALENCE OF *BRUCELLA* SPP. IN WILD BOAR AND RED DEER IN THE CENTRE OF PORTUGAL

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The aim of this study is to assess the seroprevalence and risk factors associated with anti-*Brucella* antibodies in wild ungulates in the Centre of Portugal. A cross-sectional study was conducted from 2015 to 2023. Antibodies against *Brucella* spp. were determined by a commercial ELISA. Risk factors associated with seropositivity to *Brucella* spp. were investigated by comparing the proportion between two risk groups using the Chi-square statistical test. In sera tested from 650 wild ungulates (298 wild red deer and 352 wild boar), 5.4% red deer (95% CI: 3.10-8.57%) and 35.5% wild boar (95% CI: 30.51-40.76%) were positive. Regarding age, the seropositivity in juveniles was 31.76% (95% CI: 25.8-38.16%), and in adults was 16.07% (95% CI: 12.67-19.95%). The odds of being seropositive was 6.6 higher in wild boar than in red deer (95% CI: 4.02-10.87%) (p <0.000), and the risk was 1.9 higher in juveniles than in adults (95% CI: 1.48-2.64) (p <0.000). Both wild ungulates were exposed to *Brucella* spp.. The higher seroprevalence in wild boar is in accordance with previous studies and suggests that this species may have a major contribution to the ecology of *Brucella* spp. in the Centre of Portugal.

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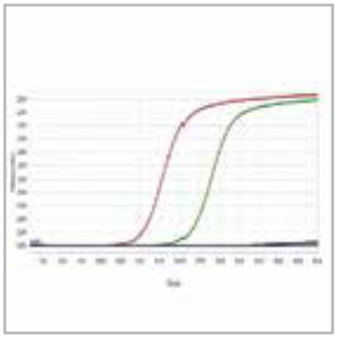

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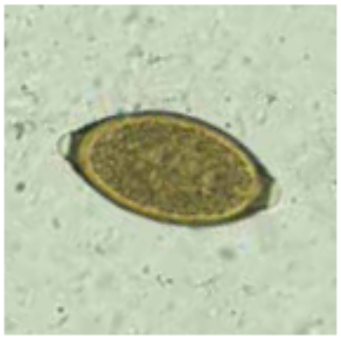

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SATURDAY, SEPTEMBER 23rd 2023

5th INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

PSC01

PSC02 IMMUNOMODULATORY EFFECTS OF CARICA PAPAYA EXTRACT AGAINST EXPERIMENTALLY INDUCED COCCIDIOSIS IN CHICKENS

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Abstract: The current study displays that *Carica papaya* has immunomodulatory properties counter to experimental coccidiosis in broiler chickens. 175 day old broiler chicks were sequestered into five correspondent groups for immunomodulatory testing. Atone week of age, a blended *Eimeria* contamination was controlled orally. Carica papaya extricate was given orally to descendants of similar age at dosages of 100, 200, and 300 mg/kg body weight in the initial three groups, distinctly. Control groups included vitamin E-treated chicks and PBS-treated chicks. PHA-P, Carbon Clearance Assay, and Dinitrochlorobenzene (DNCB) tests were utilized to reconnoiter cell-intervened resistance. The hemagglutination test was utilized to evaluate cell imperviousness. The study's finding revealed that *Carica papaya* extract treated groups had a morestronger cellular and humoral insusceptible reaction to coccidiosis. The immunological reaction of groups particular Carica papaya at the most extreme portion, 300 mg, was more noteworthy than that of lower dosages and affected contaminated chicks.
Key words: *Carica papaya*, Immunity, chicken, infection, coccidiosis

PSC03 GOOD NEWS: STRONG DECLINE IN THE PREVALENCE OF THIRD-GENERATION CEPHALOSPORIN-RESISTANT ESCHERICHIA COLI IN BROILERS AND MEAT THEREOF

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Since the beginning of the 21st century, third generation cephalosporin-resistance in Gram-negative bacteria, such as *Enterobacterales,* has emerged in human medicine. From the One Health perspective, there have been food safety concerns about whether food-producing animals can act as reservoirs for third generation cephalosporin-resistant *Escherichia coli* (3GC-R-Ec), which could then reach consumers via contaminated meat. Therefore, in 2014, analyses on the prevalence of 3GC-R-Ec in livestock and meat were included in the European harmonised antimicrobial resistance monitoring program. In the past, 3GC-R-Ec were detected in all livestock species and meat thereof, but with significant differences between animal species and countries. In Switzerland, broilers were the livestock species with the highest prevalence of 3GC-R-Ec in caecal samples (2016: 52%) and very high contamination rates were found in Swiss chicken meat (2016: 41.9%). However, since 2016, a significant decrease in the prevalence of 3GC-R-Ec in broilers' ceacal content and meat has been observed. In fact, the prevalence of 3GC-R-Ec decreased to 4.3% in Swiss broilers and 4.2% in Swiss chicken meat in 2022. It has been shown that 3GC-R-Ec are introduced into parent broiler hatcheries via imported day-old chicken that have already been colonised. The sharp decline in 3GC-R-Ec in national broiler production today is most likely due to the production and sale of 3GC-R-Ec-free day-old chicken. Although there is no evidence on the measures taken by international breeding companies, the reason for this decline could be that breeding companies have refrained from the prophylactic use of modern cephalosporins in recent years.

PSC04 CAN PHENOLIC COMPOUNDS FROM PYRUS COMMUNIS L. HELP IN THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE?

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Pears (*Pyrus* spp.) are consumed all around the world. In Portugal, pears represent the second most produced fruit; in 2021 production reached 225.4 thousand tons (+72%). Unavoidably, a large amount of pear fruits is annually wasted, due to low fruit quality or logistical issues. Fruit processing also generates huge quantities of by-products/wastes (i.e., leaf, peduncle, peel, and core). Food industry by-products and waste are frequent sources of phenolic compounds. These compounds are linked to several biological

functions such as antioxidant, anti-inflammatory and antimicrobial activities. However, although some of their biological activities have been explored by the pharmacological, food, cosmetics, packaging, and textile industries, only a few studies focused on their antibacterial potential. Therefore, this study aims to give a better insight into the antibacterial properties of phenolic compounds from *P. communis* L.The bibliographical review of the studies carried out in this theme, revealed that two different Polish studies reported that phenolic compounds from the leaves of P. communis L. were able to inhibit the growth of several bacteria, such as *S. aureus*, MRSA, *B. subtillis*, *H. pylori*, *E. faecalis*, *E. coli*, ESBL-*E. coli*, and *P. aeruginosa*. Phenolic compounds from *P. communis* L. flesh have also shown antimicrobial properties against *S. aureus*, *B. subtillis*, *P. aeruginosa*, *E. aerogenes*, *B. licheniformis*, *K. pneumoniae*, *B. megaterium*, and *E. coli*. Both Polish studies linked the antimicrobial activity of the extracts to the presence of hydroquinone, revealing that this phenolic compound could be used for its antimicrobial properties. **Acknowledgments:** This work was supported by the projects UIDP/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT) and by LAQV-REQUIMTE, which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). The authors are also grateful to FCT for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2020). L. Barros is grateful for the national funding from FCT, through the institutional scientific employment program contract for her contract, and F. S. Reis thanks FCT for her individual employment program contract (2021.03728.CEECIND).

