SCIENTIFIC ABSTRACTS

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Submitted Abstracts

Invited Speakers

(IS1) EMERGING ZOONOTIC VIRUSES: NAVIGATING THE RED QUEEN EFFECT WITH ONE HEALTH STRATEGIES

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In the ever-evolving landscape of zoonotic viral diseases, we are constantly engaged in a catch-up game akin to the *Red Queen effect*, where we must "*run as fast as we can just to stay where we are.*" Multi-host RNA viruses, such as coronaviruses and influenza viruses, have a broad species tropism due to their ability to utilize host receptors conserved across multiple species. As these viruses move between species, they adapt and evolve, presenting ongoing challenges for detection, control, and prevention. The extensive animal-human circulation of SARS-CoV-2 and the recent unprecedented clade 2.3.4.4b H5N1 avian influenza virus infections in cattle, followed by subsequent cattle-to-human infections, have all surprised us. The continued evolution of SARS-CoV-2 and H5N1 viruses, along with the emergence of novel variants with altered epidemiological features, underscores the Red Queen effect challenge for effective surveillance and monitoring. This seminar will highlight recent case studies of cross-species transmission of SARS-CoV-2 and H5N1 in the USA, examine the role of host receptors in the host and tissue tropism of multi-host RNA viruses, and explore the implications for virus adaptation and evolution. The discussion will emphasize the importance of a One Health approach to monitor the transmission dynamics of multi-host zoonotic viruses and to develop proactive mitigation strategies to protect animal and human health.

Oral Presentations

(O1) OUTBREAK OF ASPERGILLUS FUMIGATUS IN SYRIAN HAMSTERS DURING A VACCINE EFFICACY STUDY

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Introduction: Like most emerging fungi, *Aspergillus fumigatus* is considered to be an opportunistic pathogen and to be non-transmissible between hosts. Disease results from the airborne uptake of conidia from the environment. In animals, *A. fumigatus* is primarily a respiratory infection that may become generalized. Although hamsters have been used as an animal model for fungal infections, spontaneously occurring fungal disease in hamsters is rare. We describe an outbreak of *A. fumigatus* among Syrian hamsters, which were part of a vaccine efficacy study for SARS-CoV-2.

Case: At Day 50 of the trial, several hamsters in both the placebo as well as the vaccinated group, started to show lesions around the mouth, weight loss and reduced responsiveness. Several hamsters died after anesthesia. Post-mortem investigation including histopathology was performed and a pyogranulomatous pneumonia and invading septate hyphae in the lungs were observed. *A. fumigatus* was cultured from lung specimens. As several factors would interfere with the validity of the study, the study was terminated on Day 151.

Discussion: Following the diagnostic findings, an investigation was started, but the source of infection could not be identified. We hypothesize that the duration of this study might have contributed to the development of aspergillosis on a clinical and pathological level, as the first clinical signs were observed around Day 50. It is possible that the relatively long study allowed the aspergillosis to develop, but the source and time of exposure as well as other contributing factors still remain unclear.

(O2) AN OUTBREAK OF SERRATIA MARCESCENS IN A VETERINARY HOSPITAL

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The objective of this work is to describe a recent outbreak of *Serratia marcescens* in a small animal referral hospital, including transmission of infection and control measures implemented. Case records, standard microbiological culture and antimicrobial resistance phenotyping using VITEK 2™ were used to identify a possible cluster of infection with *Serratia marcescens* in cats in the UCD Veterinary Hospital (UCDVH) . Subsequent spread to dogs and environmental contamination were monitored using routine culture methods supplemented with the use of Serratia CHROMagar™. Selected isolates were subjected to whole genome sequencing (WGS) to investigate genetic relatedness.

Serratia marcescens was detected in a total of 34 animals, 13 cats and 21 dogs. Examination of culture results and case records suggested 13 clinical infections, 7 probable infections and 14 cases of colonisation. Contamination of the detergent and disinfectant solutions was detected and was likely a major factor in the spread of this organism. The original source of contamination was not identified. WGS typing of selected isolates confirmed the clonal nature of many clinical and environmental isolates; some environmental isolates were unrelated. Control measures included adoption of new cleaning and disinfectant products, commercial deep cleaning and upgrades to infrastructure, including sinks. The associated costs to the hospital were substantial.

The occurrence of this outbreak suggested that compliance with protocols, including those for hand hygiene and for decanting of cleaning and disinfectant products, had deteriorated. This poor compliance, together with some deficiencies in infrastructure were likely in large part responsible for the outbreak.

(O3) EPIDEMIOLOGICAL INVESTIGATIONS AND MANAGEMENT OF A SALMONELLA OUTBREAK IN THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF TURIN (ITALY)

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Salmonella enterica subspecies *enterica* includes numerous non-typhoidal Salmonella (NTS). *Salmonella* serovar Kentucky, initially isolated from poultry, has been found in animals and humans globally. While often causing self-limiting gastroenteritis, it poses severe risks to vulnerable individuals. Sequence type (ST) 198, an ESBL+ multi-drug resistant (MDR) strain, is particularly concerning and has been identified as frequent in human cases. NTS spreads through faeces and body fluids of carriers and infected subjects. It thrives in wet, dark conditions but can survive in dry environments if organic material persists.

This study investigates a *Salmonella* Kentucky outbreak and describes communication obstacles in absence of an early-warning system.

From September 2022 to May 2023, more than ten canine, feline, and equine specimens at the University of Turin's Veterinary Teaching Hospital tested positive for *Salmonella* spp. Clinical and environmental samples were analysed using selective agars, biochemical reactions, MALDI-ToF spectroscopy, and serotyping. Antimicrobial resistance (AMR) was assessed via agar disk diffusion and minimum inhibitory concentration. Ten isolates underwent whole-genome sequencing (WGS) at the National Reference Center for Salmonellosis. Core-genome Multi Locus Sequence Typing was performed.

The outbreak was confirmed by identical AMR profiles and phenotypic evidence: all isolates were S. Kentucky, resistant to aminopenicillins, cephalosporins, fluoroquinolones, tetracyclines, and gentamycin,

but susceptible to carbapenems, amikacin, chloramphenicol, and sulfamethoxazole-trimethoprim. WGS confirmed all isolates as MDR, ESBL+ ST198 strains, with ≤5 allelic differences.

The outbreak's persistence was linked to environmental resistance on difficult-to-disinfect surfaces. Countermeasures were taken in accordance with clinicians to resolve the outbreak and prevent further spread.

(O4) CHARACTERIZATION AND COMPARISON OF CLOSTRIDIUM PERFRINGENS ISOLATES FROM AN OUTBREAK OF ACUTE HEMORRHAGIC DIARRHEA IN DOGS IN NORWAY

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In the fall 2019, an unusual rise in severe acute hemorrhagic diarrhea (AHD) in dogs occurred in Southeastern Norway. The outbreak received broad public attention and investigations focused on the identification of a common cause. Two bacteria were most prevalent: *Providencia alcalifaciens* and *Clostridium perfringens*. Whole genome sequencing (WGS) of *P. alcalifaciens* revealed that most strains belonged to the same clone and the investigations concluded that it is thus the most likely cause. Nevertheless, *C. perfringens*, particularly strains capable of producing entero- and NetF-toxin, reportedly play a role in AHD syndrome. Therefore, this study aimed to characterize *C. perfringens* isolates from dogs from the AHD outbreak and a healthy control group to determine if a predominant *C. perfringens* clone contributed to the outbreak.

Isolates from 62 diseased dogs and 18 healthy controls were included. All isolates were subjected to real-time PCR targeting genes encoding the toxins alpha, beta, entero, NetF and NetG, and WGS was conducted on 20 isolates. The Bifrost pipeline was used for genotyping and multi-locus sequence typing, and core gene analysis was performed using the ALPPACA pipeline to explore phylogenetic relationships.

Isolates from diseased dogs had a higher prevalence of the enterotoxin gene than isolates from healthy dogs, with some also carrying genes encoding NetF and NetG. Phylogenetic analysis revealed substantial genetic diversity among the isolates, showing a wide range of sequence types. The phylogenetic diversity and absence of a dominant virulent clone indicated that *C. perfringens* was not a main driver of the 2019 AHD outbreak.

(O5) CUPRIAVIDUS GILARDII IN CALF DIARRHEA OUTBREAK: A POTENTIAL ZOONOTIC PATHOGEN (?).

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Objectives: *Cupriavidus gilardii* is an aerobic, gram-negative, motile, glucose-nonfermenting bacillus, first described in 1999. Typically, it exhibits low pathogenicity in humans, causing opportunistic infections primarily in individuals with compromised immune systems. This bacterium has been also found in various environmental sources such as plants and contaminated soils. Notably, there have been no documented cases of *C. gilardii* infections in animals. This study aims to report the first isolation of *C. gilardii* in a bovine neonatal diarrhea outbreak that occurred in Northern Greece.

Methods: During the outbreak, fecal samples were collected from 5-day-old calves and transported to the laboratory for further examination. The samples were cultured for bacterial isolation and the presence of *C. gilardii* was confirmed using Oxford Nanopore Technology sequencing. Following its identification, an autogenous vaccine was produced from the isolated strain and administered to the cows in the affected farm.

Results: *C. gilardii* was the only species isolated at higher dilutions in the fecal samples. Post-vaccination monitoring revealed a progressive reduction in the incidence of calf diarrhea and mortality, culminating in the complete resolution of the outbreak.

Conclusions: This study presents the first documented case of *C. gilardii* isolation from bovine neonatal diarrhea and any animal sample. The successful mitigation of the outbreak following vaccination suggests that *C. gilardii*, while previously considered an opportunistic pathogen in humans, can contribute to clinical symptoms in animals. These findings highlight the need for further research into the pathogenic potential of *C. gilardii* in veterinary contexts and its role as a zoonotic pathogen.

(O6) HOST-PATHOGEN INTERACTIONS IN CALVES CO-INFECTED WITH INFLUENZA D VIRUS AND MYCOPLASMA BOVIS: PROTEOMIC AND LIPIDOMIC INSIGHTS

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Calves experimentally co-infected with Influenza D virus (IDV) and *Mycoplasma bovis* (M. *bovis*) showed increased clinical signs and more extensive pathological lesions compared to calves infected with either pathogen alone. This study aimed to investigate host-pathogen interactions through proteomic analyses (PA) and lipid mediator kinetics during these infections. PA were performed on bronchoalveolar lavage (BAL) samples collected 2 days post infection (dpi) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Additionally, lipidomic analyses were conducted on BAL samples collected at 2, 7, and 14 dpi using LC-MS/MS. Whereas M. *bovis* induced the expression of proteins involved in fibrin formation, co-infection with IDV counteracted this coagulation mechanism and downregulated other proteins of the acute phase response, such as complement component 4 (C4) and plasminogen (PLG). The reduced inflammatory response to M. *bovis* and associated to IDV co-infection likely resulted in enhanced M. *bovis* replication and delayed M. *bovis* clearance, leading to significantly elevated oxylipid levels in co-infected calves. The identified induced oxylipids mainly derived from arachidonic acid; were likely oxidized by COX-1, COX-2, and LOX-5; and peaked at 7 dpi. This study provides information regarding the proteome and lipid mediator kinetics in response to IDV and M. *bovis* infections in cattle but also insights into the role of IDV as a co-pathogen in bovine respiratory disease.

(O7) EVOLUTIONARY DYNAMICS OF THE GI-19 LINEAGE OF INFECTIOUS BRONCHITIS VIRUS (IBV): A COMPARATIVE ANALYSIS OF GLOBAL SPREAD, EVOLUTION AND CONTROL STRATEGIES

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Infectious bronchitis virus (IBV), a member of the species *Avian coronavirus*, is a rapidly evolving virus whose variants are classified into lineages and genotypes. Among those, the GI-19 lineage is one of the most impactful. Originating in China several decades ago, it has consistently spread and evolved, forming independent clades in regions featured by distinct production systems and control strategies. Through the analysis of the complete S1 sequence, four datasets were identified, comprising strains of monophyletic clades circulating in different continents or countries (e.g., Asia vs. Europe and China vs. Thailand), indicative of single introduction events and independent evolution. The population dynamics, evolutionary rate, and selective pressures were compared across these datasets to explore how different environments may influence virus evolution. Since the lineage origin, a more persistent and stable viral population was estimated in Asia and China, while in Europe and Thailand a sharp increase following the introduction was followed by a rapid decline. This evidence, evaluated considering the variable control strategies implemented in different areas, suggests a strong link between effective, systematic vaccine implementation and infection control. Moreover, more focused and stronger pressures were evident in both the European and Thai strains, likely reflecting a more intense vaccine-induced selection. However, a significant inverse correlation was found between viral population size and the rate of viral evolution over time. Therefore, despite the stronger selective pressure imposed by vaccination, effectively constraining the viral population size through adequate control strategies can efficiently prevent viral evolution and the emergence of vaccine-escaping variants.

(O8) DETECTION OF MERS-RELATED BETACORONAVIRUS IN DOMESTIC HEDGEHOGS (HEMIECHINUS AURITUS) IN ITALY

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The recent discovery of MERS-related coronaviruses (CoVs) in wild hedgehogs belonging to the genus *Erinaceus* has suggested the need of CoVs surveillance among hedgehog species. This study aimed to investigate the presence of CoVs in hedgehogs kept as pets in Italy. Fecal samples were screened using a pan-CoVs PCR targeting partial sequence of the RNA-dependent RNA polymerase (RdRp) gene. One CoV-positive sample was submitted to whole genome sequencing using Illumina RNAseq. After quality filtering, reads were assembled using coronaSPAdes. Maximum likelihood phylogenetic trees were reconstructed using IQ-TREE. RDP5 was used to detect recombination events. CoV positivity was observed in 2/25 (8%) hedgehogs. Particularly, CoVs were detected in 2/3 (66.7%) *Hemiechinus auritus*, (long eared hedgehog) while all the 22 tested *Atelerix albiventris* (African pygmy hedgehog) were negative. The complete CoV genome obtained in this study had the closest nucleotide similarity (85.3%) with *Hedgehog coronavirus 1* (EriCoV) from *Erinaceus europaeus* (west European hedgehog) previously reported in Europe. Complete genome and RdRp gene phylogeny showed that the CoV clustered with other representative species of EriCoV identified in Europe, within subgenus *Merbecovirus* of the genus *Betacoronavirus*, whereas complete Spike gene phylogeny showed that the CoV clustered with other EriCoVs identified in China in *Erinaceus amurensis (Amur hedgehog)*. No recombination events were observed. Our results demonstrating the presence of MERS-related CoVs in *Hemiechinus auritus* support the circulation of CoVs along a phylogenetic continuum among different species of hedgehogs and geographic locations, suggesting the need of further CoVs surveillance in both domestic and wild animals.

(O9) ESSENTIAL ROLE OF MATURE NS3 IN GENOME PACKAGING OF CLASSICAL SWINE FEVER VIRUS (CSFV)

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Classical swine fever virus (CSFV) is a member of the genus *Pestivirus* within the family *Flaviviridae*. The enveloped particles contain a plus-stranded RNA genome encoding a single large polyprotein. Throughout the infection cycle, the processing of this polyprotein undergoes dynamic changes. NS3 is a multifunctional viral enzyme exhibiting helicase, NTPase, and protease activities crucial for viral replication. The release of mature NS3 from the polyprotein is mediated and regulated by the NS2 autoprotease and a cellular co-factor, restricting cleavage to the early phase of infection. The release of mature NS3 fuels replication, while unprocessed NS2-3 precursors are vital for progeny virus production in later phases of infection. Thus far, no packaging signals have been identified for the pestivirus RNA molecule. To explore the prerequisites for particle assembly, trans-packaging experiments were conducted using CSFV subgenomes and coreless CSFV strains. Intriguingly, we discovered a significant role of mature NS3 in genome packaging, effective only when the protein is encoded by the RNA molecule itself. This finding was reinforced by employing artificially engineered CSFV strains with duplicated NS3 genes, separating uncleavable NS2-3 precursors from mature NS3 molecules. The model for the genome packaging of pestiviruses appears to be much more complicated than anticipated, involving distinct functions of the mature NS3 and its precursor molecule NS2-3. Moreover, the reliance of genome packaging on cis-encoded NS3 may act as a downstream quality control mechanism, averting the packaging of defective genomes. Understanding the viral genome packaging mechanisms might uncover new targets for antiviral therapies.

(O10) NOVEL PAPILLOMAVIRUSES IN HORSES WITH AURAL PLAQUES

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Equine aural plaques (EAPs) are caused by papillomaviruses. At least ten equine papillomavirus (*Equus caballus* PV, EcPV) types have been described thus far, although only EcPV-1, -3, -4, -5, and -6 have been associated with EAPs. In this study we report the discovery of novel types of EcPVs in horses with EAPs. Samples were collected from the internal pinnae of 22 horses, displaying both unilateral (n=19) and bilateral (n=3) lesions. DNA extracts from 25 EAP samples were screened using three pan-papillomavirus PCR protocols (targeting the L1 and E1 genes) and the amplicons were sequenced. Samples of interest were enriched with rolling circle amplification (RCA) and sequenced with the Oxford Nanopore Technologies (ONT™) platform. All samples were re-screened using qPCR assays specific to the detected EcPVs. Overall, 72% (18/25) of the samples were positive for EcPVs by pan-papillomavirus PCRs. Sequencing of the amplicons identified EcPV-6 (8/18; 44.4%), EcPV-4 (5/18; 27.8%), and EcPV-3 (5/18; 27.8%) as the most common types. Whole genome sequences of 16 EcPV strains were generated. Nine strains showed high nucleotide (nt) identity to known EcPVs (EcPV-3, EcPV-4, EcPV-6), and seven strains were classified into five novel putative EcPV types. By qPCR, the infection rate reached 92% (23/25), with 91.3% of EcPV-positive samples being co-infections of different types. Based on our findings, at least 20 types of PVs are present in horses, including three bovine PVs and two donkey PVs. Epidemiological investigations are required to assess the epidemiological and clinical relevance of these novel EcPVs.

(O11) ISOLATION, IN VITRO CHARACTERIZATION AND EFFICACY ASSESSMENT IN THE GALLERIA MELLONELLA MODEL OF FOUR BACTERIOPHAGES TARGETING AEROMONAS SALMONICIDA

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Objectives: The Gram-negative bacteria *Aeromonas* (*A.*) *salmonicida* is a primary fish pathogen that causes furunculosis in salmonids as well as septicemia in a variety of fish. As this disease is responsible for significant losses in aquaculture worldwide, the aims of this study were to isolate new bacteriophages (phages) targeting this bacteria, phenotypically and genomically characterize them and finally assess their potential for phage therapy in a preliminary *in vivo* model.

Methods: Different water sources were collected in Belgium and France to isolate *A. salmonicida*-specific phages. Isolated phages were characterized *in vitro* for their ability to infect other *A. salmonicida* strains and their stability at different temperatures and pH. Phage morphologies and genomes were investigated by electron microscopy and Illumina sequencing. The safety and efficacy of these phages were finally evaluated using *Galleria (G.) mellonella* larvae.

Results: In 2022, four new phages targeting *A. salmonicida* were isolated. Among the characterization results, genomic analysis showed that 3 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. All four presented a myovirus morphotype. Four-day efficacy experiments in *G. mellonella* larvae showed that three of these phages were responsible for a significant extension in the larval survival time at the 2 treatment doses tested (MOI 10 and 100).

Conclusion: In light of these results, these phages targeting *A. salmonicida* could represent potential new candidates for the development of anti-furunculosis phage treatments in aquaculture.

(O12) FUNCTIONAL GUT MICROBIOME CHANGES AT DIFFERENT STAGES OF SWINE DYSENTERY

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Swine dysentery (SD) is one of the most relevant enteric porcine diseases, characterized by a mucohaemorrhagic diarrhoea associated mostly with *Brachyspira hyodysenteriae*. Little is known about the changes that occur in large intestine microbiome during the disease. The objective of this study was to characterise the functional differences of the gut microbiome in early and acute SD.

Thirty-two 7-weeks piglets were randomly allocated into control (n=16) and infected (n=16) groups, the last orally challenged in three consecutive days with 10^8 *B. hyodysenteriae*. Faeces and apex content from early infection (n=8), 24h after the first qPCR positive faeces to *B. hyodysenteriae*, or by the onset of mucohaemorrhagic diarrhoea, acute infection (n=8), were sequenced by shotgun metagenomics.

Structural analysis of vector spatial distribution regression ($P<0.05$) and PERMANOVA ($P=0.001$) tests showed the influence of group in the sample ordination. Ordination differences were observed between control and early infection compared to acute infection group. Acute infection group showed an increase of microbiota functions associated with inflammation in colon, such as Fatty Acid Biosynthesis FASII,

Histidine Biosynthesis or Branched-Chain Amino Acid Biosynthesis, as well as Multidrug Resistance Efflux Pumps, Ton and Tol transport systems, Rhamnose containing glycans or D-Galacturonate and D-Glucuronate Utilization, function associated with bacterial colonization and adaptability.

The differences found in the acute infection group provide relevant information for the knowledge of the pathogenesis and involvement of the microbiome in pigs suffering SD, showing the functional impairment of the intestinal microbiota composition and functionality.

(O13) MICROBIOTA ANAEROBIC BACTERIA VS BRACHYSPIRA HYODYSENTERIAE: THE BREAKTHROUGH OF NEW PROBIOTICS.

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Brachyspira hyodysenteriae (*B. hyo*) is the etiological agent of swine dysentery (SD), characterized by severe mucohaemorrhagic diarrhoea and significant economic losses due to mortality, production losses, and treatment costs. Increasing antimicrobial resistance has prompted research into new 'next generation probiotic' strategies as a tool to prevent enteric infections. This study preliminarily evaluated the probiotic potential of commensal anaerobic bacteria from pigs and wild boars both in vitro and in vivo using a competitive exclusion model.

22 isolates were co-cultured with *B. hyo* B204 strain. Isolates with higher inhibitory effects were further evaluated for the efficacy of their bacterial-free supernatants (CFS) on *B. hyo* growth. Six isolates including *Bifidobacterium thermoacidophilum* C2/23-YB69, *Lactobacillus mucosae* C2/23-CM96, *Intestinibaculum porci* C2/23-CM6, *Megasphaera elsdenii* C6/22-ME7, *Clostridium butyricum* CECT361, and *Christensella minuta* DSMZ-22607, were selected for an in vivo assay in pigs (n=15) to analyse their colonization capacity with and without a prebiotic supplementation (inulin).

18 of 22 isolates inhibited *B. hyo* growth in broth co-culture. Seven isolates, such as C2/23-YB69 and CECT361, limited *B. hyo* growth by over two log units. Five isolates reduced *B. hyo* by one to two log units in CFS co-culture. In the in vivo assay, four of the six strains showed significant differences between probiotic+inulin and inulin-only groups compared to controls. Only C2/23-YB69 strain showed significant differences between probiotic+inulin and inulin-only groups.

This study demonstrates the competitive activity of certain anaerobic gut commensals against *B. hyo*, potentially through nutrient competition and antimicrobial metabolites. However, the tested isolates showed poor colonization compared to the microbiota-promoting effects of inulin. Alternative probiotic supplementation strategies should be explored to assess these strains' competitive exclusion abilities under *in vivo* conditions.

(O14) TEN-YEAR ANTIMICROBIAL RESISTANCE TREND IN UROPATHOGENIC ESCHERICHIA COLI (UPEC) ISOLATED FROM DOGS AND CATS

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Urinary tract infections (UTIs) are a common occurrence in cats and dogs. A comprehensive awareness of antibiotic resistance trends is essential for the prudent selection of suitable antimicrobial agents. However, there is limited available data on this matter in Italy. This retrospective study aimed to investigate the trends of antimicrobial resistance in uropathogenic Escherichia coli (UPEC) isolated from cats and dogs over a ten-year period (January 2014 to October 2023).

A total of 339 UPEC strains were isolated from urine samples submitted to the Veterinary Teaching Hospital of Torino (Italy). Antimicrobial susceptibility testing was conducted via agar disk diffusion for up to

11 classes of antibiotics, categorized in four categories (A, B, C and D) following the EMA guidelines for prudent antimicrobial use in animals.

As expected, the results reveal a higher resistance towards compounds in categories C and D. Conversely, more favourable outcomes were observed in categories B, and, notably, A. The resistance has steadily increased since 2014. However, it is noteworthy that, starting from 2019/2020, a significant decline in resistance was observed in categories A to C.

These findings suggest that either antimicrobial-use regulation enforced in our country and education aimed to increase awareness of the threat posed by antimicrobial resistance among veterinarians contributed to mitigating antibiotic resistance in companion animals.

(O15) ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF EUCALYPTUS GLOBULUS LEAVES EXTRACT AND ITS MAIN COMPOUNDS ON MASTITIS PATHOGENS OF DAIRY COWS.

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Antibiotics are the main therapy for bovine mastitis, but the increase in antimicrobial resistance has improved the use of alternative molecules. In this study, were evaluated the Minimal Inhibitory Concentration (MIC) and Minimal Inhibitory Biofilm Concentration (MBIC) of *Eucalyptus globulus leaves* (EGL-L) extract, Ursolic (UA) and Asiatic acid (AA) against *Staphylococcus aureus* (SA), *Streptococcus uberis* (SU), *Streptococcus agalactiae* (SAG), and *Enterococcus* spp. (EN) reference and field strains isolated from infected cows. The ability to produce biofilm was assessed for every strain. Statistical analysis was conducted to evaluate differences in MIC values among bacterial groups for each compound using SPSS software, employing the Kruskal-Wallis non-parametric test and post-hoc analysis with pairwise comparisons of ranks means using Bonferroni correction. No significant differences were found for MIC values of EGL-L among the bacterial groups. For UA significant differences were observed, between SA and SU/SAG groups ($P<0.001$), while for AA, between SU and SA groups ($P<0.001$). The highest number of moderate and strongly adherent strains was found in SA (6/15) and SAG (8/17) groups. Regarding antibiofilm activity, only 5/32 strains had MBIC80 values above 256 µg/mL (maximum tested) for both UA and AA. However, 12/32 and 11/32 strains showed MBIC80 ≤ 8 µg/mL for UA and AA, respectively. For EGL-L, 17/32 strains exceeded 2000 µg/mL (maximum tested) and 6/32 ≤ 64 µg/mL. In conclusion, all compounds exhibited antimicrobial activity; however, UA and AA were more effective on Streptococci than Staphylococci. Additionally, both UA and AA were effective on moderate and strong biofilm producer strains.

