Book of Abstracts

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Keynote Lecture / 157

Viral Zoonoses in Germany: What Lurks in the Reservoirs?

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Viral zoonoses pose a persistent and evolving threat to public health in Germany, where various animal reservoirs harbor significant pathogens. Wild birds play a central role in the ecology of highly pathogenic avian influenza viruses, such as H5N1. Recurrent outbreaks affect wild birds and domestic poultry, and spillover infections in different mammalian species occur. Swine are important reservoirs for swine influenza viruses, which have the potential to reassort and transmit across species. Birds and mosquitoes maintain the circulation of the West Nile virus, a pathogen that has become established in parts of Germany in recent years. Additionally, shrews are recognized as natural hosts of Borna disease virus 1, a rare but severe zoonotic pathogen. Ticks and small mammals sustain the transmission cycle of the tick-borne encephalitis virus, an endemic flavivirus with an increasing incidence in humans in Germany. Beyond these established risks, novel threats may emerge. For instance, biting midges (Culicoides spp.) and ruminants could transmit exotic arboviruses, such as the Oropouche and Shuni viruses. These potential incursions raise concerns under changing climatic, ecological, and globalization conditions. In this context, we will discuss the various zoonotic risks, emphasizing the indispensable role of the One Health approach in surveillance, prevention, and research efforts.

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The commensal to pathogen transition of Candida albicans

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Candida albicans is a pathobiont in warm-blooded animals that can cause various mucosal infections as well as life-threatening disseminated disease. Diagnosis of systemic candidiasis is challenging due to unspecific symptoms, low sensitivity of blood culture, and lack of standardized biomarkers differentiating colonization and infection. Delayed diagnosis and limited treatment options result in high mortality rates, which led to C. albicans being assigned to the Critical Priority Group of fungal pathogens by the WHO. Although case reports suggest that mucosal and systemic candidiasis occur in different animal species, resembling infections in humans, lack of awareness in veterinary medicine likely results in underdiagnosis.

Here, I will summarize the current knowledge on why and how C. albicans shifts from a commensal lifestyle to invasive growth, and highlight how tissue-specific difference shape host-pathogen interactions on different mucosal sites. By investigating different C. albicans strains, we found that strain heterogeneity impacts adaptation to and survival on different mucosal surfaces, with consequences for virulence but also immunological responses to colonization. The later in turn affects host immunity to systemic candidiasis caused by colonizing C. albicans strains. Furthermore, it can reduce susceptibility to bacterial infections, raising the question whether eradicating C. albicans colonization is a desirable goal.

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Paramyxovirus infections in small animals and rodents: an update

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Paramyxoviruses have significant impact on veterinary medicine and global public health. The family Paramyxoviridae includes significant pathogens such as the Measles virus (the causative agent of measles) and Rinderpest virus, which has now been eradicated worldwide. The relevance of these enveloped, single-stranded RNA viruses is further highlighted by the global impact of Canine Distemper Virus (CDV) on carnivores. The epidemiological and clinical implications of these viruses are far-reaching, affecting a wide range of host species, including companion animals (such as ferrets), rodents (mice), poultry, and marine mammals.

The zoonotic potential of selected paramyxoviruses like Hendra virus (HeV) and Nipah virus (NiV), which can be transmitted to humans from intermediate animal hosts like horses and pigs, emphasizes the "One Health" paradigm and the interconnectedness of animal, human, and environmental health. Understanding infection dynamics requires elucidating their complex transmission routes, including direct contact, aerosol transmission, and indirect environmental pathways. Prominent paramyxoviruses in veterinary practice include the Feline Morbillivirus (FeMV), which is frequently associated with renal pathologies in cats, and the Canine Parainfluenzavirus (CaPIV), a key etiological agent in canine respiratory disease complexes. Other examples, like the Sendai virus in mice, highlight the phylogenetic diversity that continues to gain relevance with the discovery of new viruses like the Jeilongvirus, an emerging virus with implications for research integrity and animal welfare.