PSC05 EXPLORING ANTIBIOTIC RESISTANCE IN *E. COLI* STRAINS ISOLATED FROM SOIL SAMPLES

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Overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria, including *Escherichia coli*. While most research on antibiotic resistance has focused on clinical settings, there is growing evidence of antibiotic resistance in environmental settings such as soil samples. So understanding antibiotic resistance in soil *E. coli* is crucial for developing effective strategies to combat this threat. Samples were collected from three sites. Three meter-squared plots were randomly selected at each site, and topsoil was collected by randomly selecting ten points in each plot. 10 g of soil were pre-enriched in 90 mL of Trypticase soy broth for the enrichment of *E. coli*, and 24h after, streaked on selective media for the isolation of *E. coli*. Antibiograms were performed for 17 antibiotics according to EUCAST guidelines. Extended-spectrum β-lactamases (ESBL) production was tested by the double-disc synergy test. A total of 14 *E. coli* were isolated from the soil samples of the three sites. From the ESBL production test, we did not identify ESBL-producing *E. coli*. All strains were resistant to ampicillin and gentamicin. All strains were susceptible to cefoxitin, cefepime, ceftazidime, cefotaxime, aztreonam, meropenem, ertapenem, imipenem, and chloramphenicol. We can conclude that antibiotic resistance is present in the environment and may pose a risk to animal health since *E. coli* strains in the soil samples were resistant to ampicillin and gentamicin, which is concerning for veterinary medicine, as these antibiotics are commonly used to treat animal infections. Acknowledgments: This work was supported by the projects UIDP/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). National funds support Carolina Sabença through FCT under the Ph.D. grant 2020.06967.BD.

PSC06 EVALUATION OF BIOFILM FORMATION IN KLEBSIELLA PNEUMONIAE FROM HUMAN SAMPLES

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Klebsiella pneumoniae is a gram-negative bacterium that can cause infections in hospitalized patients by producing biofilm, a microbial community embedded in an extracellular matrix which is often found in catheters and implants. 60 *K. pneumoniae* strains were isolated from different sources, such as urine, blood, sputum, and others, at the hospital center of Trás-Os-Montes and Alto Douro between December 7, 2021, and August 11, 2022. This study used the crystal violet method, according to the O'Toole protocol. The interpretation of the biofilm formation was based on the optical density (OD) measurement using a spectrophotometer at 570 nm. Of the 60 *K. pneumoniae* clinical isolates, 1.66% (n=1) is classified as a strong producer of biofilm, 25% (n=15) are moderate producers, 61.66% (n=37) are weak producers, and lastly, 11.66% (n=7) are non-producers of biofilm. Thus, these results indicate that the majority of *K. pneumoniae* clinical isolates are weak producers of biofilm. Moderate producers were also identified in a significant number of isolates, while strong producers were relatively rare. Additionally, a small percentage of isolates were found to be non-producers of biofilm. Since *K. pneumoniae* is a significant pathogen in animals, the findings of this study could have implications for the diagnosis, treatment, and control of infections in veterinary medicine. Specifically, the identification of weak and moderate biofilm producers among *K. pneumoniae* clinical isolates could suggest that some strains may be less virulent in animals and easier to treat with antibiotics or other therapeutic measures.
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PSC07 ISOLATION AND PHENOTYPIC CHARACTERIZATION OF ESCHERICHIA COLI AND KLEBSIELLA SPP. IN RIVER WATER SAMPLES

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The study of *E. coli* and *Klebsiella spp.* bacteria in river water samples help monitor antibiotic resistance and understand the importance of water quality and associated health risks. Identifying the source of antibiotic use in humans and animals can improve waste management practices, and prevent environmental pollution, potentially slowing the spread of antibiotic resistance. Water samples (300mL) from 12 Portuguese rivers were filtered, cultured, and tested for resistance to 16 antibiotics using EUCAST guidelines. Extended-spectrum β-lactamases (ESBL) production was tested with the double-disc synergy test. In total, 25 strains of *E. coli* and 30 strains of *Klebsiella* spp. were isolated. None of the strains produced ESBL. *E. coli* strains were entirely resistant to ampicillin (100%), while none of them were resistant to amoxicillin-clavulanic acid, ceftazidime, aztreonam, cefotaxime, meropenem, ertapenem, and amikacin. *Klebsiella* spp. strains showed high resistance to ampicillin (83.3%) and gentamicin (56.7%) and low or no resistance to the rest of the antibiotics tested. These findings suggest that the prevalence of antibiotic-resistant *E. coli* and *Klebsiella* spp. strains in water sources are concerning and highlight the importance of effective surveillance and control measures. These findings also underline the potential for transmitting antibiotic-resistant bacteria from water sources to animals. Contaminated water sources may expose animals to antibiotic-resistant bacteria, which can spread to other animals or humans through direct contact or consuming contaminated animal products.
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PSC08 ANTIBIOTIC RESISTANCE IN PSEUDOMONAS AERUGINOSA ISOLATED FROM HUMAN URINE SPECIMENS: AN EMERGING HEALTH CONCERN

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Antibiotic resistance in *Pseudomonas aeruginosa* is a growing concern in healthcare, particularly in the context of urinary tract infections. *P. aeruginosa* is a type of bacteria that commonly causes infections in the urinary tract, and it has become increasingly resistant to antibiotics over time. The aim of this study was to investigate the susceptibility of *P. aeruginosa* present in urine samples to antimicrobial agents. In order to evaluate the vulnerability of the microorganisms, we conducted Kirby Bauer diffusion assays, following EUCAST guidelines. None of the isolates were found to be resistant to multiple antibiotics. The results showed that Imipenem had the highest resistance rate at 17.3%, followed by Ceftazidime at 13.6%, Piperacillin at 10.2%, Trobamicin at 7.1%, and Amikacin at 5.2%. The majority of microorganisms exhibited susceptibility to the antibiotics tested, with Amikacin and Ciprofloxacin demonstrating the highest rates of susceptibility at 94.8% and 92.9%, respectively. In conclusion, antibiotic resistance in Pseudomonas aeruginosa poses a significant challenge in the management of urinary tract infections. This study highlights the need to continue monitoring the susceptibility of *P. aeruginosa* to antimicrobial agents to ensure effective treatment. The results demonstrate that while some antibiotics showed higher resistance rates, the majority of the microorganisms were susceptible to the antibiotics tested.
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PSC09 EXPLORING SYNERGISTIC POTENTIAL OF IMIPENEM+CILASTATIN AND TETRACYCLINE IN *PSEUDOMONAS AERUGINOSA* ISOLATES FROM SEPSIS: A PROMISING STRATEGY FOR COMBATING ANTIMICROBIAL RESISTANCE

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Pseudomonas aeruginosa is a gram-negative bacterium that causes severe infections, especially in those with weakened immunity or who have undergone invasive medical procedures. Its rapid development of resistance to multiple antimicrobial agents, including common antibiotics, is a significant challenge. This resistance is due to its ability to acquire resistance genes through horizontal gene transfer and to pump out antibiotics from its cells. Consequently, treating *P. aeruginosa* infections is difficult, necessitating new strategies to combat this multidrug-resistant pathogen. Thus, the objective of this work was to study the synergistic effect of the antibiotic's imipenem+cilastatin and tetracycline on *P. aeruginosa* isolates from sepsis. The susceptibility testing of imipenem+cilastatin and tetracycline by the microdilution method

(MIC) is determined according to the EUCAST 2022 guidelines. The degree of synergy was determined by computing the Fractional Inhibitory Concentration Index (FICI). Various combinations have been demonstrated to exhibit efficacy against distinct isolates. These combinations include: 16 μg/mL tetracycline and 2 μg/mL imipenem+cilastatin, 32 μg/mL tetracycline and 2 μg/mL imipenem+cilastatin, 8 μg/mL tetracycline and 4 μg/mL imipenem+cilastatin, 16 μg/mL tetracycline and 16 μg/mL imipenem+cilastatin, and 8 μg/mL tetracycline and 64 μg/mL imipenem+cilastatin. Possible differences in FICI values between isolates could arise due to various factors such as the genetic variability of the isolates, differences in their metabolic activity, and their individual susceptibility to the antimicrobial agents being tested. In conclusion, *P. aeruginosa* is a formidable pathogen that poses a significant challenge in treating infections due to its rapid development of multidrug resistance.