(O16) PRESENCE OF NINE RESPIRATORY PATHOGENS IN CALVES THAT DIED DUE TO MANNHEIMIA HAEMOLYTICA POLYSEROSITIS.

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Polyserositis in calves is often caused by *Mannheimia haemolytica* serotype 2. Since *M. haemolytica* serotype 2 is commonly known as relatively non-pathogenic, it was questioned if other respiratory pathogens might be involved in inducing invasion of this bacterium.

To answer this question, lower airway samples were taken from 51 deceased calves that were diagnosed for having *M. haemolytica* polyserositis and multiplex PCR testing was performed. As control, the same PCR-testing was done of 49 dead calves with another diagnosis. In controls, the prevalences were; *M. haemolytica* serotype 2: 8% , *M. haemolytica* serotype 1/6: 23%, *Mycoplasma bovis*: 33%, *Pasteurella multocida*: 21%, *Histophilus somni*: 0%, bovine coronavirus: 19%, bovine respiratory syncytial virus: 18%,

influenza-D virus: 6% and parainfluenza virus type-3: 20%. The prevalences in calves with polyserositis were not significantly different for most of the pathogens, higher for *M. haemolytica* serotype 2 ($p<0.01$) and *P. multocida* ($p<0.01$) and lower for *M. haemolytica* serotype 1/6 ($p<0.01$). In controls, presence of *M. haemolytica* serotype 1/6 was correlated with *P. multocida*, bovine coronavirus with *P. multocida* and BRSV with PI3 virus.

The different respiratory pathogens, and especially *M. bovis* were widely distributed in veal calves. Only *P. multocida* presence was increased in polyserositis cases, but if *P. multocida* is causal in the induction of *M. haemolytica* polyserositis remains to be elucidated.

(O17) NANOPORE ADAPTIVE SAMPLING TO EVALUATE GAMMARETROVIRUS DIVERSITY IN DOMESTIC CATS

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Domestic cats are infected by both exogenous (XRV) and endogenous (ERV) gamma-retroviruses. The exogenous viruses are represented by Feline Leukemia Virus (FeLV), which has a worldwide distribution and can be horizontally and vertically transmitted among domestic cats. FeLV causes a wide range of pathologies, primarily associated with hematopoietic disorders and neoplasia. The endogenous (copies of virus in the cat genome) include ERV-DC (Endogenous Retrovirus Domestic Cats), enFeLV (endogenous Feline Leukemia Virus) and FcERV-gamma4 (*Felis catus* endogenous gammaretrovirus 4). Although these viruses cannot be transmitted or cause disease by themselves, they can recombine with exogenous viruses, producing more virulent variants with worse clinical outcomes (FeLV-B, D, E, XR).

This study applied the nanopore adaptive sampling (NAS) strategy using Oxford Nanopore Technologies sequencing platforms to enrich and identify complete genomes of gamma-retroviruses in Chilean domestic cats. Sequences of ERV-DC, FeLV-A, FeLV-B, enFeLV and FcERV-gamma4 were identified. FeLV-A sequences showed 94% similarity with reference sequences. Endogenous sequences were consistent (90-98% similarity) with reports from cats in Japan and the United States. EnFeLV showed a higher level of sequence variation compared with ERV-DC, likely because enFeLV is a more recent endogenization event compared to ERV-DC and is polymorphic between cats.

Recombination between XRV and ERV is determined by the expression of ERVs and by the chance of interaction with XRV during infection. The high prevalence of FeLV in Chilean cats, may increase the number of recombinants detected in this population with analysis to detect these ongoing.

(O18) DISCOVERY OF A NOVEL ERYTHROPARVOVIRUS IN CATS RELATED TO HUMAN PARVOVIRUS B19

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Erythroparvoviruses (EPVs) have been identified in humans (parvovirus B19), nonhuman primates, seals, squirrels, and cows. In healthy immunocompetent children, parvovirus B19 causes infectious erythema (fifth disease), while in adults it can be associated with acute polyarthropathy. In the present study, we report the identification of a novel EPV in cats. Two feral cats from the same colony were presented to the veterinary clinic for weakness, weight loss, and anorexia. The cats were part of a study on feline hepatotropic viruses (collection A, 43 animals). On metaviromic investigation, parvoviral reads were identified in the sera of the two cats. The feline EPV (FeEPV) genome was 5.3 kb long and had an organization similar to other EPVs. In the ORF1 (nonstructural proteins) and ORF2 (VP1/VP2 precursor) the feline virus displayed 44.3% and 48.1% nt identity to human parvovirus B19. Based on genome

comparison, the two FeEPV strains were highly related (>99.9% nt identity) to each other and clustered with chipmunk parvovirus on phylogenetic analysis. Using a quantitative PCR assay, FeEPV was also identified in an additional ten cats (prevalence 27.6%, 12/43) from collection A and in 15/1150 (1.3%) of archival sera collected for presurgical or routine clinical evaluations (collection B). The findings of our study extend the list of known parvoviruses in the feline host. Since EPVs have been associated with a broad range of clinical signs in human and non-human primates, alone or in conjunction with immuno-suppressive pathogens/conditions, it will be important to investigate the pathogenic role of FeEPV in cats.

(O19) IDENTIFICATION OF THE FELINE CIRCOVIRUS 1 IN DOMESTIC CATS: A MULTICENTRIC EUROPEAN EPIDEMIOLOGICAL STUDY.

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Members of the family *Circoviridae* (circoviruses) are small, non-enveloped viruses with a circular, covalently closed DNA genome of about 1.7 to 2.1 kb length. The family *Circoviridae* includes the genera *Circovirus* (CV) and *Cyclovirus* (CyV), which have been identified in several animal species and in human specimens. CVs are associated with severe disease in pigs and birds and with respiratory and gastrointestinal disorders and systemic disease in dogs.

Feline CV-1 (FeCV-1) was first reported in 2023 in fecal samples of cats from different geographical areas of Italy. FeCV-1 was distantly related genetically to a mongoose CV (<79% nt identity) and it was suggested that cats could be a primary host for this CV. The present epidemiological study investigated the prevalence of FeCV-1 in samples (rectal and oropharyngeal swabs, and blood) collected from domestic cats from Greece, Romania, and Portugal between 2015 and 2023. A quantitative PCR assay specific for feline CV-1 was used for molecular screening of samples.

Overall, 20 (10.7%) out of 186 samples tested positive for FeCV-1 with a prevalence of 20.4% (10/49) in Greek samples, 11.4% (5/44) in Romanian samples and 5.4% (5/93) in Portuguese samples. FeCV-1 was repeatedly detected in fecal, respiratory, and blood samples. The whole genome sequence was generated for eight strains. The FeCV-1 strains shared 95.2% to 99.6% nt identity, forming a well-conserved clade, regardless of the geographic origin. Further research would be required to understand the pathogenic role, if any, of FeCV-1 in cats.

(O20) SHEDDING PATTERNS OF DOMESTIC CAT HEPADNAVIRUS IN JUVENILE CATS

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The *Hepadnaviridae* family comprises circular DNA viruses, including human hepatitis B virus (HBV) and the recently discovered domestic cat hepadnavirus (DCH), associated with chronic liver disease in cats. The dynamic of acute DCH infection in feline populations is still unclear. In this study we investigated DCH infection in cats in the early reproductive stage (6-12 months old), assuming that cats positive at this age are likely in the acute phase of the infection. During spaying surgery, serum, mucosal swabs (oral, nasal, rectal, vaginal), and ovarian tissue were collected from 77 animals. Samples were screened for DCH by a specific real-time PCR. Five cats tested positive in the serum (15.7-27.8 Ct). DCH DNA was also detected in the mucosal swabs (26.2-42.1 Ct) of the five cats and in the ovarian tissues of a single animal. All the animals remained positive in the sera (17.9-24.5 Ct) and swabs (32.9-41.1 Ct) when re-screened after 3 months. Based on whole genome sequencing, the four strains fell into two different sub-clusters within clade II. The prolonged duration of acute infection in cats resembles the patterns observed in HBV-infected

human patients. Likewise, the detection of DCH in genital swabs suggests that the virus may be shed via the genital route for long periods, implying possible sexual and perinatal transmission, as observed for HBV. To summarize, this study provides evidence for prolonged DCH shedding in young cats, suggesting that screening in early reproductive stages could be helpful for effective prevention and control strategies.

(O21) ANTIVIRAL ACTION OF FUNGAL METABOLITES AGAINST CANINE CORONAVIRUS INFECTION

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Objectives: Canine coronavirus (CCoV), an alphacoronavirus, causes self-limiting enteritis in dogs. Due to remarkable plasticity of CoVs, mutation and recombination events can develop generating new variants/strains. In the last years, extremely virulent strains have been detected in puppies, and the recent identification of new recombinant canine-feline alphacoronaviruses isolated from humans highlights the ability of CCoV to overcome the species barrier. In this context, developing antiviral compounds certainly may have potential applications in the treatment of CoVs infection, and fungi represent a promising source of novel drugs with a broad spectrum of activities. Funicone-like compounds are a homogeneous group of fungal secondary metabolites (i.e., 3-O-methylfunicone, penisimplicissin and vermistatin), obtained from an isolate of Talaromyces pinophilus, with extraordinary biological activities that have promoted its possible pharmaceutical use. The present study provides data on the antiviral properties of these compounds towards CCoV-II infection in canine cells (A72).

Methods: Chromatographic techniques, bioscreen *in vitro*, cytomorphological analysis, immunofluorescence assay, molecular docking.

Results: the non-toxic dose of these funicone-like compounds increased features of cell viability and reduced virus yield. In addition, a significant down-regulation in the expression of viral nuclear protein and a strong inhibition of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor which is activated during CCoV infection, were found. Interestingly, funicones interact with the same binding pocket recognized by CH223191, a known chemical AhR inhibitor.

Conclusions: Taken together, our findings show the potential antiviral properties of the tested molecules during CCoV infection, identifying AhR as a possible candidate target for antiviral therapy.

(O22) EXTENDING THE HOST RANGE SPECTRUM OF PROTOPARVOVIRUS CARNIVORAN 1 SPECIES

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The species *Protoparvovirus carnivoran 1* includes feline panleukopenia virus (FPV) and canine parvovirus (CPV-2), two highly contagious pathogens, causing acute and often fatal diseases in both domestic and

wild carnivores. To better understand the role of wildlife in the epizootiology of FPV and CPV-2, we investigated the circulation of *Protoparvovirus carnivoran 1* in wild carnivores.

Eighty-nine samples from carcasses of wild carnivores and omnivores retrieved in the Italian regions of Abruzzo and Molise were screened for CPV/FPV DNA. Viral genome sequences were generated using a multiplex PCR protocol amplifying fifteen PCR-tiling amplicons and sequencing using MinION Mk1C platform.

Overall, 52/89 (58.4%) samples tested positive for CPV/FPV DNA, including the samples of a Marsican brown bear and a crested porcupine. Both the bear and porcupine viruses were characterized as FPV and the complete genomes were generated for both FPV strains. Sequence analyses revealed two aa mutations in the VP2-genomic region, 103-Val to Ala in both capsid sequences and 232-Val to Ile in the porcupine capsid. On phylogenetic analysis, the two FPV strains formed a distinct cluster among the FPV and CPV-2 strains. Immunofluorescence analysis detected parvoviral antigens in the mesenteric lymph node of the crested porcupine. To our knowledge, no parvovirus infection has ever been documented in non-carnivore hosts. CPV-2 and FPV seem to be endemic in wildlife populations. Continuous surveillance for parvoviruses in wildlife animals could help understand better the role of these animals in the evolution and epidemiology of parvoviruses.

(O23) POPULATION-BASED STUDY OF KLEBSIELLA PNEUMONIAE IN COMPANION ANIMAL INFECTIONS IN PORTUGAL: ANTIMICROBIAL RESISTANCE PROFILES AND CLONAL LINEAGES

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The spread of *Klebsiella pneumoniae* in companion animal infections is a growing public health concern due to its antimicrobial resistance. Understanding these factors in animals is crucial for developing effective control and treatment strategies. This study aimed to determine the antimicrobial resistance profiles and clonal lineages of *K. pneumoniae* in pets infections in Portugal.

From July 2022 to September 2023, 71 *K. pneumoniae* strains were isolated from pet infections, with urinary tract infections being the most common. Species identification was performed using VITEK, and minimum inhibitory concentrations (MICs) were determined for 19 antibiotics. Resistance and virulence genes were investigated by PCR. Pulsed-field gel electrophoresis (PFGE) was conducted on all isolates and 39 isolates were selected for multilocus sequence typing.

Of the 71 isolates, 24 were classified as extended-spectrum beta-lactamase (ESBL) producers. All isolates were sensitive to amikacin and resistant to ampicillin. Thirteen isolates were resistant to aminoglycosides, 38 to first- and third-generation cephalosporins, 35 to fluoroquinolones, 23 to tetracyclines, 31 to phenicols, and only three to carbapenems. These isolates carried several resistance genes, including *aad*A, *amp*C, *par*C, *bla*$_{CTX}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{KPC}$, *bla*O$_{XA}$, *sul2*, *tet*A, and *tet*B. Several virulence genes were also detected, such as *pap*C and *bfp*. Regarding genetic lineages, a high diversity of STs was identified (*n* = 26), with ST307 and ST147 being the most common.

This study revealed a concerning prevalence of multidrug-resistant *K. pneumoniae* in companion animals across Portugal, with diverse clonal lineages, highlighting the need for stricter antimicrobial stewardship and further investigation into transmission dynamics.

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(O24) THE ROLE OF EDIBLE MOLLUSCS IN VANCOMYCIN RESITANT ENTEROCOCCI (VRE) SPREAD TO HUMANS: PRELIMINARY RESULTS.

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Objectives: Vancomycin resistant enterococci (VRE) represent an increasing cause of nosocomial infection. Food of animal origin is highly suspected for their diffusion. Our study investigate edible molluscs as potential source of VRE and/or resistance to other Critically Important Antimicrobials (CIAs).

Methods: We analysed 77 samples, each consisting of 25 grams of molluscs. Selective microbiological analysis allowed Enterococci isolation. Species was identified with MALDI-TOF. Antimicrobial profiles were determined with broth microdilution method. Minimal Inhibitory Concentration (MIC) results were interpreted according to CLSI or, when not present, EUCAST breakpoints. Strains phenotypically resistant to vancomycin were screened for *van* genes presence with a multiplex PCR and whole genome sequenced (WGS).

Results: We isolate 58/77 (75.32%) *Enterococcus* strains, belonging to 5 different species. The most common were *E. faecium* (31/58, 53.45%) and *E. hirae* (16/58, 27.59%). Only one *E. hirae* isolate was sensitive to all antimicrobials. Sixteen/58 (27.59%) strains were multridrug resistant (MDR) from 3 to 7 different molecules. The most common resistance frequencies were to quinupristin/dalfopristin (47, 81.03%), erythromycin-linezolid (28, 48.27% both), ciprofloxacin (25, 43.10%), daptomycin (24, 41.38%), tetracycline (16, 27.59%), tigecycline (13, 22.41%), chloramphenicol (9, 15.52%) and gentamycin high dosage-teicoplanin (1, 1.72% both). Resistance to ampicillin was not observed. Notably one MDR *E. faecalis* was resistant to vancomycin (MIC=>128 µg/ml) and carried *vanA* gene, confirmed with WGS.

Conclusions: The study underlined the potential AMR risk represented by molluscs, which were highly associated with MDR, mostly to CIAs. Notably, we isolated one VRE strain, suggesting molluscs could contribute to vancomycin spread to humans.

(O25) HIGH LEVEL BETA-LACTAM AND CARBAPENEM RESISTANCE IN CORYNEBACTERIA IS DUE TO PBP2C CARRYING MOBILE ELEMENTS

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Introduction:. Recently, several *Corynebacterium diphtheriae* isolates displaying high-level resistance to penicillin have been described. Resistance to β-lactams in corynebacteria has been attributed to different genes including putative β-lactamase BlaB without experimental prove.

Objective: To show that high level β-lactam resistance in multiple corynebacterial species is due to a transpeptidase (Pbp2C) whose expression is induced by HdfR and BlaB encoded together in mobile elements.

Methods: The transpeptidase regulon was expressed in β-lactam susceptible *Corynebacterium auriscanis* 23KM1744 (ampicillin MIC=1mg/L), *Corynebacterium glutamicum* DSM20300^T and *Corynebacterium rouxii* 23KM0776 (ampicillin MIC=0.5mg/L). Five different plasmids were constructed. pmrdA_1 encoding the transpeptidase with its natural promoter region but without the putative upstream regulatory genes, pmrdA_2 encoding the whole regulon, pmrdA_3 only encoding *hdfR* and *blaB*, pmrdA_4 encoding *hdfR* and *pbp2C* and pmrdA_5 with *pbp2C* preceded by an alternate promoter.

Results: pmrdA_1, 3 and 4 had no clear effect on MICs. pmrdA_2 (encoding the whole regulon) led to ampicillin MIC >64mg/L and meropenem MIC >=16 in *C. auriscanis* and in *C. rouxii* with a smaller effect (MIC=4mg/L) in *C. glutamicum*. pmrdA_5 induced a lower but significant MIC increase.

Conclusion: Our results indicate that *blaB* does not act as beta-lactamase but rather as a regulatory component to activate *pbp2C* expression which leads to penicillin, cephalosporin and carbapenem resistance. However, MIC increase is markedly higher when the whole regulon is present as compared to *pbp2C* alone. Further research is needed to determine whether this is due to a higher transpeptidase expression or a synergistic effect with *blaB* in peptidoglycan synthesis.

(O26) GENOMIC PROFILING OF STAPHYLOCOCCUS AUREUS FROM HEDGEHOGS UNCOVERS EXTENSIVE CLONAL DIVERSITY, MECC-MEDIATED ANTIMICROBIAL RESISTANCE, AND VIRULENCE

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Methicillin-resistant Staphylococcus aureus (MRSA) poses a significant challenge in antibiotic resistance. Found in both humans and animals, including wildlife, *S. aureus* presents a public health issue due to potential zoonotic transmission. This study investigates the prevalence, genetic diversity, and antimicrobial resistance profiles of *S. aureus* from hedgehogs.

Swab samples from 110 hedgehogs were inoculated into BHI broth with 6.5% NaCl, then streaked onto Baird-Parker agar and ChromAgar MRSA to isolate Staphylococcus aureus and MRSA. Whole-genome sequencing (WGS) was conducted to investigate resistance genes, virulence factors, clonal lineages, and mobile genetic elements.

From the 110 samples, 22 *S. aureus* isolates were obtained, including five MRSA carrying the *mec*A or *mec*C genes. The isolates exhibited high genetic diversity, with 10 sequence types (STs) and at least 9 *spa*-types, including 5 new ones. Most MSSA isolates were susceptible to all antibiotics, while 3 showed resistance to penicillin, conferred by the *bla*Z gene. The *mec*A-MRSA isolates were resistant to penicillin, erythromycin, clindamycin, and ciprofloxacin, carrying the *bla*Z, *erm*C, and *vga*A genes, with point mutations in *grl*A, *grl*B, and *gyr*A. A total of 20 virulence genes were detected, with all isolates harboring genes for 8 common virulence factors. Additionally, 10 plasmids were identified, with rep16-type and rep10-type plasmids carrying the *bla*Z and *erm*C genes, respectively.

The presence of diverse and antibiotic-resistant *S. aureus* strains in hedgehogs highlights the potential role of wildlife in the spread of MRSA. These findings underscore the need for ongoing surveillance and control measures to mitigate the risk of zoonotic transmission.

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(O27) CROSS-SECTIONAL STUDY CHARACTERIZING THE PORCINE FAECAL MICROBIOMES IN COMMERCIAL FARMS

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Objectives: With the increasing interest in porcine gut microbiome, it is important to characterise changes in microbiome within and between farms. Farm characteristics like productive performance or antimicrobial use are relevant factors to explain such differences. Using shotgun sequencing, we characterized the faecal microbiome of pigs at different production stages in 18 Irish farms.

Methods: Faecal samples were collected at four stages: at weaning, before and after transfer to the finisher stage and prior to slaughter (at 5, 11, 13 and 23 weeks of age, respectively). Farm data included mortality, growth, *Escherichia coli* antimicrobial resistance, *Salmonella* spp. presence, zinc oxide and in-feed medication use (ZnOAB) and biosecurity. Microbiota beta and alpha diversity were analysed, as well as their associations with farm characteristics.

Results: Microbiota composition differed across stages, especially between weaned pigs and older animals ($p<0.001$). The former had the lowest average number of species and the highest Simpson diversity index, indicating reduced diversity ($p<0.05$). Age was positively correlated with diversity ($p<0.05$). Microbiota composition of weaned pigs differed based on ZnOAb, while that of finisher pigs was associated with piglet mortality.

Conclusions: Faecal microbiota is in constant change along the pig production cycle. The contrasting microbiota composition of weaned pigs, despite expected, is an undesirable consequence of a stressful transition at weaning.

(O28) BOVINE ANTHRAX: MULTI-INSTITUTIONAL CASE SERIES IN THE AMERICAS

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Anthrax, caused by *Bacillus anthracis*, is a zoonotic disease that primarily affects herbivores such as cattle, and poses a significant public health risk. A rapid and accurate diagnosis is therefore essential to minimise cattle losses and prevent human exposure. While a presumptive diagnosis can be made based on clinical history and signs, and postmortem findings, the confirmation relies on laboratory testing. This study analyses epidemiologic data, postmortem findings and diagnostic tests performed in 116 cases of bovine anthrax from Central Argentina (93) and California, USA (23). All cases occurred in beef cattle and 68% occurred during summer months. Postmortem examination was performed on 31 carcasses, all showing gastrointestinal haemorrhage of variable severity. Splenomegaly was observed in 21/26 carcasses (80%), while bloody fluid from the nostrils or mouth was present in 13/16 carcasses (81%). Additionally, enlarged lymph nodes were observed in 7/10 carcasses (70%). Cardiac hemorrhages and abdominal cavity fluid were observed in 7/13 (53%) and 8/24 carcasses (33%), respectively. All 116 cases were confirmed by

bacterial isolation> there was a shift in the use of complementary tests over time. While M'Fadyean staining on blood smears was used in 48/116 (41%) cases in the past, PCR has become more common in recent years and was used in 24/116 cases (20%), improving diagnostic accuracy. In endemic areas, it is extremely important to make a quick presumptive diagnosis in the field based on the clinical history and postmortem findings and collect the appropriate samples for laboratory confirmation.

(O29) MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICES (μPADs) FOR THE DETECTION OF EMERGING ANIMAL VIRAL THREATS

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The emergence of pathogens that can cross species barriers poses significant threats to animal and human health, as evidenced by the global impact of diseases such as COVID-19 and Highly Pathogenic Avian Influenza (HPAI) H5N1. Developing portable diagnostics is increasingly crucial for rapid, effective disease management and control. Microfluidic paper analytical devices (μPADs) hold great promise as a cost-effective, portable, and affordable platform for point-of-care (POC) applications, specifically tailored for the detection of microbial pathogens. We developed colorimetric biosensing assays to detect virus specific antigens and antibodies, using μPADs fabricated by a cost-effective thermal lamination and laser cut method. The fabrication method allows the formation of fully enclosed μPADs, that are adequately resistant to evaporation from the environment and possess superior mechanical properties. We developed an indirect enzyme-linked immunosorbent assay (ELISA) using these μPADs, for detecting severe acute respiratory symptom coronavirus 2 (SARS-CoV-2) antibodies in biological fluids. The device was further standardized for the qualitative and quantitative detection using Rabbit IgG and glucose in enzyme-linked assays in a concentration-dependent manner. The 96-well μPADs are compatible with commonly used readout systems such as microplate readers and scanners. The colorimetric assays were analyzed using greyscale intensity values and the testing reliability of these μPADs could reach up to a correlation coefficient of $R^2 = 0.94$. We believe that the refined version of this μPAD based assay has the potential to develop a high throughput screening system for emerging high consequence animal pathogens such as avian influenza and African swine fever viruses.

(030) ANTIMICROBIAL RESISTANCE PROFILE AND BIOFILM-FORMING ABILITY OF ESBL-PRODUCING KLEBSIELLA SPP. ISOLATED FROM URINE SAMPLES OF COMPANION ANIMALS IN ITALY

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Urinary tract infections (UTIs) caused by extended-spectrum β-lactamase- (ESBL-) producing *Klebsiella* spp. are of concern for both human and veterinary medicine. This retrospective study investigated the presence of *Klebsiella* spp. in urine samples collected from 2021 to 2023 by cystocentesis or catheterization from dogs and cats. *Klebsiella* spp. isolated from urine samples were identified by MALDI-TOF and submitted to bacteriological and PCR analysis for the detection of major ESBL, AmpC, carbapenemase and colistin resistance encoding genes. ESBL-producing *Klebsiella* spp were subjected to minimum inhibitory concentration analysis determined by broth microdilution method and biofilm production using the microtiter plate method. Overall, 32/1638 (1.9%) urine samples were positive for *Klebsiella* spp. corresponding to 5.5% (31/582) of the positive samples. *K. pneumoniae* complex was the most prevalent species (31/32; 96.9%) followed by *K. oxytoca* (1/32; 3.1%). ESBL-production was detected in 17/32 (53.1%) of the isolates harboring $bla_{\text{CTX-M}}$ (15/17; 88.2%), bla_{TEM} (15/17; 88.2%), and bla_{SHV} (15/17; 88.2%) genes whereas all strains were negative for bla_{CMY}, bla_{KPC}, bla_{VIM}, bla_{NDM}, $bla_{\text{OXA-48}}$ and *mcr1-10* genes. Multidrug-resistance and biofilm-forming ability was detected in all the ESBL-producing isolates. Strong, moderate and weak biofilm producers were identified in 11 (64.7%), 5 (29.4%) and 1 (5.8%) strains,

respectively. Results showing a high presence of ESBL-producing *Klebsiella* spp. along with multidrug-resistance and biofilm-forming ability are of concern. Further studies are needed to define risk factors in pets and potential risk of transmission to humans and the environment.