This overview aims to deepen the scientific and clinical understanding of paramyxovirus infections in small animals and rodents. It seeks to foster improved veterinary surveillance, effective disease control, and enhanced public health preparedness.

Bacterial Pathogenicity - Board: 1 / **24**

Highly variable Receptor Binding Proteins in Tequatrovirus phages targeting Escherichia coli contribute to their host specificity

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Bacteriophages, particularly those targeting E. coli, are known for their specificity. The narrow host ranges of four isolated Tevenvirinae phages targeting E. coli prompted us to investigate their Receptor Binding Proteins (RBP), which binds to bacterial receptors during adsorption. A better understanding of the factors determining the host range is crucial to select phages for treatments.

The serotypes of 53 strains isolated from bovine mastitis was determined and associated with the host range. To identify RBPs, the phage genomes were annotated and aligned with their 5 closest homologs in databases. Proteins with low nucleotide identities and located in the tail were further analyzed using phageDPO to detect depolymerase activity. Finally, the 3D structure of the selected proteins were predicted and the normalized RMSD scores were calculated.

The host range showed limited dependance on the serotype. In all phages, both long and short tail fibers were identified as RBPs and displayed depolymerase activity. Analyze of the 3D structure and the RMSD revealed a highly specific reversible attachment to the distal subunit of the long tail fiber, followed by a less specific irreversible attachment to the short tail fiber.

In conclusion, although phages from the same genus have the same located RBP's, mosaïcism drives their specificities. Further investigations should try to identify the bacterial receptors to predict the interaction between the RBP and its receptor.

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Capsular polysaccharide promotes a stealth-like immunological state towards Mycoplasma mycoides

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Mollicutes are minute cell wall less bacteria encompassing important pathogens. We show that pathogenic *Mycoplasma mycoides* can switch expression of capsular polysaccharide (CPS) which creates phenotypic diversity and had dramatic repercussions on immune responses. For characterizing the immune responses, we employed the highly virulent wild-type GM12 as well as its engineered CPS-deficient mutant strain in a set of assays employing primary blood cells from its native ruminant host.
Primary blood cells stimulated with GM12 showed only very moderate effects on apoptosis as well as activation marker expression supporting an immunological stealth-like lifestyle. Interestingly, GM12 showed the capacity to survive and replicate inside monocyte-derived macrophages (MDMs), which fosters dissemination and persistence in the host. Stimulation with the CPS-deficient mutant strain, which exposes surface proteins including lipoproteins, increased apoptosis, strongly suppressed expression of major histocompatibility complex on antigen-presenting cells and induced secretion of several pro-inflammatory cytokines/chemokines which is a clinical hallmark in infected animals. Moreover, the CPS-deficient strain elicited apoptosis in MDMs. In conclusion, we showed that M. mycoides can switch the expression of CPS, which leads to different immunological trajectories paving the way for clinical disease, dissemination and persistence in the host.

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The global epidemiology of Streptococcus canis identifies genomic features of host adaptation, virulence and antimicrobial resistance

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Streptococcus canis can cause severe infections mainly in dogs, cats, and cattle with symptoms such as keratitis, dermatitis, endocarditis, sepsis, or mastitis, but also occasionally causes zoonotic disease in humans. Infections of companion animals have dramatically increased in Europe over the last decades leading to increased antibiotic usage due to the lack of alternative treatment options.
To better understand host adaptation, antimicrobial resistance, and evolutionary dynamics, we analysed the genomes of over 800 S. canis isolates from different host species and geographical locations. Lineages tended to be comprised of either one of two S. canis M (SCM) protein types, one of the pathogen's most important virulence factors. In addition, bovine S. canis isolates significantly clustered together on the phylogenetic tree suggesting a degree of host adaptation. The isolates typically had around six antimicrobial resistance genes mostly belonging to the classes tetracyclines, macrolides and aminoglycosides. We did not detect any beta-lactam resistance, but penicillin-binding-protein (pbp) genes exhibited different allele patterns.
In conclusion, this work provides fundamental knowledge on the transmission and host adaptation of S. canis for establishing a prediction pipeline to assist diagnostic labs in genomic epidemiology studies. Although no beta-lactam resistance is reported in S. canis, variation in pbp alleles might assist in selection for resistance in the future.