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PSC10 IDENTIFYING PSEUDOMONAS AERUGINOSA BIOFILMS IN DOGS: A COMPREHENSIVE APPROACH

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Pseudomonas aeruginosa is a bacterium commonly found in soil and water, and it can also infect animals, including dogs. Studying biofilms in *P.* aeruginosa in samples from dogs is crucial as these biofilms play a significant role in chronic infections, treatment resistance, and transmission of infectious agents, highlighting the need for effective detection and management strategies. The objective of this study was to assess the formation of biofilms by *Pseudomonas aeruginosa* in samples obtained from dogs. These strains were obtained from the INNO-Veterinary Laboratory and were isolated using VITEK 2® COMPACT by bioMérieux. The identification of the strains was verified through the selective medium *Pseudomonas* CN selective agar. The technique used to study biofilms was the crystal violet method in 96-well sites. The results match the absorbance requirement for each isolate. The results obtained from the analysis of 82 samples of dogs reveal important information about the production of biofilms by this pathogen. Among the remaining 82 samples, the proportion of non-producers of biofilms was found to be 26.8%, which means that in these cases, the bacteria did not form any detectable biofilm. Only 1.2% of the samples were classified as strong biofilm producers, indicating that in most cases, the bacteria did not form very strong or robust biofilms. The results suggest that in most cases, the biofilms formed by this bacterium in dogs are weak, which may present opportunities for effective treatment with appropriate antimicrobial therapies and other biofilm-targeting strategies.

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PSC11 PSEUDOMONAS AERUGINOSA INFECTIONS IN DOGS: UNDERSTANDING MULTIDRUG RESISTANCE

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Pseudomonas aeruginosa in dogs can cause skin, ear, and respiratory infections and can develop multidrug resistance, limiting treatment options and causing more severe infections. Underlying health conditions and exposure to antibiotics in hospital or shelter environments increase the risk of multidrug-resistant infections. Appropriate measures must be taken to prevent the spread and treat infected dogs. The objective of this study is to investigate the phenotypic resistance of *34 P. aeruginosa* strains to various classes of antibiotics. These strains were obtained from the INNO-Veterinary Laboratory and were isolated using VITEK 2® COMPACT by bioMérieux. The identification of the strains was verified through the selective medium *Pseudomonas* CN selective agar. To assess the susceptibility of the microbial agents, both Kirby Bauer diffusion tests and minimum inhibitory concentration (MIC) tests were conducted in accordance with EUCAST standards. The results of the study indicated that all of the 34 isolates were non-multidrug resistant. However, some variations in resistance were observed among the strains. The study revealed that tobramycin had the highest resistance level among the *Pseudomonas aeruginosa* strains, at 14.71%. Similarly, the resistance rates were 94.12% for ceftiofur and 91.18% for cefovecin. On the other hand, the antibiotics with the highest susceptibility rates were Meropenem (94.12%), Imipenem (97.06%), Amikacin (100%), and Gentamicin (94.12%). Although the tested *Pseudomonas aeruginosa* strains were not multidrug resistant, their susceptibility to antibiotics varied. However, regional and temporal susceptibility patterns can vary, so monitoring antibiotic resistance is important for appropriate treatment decisions.
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PSC12

PSC13 ACTIVE SURVEILLANCE AS A TOOL TO FIGHT ANTIMICROBIAL RESISTANCE IN SMALL ANIMAL PRACTICE: COMPARISON BETWEEN AN ITALIAN AND A SPANISH VETERINARY UNIVERSITY HOSPITAL.

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Objectives: To compare the results of an active surveillance program for the detection of antimicrobial resistant (AMR) bacteria between two Small Animal Veterinary University Hospitals (VUHs) from two different countries (Italy and Spain). **Methods:** Active surveillance was executed in patients, environment, and staff. In patients, pulsed sessions of 25 patients each were executed by sampling patients hospitalized for >48 hours, at admission and before discharge. Swabs were cultured into selective media for carbapenem-resistant (CR) and Extended-spectrum beta-lactamases producing (ESBL) gram-negative bacteria (perirectal swabs) and for methicillin-resistant Staphylococci (MRS, oral swabs). Environmental surveillance was performed by sampling hospital surfaces, devices and staff, with subsequent culture in selective media for CR, ESBL and MRS. Positive cultures were identified with matrix-assisted laser desorption/ionization (MALDI-TOF). **Results:** At the Italian VUH, 125 patients were sampled, while 50 in Madrid. Admission prevalence for MRS, CR and ESBL was considerably higher in Italy (22%, 14% and 30% vs 12%,2% and 18%, respectively), as well as the acquisition rate during hospitalization (13%, 25% and 30% vs 4%, 4% and 22%). Environmental and staff surveillance showed that in both settings, a high MRS prevalence (31% and 33%, respectively) was found in personnel's hands. **Conclusions:** An active surveillance program on patients, environment and staff is a useful tool to evaluate and to compare the specific critical points for AMR spread in veterinary settings. Differences highlighted between the two VUHs can be due to population size, epidemiological, structural and management reasons, and should serve to address specific preventive measures.

PSC14 MEDIUM-CHAIN FATTY ACID SUPPLEMENTATION DURING THE WEANER PHASE RESULTS IN IMPROVED DAILY GAIN AND INCREASED LACTOBACILLUS SPP. IN PIGLETS.

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Medium-chain fatty acids (MCFA) are often applied during the weaner phase in piglets, as an alternative to antibiotics and pharmacological levels of zinc-oxide. The objective of this study was to investigate the efficacy of a blend of sustainably sourced MCFA on piglets' growth and its impact on the fecal microbiome.
At weaning, 448 piglets (TN70xTempo; housed 8 piglets/pen) were randomly distributed to two different treatment groups: 1) control diet or 2) a diet containing 1 kg/T MCFA. Treatment duration was nine days after weaning. At day 9, piglets were individually weighed and the average piglet per pen was selected from which fecal samples were collected for 16S rRNA gene sequencing for a total of 56 samples (28 per treatment). MCFA supplementation resulted in improved average daily gain during the weaner phase (180 vs 144 g/d; P=0.12). In addition, piglets' growth was positively correlated with fecal *Lactobacillus spp.* abundance (R^2=0.23; P=0.09). Alpha-diversity was not significantly different on genus (P=0.43) nor species level (P=0.41). Beta-diversity was calculated to define microbial composition dissimilarities between the treatment groups. Permutational ANOVA displayed that fecal *Lactobacillus spp.* abundance was significantly increased in the MCFA group compared to the negative control group (+40%; P=0.0001). This upregulation was mainly due to the upregulation of *Lactobacillus amylovorus* (+87%; P= 0.0001), a species which is considered probiotic. *Lactobacillus spp.* are considered to be one of the core genera in healthy pigs. This study demonstrated that sustainably sourced MCFA can enrich *Lactobacillus spp.*, resulting in healthier piglets which gain more weight.