(O31) DYNAMICS OF DIRECT TRANSMISSION OF EXTENDED-SPECTRUM CEPHALOSPORIN AND CARBAPENEM RESISTANT ENTEROBACTERALES BETWEEN HUMAN FAMILIES AND THEIR COMPANION ANIMALS DURING DIFFERENT TYPES OF ANIMAL INFECTION

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Objectives: This study aimed to evaluate the transfer of extended-spectrum beta-lactamase (ESBL) and plasmid-mediated cephalosporinase (AmpC), and carbapenemase-producing Enterobacterales between companion animals and their cohabitant humans in Portugal (PT) and the United Kingdom (UK) during animal infections.

Methods: From 2018 to 2021, faecal samples and nasal swabs were collected from dogs (n=60) and cats (n=5) with urinary tract infection (UTI) or skin and soft tissue infection (SSTI), and their cohabitant humans (n=102). ESBL/AmpC- and carbapenemase-producing strains were identified using selective media. Whole-genome sequencing (WGS) was performed to determine clonal relatedness between animal and human strains.

Results: ESBL/AmpC-producing Enterobacterales were detected in companion animals (PT=55.8%, UK=36.4%) and humans (PT=35.9%, UK=12.5%). Carbapenemase-producing Enterobacterales were found in one dog from Portugal (2.6%) and one from the UK (4.5%). Transmission of index ESBL-producing *Escherichia coli* strains (causing an UTI in a cat, and a SSTI in a dog), and *Klebsiella pneumoniae* strains (causing an UTI in a dog) occurred to cohabitant humans in Portugal (6.9%). In other two additional Portuguese households and two households from the UK, ESBL/AmpC-producing *E. coli* fecal isolates were shared between companion animals and humans, confirmed by WGS SNPs analysis. Additionally, multidrug-resistant ACT-24-producing *Enterobacter* spp. strains were shared in another Portuguese household.

(O32) EVALUATION OF A DECISION SUPPORT TOOL FOR TREATMENT OF SPORADIC URINARY TRACT INFECTIONS IN COMPANION ANIMALS

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Objectives: A decision support tool for treating sporadic urinary tract infections, in the form of a poster was distributed to general practice veterinary clinics in Victoria, Australia. A post intervention survey was conducted to evaluate the effects of the tool on veterinarians' prescribing behaviour.

Methods: The decision support tool was developed based on the ISCAID guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. Ten general practice veterinary clinics in Victoria, Australia were given access to free culture and sensitivity testing (C&S) for urine samples and

provided with the decision support tool. At the end of the study, a survey was distributed to the veterinarians at the participating clinics.

Results: In the survey, most veterinarians (65%) stated that they followed the recommendations on the decision support tool at least half of the time. Many respondents (61%) felt that the tool changed the way they prescribed antibiotics, and 74% prescribed empirical antibiotics based on the recommendations.

Conclusion: Decision support tools could improve antimicrobial stewardship in veterinary practice and should be evaluated in a larger implementation trial.

(O33) EFFECT OF BLUE LIGHT TREATMENT ON PSEUDOMONAS AERUGINOSA ISOLATED FROM EAR INFECTIONS IN DOGS

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Canine otitis externa caused by *Pseudomonas aeruginosa* is a common and often recalcitrant infection, with enrofloxacin (EFX) resistance being a growing concern. This study investigates the potential of antimicrobial blue light (aBL) as a novel treatment for *P. aeruginosa* infections in dogs with otitis externa.

Objectives: To investigate the antimicrobial effect of blue light (aBL) against *Pseudomonas aeruginosa* isolates from canine otitis externa, and to evaluate the potential of aBL alone and in combination with enrofloxacin (EFX) therapy.

Methods: Six clinical strains of *P. aeruginosa* were exposed to aBL at various wavelengths (375-450 nm) with or without EFX. Viable counts were determined by CFU/mL reduction at 24 h after treatment. Genome analysis was performed to identify antimicrobial resistance and virulence genes.

Results: aBL significantly reduced viable counts of *P. aeruginosa*, with the greatest effect observed at 405 nm. The combination of aBL with EFX resulted in a synergistic effect, achieving greater reductions in bacterial viability than either treatment alone. Genome analysis revealed that EFX-resistant strains harboured mutations in the *gyr*B gene, while aBL treatment did not increase resistance.

Conclusions: Our study demonstrates the potential of aBL as a novel antimicrobial therapy for canine otitis externa caused by *P. aeruginosa*, particularly in cases where EFX resistance is a concern. The synergistic effect of aBL with EFX suggests that aBL may be a useful adjunct to traditional antimicrobial therapy, offering a new strategy for combating antimicrobial resistance in veterinary medicine.

(O34) DIVERSITY OF AUSTRIAN CLOSTRIDIUM PERFRINGENS ISOLATES OF ANIMAL ORIGIN ANALYZED WITH NOVEL AND ESTABLISHED TYPING METHODS

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Objectives: *Clostridium perfringens (CP)* is an important pathogen causing neonatal diarrhea and/or tissue damaging infections (gas gangrene) in several animal species and humans. In recent years, new extracellular toxins and hydrolytic enzymes were described additionally to the existing toxin-gene-based

typing-scheme. Hence, two novel toxinotypes were added. As routine diagnostics typically only perform major-toxin typing, data on the prevalence of newly described virulence factors is missing.

Methods: The novel microarray-based assay for *CP* toxin-gene-typing allows the simultaneous analysis of 16 toxin-genes within a single reaction scheme. To validate this method, 109 non-repetitive clinical *CP* isolates from Austrian animals and seven reference strains were analyzed. Twenty selected isolates were characterized using Whole-Genome-Sequencing (WGS) and subsequently, core genome (cg) Multi-Locus-Sequence-Typing (MLST) was performed. The remaining 89 isolates were typed by MLST.

Results: Genes detected by microarray analysis showed significant homology with the genomes obtained or previously published. Individual *CP* strains assigned to the same toxin-gene-types displayed different toxin-gene-patterns (i.e. minor-toxins) besides the typing-toxin-genes (i.e. major-toxins). MLST and cgMLST results yielded at least 11 novel sequence types and several new alleles. The cgMLST showed a high degree of diversity among isolates without defined clusters.

Conclusion: Compared to the current single or multiplex PCR-based routine diagnostics for *CP* toxin genes the novel microarray-based multiparameter assay is less expensive, less laborious, less time- consuming, and provides information on the presence or absence of 16 toxin-genes in one single assay. Together with the WGS based validation of the microarray results, the novel assay represents a reliable and superior alternative to conventional routine PCR toxin-gene- typing.

(O35) DETECTION OF "BEAVER LEUKOCIDIN"-POSITIVE STAPHYLOCOCCUS AUREUS AND DIPHTHERIA TOXIN-POSITIVE CORYNEBACTERIUM ULCERANS IN EURASIAN BEAVERS FROM GERMANY

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Objectives: Although considered as source of zoonotic pathogens wildlife animals also suffer health consequences from emerging pathogens. Here, we report *Staphylococcus aureus* and *Corynebacterium ulcerans* infections in beavers with purulent lesions and abscesses.

Methods: Between 2015 and 2024, twenty-one *S. aureus* and two *C. ulcerans* were collected from fourteen beavers and investigated for their antimicrobial resistance and genotypic virulence properties. Expression of Panton-Valentine leucocidin (PVL) in *S. aureus* and toxin production in *C. ulcerans* were determined by PVL lateral flow device and modified Elek test, respectively.

Results: So far, eleven *S. aureus* isolates have been further characterized. The most common *spa* type was t3058, all belonging to clonal complex (CC) 1956, while t208 and CC49 were identified only once. Interestingly, all *S. aureus* carry and express beaver-specific variants of the PVL genes (*lukS-BV*; *lukF-BV*). Both *C. ulcerans* isolates belonged to sequence type (ST)332, carrying and expressing the diphtheria toxin gene. *S. aureus* isolates were susceptible to all antimicrobials tested and negative for the respective resistance genes. Both *C. ulcerans* were only clindamycin resistant.

Conclusions: We found that *S. aureus* isolated from fatally diseased beavers harbour distinct *lukS/F* genes, which are closely related to the PVL genes from humans and induce the same pathology in beavers as PVL-positive *S. aureus* in humans. Both *C. ulcerans* are classified as toxigenic and ST332, which was rarely found in beavers, hedgehogs and humans. These results indicate a more common occurrence of these pathogens with a possible risk of zoonotic transmission between wildlife and humans.

(O36) RAPID DIAGNOSTIC TESTS FOR INFECTIOUS DISEASES AND ANTIMICROBIAL RESISTANCE

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Objectives: Accurate and rapid diagnostic tests are crucial for halting the spread of infectious diseases and enabling the appropriate selection of treatments, thereby reducing the emergence of antimicrobial resistance (AMR). The current gold standard tests for the detection of most infectious diseases and AMR rely on culture and phenotypic tests or PCR, but these are time-consuming and/or require expensive and specialised lab-based equipment. Therefore, there is an urgent need for rapid tests that can be performed with minimal equipment and training.

Methods: We developed a number of rapid diagnostic tests for infectious diseases and AMR detection, that utilise loop-mediated isothermal amplification (LAMP) technology. To accurately read colorimetric LAMP results, we developed and validated a portable smart diagnostic device, with automated image acquisition, in collaboration with Vidiia Ltd.

Results: The LAMP assays designed for rapidly detecting microorganisms' responsible infectious diseases in animals demonstrated high sensitivity and specificity. AMR markers were also accurately detected in less than 1h. The Vidiia Hunter device, embedded with an Artificial Intelligence model, successfully improved colorimetric LAMP tests outputs, by removing the subjectivity associated with the interpretation of results.

Conclusions: The diagnostic tests developed in this study identified their target genes with high sensitivity and specificity, in less than 1h. When combined with the Vidiia platform, they could provide timely and cost-effective diagnostics for infectious diseases and AMR, in resource-limited settings.

(O37) GENETIC DIVERSITY OF KOBUVIRUSES IN CATTLE

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The genus *Kobuvirus* (KoV), family *Picornaviridae*, comprises small, non-enveloped single-stranded, positive-sense RNA (8.2-8.4 kb) viruses, also known as *Aichivirus* (AiV) species A to F. AiVs have a wide host range and have been associated with gastroenteric signs, with severe enteritis in children and immunocompromised patients. In this study, we investigated the prevalence of KoVs in Italian cattle, screening 38 stool samples collected from healthy (n=21) and diarrheic (n=17) animals, ranging from 20 days to 96 months old. Using a KoV-specific RT-PCR, KoVs were detected in 10/38 (26.5%) samples. On sequence analysis of the amplicons, eight strains were related to species AiV B, commonly found in cattle. In contrast, two strains showed the highest nucleotide (nt) identity (up to 97.1%) to cattle, yak, and goat AiV D strains. Six strains (including the two AiV D strains) were from animals with enteric signs. All samples were prepared using the NetoVIR protocol for enrichment of viral sequences. Analysis of NGS was performed with Genome Detective and Geneious Prime® 2024.0.5. About 17% of the reads classified as viral and KoV-related reads were identified in 4/38 (10.5%) samples. On whole genome sequencing, the AiV D strains (ITA/2019/572-1 and ITA/2020/30-2) displayed 94.7 nt % identity to each other and 89.1-89.6% nt identity to the bovine strain BKV5/2021/CHN. These findings suggest that AiV D KoVs are common components of bovine enteric virome. Understanding the genetic diversity of KoVs in animals will be useful to improve the diagnostics and fill up epidemiological gaps.

(O38) NEW DELHI METALLO B-LACTAMASE-PRODUCING ENTEROBACTERALES IN COMPANION ANIMALS: RESULTS FROM SURVEILLANCE IN AN ITALIAN UNIVERSITY VETERINARY HOSPITAL

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Carbapenems are considered one of the most important last-resort antibiotics classes, and the spread of carbapenem-resistant Enterobacterales (CRE) represents a serious concern worldwide. From a One Health point of view, reports on CRE in companion animals are increasing, requiring attention in relation with their role on maintenance and direct transmission to humans. The aim of this study was to assess the frequency of detection at admission and the in-hospital acquisition of CRE isolated from perinal swab of 150 dogs and cats hospitalized for more than 48 hours in an Italian Veterinary Teaching Hospital, and the rate of CRE infections in the same patients. From 150 patients sampled, 11.3% (n=17/150) were CRE carriers at admission, 25.6% (n=34/133) acquired CRE in their commensal flora during the hospital stay, and 2% (n=3/150) developed an infection sustained by CRE, all of them with fatal outcome. Genotypical analysis showed that in 100% of the isolates (78/78) carbapenem-resistance was conferred by *bla*$_{NDM}$, suggesting an endemic presence of such gene within the hospital. Other β-lactamases-encoding genes (*bla*$_{CTX}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA-1-like}$, *bla*$_{CMY\ 2-7,\ 12-18,\ 21-23}$, *bla*$_{LAT\ 1-3}$, *bla*$_{BIL-1}$) were found on most of the isolates. Risk factors associated with CRE in-hospital acquisition were the length of hospitalization (p=0.0002) and treatment with piperacillin-tazobactam (p=0.0380), indicating a potential cross-selection of CRE. The results reinforce the suspect that companion animals could silently contribute to the maintenance and dissemination of CRE in the local community, posing a threat for the global health.

(O39) PROMOTING THE USE OF FIRST LINE ANTIBIOTICS SULFONAMIDES AND TRIMETHOPRIM BY IMPROVING ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR IMPORTANT PATHOGENS IN LIVESTOCK

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Targeted interpretation of antimicrobial susceptibility testing (AST) is highly dependent on the availability of appropriate interpretative criteria, such as clinical breakpoints (CBP) or epidemiological cut-off values (ECOFF). However, these criteria have not yet been defined for some important pathogen-antibiotic combinations, like livestock pathogens and sulfonamides as well as for trimethoprim. Therefore, we determined minimum inhibitory concentration (MIC) distributions for *Actinobacillus pleuropneumoniae*, *Escherichia coli*, *Mannheimia haemolytica* and *Pasteurella multocida* for trimethoprim (TMP), sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfadimethoxine (SDM), and sulfamethoxazole-trimethoprim (SXT) combination.

MIC determination was carried out applying the broth microdilution method in accordance with ISO standard 20776-1 and EUCAST guidelines. The MIC distributions were analysed as to whether they meet the acceptance criteria for ECOFF setting laid down in the SOP 10.2 by the EUCAST steering committee.

MIC distributions met the acceptance criteria for all organisms for TMP and the SXT combination. For SMX all bacterial species, except for *Pasteurella multocida,* met the criteria. For SDZ and SDM, only *Actinobacillus pleuropneumoniae* and *Escherichia coli* met the criteria, with *Mannheimia haemolytica* and *Pasteurella multocida* not reaching acceptance. In all instances where MIC distributions did not meet the acceptance criteria, there was truncation at the high-end of the concentration range (i.e. >256 mg/L or >1024mg/L).

Further studies with extended concentration ranges and new sulfonamide-trimethoprim combinations are in progress. The acceptable MIC distributions will form the basis for a 5-lab study with the goal to generate aggregated MIC data to be submitted to the EUCAST steering committee for ECOFF setting.

(O40) FROM HETERORESISTANCE TO RESISTANCE: A SINGLE NUCLEOTIDE POLYMORPHISM (SNP) HOMOGENIZES POPULATION PLASTICITY OF GENE AMPLIFICATION BASED HETERORESISTANCE

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Introduction: Heteroresistance (HR) describes the ability of a subpopulation to grow in the presence of inhibitory antibiotic concentrations. We found HR to ceftazidime (CAZ) in a clinical *Enterobacter cloacae* complex (ECC) strain (IMT49658).

Material & Methods: We performed extensive phenotypic (population analysis profiles, stability analysis of resistance, ScanLag) and molecular microbiological techniques (qRT-PCR, whole genome sequencing, raw read analysis) in order to show the plasticity and mechanism of HR in this ECC strain. We re-investigated the genome and phenotype of IMT 49658 after long-term evolution in 32 g/ml CAZ.

Results: WGS detected a plasmidal gene amplification with β-lactamase ampC bla_{DHA-1}. qRT-PCR showed a high genomic copy number of bla_{DHA-1} in resistant subpopulations, decreasing when they reverted to susceptibility. Gene amplifications varied in single cells of one colony (raw read analysis). Resistant subpopulations showed heterogeneous lag times in ScanLag. After evolving ECC for 21 days in CAZ, we discovered a SNP in *dacB*, encoding for a stop codon. This mutant displayed low amplification levels but resistance in disk diffusion and homogenous lag times.

Conclusion: Long-term evolution in antibiotic niches drives the emergence of new resistant mutants, balancing the fitness costs of e.g., gene amplifications. Comprehension of the transition from HR to resistance is inevitable for successful treatment of infections from zoonotic bacteria.

(O41) CARBAPENEM EMERGING RESISTANCE IN THE ENVIRONMENT AND STAFF OF SMALL ANIMAL VETERINARY PRACTICES

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Introduction: The transmission of carbapenem-resistant bacteria in Veterinary Healthcare is poorly understood. This study aimed to assess environmental contamination and staff colonization in small animal veterinary practices (SAVPs) in Portugal.

Methods: Eighteen SAVPs were enrolled, with environmental samples collected from critical surfaces and nasal, hand and rectal swabs voluntarily obtained from staff. The samples were analysed for carbapenem-resistant bacteria using specific media and whole-genome sequencing.

Results: Out of 18 SAVPs (A to M), eight (A-H) (44.4%) had at least one surface contaminated with a carbapenem-resistant bacteria (range of Imipenem/Meropenem MIC values 8->32 mg/L). In SAVP-A, four surfaces were positive for OXA-23-producing *Acinetobacter* spp. In SAVP-B and C, carbapenem-resistant *Pseudomonas aeruginosa* by *OprD* mutations were found (ST267 and ST253, respectively). Also, on SAVP-C, IMP-8-producing *Pseudomonas juntendi* was found on two surfaces of the canine ICU. On SAVPs C-H, *Stenotrophomonas maltophilia* was detected on different surfaces: ST120 was shared on 2 different surfaces of SAVP-C, while ST115 was shared on 2 fomites of SAVP-E. Regarding staff samples, two members of SAVP-G shared carbapenem-resistant *P. aeruginosa* ST274 with mutations on *OprD* and *nalC* (one hand and one rectal swab). On SAVP-E, one veterinarian and one auxiliary member shared *S. maltophilia* ST115 on their hands. A veterinarian from SAVP-C had carbapenem-resistant *Klebsiella pneumoniae* ST11, by Omp36K mutation, on her nasal sample.

Conclusions: Discovery of carbapenem-resistant isolates on essential surfaces within SAVPs and among veterinary staff underscores the critical necessity for the development and implementation of IPC guidelines customized to Veterinary healthcare.

Acknowledgements: This project was funded by CIISA and FCT Project UIDB/00276/2020 and LA/P/0059/2020 – AL4AnimalS. This project was funded by national funds through FCT - Fundação para a Ciência e Tecnologia I.P., within the project 2022.08669.PTDC. JMS, JM and LF were supported by a Fundação para a Ciência e Tecnologia (FCT) PhD fellowship (2020.06540.BD; 2020.07562.BD; UI/BD/153070/2022, respectively).

(O42) IMPACT OF FREE CULTURE AND SENSITIVITY TESTING FOR URINARY TRACT DISEASE ON ANTIMICROBIAL PRESCRIBING IN COMPANION ANIMAL PRACTICE

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Objectives: Patients with clinical signs of urinary tract disease should have urine culture and sensitivity testing (C&S) performed to confirm the presence of infection and aid in antimicrobial selection to ensure the most appropriate drug is used. Veterinarians often cite the cost of C&S as a prohibitive factor. This study aims to determine if the cost of C&S is a barrier to antimicrobial stewardship in veterinary general practice and investigate if free C&S for urinary tract disease affected antimicrobial prescribing behaviour.

Methods: From January to December 2022, 10 general practice veterinary clinics in Victoria, Australia were given access to free urine C&S as part of an intervention study. At the end of the study period, a survey was distributed to the veterinarians at the participating clinics.

Results: A total of 480 urine C&S submissions were received in the 11-month study period. Several clinics had increases in submissions, with the largest increase 28 times compared to a previous 12-month period. Most respondents (80%) in the survey reported that they submitted more urine samples for C&S compared to before the study and the remaining felt their number of submissions remained the same. Many of the veterinarians (74%) also said they delayed prescribing antibiotics while awaiting C&S results.

Conclusion: Laboratory testing is important to determine the appropriate antimicrobial to use but cost of laboratory testing is a barrier to susceptibility testing being undertaken. Removing the barrier of cost resulted in reported positive changes to antimicrobial prescribing.

(O43) COMPARISON BETWEEN IN-CLINIC AND LABORATORY DISC DIFFUSION ANTIMICROBIAL SUSCEPTIBILITY TESTING: PRELIMINARY RESULTS

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Antibiotic resistance is a significant worldwide health concern caused by antibiotic abuse or overuse. The majority of pet antibiotic prescriptions are empirical and antimicrobial susceptibility testing (AST), even when indicated, is rarely performed, probably due to its high cost and lengthy duration. Conversely, chromogenic (color-forming) Mueller Hinton culture media have become readily available, potentially enabling less costly in-clinic (point-of-care) AST. This study compared standard microbiological laboratory and in-clinic disc diffusion AST based on ready-to-use Chromagar MH Orientation agar. We collected 39 canine and feline specimens, 21 skin and 11 ear swabs, and 7 urine samples. After inoculation, the addition of five (mostly first-line) antibiotic discs, and 18h incubation, bacteria were identified and the AST was executed according to EUCAST. Finally, the same agars with bacteria were sent to the laboratory for standard AST. We detected 45 bacteria, including 19 *Staphylococcus pseudintermedius* and 15 *Enterococcus faecalis*. The overall agreement in identification was 88.9% (40/45). The misidentifications included three undetected bacteria (two by the laboratory) and two *Staphylococcus coagulans* being mistaken for *S. aureus*. In AST, the agreement was 84.1% (164/195). In-clinic tests seemed more strict since 12 laboratory-based susceptible bacteria (including increased exposure) were labeled resistant, but only five in-clinic susceptible bacteria were resistant in the laboratory. Almost one-third of AST disagreements (9/31, 29.0%) were related to “sensitive” (in-clinic) vs. “sensitive, increased exposure” (laboratory) *Enterococcus faecalis*, probably indicating different breakpoints used. To conclude, in-clinic chromogenic culture media-based AST could improve empirical antibiotic prescriptions by identifying bacteria and their basic susceptibility.

Acknowledgments: The study was self-funded. We thank the personnel at the veterinary clinic Zamba (Vets4science Ltd) for helping us collect samples.

(O44) DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR ANAPLASMATACEAE DETECTION AND ITS APPLICATION IN HEDGEHOGS SCREENING.

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Bacteria belong to Anaplasmataceae family are pathogens to be approached from a One Health perspective. To assess their environmental spread, vectors (ticks), animal reservoirs, and accidental hosts (animals and humans) must be considered. Molecular tests are used to detect hosts infection, estimate the risk of transmission, and genetically characterize the pathogens. This study aimed to develop a multiplex real-time PCR (mqPCR) assay for the simultaneous detection and differentiation of *Anaplasma phagocytophilum*, *A. platys*, and *Ehrlichia canis* DNA. The developed mqPCR was used to test 70 hedgehogs (*Erinaceus europaeus*) sampled in 2023 from Italy, a species identified as a potential reservoir of Anaplasmataceae. The mqPCR showed good efficiency, with a limit of detection of 10 target DNA copies/µl for *A. phagocytophilum* and *E. canis*, and of 1 copy/µl for *A. platys*, great specificity and low intra- and inter-assay variability. Fourteen of 70 (20%) hedgehogs tested positive for *A. phagocytophilum* DNA, while none tested positive for the other pathogens. Phylogenetic analysis of the groEL gene showed clusterization of *A. phagocytophilum* strains detected in this study with strains identified in animals (including dogs from the same geographic area), ticks and humans, suggesting the zoonotic potential of the bacteria circulating in hedgehogs. Further investigations, in hedgehogs and in other wild species, will be necessary to assess the prevalence of these pathogens and to identify potential reservoir hosts that could act as infection source for humans. Supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

(O45) ANIMAL TRADE AND DISEASE: MULTIPLE INFECTIONS IN CAIMAN LIZARDS (*Dracaena guianensis*) IMPORTED INTO FRANCE FROM PERU

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Ferlaviruses are important respiratory and systemic pathogens of reptiles, especially snakes. Atadenoviruses and cryptosporidia are found regularly in squamate reptiles. The animal trade is one factor in transmission of infectious agents over wide distances. A group of 58 juvenile caiman lizards (*Dracaena guianensis*) were imported into France from Peru. All died within five months of import with clinical signs including prostration, pronounced lethargy, dyspnea, and anorexia. Six animals that died or were euthanized were examined by histopathology, PCR for the detection of ferlaviruses, adenoviruses, reoviruses (n = 3), and cryptosporidia, and virus isolation in cell culture (n = 4). The complete genomes of two virus isolates were sequenced using Oxford Nanopore Technology (PathoSense). Histopathology showed interstitial proliferative pneumonia in five and lymphocytic multifocal pancreatitis with acinar atrophy in all six animals. PCRs were positive for ferlaviruses (4/6 lizards), adenoviruses (2/6), and cryptosporidia (2/6). Ferlaviruses were isolated from four animals and subsequent genome sequencing showed the virus to be closely related to a ferlavirus previously described in anacondas (*Eunectes murinus*) in Hong Kong. Sanger-sequencing of the adenovirus PCR products indicated that these were a novel atadenovirus. While multiple pathogens were detected in these animals, the ferlavirus is the most likely cause of the severe disease outbreak. Stress and other pathogens likely contributed to the severity of disease. This is an example of the role the pet trade can play in the international movement of pathogens, and confirms again that lizards can be affected by ferlaviruses.