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Investigation of the clade-specific pathogenic potential of Campylobacter coli

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Human campylobacteriosis is a major foodborne disease, with ~11% due to *Campylobacter coli* (*C. coli*) infections. Most *C. coli* strains isolated from human cases belong to clade 1A. In contrast, clade 2 and 3 strains are less frequently identified in human cases, but are widespread in the environment. This study aims to investigate whether *C. coli* strains of clades 2 and 3 exhibit lower pathogenic potential than clade 1A strains. Human colonic cell lines (HT-29/B6, T84) were used for *in vitro* assays to determine the cytotoxicity (WST-1-assay), as well as the adhesion- and invasion-ability of *C. coli* strains belonging to clades 1A, 1C, 2 and 3, respectively. All *C. coli* strains were able to adhere to and invade both cell lines, with strain-dependent variances. The cytotoxic potential of clade 3 strains was exceeding those of the other clades, as they reduced the metabolic activity of HT-29/B6 cells as early as 18h after infection. A similar reduction induced by most strains from other clades was observed only after 48h. However, reduction of the metabolic activity of T84 cells was exclusively measurable after infection by clade 3 strains after 48h. In conclusion, our results indicate a higher cytotoxic potential for *C. coli* clade 3 strains, whereas no apparent difference in the adhesion or invasion ability could be detected. Therefore, the lower prevalence of clade 3 strains in human cases appears to depend on factors other than those investigated in this study.

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Efficiency test of a live, attenuated Mycoplasma hyorhinis vaccine candidate strain

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Background: Mycoplasma (M.) hyorhinis causes significant economic losses in swine. Prevention and treatment rely on antibiotics, as no vaccines are available in Europe. However, antibiotics cannot eliminate the bacteria. Applying an efficient vaccine would provide a long-term control method, reducing the economic losses.
Materials and methods: A temperature-sensitive M. hyorhinis strain was developed via 1-methyl-3-nitro-1-nitrosoguanidine treatment. The immunogenicity and efficacy of the adjuvanted, attenuated vaccine candidate were tested. Three-week-old piglets were immunized, and the vaccination site was monitored daily. At six weeks, the pigs were challenged on two subsequent days. Clinical exams were conducted daily, and blood and nasal swabs collected weekly for M. hyorhinis ELISA, real-time PCR, and isolation. Three weeks post-challenge, animals underwent gross and histopathological examinations. Body temperature was recorded daily, and body weight was measured at arrival, six, and nine weeks.
Results: Vaccination reduced clinical ($p=0.001$), gross pathological ($p<0.001$), and histopathological ($p<0.001$) lesions. The vaccinated group showed earlier, higher M. hyorhinis-specific antibody levels post-challenge. However, vaccination did not prevent weight gain reduction.
Discussion: Overall, the adjuvanted, attenuated strain provided adequate protection. The attenuated strain was patented under number P2500036 at the Hungarian Intellectual Property Office.

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A case report of equine infectious anemia in the Netherlands

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Equine infectious anemia (EIA) is a notifiable viral disease in equids caused by a vector-borne lentivirus. In March 2025, EIA was diagnosed in a horse in the Netherlands for the first time since 2017. The animal showed no clinical signs of illness and originated from Eastern Europe, where EIA is endemically present. The infection came to light when the horse was tested for antibodies against EIAV as part of an export screening. Official samples were collected and tested positive according to the ELISA and Coggins test.

Consequently, necropsy was performed and tissue samples were sent to the European Reference Laboratory for EIA (ANSES, France). EIAV genomic DNA was detected in samples from the liver, spleen and mesenteric lymph nodes by realtime-PCR, while RNA detection was unsuccessful. Molecular characterization of the isolated strain is ongoing.