PSC15 ANALISYS FOR ANTIMICROBIAL RESISTANCE IN ESCHERICHIA COLI ISOLATED FROM ANIMAL SAMPLES IN SICILY, ITALY

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In *Enterobacteriaceae*, the spread for antimicrobial resistance (AMR) through extended-spectrum beta-lactamases (ESBLs) production is already an alarming problem for public health, for increasing the diffusion of AMR which limits the control of infectious diseases in both human and animal health. To control AMR spreading, a multidisciplinary approach (One Health) has been recommended worldwide for a deeper knowledge of the problem in animals, plants, and environment. We analyzed for antibiotic resistance by phenotypic and genetic characterization routinely isolates of *Escherichia coli* from clinical cases of pets and livestock. A total of 120 *E. coli* isolates from different animals and/or different organs of single individuals were included in the study. Antimicrobial resistance was determined through Kirby–Bauer method with the following antibiotics: ceftiofur, enrofloxacin, amoxicillin, sulfamethoxazole/trimethoprim, gentamycin, tetracycline, cefovecin, and cefadroxil. The genetic analysis was performed through multiplex PCRs (MPX): two for ESBLs and two for virulent genes. Almost 40% of *E. coli* isolates were resistant to at least one antibiotic; virulent genes were detected only in animals with diarrheic problems. The presence of ampicillin resistance blaTEM gene was detected in 60% of isolates from single animals. However, in two animal carcasses different genotypes were present in different organs of the same individual. In Sicily, multidrug resistance resulted more frequent in pets compared to livestock, which is probably related to an inappropriate use of antibiotic from pet owners without veterinarian consultation. Educational campaigns involving animal owners should be considered to address MDR control in the veterinarian field.

PSC16 OCCURRENCE AND ANTIMICROBIAL CHARACTERISTICS OF *ESCHERICHIA COLI* IN RABBITS PRODUCTION IN PORTUGAL

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Antibiotics have been used routinely in farm animal production, during intensive farming, to keep animals healthy and to increase the productivity in farms. Antimicrobial resistance is one of the major threats to human and animal health and antimicrobials are widely used in food-producing animals, antimicrobial resistance has emerged globally with consequent concerns in veterinary and human medicine. The main objective of this work was to determine the antibiotic resistance profiles of *Escherichia coli* isolates from

20 *slaughterhouses and* determine the presence of antibiotic resistance genes. Antimicrobial susceptibility was performed by a Kirby–Bauer disk diffusion method against 16 antibiotics according EUCAST (2022) guidelines and the presence of resistance genes ((*tetA, tetB, bla*CTXu, *bla*CTX-9 and *bla*CTX-3G) was tested by PCR sequencing. Two hundred ninety-five fecal samples were collected and plated on Chromocult coliform agar supplemented with 2μg/ml cefotaxime and 48 samples were selected and identified as *E. coli.* All the isolates presented a multi-resistance profile, with high levels of resistance to β-lactams (100%), aminoglycosides (93.75%), cephalosporines (100%) and tetracycline (91.6%). Three different *bla*CTX-M variants were detected*: bla*CTXu (95.8%), *bla*CTX-3G (72.9%) and *bla*CTX-9 (56.25%). Furthermore, *tetA* and *tetB* were detected in 66.6% and 35.4%, respectively. Overall, this study has revealed a high incidence of resistance to commonly used antimicrobials in animal production and human medicine, such as tetracyclines, β-lactams and aminoglycosides, which suggested widespread antimicrobial resistance pollution and of *E. coli* isolates with genes with the potential risk. Consequently, a possible transmission path of potential multi-resistant pathogens to human via food chain.
Acknowledgments: This work was supported by the projects UIDP/ CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry—LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). Adriana Silva is grateful to FCT (Fundac¸ão para a Cieˆncia e a Tecnologia) for financial support through the PhD grant SFRH/ BD/04576/2020.

PSC17 BACTERIAL FITNESS AND GENOMIC ANALYSIS OF PHAGE RESISTANT ESCHERICHIA COLI K1

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Several virulence factors are expressed by extra-intestinal *Escherichia coli*, which enable them to effectively colonize and survive in diverse locations. Of these factors, the K1 capsular type has been found to be involved in several types of human infections, including meningitis, urinary tract infections and bloodstream infections. These strains can also be found in animals and particularly in poultry where they are responsible for colibacillosis. Phage therapy is a promising alternative to antibiotics and the understanding of the bacterial resistance mechanisms to phages has implications for the development of phage-based therapies. The objective of this study was to investigate the resistance of *E. coli* K1 to ULINTec4, a K1-dependent bacteriophage. Resistant bacterial colonies were isolated from an avian pathogenic *E. coli* strain APEC 45 and the human strain C5, both previously exposed to the ULINTec4. After confirmation of their resistance, the individual growth of the bacterial isolates was analyzed in the presence and absence of phages using growth curve analysis. One of the resistant isolates exhibited a significantly slower growth rate suggesting the presence of a resistance mechanism altering its fitness. After genomic sequencing using Nanopore technology, the comparative genomic analysis revealed the presence of insertion sequences in the capsular genes cluster. In conclusion, a phage resistance mechanism was detected at the genomic level and decreased the fitness of *E. coli* K1 in-vitro. Further research are now needed to identify other resistance mechanisms at the genomic level.
The project was financially supported by Wallonia in the framework of the call for projects organised by BioWin competitiveness cluster (Project Inteliphages)

PSC18 THE OCCURENCE OF ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA COLI* ISOLATED FROM DAIRY CALVES

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Calf diarrhea causes enormous economic losses worldwide. Diarrhea commonly occur in the first week of life and the most frequent infectious cause is *Escherichia coli*. Since bacterial infections are treated with antimicrobials, resistance and the increasing incidence of multidrug-resistant (MDR) *E. coli* isolates represent a serious problem in veterinary and human medicine. To assess the occurrence and characteristics of *E. coli* from Slovenian dairy farms, we collected fecal samples from diarrheic and non-diarrheic calves aged up to seven days from five farms. To determine the presence of resistant *E. coli*, all samples were plated on selective culture media (MacConkey agar supplemented with 1 μg/L cefotaxime [Biolife] and CHROMID Carba Smart Agar [BioMerieux]) after overnight enrichment in peptone water. The obtained isolates underwent whole genome sequencing (WGS) to assess their resistance profiles and pathogroups. ESBL/AmpC *E. coli* isolates were obtained from 28/39 calves. Up to two different morphological types were WGS-typed. Carbapenem-resistant *E. coli* were not detected. The results showed a high prevalence of antimicrobial resistance on dairy farms. Resistant *E. coli* strains represent a reservoir for the dissemination of resistance genes to other virulent strains, leading to difficulties in the treatment of calves.

PSC19 ANTIMICROBIAL RESISTANCE AND BIOFILM FORMATION OF STAPHYLOCOCCI ISOLATED FROM WILD BATS

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Antibiotic resistance is a public health concern and a global One Health issue involving humans, the environment and animals, including wildlife. The role wild animals as reservoirs of antibiotic resistant bacteria needs more attention. Bats are one of the potential vectors for the transmission bacterial zoonotic pathogens. Therefore, we aimed to investigate the presence of staphylococci in bats as well as their antimicrobial resistance profile and biofilm formation capacity. Nose and mouth swab samples were collected from 105 wild bats from 18 different species. Staphylococci were isolated using BHI broth with 6.5% of NaCl and Chromagar MRSA plates. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method. Evaluation of biofilm formation was conducted by the microplate assay and the biofilm biomass was quantified using the crystal violet method. From the 105 samples, 19 (18.1%) staphylococci were recovered. These strains were isolated from bats belonging to 8 species: *Pipistrellus pipistrellus*, *Myotis escalerai*, *Nyctalus leisleri*, *Pipistrellus kuhlii*, *Tadarida teniotis*, *Nyctalus leisleri*, *Plecotus auratus*, *Myotis daubentonii daubentoniid* and *Myotis bechsteinii*. Among the isolates, only one was *S. aureus* which was recovered from a *Myotis escalerai* bat. Resistance to penicillin (n=2), aminoglycosides (n=2), tetracycline (n=2), clindamycin (n=10) and fusidic acid (n=14) were detected among the isolates. Nevertheless, most strains were susceptible to the majority of the antibiotics tested. Regarding the biofilm formation, only 3 strains were classified as highly biofilm producers and most of the remaining isolates were considered medium biofilm producers. No multidrug-resistant bacteria were detected in this study. However, the colonization of staphylococci resistant to clinically important antibiotics shows the need for epidemiologic studies to understand role of bats on the dissemination of these strains and resistances.
Acknowledgements: This work was supported by the projects UIDB/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT) and was also supported by LAQV-REQUIMTE, which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020).