Flash Talks/Poster Presentations

Friday 5th September 2024: Presentations and Poster Session

(FTP1) DETECTION OF MYCOPLASMAS IN BOIDAE, COLUBRIDAE, ELAPIDAE, PYTHONIDAE, AND VIPERIDAE

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Mycoplasmas are an important cause of respiratory diseases in tortoises. In snakes, evidence of mycoplasma infections has been found almost exclusively in pythons. To better understand the occurrence of these bacteria in other snake species, samples submitted for routine testing for respiratory pathogens were also tested for mycoplasma by PCR. A total of 640 samples (mostly oral swabs) from snakes of 5 different families (Boidae n=114, Colubridae n=109, Elapidae n=34, Pythonidae n=301 and Viperidae n=82) were included in the study. A genus-specific PCR developed for the detection of *Mycoplasmopsis agassizii* (PCR1) and a pan-mycoplasma PCR (PCR2) were used. PCR products were sequenced for validation and phylogenetic analysis was performed. The sampled animals were from various owners and collections, all were kept in captivity at the time of sampling. Clinical background information was not provided. Using PCR1, mycoplasmas were detected in 175 (175/640, 27%) samples (Boidae: 7/114, 6%; Colubridae: 3/109, 3%; Elapidae: 8/34, 24%; Pythonidae: 155/301, 52%; Viperidae: 2/82, 2%). A higher percentage of positive results were obtained using PCR2 (258/640, 40%; Pythonidae: 172/301, 57%; Boidae: 9/114, 8%; Colubridae: 25/109, 23%; Elapidae: 19/34, 56%; Viperidae: 33/82, 40%). The detected bacteria can be divided into at least 6 genetically diverse clusters representing different genera and species based on multiple sequence alignment and phylogenetic analysis. These results show that diverse mycoplasmas are found in pythons and other snakes. Further investigations are necessary to evaluate the role of various mycoplasmas in respiratory diseases in snakes.

(FTP2) STABILITY AND LYTIC ACTIVITY ASSESSMENT IN MILK OF BACTERIOPHAGES TARGETING *ESCHERICHIA COLI* CAUSING BOVINE MASTITIS

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Bovine mastitis is a major production disease in dairy cattle and complementary treatments to antimicrobials are urgently needed. Intramammary phage therapy is a promising approach but characterizing isolated phages in milk is a crucial initial step. This study aimed to compare the stability and lytic activity of 10 phages targeting *E. coli* in milk and assessing their stability at different temperatures and pH.

Ten bacteriophages isolated from wastewater were spotted on 53 *E. coli* strains isolated from bovine mastitis to evaluate their host range. The phage stability was evaluated across different pH (2-12) and T°C (25-60°C). Stability in milk was assessed after 6h of incubation at 37°C. *In vitro* efficacy assays involved inoculating milk with *E. coli* and phages and tracking bacterial titers at different timepoints in raw, heat-treated and UHT milk.

A narrow host spectrum was observed and phage stability was maintained at pH ranging from 4 to 10 and temperatures ranging from 25 to 45°C. At 60°C, only 5/10 phages persisted but with a significant

degradation. Stability analysis in milk showed that all phages remained stable in raw and heat-treated milk. Lytic activity assays demonstrated a bacterial decrease with all phages, but for 5/10 phages, bacterial regrowth occurred after 5h of incubation.

In conclusion, milk components are not an obstacle for phage therapy to control bovine mastitis. However, bacterial regrowth suggests the presence of resistances that could be bypassed with the use of phage cocktails. DNA sequencing of the phages will be performed to ensure their safety.

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(FTP3) EFFECTS OF A DIET CONTAINING 7 PROBIOTIC STRAINS ON THE PHYSIOLOGY AND IMMUNE RESPONSE OF WORKER HONEYBEES: ANALYSIS OF HAEMOLYMPH CYTOLOGY, PHENOLOXIDASE ACTIVITY AND THE GUT MICROBIOME

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This study assessed the impact of a multistrain probiotic supplement on A. mellifera ligustica worker honeybees. The effects on intestinal metabolism were compared to a control group. Seven bacterial strains, isolated from a honeybee population in Roti Abbey (Matelica, Marche Region, Italy), were used. The bees received the nutritional support ad-libitum daily for one month.

To determine if the probiotic altered the immune response, phenoloxidase activity and hemolymph cellular subtype counts were measured from hemolymph samples of 10 bees from each of the two families (A and B) at the end of the trial. Gut microbial community genomic data were obtained using next-generation sequencing (Shotgun). Pools of four intestinal segments (crop, midgut, ileum, and rectum) from 20 bees in hives A and B were analyzed at the start (T0) and end (T1) of the study.

The findings indicated that probiotic supplementation was safe and well-tolerated. Hive B showed a significant decrease in phenoloxidase activity compared to hive A, suggesting improved well-being and a reduced need for immune defense activation. The probiotic also modulated the gut microbiota composition in healthy honeybees, retaining core microbiota components. Several genes, particularly KEGG genes related to amino acid and carbohydrate metabolism, were more abundant in the probiotic group, indicating effective nutritional support.

In conclusion, the probiotic improved honeybee health by promoting a balanced intestinal microbiota and enhancing metabolic activities related to digestion. This probiotic formulation appears to be a beneficial strategy for enhancing the overall health and well-being of honeybees.

(FTP4) ROTAVIRUS A INCREASES MICROBIOTA CHANGES ELICITED BY ETEC IN POST-WEANING DIARRHOEA IN PIGLETS

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Post-weaning diarrhea (PWD) is a multifactorial disease associated with high morbidity and mortality, if not treated. Different pathogens may be involved in the PWD and the microbiota dysbiosis may play an important role. Given that some species of rotavirus, especially Rotavirus A (RVA), could be involved in this pathological process, the present study aims at deciphering the interaction of this virus with the intestinal microbiota in piglets at the time of weaning.

For this purpose, 36 DNA/RNA faecal samples from a previous study were re-re-analysed. Samples came from four farms, part had normal consistency and part with diarrhoea, and enterotoxigenic *Escherichia coli* was previously diagnosed in most of them. The presence and quantification of RVA was diagnosed by RT-qPCR. The RVA results were analyzed together with metagenomics data sequenced through shot gun metagenomics to evaluate the impact of the RVA infection in the microbiome composition.

Thirteen of the 36 samples were RVA positive by RT-qPCR, all except one ETEC positive. Eight showed clear amplification and another 5 with weak amplification. Diversity analyses showed that RVA infection modified the intestinal microbiota of piglets increasing significantly ($p<0.05$) the species richness and also influencing sample ordination in ß-diversity analyses. Both diarrhea and RVA infection increased faecal dissimilarity between individuals. Specific changes in abundance were also determined, highlighting a significant increase of *Akkermansia muciniphila*, a mucin-degrading bacteria, relative abundance ($p=0.026$.

Altogether these results demonstrate the potential implication of RVA in PWD microbiota alteration underlining its importance within the multifactorial process of post-weaning diarrhoea.

(FTP5) A SIMPLE AND VERSATILE METHOD FOR EX VIVO MONITORING OF GOAT VAGINAL MUCOSA TRANSDUCTION BY VIRAL VECTORS

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Goat represents a valid large animal model for human genital pathogens and new vaccines testing. Viral vectors have been employed successfully for goats immunization. However, no data concerning the vaginal route are available. The capability of the viral vector to transduce the site of inoculation, it is of primary interest. In this work a fast and reliable ex vivo assay for testing the transduction capability of Ad5-based vector when intravaginal administered has been developed. An Ad5 vector delivering an expression cassette with a bicistronic reporter gene, Ad5-CMV-turboGFP-IRES-Luc2, was constructed. Ad5-CMV-turboGFP-IRES-Luc2 virus shown replication competence in HEK293 cells and transducing competence, as well as replication incompetence, in on caprine cells because defective for E1A and E1B genes. Taking advantage of Luc2 delivering, it was possible to show Ad5- CMV-turboGFP-IRES-Luc2 ability to transduce caprine vaginal mucosa by ex vivo bioluminescent imaging (BLI) employing a simple CCD camera apparatus for chemiluminescence western immunoblotting. Pieces of goat's vaginas maintained in culture were infected/transduced with Ad5- CMV-turboGFP-IRES-Luc2. Seventy-two hours post infection luciferin substrate was added to the culture and exposed to the CCD camera apparatus (ChemiDoc XRS+, BioRad). Indeed, it was possible to ex vivo visualize and quantify Ad5-CMV-turboGFP-IRES-Luc2 gene delivery in the vagina mucosa surface. These data, although simple, are very informative in terms of immunization strategy through the vaginal route, for pathogens inducing genital diseases, when a viral vector-based vaccine is going to be employed.

(FTP7) ANTIMICROBIAL SUSCEPTIBILITY OF ESCHERICHIA COLI ISOLATED FROM CANINE AND FELINE URINARY SAMPLES

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Objectives: Ten Victorian general practice clinics were provided access to free urine culture and sensitivity testing from January to December 2022. E. coli isolates were cultured from canine and feline urine samples submitted through this novel antimicrobial stewardship trial. The aim of this study was to determine the antimicrobial susceptibility of these urinary E. coli isolates to commonly used antimicrobials in clinical practice.

Methods: The E. coli isolates were tested for minimum inhibitory concentrations (MIC) using broth microdilution (Sensititre™). The MICs were interpreted based on clinical breakpoints published in the CLSI VET01S ED7:2024 guidelines.

Results: E. coli was the most commonly isolated bacterial species. A total of 122 E. coli isolates (42%) were obtained from 289 urine samples. The canine isolates reported high susceptibility to “Highly important” (WHO categorization) antimicrobials like Ampicillin (81%), Trimethoprim-sulfamethoxazole/TMS (95%) and Amoxycillin-clavulanic acid (92%). The feline isolates reported similar rates of 87% for Ampicillin, 100% for TMS and 98% for amoxycillin-clavulanic acid.

Conclusion: There were high susceptibility of the E. coli isolates to “Highly important” (HI) rated antimicrobials like TMS and amoxicillin. This is supportive of guideline recommendations for using TMS and amoxicillin as the empiric treatment of choice for sporadic cystitis.

(FTP8) *IN VITRO* ANTIBACTERIAL PROPERTIES OF CANINE PLATELET-RICH FIBRIN

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Abstract: Platelet-rich fibrin (PRF) is the second generation of activated non-transfusional hemo-components used to stimulate tissue regeneration. To date, few researchers have described its antibacterial properties.

Objectives: The *in-vitro* study aimed to evaluate the: -antibacterial activity of canine Advanced-PRF (A-PRF) and Injectable-PRF (I-PRF); -time–kill assays towards bacteria isolated from canine otitis, in relation to Gram’s stain affinity and antibiotic susceptibility pattern.

Methods: Blood samples were processed for A-PRF and I-PRF preparations (gel, membrane, liquid) and tested on bacterial strains (*Staphylococcus aureus* subspecies *aureus, Staphylococcus pseudintermedius, Streptococcus canis, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter cloacae*) isolated from canine otitis, identified by MALDI-TOF MS and classified as susceptible (S), multidrug- (MDR), extensively-resistant (XDR) towards a known panel of veterinary and human antibiotics. Kirby–Bauer, 96-well-plate micro-inhibition in broth, Colony Forming Units (CFU/mL) methods, in absence (Control) and in presence of A-PRF and I-PRF, at different dilutions (1:1-1:2-1:4-1:8) and times (T_0-T_3-T_6-T_{12}-T_{18}), were performed. Inhibition areas were evaluated by imitoMeasure software. One-Way ANOVA, Kruskal-Wallis and linear regression were calculated using GraphPad Prism-10. $P<0.05$ was considered statistically significant.

Results: I-PRF 1:1 recorded the best antibacterial effect, although at all dilutions showed a significant reduction in CFU/mL mean loads for almost 12hrs (P<0.0001). I-PRF showed a pronounced activity against Gram positives until 6hrs (P=0.0174), Gram negatives until 12hrs (P=0.0026), and MDR bacteria within 6hrs (P=0.0056). After 24hrs of incubation, both hemo-components demonstrated inhibition areas around the membrane and gel.

Conclusions: Canine I-PRF exhibited bacteriostatic results. PRF could be a promising biological material for ear infection treatment in dogs.

(FTP9) DRUG REPURPOSING FOR TREATMENT OF PROTOTHECA INFECTION

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Objectives: Protothecosis, caused by the emerging pathogen *Prototheca*, is an increasingly reported infection of humans and animals. *Prototheca* is inherently resistant to most antimicrobial drugs and sanitizers. Amphotericin B, currently used to treat *Prototheca* infections, causes severe side effects and shows low efficacy. Therefore, finding new efficient drugs targeting *Prototheca* is urgently needed.

Methods: A library of 400 drug-like molecules already in clinical development was screened for novel anti-*Prototheca* compounds, using adapted EUCAST yeast MIC methodology. The best hits were tested for MIC and killing against a panel of *Prototheca* strains, evaluated for synergy with amphotericin B (checkerboard method), and for the ability to destroy *Prototheca* biofilms (crystal violet and MTT methods). An additional panel of functional assays was performed to identify the drugs' mechanism of action.

Results: After screening, we found 5 potentially active drugs: alexidine, tafenoquine, ciclopirox, eberconazole and OSU-03012. All compounds had good activity, with MIC in 0.5 – 16 µg/ml range, and ability to directly kill *Prototheca*. In synergic testing, tafenoquine had interactive effect in combination with amphotericin B. In addition, alexidine and ciclopirox displayed effective eradication of biofilm mass, while all compounds, except for eberconazole and OSU, displayed effective inhibition of biofilm metabolism. Tafenoquine, eberconazole, and ciclopirox were found to target cell membranes, while ciclopirox also acted through chelation of metals needed for *Prototheca* growth. Mechanism of action of other compounds is still under investigation.

Conclusions: By using EUCAST antifungal methodology, we demonstrated high potential of identified drugs to be repurposed for treatment of *Prototheca* infections.

Posters

Poster Session 1: Friday 6th September 2024

(P1) ASSESSING GENETIC DIVERSITY AND COINFECTION OF TICKBORNE PATHOGENS IN SMALL AND LARGE RUMINANTS OF PUNJAB, PAKISTAN

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OBJECTIVE: Pakistan faces a significant economic threat from ticks, where two specific species, *Rhipicephalus microplus,* and *Hyalomma anatolicum*, act as vectors for various pathogens that pose a significant burden on livestock production. These tick-borne pathogens, responsible for endemic diseases, have never been studied in ticks and blood of tick-infested animals simultaneously.

METHODS: In 2022, a cross-sectional study of tick-borne pathogens' occurrence, diversity, and co-infection. We collected blood samples from 224 cattle, 224 buffalo, 69 goats, and 56 sheep from 112 farms in the seven districts of Punjab, and 476 ticks that were attached to these animals. The samples were processed using conventional and microfluidic PCR.

RESULTS: The most commonly collected tick species were *R. microplus* (infecting 38.65% of all animals), *H. anatolicum* (31.93%) and *R. decoloratus* (8.40%). Pathogens detected in the collected ticks included *Theileria annulata* (18.4%), *Anaplasma ovis* (15.79%), *Anaplasma centrale* (13.16%), and *Rickettsia slovaca* (13.16%). In blood samples, the most frequently detected pathogens were *T. annulata* (*n* = 8), *Babesia bovis* (*n* = 7), *A. centrale* (*n* = 6), and *Babesia bigemina* (*n* = 5). 14 different pathogen species were detected in the collected ticks, including *T. annulata, A. ovis, R. slovaca, A. centrale, R. massiliae, A. marginale, B. bigemina, Theileria sp., T. ovis, B. bovis, A. capra, Ehrlichia sp., R. hoogstraalii, T. orientalis, and A. bovis.* While in blood samples, where 13 pathogens were identified, including *T. annulata, B. bovis, A. centrale, B. bigemina, A. marginale, T. ovis, T. orientalis, A. ovis, R. slovaca, A. capra, Rickettsia sp., Ehrlichia sp*., and A. bovis. PCattle and buffaloes could be co-infected with *B. bovis*, *T. annulata*, and *A. centrale*.

CONCLUSION: Co-infection of multiple pathogens highlights the complexity of the situation. These findings contribute to understanding the epidemiology and distribution of tick-borne diseases provide valuable insights into the circulation of TBPs in livestock and highlight the need for further research on the epidemiological risk that these pathogens pose to ruminants in Pakistan

(P2) GENOMIC CHARACTERIZATION OF E. COLI FROM AN ONE HEALTH PERSPECTIVE

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The One Health concept emphasizes the interconnectedness of human, animal, and environmental health. *Escherichia coli* is an ideal model organism for understanding this nexus, with its presence in various hosts and ecological niches. Understanding the genomic characteristics of *E. coli* across these domains can provide information on the patterns of *E. coli* transmission and its antimicrobial resistance and, therefore, provide public health interventions. Wildlife, water, and soil samples were collected between July 2022 and April 2023, and *E. coli* isolates were recovered using selective media. Antibiograms were performed for 17 antibiotics according to EUCAST guidelines, and extended-spectrum β-lactamases (ESBL) production was tested by the double-disc synergy test. PCR was performed to detect the resistance genes. Three *E. coli* isolates were recovered from wildlife, twelve from water samples, and ten from soil samples. Wildlife isolates were primarily resistant to ampicillin, and one was susceptible to all antibiotics. All water and soil isolates were resistant to ampicillin, and tetracycline was the next most common antibiotic in both water and soil isolates. No ESBL was recovered. For genomic analysis, 15 *E. coli* isolates from all three niches were selected according to their phenotype, and bla_{TEM} was present in 13 of them, confirming the ampicillin resistance and *tetA* and *tetB* were present in 7 and 2 isolates, respectively, confirming the tetracycline resistance phenotype. The genomic analysis of *E. coli* isolates from wildlife, water, and soil demonstrates a robust interface between wildlife and environmental sources in disseminating antimicrobial resistance (AMR) genes. The shared resistance profiles and the presence of similar resistance genes (bla_{TEM}, *tetA*, and *tetB*) across different niches indicate potential cross-contamination common sources of AMR.

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(P3) GENOMIC CHARACTERIZATION AND VIRULENCE ASSESSMENT OF THREE *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA* STRAINS ISOLATED FROM SALMONIDS IN WESTERN EUROPE

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Objectives *Aeromonas* (*A.*) *salmonicida* subsp. *salmonicida* is the etiological agent of furunculosis, a fish disease highly aggressive for salmonids and responsible for significant economic losses in aquaculture industry worldwide. The aims of this work were to study the antimicrobial resistance profile, perform an in-depth genomic characterization, and assess the bacterial virulence in a preliminary *in vivo* model of three European isolates of this subspecies originating from salmonids displaying symptoms of furunculosis.

Methods *A. salmonicida* isolates were tested against 24 antibiotics before being sequenced through Illumina technology to explore their phylogenetic relationships and their composition in mobile genetic elements (MGEs), as well as in virulence and antibiotic resistance genes. Bacterial virulence was finally assessed in an alternative *in vivo* model using *Galleria* (*G.*) *mellonella* larvae.

Results Two of these *A. salmonicida* isolates exhibited a multi-drug resistance profile to commonly used antibiotics for treating furunculosis. Genomic analyses revealed that the chromosomes of the three strains were closely related to the European reference strain A449 of *A. salmonicida* and they harbored multiple plasmids identified to display antibiotic resistance genes, such as the pRAS3.5, pAB5S9b and the pAsa4b. The three strains showed a marked virulence from a dose of 10^2 CFU/10µl in the *G. mellonella* model.

Conclusion Overall, these findings contribute to enrich the European collection of characterized genomes of *A. salmonicida* subsp. *salmonicida* and support the use of *G. mellonella* larvae as a new infection model for the study of this pathogenic subspecies causing furunculosis in salmonids.

(P4) IDENTIFICATION OF CORYNEBACTERIUM ULCERANS ISOLATED FROM EUROPEAN HEDGEHOGS (ERINACEUS EUROPAEUS) IN WALLONIA AND CHARACTERIZATION OF ITS ZOONOTIC POTENTIAL

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Corynebacterium ulcerans is an emerging zoonotic pathogen that has been isolated from hedgehogs, mainly on skin wounds. The objectives of this study were (1) to estimate the prevalence of *Corynebacterium ulcerans* from wounded hedgehogs in Wallonia and (2) to clarify its zoonotic potential.

Systematic sampling of skin lesions and internal organs was carried out during necropsy of 80 wounded hedgehogs that died in wildlife rehabilitation centers during spring and summer 2020- 2021. The detection of corynebacteria was performed by qPCR, targeting rpoB and Tox genes, and by bacteriological methods using Hoyle's agar medium. Identification was conducted using MALDI-TOF mass spectrometry. An ELEK test was performed to confirm the production of diphtheria toxin.

The results indicate a high detection of tox gene-bearing corynebacteria (80%). This detection was observed in skin lesions as well as internal organs, demonstrating the potential of dissemination of these Corynebacteria. Among the isolated corynebacteria, *Corynebacterium ulcerans* was the only representative of the diphtheriae complex. Most (93%) were tox gene- bearing and toxigenic.

Our study presents the first detection of *Corynebacterium ulcerans* from Hedgehogs in Southern Belgium and highlights its ability to produce diphtheria toxin, the major virulence factor responsible for diphtheria in humans. The fact that tox gene-bearing corynebacteria were detected in 80% of wounded hedgehogs that were treated/manipulated in the centers led to recommendations to limit the exposure of handlers in care centers.

(P5) TOWARDS ONE HEALTH-SURVEILLANCE SYSTEMS FOR INDICATOR-PATHOGENS IN WASTEWATER

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Objectives: Wastewater-based epidemiology (WBS) is an upcoming tool for the surveillance of various pathogens (1) relevant to both human and veterinary medicine. The aim of this study is to develop a culture-based detection method for extended spectrum ß-lactamases (ESBL)- and carbapenemase-producing Enterobacterales, which will subsequently undergo whole-genome sequencing for characterization and phylogenetic analysis fostering integrated surveillance systems.

Methods: Wastewater samples processed by serial dilution or filtration methods are plated onto different media. *Escherichia coli* and *Klebsiella pneumoniae* are among the indicator organisms frequently isolated

from clinical samples of human and animal origin. Suspect colonies are identified using MALDI-TOF-MS and the phenotypic susceptibility profile is determined using Vitek 2-compact. The production of ESBL or carbapenemases is confirmed following CLSI guidelines (2).

Results: Preliminary results show that both methods of sample processing are suitable for isolating ESBL- and carbapenemase-producing Enterobacterales. *E. coli* is the dominating ESBL-producing species, while *K. pneumoniae* is the most frequently isolated carbapenemase-producing Enterobacterales identified.

Conclusions/outlook: WBS is a promising tool towards an One Health integrated system. With sample processing methods and media as well as culture conditions adapted to the target organism, the analysis of wastewater can be used to detect clinically relevant pathogens, emerging and re-emerging pathogens, and antimicrobial resistance.

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(P6) FACTORS ASSOCIATED WITH BACTEREMIA IN CRITICALLY ILL CALVES

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Objective: Sepsis is a life-threatening condition in calves, but its diagnosis remains challenging. Therefore, the objective of our study was to develop a predictive model for bacteremia in critically ill calves.
Methods: A cross-sectional study was conducted on 143 critically ill calves, sampled for two aerobic blood culture media. Presumed contaminants were excluded, so only calves with likely true pathogens were considered bacteremic. Multivariable logistic regression and classification and decision tree analysis were performed to determine risk factors for bacteremia.
Results: The best-performing logistic regression model identified abnormal temperature, heart frequency, absence of enteritis, hypocalcemia, and hyperlactatemia as risk factors for bacteremia, with a sensitivity of 71.4% and specificity of 93.9%. The decision tree analysis highlighted hypoglycemia, absence of diarrhea, and hyperlactatemia as risk factors, with a sensitivity of 39.4% and a specificity of 92.7%.
Pathogens isolated from the bacteremic calves were *Escherichia coli*, *Salmonella enterica*, *Raoultella ornithinolytica*, *Enterobacter cloacae*, *Klebsiella oxytoca, Campylobacter jejuni*, *Trueperella pyogenes*, *Streptococcus uberis*, *Staphylococcus xylosus*, *Staphylococcus sciuri,* and in one calf both *S. enterica* and *E. coli* were isolated.
Conclusions: Hyperlactatemia and hypoglycemia appear important to predict bacteremia in critically ill calves. Enteritis, although a frequent condition in this population, appears not to be the most important predisposing disease for bacteremia. The performance of these models remains insufficient to predict bacteremia. The isolated bacteria, both Gram-negative and Gram-positive, are similar to those in previous studies on bacteremic calves.

(P7) INITIAL ISOLATION AND IDENTIFICATION OF ESCHERICHIA COLI ASSOCIATED WITH WATERY MOUTH DISEASE

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Watery Mouth Disease (WM) is a significant cause in neonatal lamb mortality in sheep production. In this stage of life, *Escherichia coli* has a greater potential to cause diseases, especially without the proper environmental conditions and colostrum administration. This study reports the initial findings on the isolation and identification of *E. coli* associated with WM. This study assessed samples from organs (brain, lungs, liver), swabs from the gastrointestinal tract (abomasum, jejunum, ileum, and colon), and the umbilical cord of 32 neonatal lambs with clinical signs of WM. Bacterial isolation was performed with blood agar and MacConkey agar. TSI agar, indole tests, and API 20E were used to identified pure cultures of *E. coli*.

Bacterial culture and isolation results revealed *E. coli* in 96% of the gastrointestinal samples, which is expected given its presence in the normal mammalian flora. In 62.5% of the cases, *E. coli* was isolated from one or more organs. Among these, and out of the cases with complete histopathology results, 47% were confirmed as *E. coli* septicemia. Further analysis of phylogenetic groups and antimicrobial resistance will be performed utilizing PCR and disk diffusion methods. Approximately 100 E. coli strains will be further analysed with Whole Genome Sequencing. The results will provide insight into the genetic diversity, virulence factors, antimicrobial resistance genes, and pathogenic potential of the strains. Improving our understanding of Escherichia coli in the pathogenesis of WM and contributing to advancements in prevention, and antimicrobial treatment, thereby reducing the impact on antimicrobial resistance.