The horse had been residing in the Netherlands for three years and following an investigation by the authorities, 40 horses with an epidemiological link at three different locations were traced and sampled twice for serological screening. The second sampling took place after a period of 90 days, during which the horses were quarantined. In addition, movement of horses and manure was not allowed on these locations. All horses tested negative during the first and second round of sampling. This case highlights the importance of the screening of animals to prevent the introduction of infectious diseases into non-endemic areas.

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First outbreak of emerging ha-MYXV-associated myxomatosis in European hare (Lepus europaeus) in Austria

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In the spring of 2025, an unusually high number of dead European hares (Lepus europaeus) were found in a region northeast of Vienna, Austria. Pathological and histological examinations revealed typical swellings around the eyes, nose and genital tract with epithelial viral inclusions, consistent with a poxvirus infection. PCR analysis and cytopathic effects on RK13 cells confirmed the presence of the Myxoma virus, and sequencing of the M009L gene region identified the characteristic 2.8 kb insertion associated with the highly pathogenic ha-MYXV strain.

Myxomatosis is a severe disease primarily affecting wild and domestic rabbits (Oryctolagus cuniculus). Originally introduced to Europe in the 1950s, the virus has since become endemic in many countries, with variants of differing pathogenicity emerging over time. However, these variants have historically posed little threat to hares.
In 2018, the recombinant ha-MYXV strain emerged on the Iberian Peninsula, causing significant declines in Iberian hare (Lepus granatensis) populations.

This report marks the first documented outbreak of ha-MYXV-associated myxomatosis in European hares in Austria. Genome sequencing and epidemiological analyses are currently being carried out to better understand the evolution and spread of this emerging pathogen. Given the potential impact of myxomatosis, along with other infectious diseases, continuous monitoring of European hare populations is essential to mitigate future threats.

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Discovery of novel hepadnaviruses in passerine birds

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The Hepadnaviridae family comprises circular DNA viruses with hepatotropism [1]. The genome sequence of a novel avian hepadnavirus was serendipitously generated in a passerine ornamental bird while performing a sequence-independent enrichment protocol for circular DNA, based on rolling cycle amplification (RCA) [2]. An archival collection of samples was screened with a specific qPCR, with an overall prevalence of 7.9% (8/101). The presence of replicative covalently closed circular DNA (cccDNA), indicative of active viral replication, was confirmed in embryonated eggs, feather quills, and liver through RCA enrichment and inverse PCR [3]. By in-depth sequencing on Oxford Nanopore TechnologiesTM (ONT) platform, the whole genome sequence was obtained from 3 strains detected in Gouldian finch (Chloebia gouldiae), Society finch (Lonchura striata domestica) and Long-tailed finch (Poephila acuticauda). On phylogenetic analysis, the viruses were genetically distinct from other known avian hepadnaviruses, thereby forming a novel viral clade. These findings expand the known host range of hepadnaviruses to passerine birds. More importantly, they suggest potential vertical and feather-based transmission routes, as observed for other avian viruses [4,5]. The identification of hepadnavirus DNA in feather quills also represents a valuable, non-invasive method for future epidemiological surveillance in wild and domestic avian populations.

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Case report of a cat infected with EBLV-1 in the Netherlands

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In October 2024 an infection of European bat lyssavirus type 1 (EBLV-1, lyssavirus Hamburg) was confirmed in a domestic cat in the Netherlands. The cat started to show abnormal behavior on October 22nd. Several weeks before, the animal owners had found a dead bat in their home, which was thought to be caught by the cat. At October 25th the cat was euthanized and sent to the national reference laboratory for veterinary rabies of the Netherlands, Wageningen Bioveterinary Research. Brain material of the cat tested positive in the fluorescent antibody test. Subsequently, the genotype specific Realtime-PCR (RT-PCR)test for EBLV-1 tested positive. Additionally, salivatory gland material and swab material from the mouth of the cat tested positive for EBLV-1 in the RT-PCR, indicating potential infectiousness of the cat. Histopathology of formalin-fixed and paraffin-embedded sections of the brain showed a viral encephalitis with positive immunohistochemical staining against rabies nucleocapsid protein. Using Oxford nanopore technology, the entire genomic sequence could be determined.
Persons exposed to the cat received post-exposure prophylaxis and domestic animals from the same household were quarantined. Pet owners in the same (rural) municipality were informed and were requested to report behavioral changes of pets immediately to a veterinarian. This case stresses the need for vigilance of rabies infections of pets in countries where lyssavirus infections in bats are endemic.