PSC20 PREVALENCE AND ANTIBIOTIC RESISTANCE PROFILE OF MASTITIS-ASSOCIATED PATHOGENS IN SELECTED DAIRY FARMS IN SERBIA

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Mastitis is a major veterinary health problem in intensive livestock production, affecting public health. Antibiotic overuse and misuse in mastitis in dairy cows can lead to transmission of resistant bacteria in food chain and ecosystems. Bacteria vary by region and over the time, so choosing the right antibiotic requires insight in the current local data on the prevalence and susceptibility. Given the global significance of AMR (antimicrobial resistance) and the challenges posed by mastitis in veterinary practice, this study aimed to assess the prevalence of mastitis-associated pathogens on two dairy farms in Serbia; farm with 975 cows (F1) and with 1141 cows (F2), as well as to analyze their antimicrobial susceptibility patterns. Milk samples were obtained from 181 dairy cows (n(F1)=99; n(F2)=82) affected by clinical or subclinical mastitis. Bacterial causative agents were determined by bacteriological and biochemical tests, while Kirby-Bauer disc diffusion method was employed to assess the antimicrobial susceptibility of isolated causative agents against 15 antimicrobials. Of the positive samples (n(F1)=65; n(F2)=46), the most dominant pathogens on both farms were *Streptococcus* spp. (n(F1)=19.20%; n(F2)=14.70%) and *Escherichia coli* (n(F1)=13.13%; n(F2)=26.82%), while high prevalence of resistance to penicillin, cloxacillin and novobiocin was observed. In contrast, susceptibility rate to enrofloxacin, amoxcillin/clavulanic acid and gentamicin was among the highest. The study shows a local pattern in the prevalence of mastitis-associated pathogens and AMR. Current information on culture and susceptibility testing when mastitis is suspected could ensure effective antimicrobial treatment while also minimizing the risk of AMR.
Acknowledgement: The participation in the 5th International Conference of the European College of Veterinary Microbiology is supported by COST Action 18217 European Network for Optimization of Veterinary Antimicrobial Treatment.

POSTER PSD

SATURDAY, SEPTEMBER 23rd 2023

5th INTERNATIONAL CONFERENCE OF THE EUROPEAN COLLEGE OF VETERINARY MICROBIOLOGY

PSD01 ANTIMICROBIAL RESISTANCE IN *ENTEROCOCCUS* ISOLATED FROM COMPANION ANIMALS: IMPLICATIONS FOR VETERINARY AND HUMAN HEALTH

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Abstract: Enterococciare commensal in the gastrointestinal tract of pets. However, they can produce opportunistic infections and are a primary cause of nosocomial infections in humans. Also, they are of concern due to antimicrobial resistance.
Our aim was to investigate the resistance of enterococci isolated from pets to Critically Important Antibiotics (CIA).During 2022-2023, 35 enterococci (54% *E. faecium*, 54% *E. faecalis*) were collected from clinical specimens (75% urinary-tract infections, 20% skin lesions, 5% surgical site infections) of 19 cats and 16 dogs at the veterinary teaching hospital of Turin. MIC values for ten antibiotics, employed in human therapy, were determined by microdilution method and EUCAST epidemiological cut-offs were used to distinguish wild-type (WT) from non-wild-type (nWT) isolates. All isolates were WT for linezolid and tigecycline. *E. faecalis* was also WT for vancomycin, while the 16% of *E. faecium* was nWT to this molecule. There were more *E. faecium* than *E. faecalis* nWT to ampicillin, chloramphenicol, and ciprofloxacin ($p<0.05$). No difference was observed for daptomycin, teicoplanin, tetracycline. Overall, more nWT were observed against highly important antibiotics (49%) than CIA. Among CIA, the proportion of nWT was greater to Highest Priority antibiotics than High priority. Finally, there were more *E. faecium* than *E. faecalis* nWT to HPCIA (RR=1.73, 90%CI:1.35-2.11). The results show that enterococci isolated from infections in pets deliver resistance even to antibiotics reserved for human medicine, although we cannot establish in which direction the transmission of resistance occurs. Additionally, we confirm that *E. faecium* exhibited more resistance than *E. faecalis* in pets.

PSD02 ANTIMICROBIAL RESISTANCE IN *ENTEROCOCCUS* ISOLATED FROM COMPANION ANIMALS: IMPLICATIONS FOR VETERINARY AND HUMAN HEALTH

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Abstract: Enterococciare commensal in the gastrointestinal tract of pets. However, they can produce opportunistic infections and are a primary cause of nosocomial infections in humans. Also, they are of concern due to antimicrobial resistance.
Our aim was to investigate the resistance of enterococci isolated from pets to Critically Important Antibiotics (CIA). During 2022-2023, 35 enterococci (54% *E. faecium*, 54% *E. faecalis*) were collected from clinical specimens (75% urinary-tract infections, 20% skin lesions, 5% surgical site infections) of 19 cats and 16 dogs at the veterinary teaching hospital of Turin. MIC values for ten antibiotics, employed in human therapy, were determined by microdilution method and EUCAST epidemiological cut-offs were used to distinguish wild-type (WT) from non-wild-type (nWT) isolates. All isolates were WT for linezolid and tigecycline. *E. faecalis* was also WT for vancomycin, while the 16% of *E. faecium* was nWT to this molecule. There were more *E. faecium* than *E. faecalis* nWT to ampicillin, chloramphenicol, and ciprofloxacin ($p<0.05$). No difference was observed for daptomycin, teicoplanin, tetracycline. Overall, more nWT were observed against highly important antibiotics (49%) than CIA. Among CIA, the proportion of nWT was greater to Highest Priority antibiotics than High priority. Finally, there were more *E. faecium* than *E. faecalis* nWT to HPCIA (RR=1.73, 90%CI:1.35-2.11). The results show that enterococci isolated from infections in pets deliver resistance even to antibiotics reserved for human medicine, although we cannot establish in which direction the transmission of resistance occurs. Additionally, we confirm that *E. faecium* exhibited more resistance than *E. faecalis* in pets.

PSD03 ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER* SPP: ISOLATES FROM POULTRY, SLOVENIA

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Campylobacteriosis remains the most commonly reported zoonosis, with poultry meat being the main source of infection. Antimicrobial misuse and overuse in veterinary and human medicine is partly responsible for the increase in infections with resistant bacteria. In this study, we examined 27 poultry meat samples and 28 poultry fecal samples and obtained 40 *Campylobacter* spp. isolates. MALDI-TOF MS identified 23 isolates as *C. jejuni* and 17 isolates as *C. coli*. Isolates were further characterized using whole-genome sequencing. Phenotypic antimicrobial sensitivity testing (AST) by broth microdilution was also performed and compared with genotypic (WGS-based) AST. Understanding of the genetic background and molecular mechanisms of antimicrobial resistance is important to implement targeted control measures to limit further spread of antimicrobial resistance in *Campylobacter* spp.
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PSD04 COMPANION ANIMAL OWNERS' EXPECTATIONS AND KNOWLEDGE ON ANTIMICROBIAL USE IN PORTUGAL