(P8) CHARACTERIZATION OF CORYNEBACTERIUM ISOLATES OBTAINED FROM RATS

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Objective: Recently, hedgehogs have been identified as reservoirs for zoonotic, toxigenic *Corynebacterium ulcerans* in Europe, causing hedgehog diphteric disease. It is currently unknown whether other wildlife, such as brown rats, may act as a reservoir for *Corynebacterium ulcerans* or other *Corynebacterium* spp that may carry antimicrobial resistance or virulence determinants.
Methods: Oral swabs from 201 dead wild brown rats (*Rattus norvegicus*), captured in the context of a rodenticide resistance study in Flanders, were inoculated on Hoyle's agar (24h incubation at 37°C) and purified isolates were identified with MALDI-TOF MS. MIC testing was performed using the broth microdilution method according to CLSI standards.

Results: Various *Corynebacterium* species were obtained, such as *Corynebacterium ammoniagenes, Corynebacterium callunae, Corynebacterium doosanense, Corynebacterium efficiens, Corynebacterium flavescens and Corynebacterium stationis*, though no *Corynebacterium ulcerans* was identified. The most frequently observed species, *Corynebacterium doosanense*, is poorly described and was initially derived from a wastewater treatment plant. Most of the *Corynebacterium doosanense* isolates were part of the wild type population for most antimicrobial agents, with few isolates showing increased MIC values, and therefore suspected for acquired resistance, against penicillin, tetracycline, rifampicin and enrofloxacin. Based on these results, a selection of isolates will be genetically characterized using whole genome sequencing.

Conclusion: The oral microflora of wild brown rats contains various *Corynebacterium* species, with *Corynebacterium doosanense* as most prevalent. Acquired resistance against different antimicrobial classes was documented in *Corynebacterium doosanense*, though the prevalence of acquired resistance is still quite low.

(P9) DECOLONIZATION OF PANTON-VALENTINE LEUCOCIDIN-POSITIVE S. AUREUS IN FAMILY CAT TERMINATED SKIN INFECTIONS

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Panton-Valentine leukocidin (PVL)-producing *S. aureus* (SA) cause recurrent skin abscesses in humans. In addition to antibiotic and surgical treatment, decolonization of patients is necessary to prevent reinfection and further transmission. Here, we report on an affected family (two adults and two children) whose two cats were identified as SA colonized after all family members had been previously decolonized three times and continued to suffer reinfections. Since validated strategies for decolonization of animals do not yet

exist, we aimed to develop a protocol for outpatient decolonization using systemic antibiotic treatment of cats.

SA isolates of the cats were tested for *pvl* by PCR and subjected to susceptibility testing (AST) according to CLSI. Comparative analysis of human and feline isolates was performed by whole genome sequencing. The decolonization protocol included oral therapy with amoxicillin-clavulanic acid based on the results of susceptibility testing.

SA was isolated from the oral cavity and nose of both cats. While one cat was a carrier of a *pvl*-positive isolate, only a *pvl*-negative SA strain was isolated from the second cat. Comparative whole genome analysis revealed close clonal relationship of both the *pvl*-positive isolates assigned to ST 8 and the *pvl*-negative SA (ST45) from humans and cats. After a total of 20 days of oral therapy, SA could not be isolated in any animal. Control examinations after 3 and 7 weeks also showed negative results.

Decolonizing the pets led to successful elimination of the bacteria from the household. Clinicians should consider pets as possible reinfection sources.

(P10) PREVALENCE STUDY OF BRUCELLA CANIS IN DOGS RESIDING IN UMBRIA

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Objectives: This study aims to determine the prevalence of *Brucella canis* infection in a canine population in Umbria, Italy. This is the first national study on this subject, potentially serving as a model for future regional studies.

Methods: Blood samples were collected from various groups of dogs in Umbria: 1) dogs from three sanitary kennels; 2) breeding dogs; 3) blood donor dogs; 4) dogs accompanying refugees from Ukraine; 5) dogs from a big Italian *B. canis* outbreak. Samples were tested for *B. canis* using direct (bacteriological isolation and real-time PCR) and indirect methods (serum agglutination and complement fixation).

Results: The study population consisted of diverse groups of dogs, varying widely in age, sex, and breed. Ages ranged from 2 months to 17 years, with mean and median age of 3 years. The gender distribution was nearly equal, with 64 males and 62 females, and 2 of unknown sex because of anonymized. These dogs represented more than 21 breed, with mixed breed dogs made up 25.78% of the population, followed by Golden Retrievers at 19.53%, and Yorkshire Terriers at 13.28%. This variety provides a comprehensive overview of the canine population in the region.
None of the 128 dogs tested positive for *B. canis*, implying a potential prevalence of up to 3.5% (CI 95%).

Conclusions: Despite no positive cases, the study highlights the need for continuous surveillance of *B. canis* in canine populations due to the disease's zoonotic potential, presence of risk factors of introducing this infection in the area and economic impact on breeding.

(P11) STAPHYLOCOCCUS SPP., AN EXOTIC APPROACH: CAN IT BE RESPONSIBLE OF DISEASE?

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Exotic pet ownership is on the rise globally, including in the UK, Canada, Australia, the US, Europe, Asia, and South America. Given the high demand, it's important to note that these animals can be significant sources of zoonoses, particularly for young children and immunocompromised individuals. Gram-positive

bacteria from the Staphylococcaceae family, part of the normal skin and mucous microbiota of animals and humans, can cause disease, making exotic pets potential infection sources.

The objectives of this study consist of assessing the prevalence of Staphylococcus spp. responsible of infection in exotic animals, identifying the species and evaluating the sensibility to different antibiotics.

Samples were collected from exotic animals exhibiting various pathologies. These samples were then inoculated on blood agar media. Based on cultural and morphological characteristics, the samples that contained Gram-positive cocci arranged in clusters were identified using the Vitek 2 Compact system. The same system was used for antibiotic susceptibility testing resulting in MIC concentrations, as well as the disk diffusion method.
A total of 29 cases were analyzed, consisting of 19 small mammals, 8 birds and 2 reptiles. From these cases, 34 strains of Staphylococcus spp. were isolated, with *Staphylococcus warneri* being the most frequently isolated species. The majority of strains were isolated from respiratory pathologies. Thirteen species were coagulase-negative, and only one was coagulase-positive.

The study highlights the rising trend of exotic pet ownership globally and the associated risks of zoonotic infections, particularly from Gram-positive *Staphylococcus* bacteria.

(P12) PARATUBERCULOSIS IN CATTLE: THE ROLE OF ALPINE COMMUNAL PASTURES IN THE EPIDEMIOLOGY OF THE DISEASE

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Paratuberculosis (paraTB), caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), is a chronic infectious wasting disease that is endemic worldwide and affects many animal species. ParaTB infections lead to significant economic losses due to reduced production and premature culling, affect animal welfare and are also thought to cause disease in humans. Map prevalence in dairy cattle herds in Europe varies and may exceed 50% in some regions. In Slovenia, paraTB control is based only on following voluntary hygiene guidelines; Map prevalence at the herd level is estimated at around 8%.

The aim of the study was to assess the impact of common summer pastures on the spread of paraTB among herds, as pastures are considered important Map transmission routes, as are animal relocation and trade.

Between August 2022 and July 2023, 118 pooled faecal samples were collected from 14 shared alpine pastures. On each pasture, at least three pooled samples were taken from the grazing areas frequently visited by the animals. In addition, 21 water samples from the pastures were analysed. Map was detected and quantified using a previously described TaqMan qPCR assay targeting the F57 gene. All water samples were negative, while the presence of Map could not be clearly excluded in 13 (11%) faecal samples from five (35%) pastures.

Our preliminary results indicate that alpine pastures in Slovenia are not an important risk factor for the spread of paraTB. However, further surveillance of cattle herds using common pastures is advisable.

(P13) HUMAN AND ANIMAL ORIGINS OF STAPHYLOCOCCUS AUREUS FROM COMPANION ANIMALS

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Staphylococcus (S.) *aureus* is commonly found in both humans and companion animals. The species has a clonal structure. Some clonal complexes (CC) are host-specific, while others occur equally in both humans and animals. Virulence factors such as Panton-Valentine-Leukocidin (PVL, luk PV) are associated with human strains. The animal-associated clonal complex LA CC398 can be differentiated into 3 evolutionary-epidemiological subpopulations (1. human, 2. livestock 3. veterinary clinic associated). The aim of this study was to type *S. aureus* isolates obtained from veterinary clinical samples. *S. aureus* isolates from dogs, cats, small mammals and horses (n=396) were analyzed for assignment to clonal complexes (spa gene), MRSA (mecA), PVL (lukS-PV, lukF-PV) and LA CC398 clade C (veterinary clinic associated) using molecular typing methods. In addition, resistance tests were carried out using bouillon microdilution. The isolates originated predominantly from skin, wound and ear infections. With regard to resistance and assignment to clonal complexes, a heterogeneous distribution with predominantly human clonal complexes was detected. 68 (17.2 %) isolates were assigned to the animal-associated clonal complex LA CC398, of which 20 isolates (29.4%) were assigned to clade C (veterinary clinic-associated). All LA CC398 were PVL negative. Veterinary *S. aureus* isolates and livestock-associated *S. aureus* are genetically diverse and include strains with both human and animal specific backgrounds. MRSA occurs more frequently in samples from clinically diseased animals compared to asymptomatically colonized animals. A transmission of PVL from human *S. aureus* strains to livestock associated *S. aureus* could not be proven.

(P14) MANNHEIMIA HAEMOLYTICA SEROTYPES OF ISOLATES FROM DIFFERENT TISSUES OF CALVES WITH POLYSEROSITIS OR CONTROL CALVES

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It is unknown why specifically *M. haemolytica* serotype 2 is involved in polyserositis in veal calves and not serotype 1/6. To test, if this was caused by a relatively high prevalence of serotype 2 in Dutch veal, we performed culturing and whole genome sequencing (WGS) for *M. haemolytica* from eleven locations of 54 calves with *Mannheimia* polyserositis and control calves at necropsy.

This delivered 98 *M. haemolytica* isolates from 23 cases and 19 isolates from 14 controls for GS. With serotyping based on WGS. From case calves, 96 isolates were serotype 2 and were retrieved from nasal cavity, spleen, serosa, bronchial lymph nodes and lung tissue. Two were serotype 1, which were retrieved from the nasal cavity and the retropharyngeal lymph nodes. In contrast, 15 control calf isolates were serotype 1 and four were serotype 2. These were mainly found in tonsils, nasal cavity and bronchial bifurcation.

M. haemolytica serotype 2 is widely spread in calves with *M. haemolytica* polyserositis. In contrast, in control animals, the populations are dominated by serotype 1/6, mainly found in the upper airways. This is an indication that the linkage between polyserositis and serotype 2 is not simply caused by a wider distribution of this serotype 2 in the Dutch veal calf industry but must have another cause.

(P15) NEW VARIANT STRAIN OF STREPTOCOCCUS CANIS WITH LANCEFIELD GROUP C ISOLATED FROM CANINE OTITIS EXTERNA

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Every basic course in microbiology teaches us, *Streptococcus (S.) canis* always tests positive for Lancefield group G. In human diagnostics, Lancefield grouping is still the gold standard for determining streptococcal species and establishing treatment plans. Surprisingly, we identified a strain of *S. canis* with Lancefield group C.

From the ear swab of a dog with otitis externa after lateral ear canal resection, phenotypically suspicious beta-hemolytic streptococci were isolated. One of the strains showed different morphology on a blood agar plate. The strains were grouped with a latex co-agglutination kit, where one of the strains tested positive for Lancefield group C, while the other strains tested positive for Lancefield group G. This Lancefield group C strain corresponded with the strain that showed a different morphology. MALDI-TOF and 16S PCR confirmed all strains to be S. canis. The strains were sequenced by WGS. Besides the Lancefield C *S. canis* strain, in the same ear, two other *S. canis* strains were found, all with different sequence types according to MLST analysis. Analysis of the WGS data points towards a horizontal gene transfer event between *S. canis* and *S.* dysgalactiae predicted by Alien_Hunter software. Although these species are closely related, gene transfer in this region of the genome of *S. canis* has not been described before.

This finding provides evidence that Lancefield grouping is not as distinctive for the streptococcal species as it was in the time of Rebecca Lancefield. Due to evolutionary events like mutations, genomic rearrangements and in this case, horizontal gene transfer, bacteria can adapt to overcome the host immune system and antibiotic treatment. Adopting a new serotype by exchanging the Lancefield carbohydrate will create new possibilities for infection, but will also create difficulties in diagnostic processes. The value of technologies as MALDI-TOF MS and sequencing will grow as more diverse streptococci arise.

(P16) BRONCHOALVEOLAR LAVAGE SAMPLES FROM DOGS AND CATS; A COMPARISON OF METHODS AND RESULTS OF BACTERIOLOGICAL CULTURING FROM THREE EUROPEAN VETERINARY DIAGNOSTIC LABORATORIES

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Objective: Diagnosing bacterial lower respiratory tract disease (LRTD) in dogs and cats relies on a combination of diagnostic methods such as thoracic imaging, bronchoscopy, and collection of lower respiratory tract samples, such as bronchoalveolar lavage (BAL), for cytology and microbiology. The objective of this study was to compare bacteriological culture protocols and results for BAL samples processed in different veterinary laboratories.

Methods: A retrospective analysis of pathogen prevalence was made from culture results of canine and feline lower respiratory tract samples processed in three European veterinary laboratories (L1-L3) over a period of respectively 4,5 years, 6 years, and 12 years. Also, protocols for processing such samples were retrieved and compared.

Results: Protocols for bacteriological culture of BAL samples differed. Specimens were plated directly (L1 and L3) or after centrifugation (L2). Enrichment was only used in L2. Anaerobic culture was routinely performed in L1 and L2, and upon request in L3.
In total, 976 BAL specimens were analyzed (L1, n=267; L2 n=614; L3 n=95). The prevalence of positive cultures was 45,3%, 60,7% and 51,6% for laboratories L1-L3 respectively. Many BAL samples yielded mixed cultures (13,9%, 24,9% and 15,8%). The most common pathogens were *Bordetella bronchiseptica*

(3,0 %, 5,0% and 4,2%) Pasteurella spp. *(*5,2%, 5,9% and 7,4%) and *Escherichia coli* (4,1%, 2,6 % and 7,4%).

Conclusions: Different methods for bacteriological culturing of BAL samples impact on the detection of pathogens and the prevalence of pathogens identified. The outcomes of this study represent an initial step towards the standardization of bacterial culture methodologies.

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(P17) THROUGH THE LABYRINTH: HOW SALMONELLA PENETRATES THE GASTEROINTESTINAL MUCUS

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Motility and virulence of enteropathogenic bacteria such as *Salmonella enterica* and *Escherichia coli* have been shown to connected by complex regulatory networks[1]. Apart from motility, flagellum nanomachine can contribute to bacterial chemotaxis and evasion of host immune responses[2]. For successful host infection, pathogens need to penetrate mucus layer to be able to disrupt the mucosal barrier and invade the epithelial cells in the gut. Previous studies showed the importance of the flagella driven motility in breaching of the mucosal barrier and gut colonization[3]. However, the exact bacterial navigation strategies and mechanisms of interaction between bacteria and mucus layer/underlying cells are unknown.

We aim to elucidate molecular mechanism of interaction between enteric pathogen *Salmonella* Typhimurium and the gastrointestinal mucus prior to intestinal epithelium invasion with a focus on the role of bacterial flagellar apparatus. Thus, we generated a group of GFP-expressing *Salmonella* Typhimurim ATCC 14028 mutants lacking different structural, antigenic and functional parts of flagellum such as Δ*fliB*, Δ*fliC* and Δ*flijB*/*fliC*. To this end, using optical microscopy we follow the motion of *Salmonella* Typhimurium at interfaces and within biological hydrogels such as mucus. We will give examples, how motility of *Salmonella* is affected by the properties of its environment or the *Salmonella* mutant probed.

Furthermore, by continuously recording z-stacks of samples, in which *Salmonella* interact with mucus-producing cells, we succeeded to follow dynamics of bacterial penetration through a biologically relevant hydrogel. We will show that bacterial penetration predominantly proceeds via hydrogel voids, which either already present or actively generated by bacteria.

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(P18) EVALUATING E. COLI SURVIVAL DURING COMPOSTING: IMPLICATIONS FOR SOIL AND ANIMAL SAFETY

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The use of animal manure or sewage sludge as fertilizers raises significant safety concerns, namely the potential soil contamination by *Escherichia coli*, despite their richness in essential nutrients. Composting represents a viable strategy to mitigate these risks, aiming to ensure the safety of water, food, and soil by promoting heat-related mortality in *E. coli*. This strategy allows the beneficial use of these residues while preventing the risk of illness outbreaks in humans and animals. This study assessed the survival rate of *E. coli* during the composting process of two mixtures (M1 and M2) with varying proportions of vine pruning residues and sewage sludge, in which cattle slurry solid fraction was used as inoculum. Samples were collected at four timepoints (0, 30, 60, 90 and 140 days) during composting. Approximately 25g of compost underwent incubation in sterile saline solutions at increasing dilutions (1:10, 1:100, 1:1000 w/v). The resulting samples were cultured on CHROMagar™CC. agar medium and incubated aerobically at 37°C and 44°C for 24 hours, with microbial quantities measured as colony forming units (CFU) per gram of sample. Results revealed the presence of *E. coli* at the outset of composting for both M1 (ranging from $5.2x10^3$ to $1.35x10^4$ CFU/g) and M2 (ranging from $1.5x10^3$ to $4.3x10^3$ CFU/g). *E. coli* persisted in M1 for up to 30 days and in M2 for up to 60 days. The absence of *E. coli* in both final products (140 days) highlights the effectiveness of the thermophilic phase of composting in sanitizing the studied mixtures.

(P19) FATAL INVASIVE PULMONARY ASPERGILLOSIS IN AN IMMUNOCOMPROMISED DOG

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Objective: *Aspergillus fumigatus* is a saprophytic opportunistic fungus associated with localized or disseminated infection in immunocompromised hosts. While thoroughly researched in human medicine, deep aspergillosis in dogs lacks investigation. To the best of our knowledge this case is among the few reports describing mycotic bronchopneumonia in an immunocompromised dog.

Case summary: A three-year-old Lagorai Shepherd dog was referred to the clinic due to a cough and reluctance to walk persisting for six days.

Clinical findings: On a complete blood count pancytopenia with degenerative left shift of neutrophils was detected. Tomography showed diffuse pneumopathy with regional lymphadenomegaly and pleural effusion. Cytological examination of bronchoalveolar lavage fluid (BALF) detected neutrophilic inflammation along with coccoid microorganisms and branching and septate structures consistent with fungal hyphae.

Diagnostic findings: BALF was processed with eSwab® and streaked in differential growth media including Sabouraud Dextrose Agar. The plates were incubated at 37˚C under aerobic conditions for 4 days. *Klebsiella oxytoca*, *Staphylococcus shleiferi* and *Aspergillus fumigatus* growth were observed. Identification was made by MALDI-TOF mass spectrometry. Real-time PCR assay detected *Bordetella bronchiseptica*, while on faecal examination *Angiostrongylus vasorum* larvae were also seen. Due to worsening of respiratory condition and the onset of multiorgan dysfunction, the owners elected euthanasia.
Clinical relevance: Necropsy examination confirmed mycotic bronchopneumonia. The synergistic effect of the bacterial and parasitic infections along with possible previous neutropenia probably led to the

colonization of *Aspergillus fumigatus* resulting in widespread bronchopathy. Parasitic lesions and the *Bordetella bronchiseptica* infection may have diminished local immune defence capacity of the lung.

(P20) ANTIFUNGAL ACTIVITY OF STRAWBERRIES AND BLUEBERRIES LEAVES AGAINST ANIMAL-ORIGIN *ASPERGILLUS* SPP.

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The incidence of fungal infections caused by *Aspergillus* species poses a significant challenge in veterinary medicine. These fungi are known to cause a wide range of diseases in animals, including respiratory and cutaneous infections, often resulting in substantial morbidity and mortality. Developing effective strategies for controlling these infections is crucial for animal health and welfare, as well as for preventing zoonotic transmission to humans.
Strawberries and blueberries have garnered attention not only for their nutritional value but also for their potential health benefits, including antimicrobial properties. Their antioxidant properties and other bioactive compounds have been extensively studied for their role in promoting human health.
This study aimed to evaluate the efficacy of strawberry and blueberry leaves in inhibiting the growth of *Aspergillus niger* and *Aspergillus fumigatus* isolated from animals, with the goal of contributing to the development of new strategies for controlling and preventing fungal infections in veterinary medicine.
The methodology employed in this study involved the use of macerated leaves of blueberries and strawberries. Various concentrations of plant extracts (5 mg/mL, 10 mg/mL, 20 mg/mL, and 30 mg/mL) were prepared in Potato Dextrose Agar supplemented with water. Subsequently, fungal inoculum was introduced onto each plate, and the diameter of fungal growth was measured over the course of one week.
The results revealed that blueberry leaves did not inhibit the growth of either fungus, while strawberry leaves inhibited *Aspergillus fumigatus* with a 100% inhibition at the highest concentrations. Furthermore, a dose-dependent relationship was observed, with higher concentrations exhibiting greater inhibition.

(P21) OCCURRENCE OF *ASPERGILLUS FUMIGATUS*, *A. NIGER*, *A. VERSICOLOR* AND *ASPERGILLUS FELIS* IN REPTILES FROM A PORTUGUESE ZOO

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Aspergillus fumigatus is a significant cause of morbidity and mortality worldwide, primarily due to its association with pulmonary infections. Risk factors for developing pulmonary aspergillosis include immunosuppression, chronic respiratory diseases, and, more recently, concurrent viral or bacterial pulmonary infections. Understanding the distribution and host range of *Aspergillus* spp. is essential for implementing effective mitigation measures.

A convenience sample of 42 reptiles (belonging to a Portuguese zoo) was examined for the presence of *Aspergillus* spp. in the skin. Samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25ºC and 37ºC, for 3-7 days.

Occurrence of *Aspergillus* was as follow: *A. niger* in 2 Cuban boas, 2 green iguanas, 1 Burmese python, 2 red-footed tortoises, 1 Greek tortoise, 1 Mediterranean tortoise, 1 Russian tortoise, and 2 common tegus,

31.0% (95% CI: 17.6-47.1%), *A. felis* in a green iguana and a 2.4% (95% CI: 0.0-12.6%), *A. versicolor* in red-footed tortoises, 2.4% (95% CI: 0.0-12.6%), *A. fumigatus* in a Russian tortoise and 1 golden tegu, 4.8% (0.6-16.2%).

Aspergillus spp. are a ubiquitous saprophyte in nature found in air, soil and organic matter. It can cause a wide range of illnesses ranging from allergies to invasive infections in animals and humans. Occurrence of *Aspergillus* was high in this study. Since this agent is an important opportunistic fungus, more studies are required to better understanding the relevance of the isolation of this fungus in the skin of reptiles and its significance for animal and public health.

(P22) EPIDEMIOLOGICAL STUDY ON DERMATOPHYTES IN PORTUGUESE HEDGEHOGS

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Dermatophytosis is a widespread fungal infection affecting humans and animals, commonly known as ringworm. Dermatophytosis in hedgehogs can cause clinical signs such as crusting around the face and loss of spines. Children exhibit a notable fascination with hedgehogs and display a keen interest in tactile interaction, intrigued by the unique combination of softness and spines these small mammals possess. In many cases, hedgehogs can be symptomatic and asymptomatic carriers of dermatophytes, which poses a risk of zoonotic transmission to humans. Despite the potential public health implications, there is limited information on the prevalence and epidemiology of dermatophytes in hedgehogs, in Portugal. To better understand the epidemiology of dermatophytes in hedgehogs in Portugal, a study was conducted from January to May 2024.

Fur and spines were collected from 100 hedgehogs from a hedgehog rescue and interpretation centre using the toothbrush technique. Dermatophyte culture was performed using Dermatophyte test medium®. Petri dishes were handled under sterile conditions and incubated at 28°C for up to 21 days. Of the 100 animals under study, 35.0% had clinical signs, and 7.0% presented with alopecia. The observed lesions were crusts (28.0%), erythema (21.0%) and vesicles (4.0%). The frequency of occurrence of dermatophytes was 19.0% (95% CI: 11.8-28.1%), *Trichophyton erinacei* was 7.0% (95% CI: 2.9-13.9%) and *T. mentagrophytes* was 13.00% (95% CI: 7.1-21.2%). The frequency of occurrence of *Microsporum* spp. was 1.0% (95% CI: 0.0-5.5). Considering the scarcity of epidemiological reports in Portuguese hedgehogs, these results could be a useful contribution towards diagnosis and prevention.

(P23) FUNGAL BIODIVERSITY IN BIRDS AND MAMMALS OF A ZOO - IMPLICATIONS FOR ANIMAL AND PUBLIC HEALTH WITHIN A ONE HEALTH APPROACH

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The exploration of fungal biodiversity within zoo ecosystems, particularly among birds and mammals, is essential for understanding the ecological dynamics and potential health implications associated with these environments. In this study, we investigate the fungal biodiversity inhabiting the integuments of birds and mammals within a zoo setting, shedding light on the diverse array of fungal species that coexist with these animal populations. A convenience sample of 5 birds and 16 mammals belonging to a Portuguese zoo was

examined for the presence of fungus. Samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25ºC and 37ºC, for 3-7 days.

Based on the observation of microstructures and colony morphology, the fungal isolates were identified at genus level. In this study, 12 genera were identified. The most occurrent genera were Penicillium spp. (27.3%; CI 95%: 10.7-50.2%), Fusarium spp. (27.3%; CI 95%: 10.7-50.2%), Trichoderma spp. (13.6%; CI 95%: 2.9-34.9%) and Aspergillus spp. (13.6%; CI 95%: 2.9-34.9%). This study provides the first description of mycobiota of the integument of zoo birds and mammals, showing many saprophytic genera.

Our findings also raise public health concerns as veterinarians and other professionals, especially those who are immunosuppressed, have a high risk of infection when handling samples from these species.