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Black proventriculus in broiler chicks: recent Italian clinical case and laboratory findings

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Avian colibacillosis refers to any localised or systemic infection caused entirely or partly by avian pathogenic Escherichia coli (APEC). It includes colisepticemia, air sac disease, peritonitis, salpingitis, synovitis, and, in young birds, yolk sac infection. Over the past decade, a new syndrome known as "Black proventriculus-Bp," caused by APEC, has been recognised in two different broiler breeding farms (Wang et al., 2015; Talebi et al., 2020). In Italy in 2025, we observed eleven clinical cases of Bp in broiler chicks aged three to six days. One hundred and thirty-three chicks were necropsied and submitted for bacteriology and histological examination. Thirty per cent of chicks displayed the classical Bp lesion, while the remainder exhibited a combination of pericarditis and omphalitis/yolk sac infection. Escherichia coli of different serotypes (O88, O45, O15, O1) was isolated as the sole bacteria from the mucosa of the proventriculus in Bp lesions. The strains isolated were classified as weak biofilm producers. The histological investigation in one clinical case showed catarrhal erosive bacterial proventriculitis with mild ectasia of the submucosal glands, cellular necrotic cells, and bacteria in the lumen. Vascular congestion and bacteria in the lumen of the vessels, as well as fibrinous bacterial serositis with the presence of rare macrophages and heterophils. Etiopathogenetic hypotheses will be speculated and presented at the Scientific Symposium.

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Coronavirus detection in British Red Foxes (Vulpes vulpes)

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Britain has eight species of carnivorous wild mammal which, in their international populations, are known to be susceptible to a range of Alphacoronaviruses and Betacoronaviruses (CoV's).
Previous work in 2020-2021 to determine if SARS-CoV-2 was present in UK wildlife demonstrated no detection of this pandemic virus. However, a novel mustelid coronavirus, a previously uncharacterised stoat Minacovirus, was discovered. Further to this, in 2022 a highly divergent coronavirus (*MelesCoV*) in Italian badgers (*Meles meles*) was reported, that to date, has not been found in UK animals.
Over 500 carnivore samples have been screened, the sample type dependant on the requirements of the sample provider (samples including faecal and tissue (lung and enteric lymph node) samples, as well as oronasal and rectal swabs). Samples were preserved with RNAlater. RNA was extracted using ThermoFisher's Kingfisher Apex and screened for CoV's using pan-coronavirus primers.
PCR positive results have been found in a Red Fox (*Vulpes vulpes*) rectal swab. Sanger and Illumina sequencing were conducted, and downstream bioinformatic pipelines identified the sequence as a coronavirus similar to other canid and canine viruses.
Britain's wild carnivores play an important role in ecosystems, with red foxes inhabiting both wild and urban habitats. Determining the presence of coronaviruses within these animals is critical to our preparedness for the emergence and detection of novel viruses.