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While multiple studies have focused on motivations surrounding antimicrobial prescription among companion animal veterinarians, Owners' expectations and knowledge on antimicrobials and their role on antimicrobial judicious use remains understudied. This work aimed to characterize the knowledge about antimicrobial use and antimicrobial-resistance by pet owners from Portugal. A nationwide survey directed to pet owners was conducted both online and at Portuguese companion animal veterinary practices. A total of 423 valid submissions were obtained. Although most respondents (97.9%) believed to know what an antibiotic is, 13.8% and 10.1% answered they could treat viral and fungal infections, respectively. Around 80% of respondents strongly-agreed/somewhat-agreed that it's the veterinarians' obligation to assure the antimicrobial treatment prescribed is both the most convenient and economic; however, effectiveness is favoured over cost when 87.7% of owners strongly-agree/somewhat-agree they'd prefer to spend more to identify the most appropriate antibiotic. Notably, 87% of respondents recognize that antibiotic resistance is a significant problem and 74.6% strongly-agree/somewhat-agree that antimicrobial use in pets may contribute to the development of antimicrobial-resistant microorganisms. However only 25.3% recognized that this risk could be the source of resistance in other animals and people, showing little knowledge of the interconnection between species. Moreover, 55.6% of respondents were neutral when asked if the most frequently used antibiotics in veterinary medicine were also very important for humans. These findings suggest that communication between prescribing veterinarians and pet owners should be improved to further clarify the impact antibiotic administration to pets can have to human health and vice-versa, and that this could be foreseen as an additional mean towards good veterinary antimicrobial stewardship.
Keywords: antimicrobial resistance; companion animal; owner; antimicrobial stewardship; one health; antimicrobial use
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PSD05 VERTICAL AND HORIZONTAL SPREAD OF AN INCX3 PLASMID ENCONDIG *bla*$_{SHV-12}$ AND *qnrS* GENES AMONG SLOVENIAN BROILERS

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Food-producing animals, especially poultry, are a potential source of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. In our study, we analyzed conjugative transfer and nucleotide sequence of beta-lactamase SHV-12 and plasmid-mediated fluoroquinolone resistance gene *qnrS* encoding plasmid pES, which was detected in clonal and non-clonal ESBL *E. coli* isolates, sampled at different times from different poultry farms in Slovenia. Clonality of the isolates was determined by ERIC PCR, phylogenetic groups and the presence of virulence associated genes. Several clonal and non-clonal isolates displaying the same or similar plasmid restriction pattern were analyzed. Isolates VF2017-A5 (A5) and VF2017-A6 (A6) were used as donors, and *E. coli* J53Az^{R} as recipient for initial conjugation experiments. The frequencies for the *bla*$_{SHV-12}$ encoding plasmid pES from donor A5 and A6 varied from 10^{-7} to 10^{-5} from 10^{-5} to 10^{-3}, respectively. Sequencing the plasmid DNA of selected transconjugants revealed the presence of other plasmids in donor strains A5 and A6. In addition to plasmid pES6, which is identical to plasmid pEC-243 described in the literature, plasmids encoding colicins B, M, Ia, microcin V and an 1.5 kb cryptic plasmid were detected. Our study has confirmed clonal and, at a relative high rate, plasmidic spread of an 46338 bp IncX3 plasmid, encoding *bla*$_{SHV-12}$ and *qnrS* genes, that could replace, as it was recently reported from Switzerland, the previously predominant *bla*$_{CTX-M-1}$ in ESBL-producing enterobacteria.

PSD06 SURVEILLANCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF SALMONELLA ISOLATED FROM CHICKENS IN NORTHEAST OF THAILAND

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To determine the surveillance and antimicrobial resistance pattern of *Salmonella* spp. isolated from chickens at slaughterhouses in northeast of Thailand, the total of 604 cloacal swab samples were collected and isolated for the presence of *Salmonella* spp. during 2020-2022. All samples were isolated and identified according to ISO 6579:2002. *Salmonella* spp. was detected in 109 of 604 (18.05%) samples. The

most prevalent serovars were *Salmonella* Kentucky (22.94%), Give (20.18%) and Typhimurium (7.34%). In this study, 66.97% of the isolates were resistant to at least one antimicrobial drug and 38.39% were multidrug resistant. The highest resistances were found in Nalidixic acid (49.54%), ampicillin (30.28%), tetracycline (27.52%), amoxicillin (26.61%), ciprofloxacin (23.85) and norfloxacin (19.27%). The results revealed high prevalence of *Salmonella* spp. in chickens and antimicrobial resistance patterns. Prevention and control of *Salmonella* contamination in chicken meat processing impact on health and wellness of both chickens and consumers.
Key words: antimicrobial resistance, *Salmonella* spp., chicken, slaughterhouse

PSD07 PREVALENCE AND ANTIMICROBIAL RESISTANCE OF SALMONELLA COLLECTED FROM PIGS IN NORTHEAST OF THAILAND

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This study aimed to determine prevalence and antimicrobial resistance pattern of *Salmonella* spp. isolated from pigs in slaughterhouses in northeast of Thailand. During 2020-2022, all samples were isolated and identified according to ISO 6579:2002. The total of 699 samples from rectal swab were collected and isolated for the presence of *Salmonella* spp. *Salmonella* spp. was detected in 275 of 699 (39.34%) samples.Twenty-four serovars were identified in the 275 isolates. The most prevalent serovars were Rissen (36.97%), *S. enterica* ser.4,5,12:i: (25.35%) and Typhimurium (21.33%). In this study, 76.30% of the isolates were resistant to at least one antimicrobial drug, and 38.39% were multidrug resistant. The highest resistance was found to ampicillin (69.20%), tetracycline (66.35%), sulfamethoxazole/trimethoprim (35.55%) and chloramphenicol (9.00%). High prevalence of *Salmonella* spp. in pigs and high antimicrobial resistance among the isolates were found. This indicates the need for monitoring program to control *Salmonella* contamination and to reduce the dissemination of antimicrobial resistance in pig supply chain.
Key words: prevalence, antimicrobial resistance, *Salmonella* spp., pigs

PSD08 NUTRIENT-DEPENDENT INTERACTIONS BETWEEN SALMONELLA ENTERICA SEROVAR TYPHIMURIUM AND BACILLUUS SUBTILIS IN BIOFILMS

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Salmonella enterica serovar Typhimurium is one of the most common foodborne pathogens affecting both humans and animals. *Salmonella* is ubiquitous and can also be present in nutrient-poor environments outside the host. Due to the spread of antibiotic resistance, new strategies are needed to limit foodborne pathogens. One of the approaches involves the antagonistic properties of probiotic bacteria such as *Bacillus subtilis*. The vast majority of the literature dealing with *B. subtilis-Salmonella* interactions is conducted in broilers, and although these studies are essential for the development of probiotics, they do not address the mechanisms mediating pathogen-probiotic interactions not how probiotics affect pathogen's biofilm. We tested the competition between the two bacteria, *B. subtilis* PS-216 and *S.* Typhimurium SL1344, under different environmental conditions focusing on nutrient availability. The results show that under nutrient-rich conditions *B. subtilis* PS-216 inhibits the growth of *S.* Typhimurium SL1344 and reduces its biofilm thickness. The antagonistic potential of *B. subtilis* is greatly impaired in a mutant with an inactive *pks* operon, which is responsible for the synthesis of the secondary metabolite bacillaene and acts as an antagonist against *S.* Typhimurium. In addition, the presence of *S.* Typhimurium in the coculture increased the activity of the P_{pksC} promoter, which controls bacillaene production, suggesting that *B. subtilis* senses and responds to a Gram-negative competitor. In nutrient-depleted conditions *B. subtilis* lost its antagonistic effect and entered sporulation, but in coculture *S.* Typhimurium inhibited spore formation of its competitor. The results show that the two bacteria have to be in direct cell-to-cell contact for inhibition to occur and that the sporulation inhibition rate depends on the population density of *S.* Typhimurium. Moreover, preliminary results show that upon direct contact, *Salmonella* uses type 6 secretion system (T6SS) and triggers SigB-dependent stress response in *B. subtilis*. The work reveals molecular determinants of the competition and its tight dependence on cell-cell contact and environmental conditions, highlighting the importance of evaluating probiotic strains against pathogens under conditions relevant to the intended use.
Key words: *Bacillus subtilis*, *Salmonella enterica*, probiotic, pathogen, biofilm, sporulation, nutrients
Ref. *Podnar et al., 2022. Microbiology spectrum, 10, 6:* e0183622. doi: 10.1128/spectrum.01836-22.