(P24) HEMOLYTIC ACTIVITY IN SHELTER OPPORTUNISTIC FUNGI

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Fungal haemolytic activity has been suggested as potential virulence factor, exhibiting cytotoxic effects on erythrocyte membranes, and leading to the release of iron, a critical growth factor for microorganisms, especially in the context of infection. Iron is essential for fungal growth, being crucial for metabolic and biochemical processes. The expression of haemolytic proteins capable of lysing erythrocytes has also been associated with the survival strategy of fungi during opportunistic infections. This study aims to ascertain the presence of haemolytic activity in diverse isolates of fungi from the fur of animals and from the environment of a Portuguese shelter. Microbiological cultures were performed in Potato Dextrose Agar. Hemolytic activity was evaluated in 29 isolates (*Aspergillus felis*, *A. fumigatus, A. niger, Penicillium spp., Talaromyces spp.* and *Talaromyces marneffei*). Samples were speciated based on microscopic identification using lactophenol cotton blue mount and slide culture technique. To determine haemolytic activities, fungi were subcultured on Brain Heart Infusion agar with 5% sheep blood and incubated for 3-5 days at 28°C under aerobic conditions. The occurrence of hemolytic activity in this study was 58.6% (95% IC: 38.9-76.5%): *Aspergillus felis* (2/4), *A. fumigatus* (3/6), *A. niger*, *Penicillium* spp. (10/15), *Talaromyces* spp. (1/1) and *Talaromyces marneffei* (1/1).
Since, one of the advantages for initial survival of inhaled fungal spores in the respiratory tract is the ability for iron acquisition via hemolytic factor-production. Virulence factors production among these isolates could play an important role in the pathogenesis of opportunistic fungi.

(P25) COMPARATIVE STUDY ON THE ANTIFUNGAL ACTION OF BIOCIDAL SUBSTANCES ON *ASPERGILLUS* ISOLATES FROM A SHELTER

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Aspergillus spp. has been identified as a causative agent of invasive aspergillosis and rhinosinusitis in humans, dogs, and cats. The aim of this study was to evaluate the antifungal potential of the commercial disinfectant Biocidal active substance (PT2/AL): 0.5g/100g didecyldimethylammonium chloride (CAS: 7173-51-5) against *Aspergillus felis* and *Aspergillus fumigatus* using the mycelial growth method.

Samples were collected in a Portuguese shelter from the fur of cats and the shelter environment. Sterilized

test tubes and cotton-tipped swabs, moistened with sterilized distilled water, were used to collect samples. *A. felis* (*n* = 4) and *A. fumigatus* (*n* = 6) was grown on Potato Dextrose Agar (PDA) for 3-5 days. Afterwards, a mycelial 4 mm-disk was placed in the center of a PDA Petri dish with 0.005% of active principle. The radial growth of colonies was measured along two diameters and the average of these two measurements was considered as the diameter of the fungal colony. Growth zones were measured in the third, fifth and seventh day, after incubation at 28 °C, to determine antifungal activity. The colony growth was compared to the control, converting the difference in percentage of inhibition. The average percentage of inhibition for *A. felis* was 83.4%, and for *Aspergillus fumigatus*, it was 80.7%. No differences were observed in the mean inhibition percentages between *A. felis* and *A. fumigatus* (*p* = 0.326). Research into antifungal activity is essential for reducing the spread of potentially dangerous fungi for human and animal health.

(P26) DETECTING CORONAVIRUSES IN UK CARNIVORES

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Spillover events of SARS-CoV-2 into wildlife have occurred across the world, notably in farmed mink (*Neovison vison*) and North American white-tailed deer (*Odocoileus virginianus*). Whilst a 2021 BBSRC funded investigation into the presence of SARS-CoV-2 in UK wildlife demonstrated no detection of this virus in UK wildlife, another novel coronavirus was detected - a previously uncharacterised stoat Minacovirus.

Further to this, a highly divergent coronavirus in Italian badgers (*Meles meles*) has been reported. Screening of archived and new UK badger samples is underway to determine if this coronavirus is present in the UK population. Samples have been PCR tested using broader generic coronavirus primers that target the RdRp region of the genome which is highly conserved amongst coronaviruses. No positive samples have been detected thus far; however, any positive samples will be subjected to Illumina sequencing to retrieve the full-length virus sequence of the new badger coronavirus (only partial sequence is currently available).

The virus sequences will be characterised using coronaSPAdes and other bioinformatic tools to identify any contigs of interest. The virus will then then be assembled in Geneious prime and phylogenetically categorised in IQ-Tree.

Developing our awareness of the diversity of existing wildlife coronaviruses in the UK, particularly in wild carnivores will form the starting point for an investigation of pathology in the wildlife hosts. The potential for cross species transmission or recombination of these highly divergent viruses in these wild UK predators will also be understood.

(P27) REPLICATION KINETICS OF BOVINE GAMMAHERPESVIRUS 4 IN THE PRESENCE OF PLATELET RICH PLASMA IN VITRO

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Diseases of the reproductive tract are a frequent problem in dairy herds. Herpesviruses cause several syndromes including uterine diseases. The role of bovine gammaherpesvirus 4 (BoGHV-4) in the development of endometritis has not been clearly described. Platelet-rich plasma (PRP) is an emerging therapeutic in tissue regeneration due to its enrichment in growth factors with mitogenic and anti-inflammatory potential. This study analysed the replication kinetics of BoGHV-4 in the presence of 5% and

10% PRP, compared to 10% fetal bovine serum. For this, Madin-Darby Bovine Kidney (MDBK) and primary culture-derived endometrial bovine (BEC) cells and the field BoGHV4 strain 07/435 were used. Supernatants and cells were collected at 12, 24, and 48 hours post infection (hpi) for virus titration using the endpoint titration method. A factorial model, as a function of time and treatment, testing hypotheses of absence of interaction was used. Viral titers (VT) were fitted to polynomial regression models. The extracellular VT in BEC cells were not affected by the treatment at any time-points. However, intracellular titers at 24 hpi were significantly higher with 5% PRP and decreased with 10% PRP. At 72 hpi, the VT with PRP significantly decreased compared to earlier time-points. Extracellular BoGHV-4 titers in MDBK, were significantly higher with 5%PRP at all times. Conversely, with 10%PRP, the VT decreased at 48 hpi. However, a significant increase was observed at 72 hpi. This study demonstrates that PRP has strong effects on the replication kinetics of BoGHV4, being highly dependent on the host cell.

(P28) ANALYSIS OF THE INFLAMMATORY RESPONSE INDUCED BY BOVINE GAMMAHERPESVIRUS -4- LPS IN PRIMARY CULTURE CELLS OF BOVINE ENDOMETRIUM

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Most bovine uterine diseases are associated with bacterial infections. However, when the involvement of viruses has been studied, Bovine Gammaherpesvirus 4 (BoGHV-4) has consistently been associated with postpartum endometritis in cattle. BoGHV-4 infection stimulates the secretion of Prostaglandin E (PGE) in endometrial cells, which is a mediator of the inflammatory response in bacterial infections and plays an important role in the reactivation followed by lytic replication of BoGHV-4. In this study, we analyzed CXCL-8 and Interferon-gamma (IFN-γ) because the stimulation of Toll-like receptor 4 (TLR4) by lipopolysaccharide (LPS) induces the release of cytokines necessary to activate potent immune responses and its consequent effect on latent BoGHV-4 infections. For this purpose, an analysis by RT-PCR and ELISA was carried out at different times in cell cultures infected with BoGHV-4 and/or treated with LPS. The results demonstrated that in vitro infection of bovine endometrial cells (BEC) by BoGHV-4 downregulates the TLR4 gene, while the co-presence of BoGHV-4+LPS induces early overexpression of the gene, driven by a synergistic interaction enhanced by LPS. Maximum fold-gene expression for CXCL-8 and IFN-γ in BoGHV-4-infected BEC cells coincided with the maximum viral titer (48 hpi). The increased production of CXCL-8 by BEC cells in the presence of BoGHV-4+LPS indicates a more intense inflammatory response compared to other scenarios. This study demonstrates that bacterial-viral coinfection may exert synergistic effects on endometrial inflammation.

(P29) CONTRIBUTION TO THE EPIDEMIOLOGY OF FLAVIVIRUSES DETECTED IN NATIVE WILDLIFE IN WALLONIA

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Usutu virus (USUV) is an RNA virus from the Flaviviridae family, closely related to the more pathogenic West Nile virus. Initially isolated from Culex neavei mosquitoes in South Africa in 1959, USUV has since spread across Africa, the Middle East, and Europe, primarily affecting wild birds. European Blackbirds (Turdus merula) are particularly susceptible, experiencing significant epizootics and mass mortalities.

The natural transmission cycle of USUV involves mosquitoes and birds as amplifying hosts, with humans and most mammals being incidental hosts. In Europe, USUV has been found in multiple species, including bats, and antibodies have been detected in various domestic and wild animals, including horses, dogs, squirrels, wild boar, deer, and reptiles.

In 2023, a passive surveillance program targeted 602 birds from eight wildlife rehabilitation centers, with samples analyzed using RT-qPCR, identifying 88 positive cases across eight bird orders. Samples included brain, liver, and spleen tissues. This study also conducted genomic monitoring to investigate the emergence and spread of USUV using Sanger sequencing. Additionally, sera from wild boar (Sus scrofa), red deer (Cervus elaphus), roe deer (Capreolus capreolus), and wild raccoons (Procyon lotor) were analyzed using a commercial ELISA kit. This comprehensive approach allowed for a detailed understanding of the virus's spread among different host species in Belgium.

Our findings highlight the need for ongoing multidisciplinary interventions under the “One Health” approach to monitor and prevent this emerging arboviral infection. Understanding the spread and impact of USUV through genomic and epidemiological surveillance is essential for managing its effects on both wildlife and human health.

(P30) SURVEILLANCE ON HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) H5 INFECTIONS IN DOMESTIC CATS AND RURAL STRAY CATS IN THE NETHERLANDS SAMPLED FROM 2020 TO 2023.

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Objectives: Highly pathogenic avian influenza (HPAI) H5Nx and human H1N1pdm2009 influenza viruses can infect cats. Infections in cats may result in viral adaptations or recombinant viruses, which may facilitate zoonotic transfer. Here we investigated the HPAI H5 clade 2.3.4.4 and H1 seroprevalence and risk factor analysis, in domestic cats and stray cats in the Netherlands. In addition, cats were sampled for virus detection.

Methods: Sera from 701 rural stray cats and 871 domestic cats, sampled in the Netherlands from 2020 until 2023, were analysed in ELISAs and HAIs. Swabs and lung tissue from domestic cats and stray cats were analysed using RT-qPCR.

Results: In stray cats, 83/701 (11.8%) sera were positive for HPAI H5 by ELISA and 79.3% of these were confirmed by HAI. Of those, two sera were positive in the HPAI H5 and H1 HAI. In domestic cats, 4/871 (0.5%) sera were ELISA HPAI H5 positive, but none were confirmed by HAI. Domestic cat ELISA H1 seropositivity was 40/871 (4.6%), of which 65% were confirmed by HAI. The highest HPAI H5 seropositivity was found in stray cats living in nature reserves, at dairy farms, in older cats and FIV positive cats. No influenza A virus was detected in 230 cats by RT-qPCR.

Conclusions: The higher HPAI H5 seroprevalence in stray cats compared to domestic cats suggests these are more frequently exposed to HPAI H5 virus, most likely due to foraging on wild birds. In contrast, exposure to H1 was more common in domestic cats compared to stray cats.

(P31) THE HUMORAL IMMUNE RESPONSE OF FOALS TO HEV VACCINATION

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Hendra Virus (HeV) periodically causes fatal disease in horses and humans in Australia. The Equivac® HeV vaccine induces neutralising antibodies in adult horses, however foal response to vaccination is largely undescribed. This study investigated neutralising HeV antibodies in foals in response to HeV vaccination. Serial equine serum samples were collected from two farms, one with HeV vaccinated broodmares and the second with unvaccinated broodmares. All samples were tested using a Luminex® microsphere immunoassay (MIA). A subset of 100 samples was also tested with a HeV virus neutralisation test (VNT) and a Spearman's rank correlation test determined an MIA protective threshold (MIA-PT) that correlated to the minimum protective neutralising titre of 32 (VNT-PT). Foals of vaccinated mares acquired protective levels of maternally derived antibody (MDA), although passive antibodies waned to below MIA-PT levels in most foals from 3-6 months of age. Additionally, foals of vaccinated mares had a suboptimal response to vaccination, however establishment of long-term protective immunity after completion of the primary vaccination course is unknown. Foals from unvaccinated mares had a low to moderate response to vaccination until completion of the primary vaccination course when over 80% of foals had HeV specific antibody above the MIA-PT. This study shows that vaccinated mares transfer protective levels of MDA to their foals, however MDA waning leaves foals susceptible from 3 months old. All foals had an overall poor response to initial vaccination, suggesting that foals from both vaccinated and unvaccinated mares may be susceptible to infection during the primary vaccination course.

(P32) FAECAL VIROME OF BROWN BEARS (URSUS ARCTOS) FROM SLOVENIA

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The brown bear (Ursus arctos) is one of the largest bear species and is found in North America, Europe, and Asia. It is omnivorous and feeds mainly on a variety of plant and animal species, with plants making up 90% of its diet. Understanding their virome, particularly in faeces, can provide insights into their health, diet and interactions with other species, including humans. In this study, the main objective was to investigate the composition and diversity of the faecal virome of brown bears. Understanding the virome of brown bears can provide valuable information about their exposure to pathogens, which is crucial for monitoring the health of wildlife populations.

Samples were prepared following a shotgun metagenomic NGS workflow procedure to study the complete virus population (virome) in a sample. Samples of the intestinal contents of 60 animals of different age, sex, weight and from different hunting areas in Slovenia in 2015, 2016, 2017 and 2018 were selected for analysis. Based on the viral reads, bacteriophages were the most abundant in the virome, followed by mammalian viruses, plant and fungal viruses and invertebrate viruses. Several families of mammalian viruses were found, with *Circoviridae* being the most abundant, followed by *Picornaviridae*, *Picobirnaviridae* and *Parvoviridae*.

In conclusion, the majorities of virus sequences were not from mammalian origin and actually reflect the diet and the microbiota (bacteriophages) of the investigated animals. However, there were also virus sequences belonging to virus families, to which viruses pathogenic to mammals belong (picornaviruses, circoviruses and parvoviruses).

(P33) ANTIVIRAL ACTIVITY OF FUNGAL METABOLITE 6-PENTYL-α-PYRONE DURING BOVINE CORONAVIRUS INFECTION

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Objectives: The main target of this study is the in vitro evaluation of antiviral activity of fungal secondary metabolite (SMs) 6-pentyl-α-pyrone (6PP) against bovine coronaviruses (BCoV), a betacoronavirus, as SARS-CoV-2, to obtain a translational study to SARS-CoV-2. To date, non-toxic antiviral compounds are not available against BCoV infection, but it has been demonstrated that some SMs reduce coronaviruses infection in vitro. The compound 6PP, extracted from Trichoderma strains, has demonstrated antiviral activity against canine coronavirus (CCoV) infection. Thus, in this study, the potential antiviral activity of 6PP was tested against BCoV infection in vitro in bovine kidney cells (MDBK).

Methods: Chromatographic techniques, bioscreen in vitro, cytomorphological analysis, immunofluorescence assay, molecular docking.

Results: Our preliminary results showed that non-toxic concentrations of 6PP reduced signs of morphological cell death and increased significantly cell viability of MDBK cells during BCoV infection. The expression of aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor activated by BCoV infection, and which regulates host immune response to viral infections, was downregulated by 6PP. Interestingly, a high sequence identity of the 3D structural models obtained for the two domains (PASB and TAD) of human and bovine AhRs was revealed by bioinformatics analysis.

Conclusions: The present study demonstrates that 6PP may represent a potential antiviral agent against BCoV infection. In subsequent steps, inclusion complexes with β-cyclodextrin will be prepared and characterized in order to improve the pharmacokinetics of selected SMs to test in cattle.

(P34) UPDATE OF CANINE MORBILLIVIRUS INFECTION IN WILD CARNIVORES – A MULTICENTRIC STUDY IN THREE EUROPEAN COUNTRIES

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Canine distemper is recognized as one of the most significant infectious diseases for wildlife across the globe, especially carnivores, and it's caused by canine morbillivirus. A retrospective study was performed to analyse canine distemper in wild carnivores within the European territory over the last five years (2018-2023). Cases were collected from three wildlife rescue centres from 3 countries: Wildlife Study and Rehabilitation Centre (CERAS), Quercus ANCN (Portugal), Wildlife Station Retscheider Hof e.V. (Germany), and Wildlife Conservation Centre Le Tichodrome (France). This study included 39 animals and the following species: the red fox (*Vulpes vulpes*), the European badger (*Meles meles*), the stone marten (*Martes foina*), the European polecat (*Mustela putorius*), and the raccoon (*Procyon lotor*). The country with the most cases was Germany (76.9%), when compared to France (12.8%) and Portugal (10.3%). The Canidae family (74.4%) was the most affected, as red foxes comprised most of the cases. This family was followed by the Mustelidae family (20.5%), with most cases involving the European badger, as only one stone marten and one European polecat were registered. The Procyonidae family (5.1%), which was represented by the raccoon, was the least affected. Animals were mainly diagnosed via real-time RT-PCR (43.6%) and antigen immunochromatographic rapid-tests (43.6%). Few were tested via histopathology (7.7%) and through a combination of antigen immunochromatographic rapid-tests and real-time RT-PCR (5.1%). This study highlights canine morbillivirus in European wild carnivores, emphasizing the need for comprehensive research on its impact on disease control and wildlife conservation.

(P35) DIFFERENTIAL CYTOKINE RESPONSES TO TRUNCATED AND NON-TRUNCATED NS1 PROTEINS OF EQUINE INFLUENZA VIRUS IN CANINE DH82A CELLS DURING STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS CO-INFECTION

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Equine influenza virus (EIV) is a major cause of respiratory diseases in equids globally. The severity of the disease is influenced by host and viral factors. Variations in the influenza NS1 protein may impact the host immune response and viral pathogenesis. Streptococcus equi subsp. zooepidemicus (SEZ), a commensal bacterium in horses, can cause opportunistic infections, especially when co-infected with EIV, leading to severe respiratory disease and complicating treatment strategies.

This study investigated the expression of selected cytokine (TNFα, IL-6, IL-8, IFNα, IFNβ) and TLRs (TLR2, TLR3) induced EIV strains with truncated or non-truncated NS1 proteins in a canine macrophage cell line (DH82α) co-infected with SEZ using RT-qPCR and ELISA. Two wild-type EIV strains were used: Sussex/89 with full-length NS1, and Newmarket/5/03 with a truncated NS1. DH82α cells were infected with these strains at a multiplicity of infection of 0.1 for 24 hours, followed by bacterial infection with 105 CFU/mL of SEZ, Cell RNA and culture supernatants were analysed for cytokine expression via RT-qPCR and ELISA, respectively.

M gene expression data indicated that the N/5/03 strain replicated more efficiently than the SX/89 strain ($P<0.01$). The mRNA expression levels of TNF-α, IL-6, IL-8, IFNβ, and TLR2 were significantly upregulated in co-infections with either N/5/03 or SX/89 and SEZ, or with SEZ alone compared to the mock cells, with no significant differences observed between the two viral strains. Expression levels for TLR3 and IFNα were not significantly affected by infection. Protein levels of IL-6 and TNF-α were significantly elevated in co-infections with N/5/03, SX/89, and SEZ alone compared to mock cells, with no significant differences between the two viral strains ($P<0.01$). IFNβ levels showed no significant differences post-infection with both virus and SEZ. Heat inactivation of SEZ abrogated the observed elevations of cytokine expression. These findings suggest that while the truncated and non-truncated NS1 proteins of EIV elicit similar cytokine responses, the presence of SEZ leads to more dramatic elevations in pro-inflammatory cytokines. This highlights the importance of understanding virus-bacteria interactions in managing co-infections.

Poster Session 2: Saturday 7th September 2024

(P36) CEFOTAXIME-RESISTANT *ESCHERICHIA COLI* ISOLATED FROM THE CLOACAE OF CONSUMPTION CHICKENS

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Chicken meat is highly consumed worldwide. To ensure high levels of production it is produced with the use of antibiotics, which contributes to the selection and dissemination of drug-resistant bacteria, largely

among the intestinal microbiotas. Direct and indirect contact between farmers, workers, and veterinarians with animals and the consumption and handling of raw meat can result in the transmission of antibiotic-resistant bacteria. Among the commensal microorganisms of the intestinal tract is *Escherichia coli*, which is used as an indicator of the development of antimicrobial resistance.

A total of 190 swabs were collected from the chicken's cloaca. Each sample was inoculated onto HiCrome agar supplemented with cefotaxime to isolate cefotaxime-resistant *E. coli* (CTX^R *E. coli*). The susceptibility profiles were studied by the Kirby-Bauer method in accordance with the CLSI guidelines against ampicillin, amoxicillin+clavulanic acid, cefepime, cefotaxime, cefoxitin, ceftazidime, aztreonam, doripenem, ertapenem, imipenem, meropenem, gentamicin, tobramycin, amikacin, kanamycin, streptomycin, tetracycline, ciprofloxacin, trimetopim-sulfametoxazol, and chloramphenicol.

CTX^R *E. coli* strains were isolated from five samples, representing a prevalence of 2.6%. All isolates exhibited resistance to cefotaxime, as expected, but also to ampicillin. In addition, one isolate was also resistant to streptomycin, tetracycline, and trimethoprim-sulfamethoxazole, while another was also resistant to cefepime.

The results of this study demonstrate that the use of antimicrobials in animal production has led to the inefficiency of antibiotics, specifically ampicillin. Furthermore, the study indicates that chickens represent a source of antibiotic-resistant bacteria, particularly CTX^R *E. coli* strains. This raises further concerns since these bacteria can degrade broad-spectrum β-lactam antibiotics, which are commonly used to treat numerous systemic infections. These findings serve to reinforce the necessity for the control of the use of antimicrobials in livestock and the implementation of precautions when handling or consuming these animals.

(P37) ANTIBIOTIC-RESISTANT *ESCHERICHIA COLI* IN FECAL SAMPLES FROM CHICKENS FOR HUMAN CONSUMPTIONTION

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The use of antibiotics in livestock production has contributed to the dissemination of antibiotic-resistant bacteria, which represents a serious threat to public health. The incomplete metabolism of antibiotics in the animal's gut results in the distribution of antibiotics into the environment through their faeces. *Escherichia coli* are commensal bacteria from the intestinal tract. Over the past decades, these bacteria have undergone a transformation, becoming important pathogens.

A total of 40 samples of chicken faeces were collected from Savinor (Trofa, Portugal) and inoculated onto HiCrome agar for the isolation of *E. coli*. The antimicrobial susceptibility was tested in accordance with the CLSI guidelines by the Kirby-Bauer disc diffusion method against ampicillin, amoxicillin+clavulanic acid, cefepime, cefotaxime, cefoxitin, ceftazidime, aztreonam, doripenem, ertapenem, imipenem, meropenem, gentamicin, tobramycin, amikacin, kanamycin, streptomycin, tetracycline, ciprofloxacin, trimetopim-sulfametoxazol, and chloramphenicol.

E. coli strains were isolated from 19 (47,5%) faecal samples and demonstrated resistance to ampicillin (n=17), amoxicillin+clavulanic acid (n=16), tetracycline (n=14), cefotaxime (n=11), ciprofloxacin (n=9),

streptomycin (n=6), amikacin (n=3), trimetopim-sulfametoxazol (n=3), cefepime (n=1), gentamycin (n=1), and tobramycin (n=1). Almost 85% (n=16) of the isolates exhibited multidrug resistance.

The results demonstrate that aviary chickens can act as vectors for the spread of antimicrobial-resistant *E. coli*, as they can be transmitted through faecal material into the environment, from fertilised soil, or directly from human contact or sewage. Furthermore, 16 isolates exhibited multidrug resistance, while 11 were resistant to cefotaxime. This raises further concerns since these bacteria can degrade broad-spectrum β-lactam antibiotics, which are regularly used to treat several systemic infections.

(P38) SEAGULLS AND BEACH WATER AS POTENTIAL VECTORS OF ANTIMICROBIAL-RESISTANT *ESCHERICHIA COLI*, INCLUDING *MCR*-1-POSITIVE ISOLATES, IN COASTAL AREAS OF PORTUGAL

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Due to the inherent survival characteristics of Enterobacteriaceae, their presence in environments with which humans interact represents a global problem, according to the One Health perspective. The main goal was to study antibiotic resistance and its coding genes present in *Escherichia coli* isolated from seagulls and the environmental surroundings with which they interact daily as well as their capacity for biofilm formation.

From October 2021 to October 2022, 265 samples were collected from Berlenga Grande, Aver-o-Mar, Matosinhos, and Miramar beaches, including 70 seagull feces, 80 sand, 65 saltwater, and 50 stagnant water samples. *E. coli* was isolated using Chromogenic Coliform Agar. Antibiotic susceptibility was tested with the Kirby-Bauer Method . Resistance genes were identified through PCR, and biofilm formation was assessed with the microplate method.

Of the 265 samples, 39 (23.6%) were positive for *E. coli*, with 79.4% from Aver-o-Mar, 12.8% from Miramar, and 7.7% from Matosinhos. Among the isolates, 25.6% were classified as multidrug-resistant (MDR). Genotypic characterization revealed the presence of several resistance genes, including *bla*CTX-M, *bla*VIM, *bla*SHV, *bla*TEM, *tet*A, *sul*1, *sul*2, and *mcr*-1. Notably, 22 isolates carried the *aac*(3)-II gene, predominantly from Aver-o-Mar. ESBL production was observed in 6 isolates (7%) primarily from Miramar stagnant water and Matosinhos seagull samples. All isolates demonstrated biofilm formation capabilities, with faecal-origin isolates showing stronger biofilm production than environmental samples.
This study highlights the presence of antibiotic-resistant and biofilm-forming *E. coli* in seagulls and their environment, emphasizing the need for stricter antibiotic stewardship and further research on transmission dynamics.

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(P39) ONE HEALTH APPROACH TO UNDERSTANDING ANTIMICROBIAL RESISTANCE OF *KLEBSIELLA* SPP. IN COASTAL ENVIRONMENTS

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Klebsiella spp. are commonly found in the environment, including soil, water, and the intestines of animals. While some *Klebsiella* species are harmless, others can cause opportunistic infections in humans and animals, particularly those with weakened immune systems. This study investigated the presence, antibiotic resistance profiles, and biofilm formation capacity of *Klebsiella* spp. isolated from seagulls and their surroundings including sand and water samples.