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Pathogenesis of P. larvae - 20 years of research on an important but understudied bacterial pathogen

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American foulbrood (AFB) is one of the most important and devastating infectious diseases of the Western honey bee Apis mellifera. It is caused by the gram-positive, spore-forming bacterium Paenibacillus larvae, which not only kills the brood of a colony but also the entire colony as the disease progresses. Since P. larvae is highly infectious and contagious, the disease spreads very easily within a colony and between colonies. Therefore, AFB is listed as a notifiable animal disease in many countries.
The species P. larvae is divided into several, so-called ERIC-genotypes, which also differ phenotypically. Only two of these genotypes, ERIC I and ERIC II, are currently driving the global AFB-outbreak situation. P. larvae ERIC I and ERIC II differ in their suite of expressed virulence factors resulting in variations in pathogenesis and virulence differences. Over the past two decades, we have intensively studied the species- and genotype-specific virulence factors of P. larvae, thereby deepening our understanding of the molecular pathogenesis of AFB. We have identified a chitin-degrading protein as key virulence factor of the species P. larvae, toxins and an S-layer protein as genotype-specific virulence factors, and unravelled the role of secondary metabolites during biotrophic and necrotrophic growth of P. larvae in the host. One practical result of this basic research is the recently completed development of a highly specific point-of-care immunoassay for P. larvae diagnosis.

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New vaccines against emerging zoonotic infections: From animal models to clinical evaluation

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Modified Vaccinia virus Ankara (MVA) is a licensed third-generation smallpox vaccine and a potent vector platform for developing vaccines against infectious diseases and cancer. Developed by serial passage in chicken cells, MVA lost replication ability in mammalian hosts and many orthopoxvirus virulence genes, enhancing its safety profile. MVA-based vaccines have demonstrated safety, immunogenicity, and protective efficacy in animal models, including an MVA-MERS-S candidate tested successfully in dromedary camels—the primary MERS-CoV reservoir—supporting its use as a One Health strategy to prevent zoonotic transmission. Clinical safety and immunogenicity of MVA-MERS-S were confirmed in a phase I human trial, with phase II studies underway in Europe. Recent preclinical work on MVA-based COVID-19 vaccines showed that a recombinant MVA expressing stabilized SARS-CoV-2 spike protein (MVA-SARS-2-ST) elicited superior S1 surface expression and stronger neutralizing antibody responses across variants compared to the native S protein. Intramuscular vaccination with MVA-SARS-2-ST protected mice and hamsters from disease and lung pathology upon challenge. These results support MVA-SARS-2-ST as an improved clinical vaccine candidate, highlighting the importance of membrane-bound S1 for protective immunity.

AMR & Evolution - ES / 151

Chromosomal ampR Regulates Plasmid-Mediated Antibiotic Resistance and Gene Duplication Amplification in Enterobacter cloacae

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Background and Objectives: Gene duplication and amplification (GDA) is crucial for bacterial adaptation to antibiotic pressure [1]. In Enterobacter cloacae strain IMT49658-1, ceftazidime induces GDA of a genomic region containing blaDHA and adjacent ampR(DHA) [2]. The strain also carries plasmid-borne blaTEM and blaCTX, and chromosomal blaACT with ampR(ACT). Given the regulatory role of ampR in β-lactamase expression, we aimed to investigate whether ampR(ACT) influences GDA and the expression of plasmid-borne resistance genes, thereby modulating antibiotic susceptibility and bacterial fitness.

Methods: ampR(ACT) was deleted via λ Red recombineering, confirmed by PCR and sequencing. Antibiotic susceptibility was tested by agar disc diffusion assay. GDA copy number was quantified by qPCR of genomic. Meanwhile, Expression of blaDHA and ampR(DHA) was measured by RT-qPCR. ScanLag was used to assess colony appearance and growth times.

Results: Deleting ampR(ACT) unexpectedly increased ceftazidime resistance, despite reduced GDA. RT-qPCR showed blaDHA upregulation and ampR(DHA) downregulation, suggesting altered regulation. ScanLag revealed delayed growth, indicating a fitness cost.

Conclusions: These findings indicate that ampR(ACT) functions as a key regulatory element, influencing both chromosomal and plasmid-borne resistance genes. This study provides new insights into the genetic and regulatory mechanisms shaping antibiotic resistance evolution in E. cloacae.