PSD09 MOLECULAR EPIDEMIOLOGY OF CANINE PARVOVIRUS TYPE 2 IN SICILY, SOUTHERN ITALY: A FAST-EVOLVING EPIDEMIOLOGICAL SCENARIO

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Canine parvovirus type 2 (CPV-2) is the causative agent of an acute and often fatal viral disease of domestic dogs and wild carnivores. The original CPV-2 type, to date no longer circulating but still present in some vaccines, has been replaced by three antigenic variants, namely CPV-2a, CPV-2b, and CPV-2c. Limited epidemiological data are available for Sicily, southern Italy. To fill this gap, a molecular epidemiological retrospective study was performed, testing for CPV-2 samples collected from domestic dogs in Sicily between 2019 and 2022. Samples from 346 dogs with suspected infectious gastrointestinal disease were tested by PCR for CPV-2 and other enteric viral pathogens. Near complete VP2 sequences were obtained from CPV-2 positive samples by Sanger sequencing. Sequence and phylogenetic analyses were then performed. CPV-2 was detected in 230 (66%) dogs, as a single viral agent (82%) or in co-infection with other viruses (18%). The original CPV-2 type (4%), CPV-2a (9%), CPV-2b (18%), and CPV-2c (69%) were detected. Since 2019, the relative proportion of CPV-2a/CPV-2b variants decreased, while CPV-2c showed a proportional increase in the last two years; the complete replacement of CPV-2 old strains by the Asian CPV-2c genetic mutant was observed, highlighting a possible higher fitness of this clade. Phylogenetic analyses showed the co-circulation of CPV-2 variants and viral transmission across different regions. The diffusion and transmission dynamics observed in an insular Mediterranean area of southern Italy demonstrate the rapidly evolving epidemiological scenario of CPV-2 and show the importance of molecular surveillance to monitor viral spread.

PSD10 EMERGING VIRUSES IN DOGS ILLEGALLY IMPORTED TO ITALY

WITHDRAWN

PSD11 DETECTION OF PANTROPIC CANINE CORONAVIRUS IN AUTOCHTHONOUS ITALIAN ADULT DOGS

WITHDRAWN

PSD12 SEROEPIDEMIOLOGICAL STUDY OF HEPATITIS E VIRUS (HEV) INFECTION IN LARGE GAME ANIMALS IN THE CENTRE OF PORTUGAL

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To better understand the epidemiology of the Hepatitis E virus (HEV) infection in large game animals of Central Portugal, a cross-sectional study was conducted from 2016 to 2023 in red deer and wild boar. The seroprevalence and risk factors for HEV seropositivity were evaluated. Antibodies against HEV were determined by a commercial ELISA. Risk factors associated with seropositivity to HEV were investigated by comparing the proportion between two risk groups using the Chi-square statistical test. In sera tested from 650 game animals (298 wild red deer and 352 wild boar), 9.1% red deer (95% CI: 6.3-12.9%) and 1.7% wild boar (95% CI: 0.6-3.3%) were positive. Regarding age, the seropositivity in juveniles was 1.3% (95% CI: 0.27-3.72), and in adults was 7.2% (95% CI: 4.9-10.11%). The odds of being seropositive was 5.3 higher in red deer than in wild boar (95% CI: 2.2–12.7%) ($p < 0.000$) and the risk was 5.9 higher in adults than in juveniles (95% CI: 1.72-18.11) ($p = 0.001$). Both wild ungulates were exposed to HEV. The higher seroprevalence in red deer suggests that this species may have a major contribution to the ecology of HEV in the Centre of Portugal.

PSD13 MORE THAN A THOUSAND CATTLE HERDS HAVE ALREADY STARTED THE VOLUNTARY PROGRAM FOR OBTAINING THE BOVINE VIRAL DIARRHEA VIRUS (BVDV) FREE STATUS IN SLOVENIA IN 2023

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Abstract: Bovine viral diarrhoea virus (BVDV) is one of the most devastating viral diseases of cattle with high economic impact on production. The first laboratory testing of BVDV infections started in Slovenia in 1994. In positive herds different problems are observed: infertility, abortions, foetal abnormalities, diarrhoea, persistently infected animals, mucosal disease, reduction of milk production and immunosuppression, leading to respiratory signs and increased severity of other diseases. In 2023, new rules were implemented within voluntary programme in Slovenia according to Commission Delegated Regulation (EU) 2020/689 that prescribe the conditions for recognising, acquiring, and maintaining a BVDV free status also for an individual herd. The principle is based on regular laboratory testing, culling of BVDV positive animals and biosecurity that need to be strictly implemented in an individual herd. At the beginning of the year 2023 a total of 1011 Slovenian cattle holdings has joined to participate in regular laboratory testing with the aim to be officially recognised as BVDV free. Until May 2023 a total of 535 herds have already been screened (the first testing) and 83,92 % of tested herds were BVDV antibody free, while BVDV antibody positive animals were detected in 16,08 % of tested herds. The positive herds need to identify and eliminate all BVDV positive animals if they want to obtain official BVD free status. We have the resources and capacities to eradicate BVDV in Slovenia, but the most important factor for improvement of the current situation is willingness of the cattle owners, to start the program and to prevent further transmissions of BVDV from positive herds. This step will also be important for improving the economic feasibility, sustainability and animal welfare in Slovenian cattle herds.
Keywords: BVDV, control program, diagnostics, free status, cattle

PSD14 SMALL RUMINANT LENTIVIRUS INFECTION IN SHEEP AND GOAT IN NORTH PORTUGAL: SEROPREVALENCE AND RISK FACTORS

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The aim of this research was to quantify the seroprevalence and associated risk factors for Small ruminant lentivirus (SRLV) infection in the north region of Portugal. Serological analysis was performed at Zamora Provincial Animal Health Laboratory. Infection by small ruminant lentiviruses (SRLV) of each sample was determined by a commercial indirect ELISA test (ID Screen® MVV / CAEV Indirect) following the manufacturer's instructions. Variable analysis was performed using the chi-square test (x2) to verify the association between the variables. JMP® Statistical Discovery version 7 software was used for this analysis. A significant effect was considered to $p < 0.05$. A univariate analysis was performed between the independ'nt variables according to the association between the causes of failure and the potential risk factors. Collected samples from a total of 150 herds, of which 129 (86.0%; 95% CI: 80.67% - 91.33%) had at least one seropositive animal. Out of 2607 individual blood samples, 1074 (41.2%) were positive for SRLV. The risk factors associated with SRLV infection were: specie (caprine), age (> 2 years old), herd size (> 100 animals), production system (intensive), production aptitude (milk), type of activity (professional), participation in livestock competitions (yes), buy replacement young ewe (yes) and rearing (natural). This knowledge empowers the implementation of effective preventive measures. Overall, biosecurity measures should be promoted and implemented to aim reducing viral transmission, with the main goal of reducing the prevalence of this disease.