From October 2021 to October 2022, 265 samples were collected from Berlenga Grande, Aver-o-Mar, Matosinhos, and Miramar beaches, including seagull feces, sand, saltwater, and stagnant water. *Klebsiella* spp. were identified using selective media, and antibiotic susceptibility was assessed using the Kirby-Bauer. Genotypic resistance was verified via PCR, and biofilm formation was quantified using the microplate method.

Of the 265 samples collected, 46 (17.3%) tested positive for *Klebsiella* spp., with higher prevalence in seagull feces and sand. Antibiotic susceptibility testing revealed 13 multidrug-resistant *Klebsiella* spp. isolates, predominantly from seagull feces. PCR analysis detected various resistance genes, including *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{VIM}$, and *bla*$_{SHV}$. Additionally, 12 isolates were confirmed as *Klebsiella pneumoniae*. Biofilm formation assays demonstrated strong biofilm production, particularly in isolates from seagull feces. These findings highlight the presence of antibiotic-resistant and biofilm-forming *Klebsiella* spp. in environments shared with humans, emphasizing the importance of enhanced antibiotic stewardship practices and further research into bacterial transmission dynamics. The detection of multidrug-resistant isolates and *bla*$_{CTX-M}$ and *bla*$_{VIM}$ genes raises public health concerns.

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(P40) COMPARATIVE MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BIOFILM INHIBITORY CONCENTRATIONS (MBIC) DETERMINATION OF *ENTEROCOCCUS* ISOLATES IN A ONE HEALTH CONTEXT

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The rise of antibiotic-resistant bacteria highlights the need for a One Health approach. Enterococcus spp., known for spreading resistance, are ideal for study due to their diverse environments. This study compares the MIC and MBIC of Enterococcus isolates from humans, animals, and environmental samples.

A total of 207 Enterococcus isolates were sourced from infected children, various animals, wastewater plants, and effluents. Antimicrobial resistance was determined using the Kirby-Bauer method. MICs were tested against vancomycin, tetracycline, and ampicillin. Biofilm formation capacity was investigated, with ten strong biofilm-formers subjected to MBIC tests to assess biofilm reduction after exposure to their MIC and 10x MIC of each antibiotic.

Out of the 207 Enterococcus isolates, 149 were susceptible to vancomycin, 105 to tetracycline, and 122 to ampicillin. The MICs for these susceptible isolates ranged from 0.5 to 4 µg/mL for vancomycin, 0.25 to 4 µg/mL for tetracycline, and 0.5 to 8 µg/mL for ampicillin. For the resistant isolates, the MICs ranged from 128 to >256 µg/mL for vancomycin, 16 to >256 µg/mL for tetracycline, and 16 to >256 µg/mL for ampicillin. Only 135 isolates produced biofilms, with most classified as moderate biofilm producers. Vancomycin and ampicillin reduced biofilm biomass by about 11%, while tetracycline achieved a 9.7% reduction at 10x MIC.

This study highlights the pervasive nature of antibiotic resistance and biofilm formation among Enterococcus spp. across diverse ecological interfaces. The findings emphasize the critical need for a One Health approach to effectively address and mitigate the spread of antibiotic-resistant bacteria.

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(P41) ISOLATED BACTERIA AND THEIR RESISTANCE PROFILE IN BLOOD CULTURES OF CRITICALLY ILL DOGS

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Objective: Knowledge of causative bacteria and their susceptibility patterns in critically ill dogs is crucial for appropriate antimicrobial therapy to increase survival, particularly in septic dogs. This study aimed to identify bacterial isolates from blood cultures of critically ill dogs and describe their antimicrobial susceptibility profiles.

Methods: Prospective study containing 120 critically ill dogs suspected of sepsis, admitted between 2021 and 2024 to the veterinary university hospital. Bacteria were identified from blood cultures (Bactec) using biochemical testing or MALDI-TOF MS. Antimicrobial susceptibility testing was conducted by agar disk diffusion or by establishing minimum inhibitory concentrations, executed by an accredited Belgian lab.

Results: Positive cultures were obtained in 36.7%(44/120) of blood flasks, identifying 38 pathogens. Most common were *Escherichia coli* 31.6%(12/38) *and Streptococcus spp.* 18.4%(7/38), including *S. canis, S. infantarius, S. agalactiae, and S. mitis*. *Staphylococcus spp.* 10.5%(4/38), including *S. epidermidis and S. pseudintermedius,* were also frequently observed.
Susceptibility testing results showed inconsistencies in tested antimicrobial agents and suboptimal test panels. Expected inappropriate treatment, reflecting both acquired (when available) and intrinsic resistance, was high for erythromycin 94.7%(18/19), clindamycin 75.7%(28/37), and penicillin 69.7%(23/33). Resistance levels were low to moderate for enrofloxacin 13.2%(5/38), doxycycline 23.7%(9/38), trimethoprim/sulfamethoxazole 31.6%(12/38), cephalexin 50.0%(19/38), and cefovecin 28.9%(11/38). Multi-drug resistance was relatively uncommon 19.4%(7/36).

Conclusions: The importance of *Escherichia coli* as a major cause of bacteremia aligns with previous studies on critically ill dogs. High levels of expected inappropriate treatment for certain antimicrobial agents underscore the need for careful antimicrobial selection, preferably based on reliable susceptibility testing reports.

(P42) PLANT EXTRACTS AS ALTERNATIVES FOR ANTIBIOTIC GROWTH PROMOTER IN BROILER DIETS

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Necrotic enteritis (NE) is one of the most important digestive tract disorders in poultry and is a high cost to the industry worldwide. It is caused by Necrotic Enteritis Beta toxin (NetB)-producing strains of *Clostridium perfringens* (CP). In Europe, the incidence of this disease has increased since 2006, with the ban of in-feed antibiotics. This research aimed to test the efficiency of five different feeding programs represented by plant extracts on the broiler's digestive tract health and performance. Five experimental groups (10 day-old chicken each) were exposed to CP infection, on day 10 of life (ATCC strain 12916) and treated with mixtures of garlic, thyme, cinnamon, and licorice extract, in different concentrations and quantities (100-500 g/ton), administered from day 1 to day 35. Three other chicken groups were represented by positive, negative control, and amoxicillin-treated group.

Before the challenge, feces samples were collected from each pen for PCR identification. On day 35, the chickens were weighed and samples collected were processed for *Clostridium perfringens* quantitative evaluation on the plates to determine the CFU/mL of intestine content and PCR. The necrotic enteritis lesion scoring system was also used, ranging from 0 (normal, no evidence of gross lesions) to 4 (severe, extensive necrosis). Regarding the results, CFU/mL of intestinal content for the groups treated with plant extracts was lower than the positive control group, PCR revealed no CP before the challenge and was positive after. The extent of gastric and intestinal lesions was reduced, also with similar values to the amoxicillin group.

(P43) CANINE INFECTIONS WITH *STAPHYLOCOCCUS COAGULANS* AND RETROSPECTIVE STUDY OF ANTIMICROBIAL RESISTANCE

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Staphylococcs coagulans (formerly *Staphylococcus schleiferi* subsp*. coagulans*) is occasionally isolated from healthy dogs, but it can also be associated with otitis externa and dermatitis. Our study includes strains isolated from routine samples at the Veterinary Faculty of Ljubljana. During the three-year period from 2021 to 2023, we obtained 31 samples in which *S. coagulans* was identified as the main pathogen. Most of the samples were taken from dogs, with one case involving a cat. The bacterium was primarily collected with swabs taken from the skin and ear canal, but occasionally also from the nose and conjunctiva. Identification of *S. coagulans* was performed by mass spectrometry (MALDI-TOF) and antimicrobial sensitivity testing was conducted using the disc diffusion method according to the CLSI standard. Retrospective analysis revealed that 19 (61 %) of the isolates were sensitive to all antibiotics standardly tested for staphylococci. Of the 12 resistant isolates, 10 (32 % of all isolates) were resistant only to fluoroquinolones marbofloxacin and enrofloxacin. The remaining two isolates exhibited resistance to tetracyclines, one of which was also resistant to aminoglycosides and the other to bacitracin. The relatively high rate of *S. coagulans* resistance to fluoroquinolones aligns with findings from previous studies and has been described several times. Contrary to the existing literature, which frequently documents resistance to beta-lactams, no isolates with such resistance were found.

(P44) EXPLORING ANTIBIOTIC RESISTANCE IN MRSA AND MSSA FROM HEALTHY AND DISEASED RABBITS

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Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a significant public health concern due to its resistance to antibiotics. Although it is commonly found in healthcare settings, it can also be present in livestock populations, which raises additional concerns. The objective of this research is to ascertain the prevalence of MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) in rabbits intended for human consumption, as well as their susceptibility and resistance to various antibiotics.
A total of 155 samples were collected from healthy and infected rabbits from different farms in northern Portugal. Of these, only 54 (34,8%) contained S. *aureus*, which were then tested against 14 antimicrobial agents, including penicillin, cephalosporins, fluroquinolones, aminoglycosides, macrolides, tetracyclines, oxazolidinones and other miscellaneous agents. Susceptibility testing was carried out using the agar disc diffusion method, as recommended by EUCAST and CLSI guidelines.
A total of 72 samples from healthy rabbits were analyzed, with 16.7% of these samples testing positive for MRSA and 6.9% for MSSA. Of the 38 samples of pododermatitis, 28.9% tested positive for MRSA and 13.2% for MSSA. Finally, 37 strains of mastitis were examined, with 27.0% testing positive for MRSA and 21.6% for MSSA. None of the abscess samples (8) were MRSA, while 37.5% were MSSA. Of the strains studied, 59.3% were multidrug-resistant, 81.5% were resistant to ciprofloxacin, 75.9% to penicillin, 64.8% to clindamycin and 62.96% to cefoxitin. The 54 strains didn't show any resistance to linezolid, but they did show some resistance to, erythromycin (48.2%), tetracycline (27.8%), tobramycin (12.96%), gentamicin (11.1%), kanamycin (11.1%), fusidic acid (5.6%), mupirocin (5.6%) and trimethoprim-sulfamethoxazole (3.7%).

The results highlight the importance of effective surveillance, responsible use of antibiotics and infection control measures to mitigate the public health impact of multidrug-resistant S. *aureus*. The issue of infected food animals is a major public health and agricultural concern.

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(P45) ANTIMICROBIAL RESISTANCE AND CLONAL LINEAGES OF *E. COLI* IN RABBITS AND SWINE: ENSURING SAFER ANIMAL PRODUCTION AND PUBLIC HEALTH

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Escherichia coli in livestock is becoming more resistant to antibiotics, posing a health risk. The intensification of farming and inadequate processing increases the risk of foodborne contamination, especially in swine and rabbit populations. The study aimed examined cefotaxime-resistant *E. coli* strains from rabbit farms in northern Portugal and healthy pigs in central Portugal to tackle antibiotic resistance in livestock production. Our study revealed high levels of antibiotic resistance among *E. coli* strains, with notable resistance observed to tetracycline, ampicillin, aztreonam, streptomycin, tobramycin, and trimethoprim-sulfamethoxazole. All isolates were susceptible to cefoxitin and imipenem, but multidrug resistance was widespread, with some strains resistant to up to seven antibiotic classes. It should be noted that a significant portion of antibiotics deployed in livestock management belong to the critically important antibiotics (CIA) class, which should be reserved for human therapeutic applications. The presence of high-risk clones in swine, such as ST10, ST101, and ST48 is of concern due to their virulence and increased multidrug resistance, while ST457 and ST2325 found in rabbits are important sequence types associated with ESBL-*E. coli* is isolated and spread across a variety of environments and host species. High levels of antibiotic resistance in both human and veterinary medicine pose a major challenge for One Health. Resistance to important antibiotics is widespread and concerning, impacting both sectors significantly. The study underscores the necessity for a global strategy to combat antibiotic resistance, and sustainable agricultural practices in pig and rabbit production, recommending regulatory measures and consumer education.

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(P46) ESBL-PRODUCING *KLEBSIELLA PNEUMONIAE* IN DOGS OF CAPE VERDE AND SÃO TOMÉ AND PRÍNCIPE – A PUBLIC HEALTH CONCERN

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Objectives: Antimicrobial resistance is a major health concern. Cape Verde and São Tomé and Príncipe are African countries in which antimicrobial-resistant bacteria constitute an important cause of mortality.

This study aimed to characterize ESBL-producing *K. pneumoniae* isolates, classified as being of critical priority in the WHO priority pathogens list for research and development of new antibiotics, and to assess dogs' role as reservoirs of these strains.

Methods: A collection of 35 presumptive ESBL-producing *K. pneumoniae* isolates were collected from 195 fecal samples from dogs of São Nicolau (n=19) and Praia (n=45), Cape Verde, and São Tomé (n=35) and Príncipe (n=96) Islands. Isolates identification was confirmed by MALDI-TOF MS. Antibiotic resistance was asserted through disk diffusion after confirmation of ESBL production following CLSI standards, being multiple antibiotic resistance (MAR) index calculated for each isolate.

Results: Resistance percentages of 32 isolates confirmed to be ESBL producers ranged between 0.0% and 100.0%, as follows: 100.0% were resistant to aztreonam, cefotaxime, cefpodoxime and ceftaroline; 93.8% to cefepime and ciprofloxacin; 90.6% to trimethoprim/sulfamethoxazole; 84.4% to ceftazidime; 62.5% to tetracycline and doxycycline; 53.1% to nitrofurantoin; 50.0% to gentamicin; 12.5% to amoxicillin/clavulanate; and 9.4% to piperacillin/tazobactam and chloramphenicol. All isolates were susceptible to cefoxitin, imipenem and meropenem. Additionally, all isolates were classified as multidrug resistant (MDR), and MAR indexes ranged between 0.47 and 0.74.

Conclusions: This study highlights the presence of MDR ESBL-producing *K. pneumoniae* in dogs from these countries, remarking their position as reservoirs and spreaders of resistant bacteria, which can pose a public health concern.

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(P47) *PROTEUS* SPP. FROM CATS AND DOGS WITH UTI, AN OVERLOOKED UROPATHOGEN

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Objectives: *Proteus mirabilis* is frequently associated with UTI, but there is a lack of studies focused on this uropathogenic bacteria. This study aimed to report the antibiotic resistance (AMR) of *Proteus* spp. isolated from dogs and cats with urinary tract infection (UTI).

Methods: A total of 455 (2020-2023) *Proteus* spp. were isolated from urine samples cultured in standard media and Thermo Scientific™-Brilliance™ESBL-Agar (ESBL-Agar). Antibiotic susceptibility (AST) was conducted by disk diffusion or microdilution following EUCAST guidelines. Fisher-exact test was conducted using SAS/ODA (2024).

Results: Most *Proteus* spp. were isolated from dogs (82.2%, 374/455). Ampicillin- (p=0.0014; cats:53.1%, 43/81; dogs:33.4%, 125/374) and gentamicin-resistance (p=0.0127; cats:33.3%, 27/81; dogs:20.2%, 75/374), was higher in isolates from cats. Resistance to trimethoprim/sulfamethoxazole, second-generation fluoroquinolones, amoxicillin/clavulanate and cefotaxime was present in 40.8% (185/454), 31.2% (142/455), 15.4% (70/455) and 11.9% (54/455) isolates, respectively without significant differences between host species (p>0.05). Most cefotaxime-resistant *Proteus* spp. were also resistant to amoxicillin/clavulanate (81.5%, 44/54). The Thermo Scientific™ Brilliance™ ESBL detected (90.7%, 49/54) of cefotaxime-resistant isolates while around 10% (6/55) of *Proteus* spp. isolated from the ESBL-Agar revealed to be cefotaxime-susceptible. Finally, around 85% (46/54) of cefotaxime-resistant isolates were multidrug-resistant (>3 antibiotic classes).

Conclusions: *Proteus* spp. showed a high frequency rate to the most frequently used antimicrobials to treat UTI in dogs and cats. Atencion should be raised towards monitoring this uropathogen that commonly causes complicated and recurrent UTIs.

(P48) VETERINARY INFECTION AND PREVENTION THROUGH VISUALISATION (VIPVIS): CAN INFECTION IN VETERINARY PRACTICE BE REDUCED BY USING AN EDUCATIONAL SIMULATION TOOL?

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Antimicrobial resistance (AMR) is a growing issue within veterinary practice. Despite initiatives to address the issue, there remains a need for innovative intervention-based strategies to tackle AMR, with a focus on Infection Prevention and Control (IPC). Appropriate IPC is influenced by our perception of what infection risk is, and how we interact with our surrounding environment. The focus of this study is the application of a simulation tool, Veterinary Infection and Prevention through Visualisation (VIPVis). VIPVis demonstrates the real-life interactions between humans, animals, pathogens, and the veterinary environment making 'the invisible, visible', i.e. bacterial contamination, to those using the tool. The aims of the project are to: 1) Evaluate whether use of VIPVis has a positive impact on the behaviours and attitude of veterinary staff towards IPC; and 2) Determine whether this contributes to a lower bacterial burden within veterinary practice, resulting in fewer infections and reduced use of antibiotics. This will be investigated through an intervention utilising the VIPVis App, together with questionnaires for veterinary staff to complete and the collection of environmental swabs from the veterinary practice environment. We hypothesise that VIPVis will increase the implementation of effective IPC measures and reduce the level of microbial contamination throughout the veterinary practice. We predict that this will result in lower levels of infection, decreasing antibiotics used, thus contributing to reducing the emergence of resistance. If successful, the VIPVis tool could provide universal training for veterinary staff and students in veterinary and healthcare settings, thus contributing to improving antimicrobial stewardship.

(P49) *TOWARD UNDERSTANDING C*OLISTIN RESISTANCE MECHANISMS IN *PSEUDOMONAS AERUGINOSA: PROTEOMIC INSIGHTS AND ADAPTIVE STRATEGIES*

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Pseudomonas aeruginosa, known for its adaptability and antibiotic resistance, poses a significant clinical challenge, particularly due to its resistance to colistin. A preliminary study was carried out to determine the minimum inhibitory concentrations (MICs) to different antibiotics among a panel of 11 strains. Strains from different contexts reveals intrinsic resistance to certain antibiotic classes, particularly polymyxins such as colistin. Plasmid-mediated resistance was absent in studied strains. Instead, colistin resistance was linked to genetic mutations and specific endogenous proteins, suggesting diverse mechanisms influenced by epidemiological factors. One strain displayed notably high colistin resistance, suggesting potential adaptive mechanisms. The objective of this study was to analyze the protein expression levels of three of these *P. aeruginosa* strains with different MICs, in response to colistin antibiotic treatment at three concentrations

(0, 2 and 4 mg/L). The proteomic study focused on two protein subsets, the intracellular and cell wall/debris proteomes. Separate extraction and analysis of the two sub-proteomes was aimed at obtaining a more exhaustive view of the protein expressed, to better decipher the mechanisms of colistin resistance, whether common to the three strains or specific. Protein samples were digested with trypsin and peptides were analysed using a "shotgun proteomics" approach, *i.e.* a label-free relative quantitative analysis by nano-liquid chromatography coupled to tandem mass spectrometry (nano-LC-MS/MS). Proteomic analysis showed unique protein expression patterns under colistin stress, with strain-specific responses. Clustering analysis revealed differential expression of proteins related to metabolism, stress response, and antibiotic resistance. Protein interactions suggested complex adaptive strategies, highlighting the multifaceted nature of bacterial resistance. These findings underscore the importance of understanding strain-specific resistance mechanisms in combating antimicrobial resistance and developing effective treatment strategies against multidrug-resistant pathogens like *P. aeruginosa*.

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(P50) ANTIBIOTIC RESISTANCE TRENDS IN *KLEBSIELLA* SPP. FROM COMPANION ANIMAL INFECTIONS: A FOUR-YEAR STUDY

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Klebsiella spp. is a pathogen of growing concern due to the emergence of multidrug resistant and invasive lineages. This retrospective study explores the trends in *Klebsiella* spp. resistance in companion animal (CA) infections in Portugal.

Enterobacterales isolates obtained from clinical samples of CA infections during 2020-2023 were submitted to antimicrobial susceptibility testing following CLSI and EUCAST guidelines and up-to-date breakpoints, covering beta-lactams, fluoroquinolones, aminoglycosides, and folate pathway inhibitors. Suspected carbapenemase-producing *Klebsiella* spp. were screened by PCR.

From 4394 Enterobacterales isolates, 499 (11.4%) were identified as *Klebsiella* spp.. Resistance to 3rd generation cephalosporins and/or cephamycins compatible with the possible presence of ESBL or AmpC cephalosporinases was detected in 66.5% of the *Klebsiella* spp. isolates (n=332/499), comprising 26.3% of all Enterobacterales isolates with this phenotype (n=332/1260), and remained relatively stable over time. The screening for carbapenemase genes yielded three *bla*$_{KPC-3}$ and three *bla*$_{OXA-181}$ carrying *Klebsiella pneumoniae*, the most prevalent carbapenemase genes in human nosocomial infection isolates in Portugal. Regarding the other antibiotic groups, the average percentage of resistance in *Klebsiella* spp. is higher than Enterobacterales (49.2 versus 28.3% respectively). While *Klebsiella* spp.'s aminoglycoside and folate pathway inhibitor resistance appear to be trending downward towards the levels of Enterobacterales in general however, fluoroquinolone resistance registered a slight increase in the last year in both datasets.

This study highlights *Klebsiella* spp. the ability for acquiring antibiotic resistance determinants, which might lead to a higher rate of treatment failure if CA antibiotic treatment guidelines fail to account for local variability in bacterial prevalence as well as antibiotic resistance.

(P51) RESISTANCE AND PRESENCE OF ANTIBIOTIC RESISTANCE GENES TO CATEGORY D ANTIBIOTICS IN *E. COLI* STRAINS CAUSING SWINE POSTWEANING DIARRHEA

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Spanish legislation (R.D. 666/2023) establishes categories for the antibiotic prescription in production animals. Category D includes those that should be used as “first choice”, among them penicillins, sulfonamides and tetracyclines. The spectrum of action of these antibiotics includes *E. coli*, which can cause postweaning diarrhea. The aim of this work has been to evaluate the level of resistance and the presence of antibiotic resistance genes in strains of E. coli causing diarrhea isolated from post-weaning piglets.A total of 36 strains of pathogenic *E. coli* (they encoded at least one of the following virulence factors: STa, STb, LT, F4, F18, Stx2) isolated from piglets in postweaning stage were used. An antibiogram was performed in each strain using the disk diffusion method, including the following antibiotics: Penicillins (ampicillin, amoxicillin-clavulanic acid, ticarcillin, piperacillin, piperacillin-tazobactam), Tetracyclines (tetracycline, doxicycline) and Sulfonamides (trimetropim-sulfamide). In addition, the presence of the following antibiotic resistance genes was analyzed by PCR: *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{NDM}$, *tet*A, *tet*B, *tet*M, *sul*1, *sul*2 and *sul*3. Regarding Penicllin group, 22 strains (61.11 %) showed resistance to t least one antibiotic; additionally, 16 (44.4 %) were positive to the *bla*$_{TEM}$ gene, with none detected the *bla*$_{SHV}$ or *bla*$_{NDM}$ genes. Regarding Tetracycline group, 21 strains (58.33 %) were resistant to tetracycline and/or doxycycline; additionally, 9 strains (25.00 %), 4 (11%) and 3 (8.33 %) carried the *tet*A, *tet*B, and *tet*M genes, respectively. The combination of *tet*A and *tet*B genes was detected in 2 strains, *tet*A and *tet*M in another 2 strains, and 1 strain had all three genes. Regarding Sulfonamides, 20 strains (55.50 %) were resistant in the antibiogram and 6 (16.67%) and 10 (27.78 %) of them were positive to *sul*1 and *sul*2 genes, respectively. Moreover, combination of both genes was detected in 5 strains, while *sul*3 gene was not detected. Studying the antibiotic resistance genes complements the results of the antibiogram and provides epidemiological information since it allows confirming the circulation of these genes in the farms and predicting the lack of efficacy in the antibiotic treatment. Most of pathogenic *E. coli* strains tested were resistant to Penicillins, Sulfonamides and Tetracyclines in the antibiogram, although the presence of antibiotic resistance genes to those antibiotics was lower to 50 %.

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(P52) SEASONALITY OF MASTITIS PATHOGEN ISOLATION AND ANTIMICROBIAL RESISTANCE PERSISTENCE IN IRISH BOVINE HERDS FROM 2020 TO 2023

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Mastitis is an inflammation of the mammary gland and is a cause of great economic loss to the dairy industry in Ireland due to reduced milk yield. On the other hand, while antimicrobial resistance (AMR) is an issue of significant concern to public health, it can also result in antimicrobial therapy becoming less effective, causing complications for mastitis treatment. We aimed to further understand the epidemiology from the major bacterial species causing mastitis by establishing their seasonality and AMR patterns. Culture and antimicrobial sensitivity data was obtained by the DAFM Regional Veterinary Laboratories. Our results showed a higher prevalence of *Streptococcus uberis* in late Summer and Autumn, *Staphylococcus aureus* in Autumn, and *Escherichia coli* in Winter and early Spring. On the other hand, resistance patterns were very similar to those observed in other EU countries. However, we observed that while the herds exhibited consistent resistance to penicillin in *S. aureus* and to pirlimycin in *S. uberis* across different years, resistance in *E. coli* was not consistent. These results may be attributed to the more contagious nature of *S. aureus* and *S. uberis*, while *E. coli* is considered a purely environmental pathogen. Further analyses by whole Genome Sequencing that can help gain a deeper insight into these results are granted.

(P53) A CLOSER LOOK AT GOAT SUBCLINICAL MASTITIS AND MILK PROTEIN PROFILE CHANGES IN RELATIONSHIP WITH BACTERIAL PATHOGENS

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Goat milk, as a valuable alternative for cow milk, has been gaining increasing attention in the past decades. However, due to several differences in the physiology of lactation, subclinical inflammatory processes are difficult to detect.Therefore, the aim of the present study was to evaluate the impact of bacterial pathogens on milk protein profile, described using a microfluidic chip electrophoresis. 76 Romanian Carpathian goats (2nd lactation) were included in the study and pathogens were identified and classified for a better interpretation based on microbiological testing (Vitek® 2) and molecular analysis (16s rRNA sequencing), as follows: E (enterococci), NAS (non aureus staphylococci), B (*Bacillus*), SA (*S. aureus*) and N (negative samples).