AMR & Evolution - ES / 20

Within-host evolution of Staphylococcus pseudintermedius in dogs with skin disease

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This study assessed within-host phenotypic and genotypic evolution amongst methicillin-resistant Staphylococcus pseudintermedius (MRSP) isolates from two dogs with chronic skin disease and recurrent pyoderma. Phenotypic antimicrobial susceptibility was determined by broth microdilution. Isolates underwent genome sequencing using both short read and long read platforms. Genome short reads were assessed for the accumulation of core genome single nucleotide polymorphisms (SNPs). Assembled genomes were interrogated for variations in accessory genome content. Phylogenetic, phenotypic and genotypic variation in antimicrobial resistance was evident in longitudinally isolated S. pseudintermedius from both dogs. Dog 1 had three infections with the same MRSP clone (ST316) and one infection with an unrelated methicillin-susceptible (MSSP) clone. Dog 2 had three different clones over five infection episodes: two infections were due to ST64 MRSP, two ST257 MSSP and one ST2814 MSSP. Non-synonymous SNPs accumulated in genes relating biofilm formation, rifampicin resistance, metabolism and cell wall synthesis. Sequential ST316 MRSP isolates from Dog 1 developed a mutated rpoB gene which conferred phenotypic rifampicin resistance, making the isolate extensively drug resistant. Within clonal types, plasmid content was stable while prophage content was variable. These findings provide evidence for the emergence of extensive drug resistance during recurrent S. pseudintermedius infection.

AMR & Evolution - ES / 150

Investigating the Role of the DUF445-Containing Putative Membrane Protein in Albicidin Resistance in Acinetobacter baumannii IMT51508

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Albicidin, a promising antibacterial peptide, functions by inhibiting the activity of bacterial DNA gyrase. This study aims to elucidate the mechanisms underlying albicidin resistance in ESKAPE pathogens, which are leading contributors to nosocomial infections. Specifically, we focus on investigating the resistance mechanisms in *Acinetobacter baumannii* (IMT51508), a clinically significant multidrug-resistant pathogen.

The MIC of albicidin was determined, followed by laboratory evolution, where the albicidin concentration was increased by two-fold. The evolution of bacterial strains was confirmed by MIC assays of all independently evolved replicates. Subsequently, genomic DNA was extracted from the wild-type strain and eight evolved strains, and WGS was performed. Genome analysis revealed a consistent mutation in an uncharacterized protein, containing a DUF445 domain, YjiN, in 7 out of the 8 evolved strains. Bioinformatics analysis was employed to analyze YjiN protein, suggesting its role as a 2-3 transmembrane domain containing protein. To further elucidate the operon structure of gene cluster, RT-PCR was conducted. In addition, mutations in YjiN and MATE in *Acinetobacter baumannii* were performed, and their effects were measured through MIC assays. The involvement of the YjiN protein in *Acinetobacter baumannii* will provide valuable insights into the role of this putative membrane protein in the bacterial resilience to albicidin.

Innovative Techniques / 65

Application of Machine Learning to Predict Antigenic Distance Between Newcastle Disease Virus Strains from Sequence Data

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Newcastle disease virus (NDV) remains a major vaccination challenge due to its rapid evolution and the emergence of new variants.. Although molecular and sequence data are now quickly and inexpensively produced, genetic distance rarely are a good proxy for cross-protection, while experimental studies to assess antigenic differences are time-consuming and resource-intensive. In the present study several machine learning (ML) methods were developed and compared to predict the antigenic distance between NDV strains as determined by haemagglutination-inhibition (HI) assays based on F and HN gene sequences -and corresponding amino acid features - analysis. Among the models evaluated, the random forest (RF) approach outperformed traditional linear models, achieving a predictive accuracy with an R^2 value of 0.723 compared to only 0.051 for linear models based on genetic distance alone. This significant improvement demonstrates the usefulness of applying flexible ML approaches as a rapid and reliable tool for vaccine selection, minimizing the need for labour-intensive experimental trials. Moreover, the flexibility of this ML framework allows the application of comparable approaches to other infectious diseases in both animals and humans, particularly in scenarios where prompt response and ethical constraints limit conventional experimental approaches.