PSD15 FELINE CORONAVIRUS ACTIVATES ARYL HYDROCARBON RECEPTOR DURING INFECTION

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Objectives: The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, is activated by different endogenous and exogenous molecules such as bilirubin, biliverdin, environmental pollutants (dioxin), and microbial metabolites. Recent studies have found that AhR regulates immune response to viral infections, with effects on host resistance and survival. For instance, AhR is involved in the host response to Coronaviruses (CoVs), like McoV, SARS-CoV-2, HcoV 229E, and CcoV infection. Using AhR antagonist substances results in suppression of CcoVs infection in different mammalian host cells. Herein, we show that AhR is activated by infection with Feline CoV (FcoV), an alphacoronavirus, that can infect cats provoking feline infectious peritonitis, a disease fatal if untreated. In addition, *in vitro* replication of FcoV is suppressed by pharmacological inhibition of AhR. **Methods:** Infection of FcoV RVB-1259, a serotype I FcoV strain, in Crandell-Rees Feline Kidney (CRFK) cell line was carried out in the presence of CH223191, a chemical AhR antagonist. Bioscreen, immunofluorescence, and virus yield analyses were performed. **Results:** During FcoV infection, we detect a significant upregulation of AhR, a receptor expressed in CRFK cells. CH223191, at non-toxic doses, decreased cell death features, and enhanced cell

viability. Moreover, the AhR antagonist provoked a reduction in virus yield, and a strong downregulation in the expression of viral nuclear protein.
Conclusions: Overall, our results indicate that infection with FcoV stimulates AhR. In addition, the selective antagonist of AhR decreases *in vitro* replication of FcoV. Thus AhR AhR may be a potential target for therapy against CoVs.

PSD16 DIOXIN MODULATES AHR SIGNALING DURING CCOV INFECTION

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Objectives: Exposure to the persistent pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), commonly known as dioxin, suppresses immune response and decreases resistance to several viruses. Indeed, TCDD can interfere with the replication of both human and animal viruses, like influenza A viruses, coxsackie virus B3, immunodeficiency virus type-1, cytomegalovirus, herpes simplex II, bovine herpesvirus 1 and canine coronavirus (CcoV). TCDD acts biochemically in vertebrate species through the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor and its nuclear translocator (ARNT). It has also been demonstrated that to infect host cells, AhR is regulated by coronaviruses (CoVs), including SARS, mers, SARS-CoV-2, and CcoV. Herein, we investigated how AhR signaling pathway is modulated by dioxin during CcoV infection. **Methods:** Following infection with CcoV (S378) in A-72 cells, in the presence of TCDD (0.01, 1 and 100 pg/Ml), the virus titer and the immunofluorescence staining were performed. **Results:** In uninfected cells, in a dose-dependent manner, TCDD downregulated AhR, probably due to saturation and degradation of receptor, accompanied by increased expression of its transcriptional targets CYP1A1 and CYP1B1, and ARNT1. CcoV infection induced an increase of AhR and CYP1A1, CYP1B1 and ARNT1 expression, compared to uninfected cells. These AhR signaling members were differently modulated by TCDD during infection, as a decrease of AhR and CYP1A1, and an increase of CYP1B1 and ARNT1 levels was observed. **Conclusions:** Taken together, our findings suggest that TCDD and virus may compete for AhR binding, modifying AhR signaling, a crucial pathway to explain the influence of dioxin on the promotion of CcoV replication.

PSD17 NOVEL PARVOVIRUS IN AN OUTBREAK OF FATAL ENTERITIS IN EUROPEAN HEDGEHOGS (*Erinaceus Europaeus*), ITALY, 2022

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European hedgehogs (*Erinaceus europaeus*) are common in Europe and can be found in a variety of habitats. In this study, we describe an enteric disease associated with increased mortality in immature weaned hedgehogs, from 16% the previous years to 53% in 2022, housed in a rescue center in Southern Italy. Initially in June, three animals presenting clinical signs of anorexia and haemorrhagic gastroenteritis were hospitalized and died overnight. Necropsy of the carcasses revealed inflammation of the small intestine, congestion of the mucosa, overextended intestinal loops, as well as congestion of the liver and spleen. Both parasitological and bacteriological analysis were not conclusive. Metagenomics analysis using Nanopore's MinION technology, implemented with 5' RACE protocols and a primer walking strategy, generated the genome of a parvovirus-related strain, namely ITA/2022/hedgehog/265. The genome was 4.4 kb in length and encoded three proteins: accessory protein p15, and non-structural and capsid proteins. Sequence analysis showed 90.4% nucleotide identity at the genome level to a parvovirus identified in Amur hedgehog (*Erinaceus amurensis*) in China. Phylogenetic analyses also revealed a close relatedness to chaphamaparvoviruses detected in bats and rodents. Following the initial case, more hedgehogs were hospitalized presenting similar clinical signs, and died till the end of July. The outbreak died out with the end of hedgehogs breeding season. In conclusion, we identified a novel parvovirus in European hedgehogs with fatal enteric disease. Exploring the virome of wildlife animals is currently recognized as a priority in terms of animal conservation and in the perspective of One Health principles.

PSD18 DETECTION AND MOLECULAR CHARACTERIZATION OF PSEUDORABIES VIRUS STRAINS ISOLATED FROM DOGS IN SLOVENIA

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Pseudorabies (PR) is one of the economically most important diseases in domestic pigs. Since 2010, Slovenia has been free of PR in the domestic pig population, but the disease is endemic in the wild boar population, which can pose a real threat to domestic pigs and other animal species, including dogs. Between 2006 and 2020, infections with the PR virus (PRV) were reported in two pet and three hunting dogs from Slovenia that were found to have had direct contact with wild boar or raw wild boar or pork meat. Typical clinical signs of PRV infection, including characteristic facial itching, cytopathic effect in cell cultures, positive immunocytochemistry and positive PCR results confirmed the presence of PRV in all five cases investigated. Phylogenetic comparison of the partial glycoprotein C (Gc) genomic region revealed that the Slovenian PRV isolates belong to clade A, with 95.78-100% nucleotide identity with strains isolated from dogs, domestic pigs and wild boars from Europe. Phylogenetic comparison of partial glycoprotein D (Gd) and partial glycoprotein E (Ge) genomic regions of Slovenian PRV isolates revealed 100% and 99.12%-100% nucleotide identity, respectively, indicating low diversity among PRV strains identified from dogs in Slovenia. This study provides the first molecular characterisation of PRV in dogs and suggests that similar PRV strains circulate in wild boar populations in this geographical area.

PSD19 RETHINKING HUMAN WEST NILE VIRUS PREDICTION IN NORTHERN ITALY WITH MACHINE LEARNING

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Objectives: West Nile virus (WNV) is an emerging mosquito-borne neurotropic virus, belonging to the *Flaviviridae* family and the Flavivirus genus. Neural Network (NN) and Deep Neural Network (DNN) are powerful algorithms for solving classification problems. The qualitative assessment of specific environmental indices, possible indicators for WNV outbreaks, have not been adequately evaluated. Therefore, this study implemented a model using NN and DNNs which inputted vegetation, temperature, and soil moisture indices of the Emilia-Romagna Region of Italy over a ten-year period. **Methods:** Remote-sensed images (raster) were obtained from Landsat 8 data repository from 2013 to 2022, and annual surveillance human WNV case report obtained from the ECAD Information System. Transformed mean annual spectral indices computed for vegetation cover (NDVI), surface temperature (LST), soil moisture content (SMI), and the human WNV cases were imported as matrix and vector respectively for machine learning implementation. **Results:** The designed NN attained a predictive accuracy of 70% for the classification problem of either a high or low human WNV cases for the study period in comparison with the traditional regression model with an accuracy of 7%. Using the median labelled value for output classification significantly improved the NN performance. The DNN implementation for raster images is ongoing. **Conclusion:** The findings of this study quantitatively evaluated the impact of environmental predictors of human WNV cases and provide a promising perspective to model prediction through machine learning inputting more divergent unlabelled variables. Also, it highlighted the need for continuous disease surveillance and validation.

PSD20 ORAL UPGRADED

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