Following capillary electrophoresis for protein separation, quantification and statistical analysis, several observations were made, including: lactoferrin and serum albumin recorded the highest mean value in staphylococcal infection compared to the N group ($p < 0.05$), suggesting increased vascular permeability during an intramammary infection. Immunoglobulin concentration was also significantly increased in mastitic milk. Among caseins, only β-casein registered variations, showing lower mean values in case of staphylococcal mastitis, demonstrating that this fraction is more prone to proteolysis due to bacterial enzymatic activity. Subclinical mastitis seems to not have a significant influence on milk physico-chemical parameters, except for electrical conductivity, markly increased in NAS infection. Overall, observed differences were statistically corellated with bacterial pathogenicity, major pathogens being responsible for increased inflammation and a shift in goat milk's protein profile, which could result in poor digestibility and loss of nutritional value.

(P54) ANTIBACTERIAL POTENTIAL OF GRAPE BY-PRODUCTS AGAINST FOODBORNE PATHOGENS: IMPROVING SUSTAINABILITY AND REDUCING ANTIBIOTIC UTILIZATION

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Wine production is a vital global agricultural industry utilizing grapes valued for their polyphenol content. By-products with antioxidant properties also exhibit antibacterial effects through phenolic compounds disrupting bacterial cell structures like walls, membranes, proteins, and adhesive structures This study analyzed the phenolic composition and antioxidant activity of grape by-products (skins, seeds and stems) from three Portuguese red grape varieties (Periquita, Gamay and Donzelinho Tinto). It also examined their antimicrobial activity against antibiotic-resistant bacteria like *Escherichia coli* and *Listeria monocytogenes* in food-producing animals and environments. Ten polyphenolic compounds were quantified in three red grape varieties, with specific compounds found in different parts of the grape including phenolic acids, flavonoids, and anthocyanins. Flavonoids were found abundant in seeds and stems. The ethanolic extracts from seeds of red grape varieties showed concentration-dependent of reactive species like •NO and O2•− in vitro. Gamay extract had the highest activity, followed by Donzelinho Tinto and Periquita. Regarding the antibacterial effects of by-products found that they were less affected by Gram-negative bacteria. Seed extracts showed the highest activity against foodborne bacteria, followed by stem and skin extracts. Grape seed extracts exhibit potent antimicrobial properties against *E. coli* and *L. monocytogenes*, with Gamay, Periquita, and Donzelinho Tinto extracts showing greater efficacy against Gram-positive bacteria. Grape seeds contain higher total phenolic compounds. As observe in our study, the use of wine by-products has attracted attention due to their antimicrobial properties, providing a promising approach to improve food safety and combat antibiotic resistance in food production and related sectors.

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(P55) SEROTYPING AND ANTIBIOTIC RESISTANCE PROFILES OF *SALMONELLA* AND *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM PET FOODS AND FEED

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Salmonella spp. and *Listeria monocytogenes* are foodborne pathogenic bacteria of great concern that are commonly detected in animal feeding stuff. The aim of the present study was to phenotypically confirm the presence of *Salmonella* and *L. monocytogenes* in raw pet foods and feed, through biochemical and/or serological testing of the isolates. Fifteen strains of *Salmonella* and nine strains of *L. monocytogenes* were detected and confirmed in a corresponding equal number of samples (24). Multiplex PCR for the assignment of strains to the four most prevalent and most important public health-related *Salmonella* serovars (i.e., *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Thompson) as well as to the four most frequently identified PCR-serogroups of *L. monocytogenes* (i.e., PCR-serogroups IIa, IIb, IIc, IVb), revealed that the majority of *Salmonella* and *L. monocytogenes* strains belonged to serovar Thompson (9) and PCR-serogroup IIa (4) (i.e., serotypes 1/2a, 3a), respectively. *S.* Enteritidis and *S.* Typhimurium were also detected among the isolates, while four strains were identified as *Salmonella* spp. without belonging to the aforementioned serovars. Moreover, three and one strain of *L. monocytogenes* were assigned to PCR-serogroups IVb (i.e., serotypes 4b, 4d, 4e) and IIb (i.e., serotypes 1/2b, 3b, 7), respectively. The bacterial isolates were screened against a panel of eight and seven antibiotics for *Salmonella* and *L. monocytogenes*, respectively, according to EUCAST. Antimicrobial resistance (AMR) was originally recorded for *L. monocytogenes* against trimethoprim-sulfamethoxazole (SXT; 55.5%), ciprofloxacin (CIP; 22.2%), tetracycline (TE; 11.1%), benzylpenicillin (P; 11.1%), and meropenem (MEM; 11.1%), with one strain of the pathogen showing multidrug resistance to all the above antibiotics (PCR-serogroup IIb) and one strain showing simultaneous resistance to SXT and CIP (PCR-serogroup IVb). MIC values for SXT and CIP ranged from 0 to 128 and 0.94 to 1.50 mg/L, respectively, with 4 out of 5 strains deemed resistant to SXT and no antimicrobial resistance finally inferred for CIP. MICs for TE (48 mg/L), P (4 mg/L), and MEM (4 mg/L) were above the cut-off values and thus the multidrug resistant strain of *L. monocytogenes* was finally deemed resistant to 3 antibiotics. Antibiograms for *Salmonella* isolates are to be presented also. Results presented herein should alert pet owners for the presence of major foodborne pathogenic bacteria and their public health-related serotypes showing AMR in animal feeding stuff.

(P56) PREVALENCE OF *ESCHERICHIA COLI* VIRULENCE GENES IN DIARRHEAL AND HEALTHY CALVES

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Objectives: The most common diseases in the first weeks of a calf's live are gastrointestinal diseases that lead to neonatal calf diarrhea (NCD). More than half of diarrhea occurs in the first week of life and the most common cause is *Escherichia coli*. Nine pathotypes of *E. coli* have been described, seven of which have been characterized as diarrheagenic *E. coli*, which mainly cause diarrhea through the expression of specific virulence factor-encoding genes.

Methods: Fecal samples were collected from 44 diarrheic and non-diarrheic calves up to seven days of age from seven dairy farms. To determine the presence of *E. coli,* all samples were plated on selective Drigalski agar and confirmed using the MALDI-TOF mass spectrometry method. Up to three different morphological types of colonies were determined in each sample and 92 isolates (34 from calves with diarrhea and 58 from healthy calves) were genotyped using whole-genome sequencing. Virulence genes were identified using VirulenceFinder v2.0 with default parameters.

Results: Different *E. coli* virulence factor profiles were observed. We confirmed the presence of 26 virulence genes specific for diarrheagenic *E. coli*. The presence of enterotoxigenic (ETEC) (*est*), Shiga toxin-producing (*stx1, stx2*), enteropathogenic (*eae*) and diffusely adherent *E. coli* (*afa*) specific genes were detected, but only ETEC genes were significantly associated with diarrhea ($p = 0.017$). The results showed a correlation between diarrhea and the presence of virulence genes *astA*, *fimH* and *iutA*.

Conclusions: Calves can harbor multiple *E. coli* strains with different virulence factor profiles. The presence of pathotype-specific virulence genes is not necessarily associated with the occurrence of diarrhea. Other common virulence genes and NCD risk factors, such as co-infections, poor nutrition, inadequate colostrum intake and animal hygiene, may contribute to disease outcome.

(P57) LOW SEROPREVALENCE OF SMALL RUMINANTS LENTIVIROSIS IN ALENTEJO REGION, PORTUGAL: A CASE-STUDY

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Previously known as Maedi Visna and Caprine Arthritis and Encephalitis affecting sheep and goats. Small Ruminant Lentivirus disease (SRLV) was studied for seroprevalence and risk factors in Alentejo, Portugal. The commercial indirect ELISA test (ID Screen® MVV/CAEV Indirect) detected infection, with herds positive if at least one animal was seropositive.

Samples were collected from 31 herds (22 sheep, 6 goat, and 3 mixed herds). Results showed 10 (32.26%) herds were SRLV positive: 4 (18.18%) sheep herds, 5 (83.33%) goat herds, and 1 (33.33%) mixed herd. Out of 553 individual samples, 109 (19.71%) were SRLV positive, including 29 (6.84%) sheep and 80 (62.02%) goats.

Risk factor analysis revealed significant associations ($p < 0.001$) for SRLV infection in animals from herds with over 100 animals (OR=4.55, 95% CI: 2.36-8.74), non-extensive production systems (OR=37.93, 95% CI: 20.88-68.91), goats (OR=22.24, 95% CI: 13.24-37.34), exotic breeds (OR=3.84, 95% CI: 2.35-6.27), dairy farms (OR=58.71, 95% CI: 31.52-109.35), and purchase of replacement lambs (OR=2.61, 95% CI: 1.63-4.18).

The low seroprevalence of SRLV in Alentejo's small ruminant herds compared to other regions of Portugal may be due to the predominance of extensive production methods. This indicates that more intensive animal production is a significant risk factor for SRLV infection.

(P58) SPECIES IDENTIFICATION OF *STAPHYLOCOCCUS AGNETIS* AND *STAPHYLOCOCCUS HYICUS* ISOLATES USING MALDI-TOF ANALYSIS

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Staphylococcus hyicus (S. hyicus) and *Staphylococcus agnetis (S. agnetis)* are two coagulase-variable staphylococci within the non-*aureus* staphylococci and mammaliicocci (NASM) group. Differentiating these two species using routine phenotypic and genotypic tests is challenging, due to their genetic similarity. This study aimed to validate the use of specific peptide mass fingerprint peaks obtained through Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) to differentiate *S. hyicus* and *S. agnetis*. Eighteen presumptive isolates of both species originating from bovine milk samples were analyzed. For species identification, *rpoB* and *tuf* gene sequencing was performed as the gold standard method. *rpoB* gene sequencing identified 3 isolates as *S. hyicus* whereas *tuf* gene sequencing successfully identified the remaining 15 isolates as *S. agnetis*. Subsequently, MALDI-TOF MS analysis was performed using a custom in-house reference spectra expanded database of NASM. Following Bruker's protocol, main spectrum profiles (MSPs) were created as reference spectra. Two

different MALDI-TOF methods were tested: direct transfer (DT) and extended direct transfer (eDT). The eDT method improved identification accuracy, identifying 13 *S. agnetis* and 2 *S. hyicus* isolates correctly, compared to the DT method which identified only 9 *S. agnetis* isolates correctly. Analysis of MSPs revealed a unique peak at 6136 m/z present in all *S. agnetis* isolates but absent in all *S. hyicus*, indicating the potential of this peak to be a reliable biomarker for differentiation between these two species. Importantly, this consistent presence of the peak in both DT and eDT spectra supports its utility for routine laboratory identification.

(P59) USE OF ORAL AND FAECAL MICROBIOME SIGNATURES TO ASSESS HEALTH AND PRODUCTIVITY IN DAIRY CATTLE DURING THE TRANSITION PERIOD

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The transition period, defined as the three weeks before and after calving, represents one of the most metabolically challenging phases in the dairy cow's reproductive cycle. The microflora of the bovine gastrointestinal tract plays a key role in ensuring that the cow's nutrient requirements continue to be met. Changes in the composition of the gut microflo-ra that occur during the transition period may impact nutrient availability and increase an individual animal's susceptibility to certain diseases. A number of 16S rRNA studies of the rumen have demonstrated the association between prokaryotic taxa and specific health and productivity traits however less is known about the global gut microflora composition.

In this study, the oral and faecal microbiomes were used as non-invasive proxies of the gut microbiome. Pre- and post-partum samples were collected from 150 Holstein cows and heifers on two commercial UK dairy farms. Body condition, rumen fill, and hock scores were assigned at each sampling timepoint, and health and productivity data were collected throughout the lactation period.

In an initial pilot study, DNA extracted from the oral swabs, rectal swabs and corresponding voided faecal samples of ten individuals was submitted for shotgun metagenomic sequencing. Sequencing data were processed using the SqueezeMeta pipeline and the microbiome composition and functionality of these two sample types was compared. Further analysis will investigate whether an association exists between the faecal microbiome signatures, cattle health and production parameters.

(P60) SEROEPIDEMIOLOGICAL STUDY OF SMALL RUMINANTS LENTIVIROSIS IN THE CENTER REGION, PORTUGAL

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Small Ruminant Lentivirusis (SRLV), (previously Maedi Visna and Caprine Arthritis and Encephalitis), affects sheep and goats, respectively. This persistent infection causes chronic, progressive disease, and

laboratory methods are essential for diagnosis. In Portugal's Center region, where small ruminants are vital for cheese production, a study was conducted to determine SRLV seroprevalence and associated risk factors.
The study involved 28 herds: 11 sheep, 14 goat, and 3 mixed herds. Blood samples were randomly collected, and the commercial indirect ELISA test (ID Screen® MVV/CAEV Indirect) was used to detect SRLV. A herd was deemed positive if at least one animal tested seropositive.
Results showed that 23 (82.14%) herds were SRLV positive: 10 (90.91%) sheep, 10 (71.43%) goat, and 3 (100%) mixed herds. Out of 494 individual samples, 231 (46.76%) were SRLV positive, including 96 (39.67%) sheep and 135 (53.57%) goats.
Risk factor analysis revealed significant associations ($p < 0.001$) for SRLV infection in herds with more than 100 animals (OR=2.13, 95% CI: 1.44-3.13), acquisition of animals without infection screening (OR=4.13, 95% CI: 2.40-7.10), producers without training (OR=2.06, 95% CI: 1.43-2.98), exotic breeds (OR=2.19, 95% CI: 1.50-3.19), dairy farms (OR=2.75, 95% CI: 1.42-5.31), acquisition of replacement lambs (OR=2.04, 95% CI: 1.39-2.98), non-separation of sick animals (OR=3.77, 95% CI: 2.49-5.72), and herds without veterinary care (OR=9.28, 95% CI: 4.63-18.61).
The high SRLV seroprevalence in Central Portugal is linked to intensive production methods and risk factors like inadequate animal separation, unscreened animal acquisition, and lack of veterinary care. Addressing these issues could reduce SRLV.

(P61) APPLICATION OF NEURAL NETWORKS IN MALDI-TOF MS ANALYSIS FOR REDUCING KLEBSIELLA PNEUMONIAE TRANSMISSION

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The global increase of multi-resistant Gram-negative bacteria is a threat both to veterinary and human medicine, with the spread of *Klebsiella pneumoniae* being one of the major concerns. Artificial intelligence, using neural networks, has the capability to analyze mass spectra from different isolates and form algorithms that allow the classification of these isolates, helping to prevent the increase of antibiotic resistance. To evaluate the contribution of neural networks in reducing the transmission of *K. pneumoniae* infection in a One Health context, using different clinical isolates.
A retrospective study conducted in March 2024, involving 30 different clinical isolates of *K. pneumoniae*. The analytical method for identifying and evaluating the signal intensity of each *K. pneumoniae* isolate was Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) using the MALDI Biotyper® sirius – Bruker®. The t-test was used to compare the significance of the results obtained.

A significant difference was observed between the two study populations in both signal intensity ($p < 0.05$) and antibiotic resistance ($p < 0.01$). The 10 points with the highest signal intensity were compared across the populations. The nosocomial population showed higher signal intensity at m/z. Using the t-test, a $p < 0.05$ was obtained for signal intensities, and a $p < 0.01$ for antibiotic resistance. Integrating these data with AI neural networks allows for determining bacterial virulence, defining specific patterns and algorithms for each population. The graphs were created using the SuperPlotsOfData platform.
The strains of different clinical isolates under study with clinical infection exhibit higher protein signal intensity, as well as a higher number of antibiotic resistances, compared to community-acquired infections. Based on artificial intelligence, neural networks have proven to be an emerging and highly promising tool in improving the One Health approach to combating the transmission of infections in health.

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(P62) TINY EYES, BIG PROBLEMS: OPHTHALMIA NEONATORUM AND INFECTIOUS THREATS IN SHELTER KITTENS

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Ophthalmia neonatorum is an ocular condition affecting newborn mammals, including cats, and typically occurs before or just after the opening of the eyelids in kittens, usually during the first 10-14 days of life. This case report presents a notable instance of ophthalmia neonatorum in a shelter involving a mother cat and her litter of five kittens. A 2.5-year-old Siamese queen gave birth to five kittens. At 13 days of age, the kittens exhibited ankyloblepharon (fused eyelids) and chemosis (conjunctival swelling), while the mother had a whitish vaginal discharge. Clinical examination revealed ocular discharge, conjunctival hyperaemia, and chemosis in all kittens. One kitten had exophthalmos (bulging eye) and haemorrhage in the left eye. Samples were collected from each eye of the kittens and the mother's vagina. The swabs were inoculated in different culture media according to standard microbiological techniques. *Staphylococcus* spp. (*Staphylococcus chromogenes* and *Staphylococcus lentus*) were identified in all cats, with *Streptococcus* also commonly isolated (*Streptococcus canis* and *Streptococcus agalactiae*). In one eye of one kitten, *Aspergillus* spp. was also identified. The kittens were treated with subcutaneous amoxicillin-clavulanic acid and topical ophthalmic drops containing chloramphenicol, ofloxacin, and EDTA. These cases emphasise the challenges of managing ophthalmia neonatorum in shelters, where susceptible animals can rapidly transmit infections. Prompt diagnosis, targeted therapy, and monitoring are crucial for managing outbreaks and preventing complications. This report contributes to the limited literature on feline neonatal conjunctivitis in shelters, informing on strategies to safeguard vulnerable kittens.

(P63) MALIGNANT CATARRHAL FEVER IN CATTLE FROM ARGENTINA: CASE STUDIES

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Sheep-associated malignant catarrhal fever (MCF) in cattle is caused mainly by ovine herpesvirus 2 (OvHV-2), which is endemic in sheep populations worldwide. It is fatal, although appears sporadically. MCF is included as a differential diagnosis of foot-and-mouth disease (FMD). Being FMD a major transboundary disease that threatens livestock production in South America, surveillance of erosive/ulcerative diseases is key to enhance control strategies. This work describes 10 cases of MCF affecting beef cattle older than 10-months-old, in Chubut (n=1) and Buenos Aires (n=9) provinces, Argentina, between 2008 and 2024. In all cases, a fatal clinical disease was recorded, with previous neurological and respiratory disease, associated with catarrhal nasal and ocular secretions, ocular opacity, blindness, and sialorrhea. Tissue samples were formalin fixed and processed using rutinary histopathology techniques, confirming widespread lesions characterized as severe multifocal non-suppurative necrotizing vasculitis mainly in brain, kidneys, liver, and lungs. OvHV-2 DNA was detected in samples from all 10 cases using PCR. MCF cases were associated with sheep-cattle coexistence (10/10) and OvHV-2 detection in the sheep population (2/10). Of note, two cases occurred simultaneously in neighbour farms, although no animal movement between them was recorded. Most of these MCF cases (7/10) were individual. Surveillance of erosive/ulcerative diseases in ruminants is important in the region due to the patent risk of FMD. This report confirms the occurrence of MCF in cattle from Argentina.

(P64) INFECTIOUS NEUROLOGICAL DISEASES IN CATTLE FROM ARGENTINA: RETROSPECTIVE STUDY

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Surveillance of ruminant neurological diseases is key to assess Bovine spongiform encephalopathy (BSE) status in the region. Although Argentina possesses a low geographical BSE risk, passive surveillance is negligible status. This work retrospectively revised the causes of neurological diseases in cattle at the Veterinary Diagnostic Service of INTA Balcarce, Argentina, between 2000-2023. During this period, 19,372 cases were registered, and 789 (4.07%) were neurological. Among them, 61.1% and 38.9% affected cattle younger or older than 2-years old, respectively. Neurological cases were registered in beef (84.9%) and dairy (15.1%) systems. The most frequent neurological diseases in cattle were associated with metabolic (31.4%), toxic (13.1%), infectious (11.7%), parasitological (3.8%) or miscellaneous (2.8%) diseases. The most frequent infectious aetiologies were septicaemic processes (3.4%), encephalopathy associated with *Histophilus somni* (1.7%), *Listeria monocytogenes* (1.4%), *Salmonella enterica* (0.8%), Varicellovirus bovinealpha (BoAHV) (0.6%), malignant catarrhal fever (MCF) (0.4%) or rabies (0.1%). Neurological diseases associated with lymphoma due to bovine leukaemia virus (0.8%) and granulomas due to *Mycobacterium bovis* (0.7%) were also registered. The aetiology remains unknown in 37.2% of the neurological cases. Nevertheless, in 7.7% and 4.3% of these undiagnosed cases, non-suppurative or suppurative was confirmed after histopathological studies, respectively, with a suspicion of infectious disease. These results show the most frequent infectious causes of neurological disease in cattle from Central Argentina. Regional variations are common due to the occurrence of specific diseases associated with reservoir (i.e. rabies or *Babesia*). This information is the result of the passive BSE surveillance in Argentina.

(P65) CLIMATIC CHANGES AND THEIR ROLE IN EMERGENCE OF MYCOBACTERIAL INFECTIONS AFFECTING MARINE ORGANISMS

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Access to clean water including potable, surface, ground, marine, aquaculture, rain, wetland, and swimming bath waters, is the 6th of the Sustainable Development Goals (Ensure availability and sustainable management of water and sanitation for all). This goal is increasingly challenged by the presence of emerging microbial contaminants due to urbanization, pollution, overfishing and climate change.
Over the past few decades, there has been a global surge in reports of diseases affecting marine organisms across various taxa. Climate change is exerting its influence on the marine ecosystem, already facing numerous anthropogenic disturbances. The warming climate can bolster pathogen development and their survival rates, enhance disease transmission, and increase host susceptibility. Environmental conditions not only facilitate pathogen transmission but also serve as critical risk factors for the onset of clinical diseases.
The emergence of mycobacteria in the Mediterranean Sea due to an increase in sea temperatures, is considered a causing factor to high mortalities or frequency of severe epidemics in fish populations. During mainly the last decade, professionals of the aquaculture sector in Greece report an increasing number of cases of severe outbreaks consistent with the clinical and histopathological characteristics of mycobacterial infections, especially in reared populations of sea breams and sea bass. The aquaculture industry seems to be faced with the problem of mycobacterial infections of fish with no or little regulatory support.
It is worth noting that in 2022, a question was addressed to the European Parliament with the intend to establish whether the European Commission aims to take action against the spread of *Mycobacterium pseudoshotssii* in Mediterranean aquacultures. The parties that raised the issue stated that the certain pathogen had been reported until recently only in Japan and the USA, but was detected in 2022, in Italy, a finding that they consider probably associated with the climatic change.

(P66) ANTIBIOTIC RESISTANCE IN *E. coli* FROM CATS AND DOGS WITH UTI

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Introduction: *Escherichia coli* is a common uropathogen that may be shared by companion animals and humans. Monitoring local antibiotic resistance (AMR) trends is key to identify opportunities for antimicrobial stewardship actions. This study aimed to report the AMR of *E. coli* isolated from companion animals with urinary tract infection (UTI) in Portugal.

Methods: A total of 1900 (2020-2023) isolates, from dogs (*n*=1092) and cats (*n*=808) with UTI were tested for antibiotic susceptibility (AST) by disk diffusion or microdilution following EUCAST guidelines. Urine was also plated in Thermo Scientific™ Brilliance™ ESBL Agar. AST intermediate resistance isolates were considered as susceptible. Fisher exact test was conducted to compare AMR according to the host species using a $p<0.05$ and SAS ODA (2024).

Results: Resistance to ampicillin (36.3%, 689/1899) was the highest followed by trimethoprim/sulfamethoxazole (18.8%, 356/1893; higher in dogs [20.8%, 226/1088] than in cats [16.2%, 130/805], p=0.0124), gentamicin (15.5%, 293/1891), enrofloxacin/ciprofloxacin (15.4%, 292/1893), amoxicillin/clavulanate (13.4%, 254/1898), cefotaxime (9.3%, 177/1899), and nitrofurantoin (2.9%, 54/1874). Among cefotaxime-resistant *E. coli* isolates, 29.9% (53/177) were susceptible to amoxicillin-clavulanate. Around 83.1% (147/177) of cefotaxime resistant isolates were detected in the selective Brilliance™ ESBL Agar media prior to AST.

Conclusions: First line antimicrobials still seem to be an adequate choice for UTI treatment although resistance was above 10%. Selective medium towards epidemiologically relevant resistance-mechanisms is a valuable tool for diagnostic stewardship.

(P67) GENETIC CHARACTERISATION OF INFLUENZA D VIRUS IN SWEDISH CATTLE

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Influenza D virus (IDV) has gained attention for its impact on cattle health, particularly respiratory disease in calves. The detection of IDV-specific antibodies in humans suggests possible transmission from cattle to humans. Our previous study found IDV antibodies in 35% of bulk milk samples from Swedish dairy farms in 2019-2020. However, understanding IDV's role in bovine respiratory disease and identifying phylogenetic lineages that are circulating in Sweden is still lacking. This study investigates the presence and phylogeny of IDV in Swedish cattle with respiratory disease.

We tested 1680 samples (nasal swabs, trachea, and lung tissues) from the Swedish Veterinary Agency (SVA) collected between 2021-2024. Samples were screened for other respiratory pathogens, then tested for IDV using rRT-PCR targeting the NP gene and confirmed with a second rRT-PCR targeting the PB1 gene. Samples with low Ct values (Ct<30) underwent whole-genome sequencing.

We detected 21 positive samples with a mean Ct value of 27 (range 15-37). In two samples, IDV was the only pathogen detected; the rest contained multiple pathogens. *Pasteurella multocida* was the most

common co-infecting pathogen (17/21), followed by bovine coronavirus (13/21). Phylogenetic analysis of the whole genome showed that 2021 strains belonged to the D/OK cluster, while 2023 strains were the result of reassortments of viruses belonging to D/OK and D/660 clusters.

Our data confirm active IDV circulation in Sweden with diverse strains. The co-infection rate suggests that IDV commonly acts as a co-pathogen. Overall, the prevalence of IDV was low. The identification of different clades and reassortment patterns underscores the need for continued surveillance. Moreover, the potential risk of IDV infections in humans requires further investigation.