Innovative Techniques / 56

Virome Diversity in Ticks Associated with Wild Boars: A Metagenomic Approach

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Tick-borne viruses (TBVs) include several emerging zoonotic agents with variable pathogenic potential ranging from asymptomatic to mild to severe symptoms, such as encephalitis, or meningitis. As part of the project "Ricerca Corrente 2022 – IZS ME 02/22 RC," we investigated the virome of ticks collected from wild boars in Southern Italy, a species with potential epidemiological relevance for both domestic pigs and humans. A total of 36 *Dermacentor spp.* ticks were pooled (6 pools of 6 individuals) and analyzed using a metagenomic approach based on SISPA enrichment and Oxford Nanopore Technology sequencing. Viral reads corresponding to segments L and S of Tacheng tick virus 2 (TTV-2; Uukuvirus tachengense, family *Phenuiviridae*) were detected in 3 out of 6 pools. Nearly complete genomes of Tacheng tick virus 3 (*Rhabdoviridae*), Tacheng tick virus 5 (*Chuviridae*), Orthonairovirus sulainense, and Norwavirus grotenhoutense (*Nairoviridae*) were also recovered in other pools. TTV has been detected in human patients with TBV-like febrile disease in China, and it has since been detected in several tick genera/species across Asia and Europe. Its wide vector range suggests potential for ecological adaptation and cross-species transmission. These findings highlight the diversity of tick-associated virome in wildlife and the importance of surveillance.

Innovative Techniques / 37

NOVEL STRATEGY FOR THE SEQUENCING AND DISCOVERY OF CIRCULAR DNA VIRUSES

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The emergence of COVID-19 and recent zoonotic outbreaks highlight the need of early pathogen detection, thus the development of quick and reliable diagnostic strategies. In this study, we developed an agnostic and cost-effective unbiased sequence-independent enrichment (USIE) protocol for the complete genome sequencing of circular DNA viruses.

DNA extracts from different hosts and biological samples are enriched by multiply primed rolling circle amplification (RCA). RCA products are debranched using T7 endonuclease and used as input for libraries and sequencing by Oxford Nanopore Technology™. The generated reads are analyzed using different metaviromic tools and customized pipelines.

Thus far, this diagnostic strategy has been successfully used for the complete genome sequencing and discovery of the following viruses; hepadnaviruses in domestic dogs (Diakoudi et al, 2022), Iberian lynxes (Diakoudi et al., 2025), and passerine birds (unpublished data), emerging papillomaviruses in horses (unpublished data), several CRESS DNA viruses in cats (Vasinioti et al., 2023), squamates (Capozza et al, 2022), Iberian lynxes (Castro-Scholten et al., 2024), and wolves (unpublished data), and avian polyomaviruses (unpublished data).

Overall, the USIE protocol is a host- and tissue-independent strategy that can be used to sequence circular DNA viruses. Updating and implementing the diagnostic algorithms is crucial for the effective prevention and control of emerging and re-emerging viruses.

AMR - genetic basis / 23

A novel macrolide-lincosamide resistance gene in Actinomyces bowdenii isolated from an abscess in a dog

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Acquired resistance in actinomycetes infecting companion animals is rare. Usually β-lactams, tetracyclines and clindamycin are considered for treatment. There are however studies about human isolates reporting acquired resistance, especially against clindamycin.
Over the last five years three out of 98 actinomycetes isolated from dogs and cats in our diagnostic laboratory were resistant to clindamycin. One *Actinomyces bowdenii* strain (21MD1404) isolated from a dog abscess was subjected to whole genome sequencing to determine the genetic basis of resistance. Analysis with ResFinder-4.7.2 revealed no known resistance genes. However, a genome alignment with clindamycin susceptible strain 07KM1036 using mauve 1.1.3 led to the discovery of a potential rRNA-methylase gene. The gene was related to known *erm* genes which usually confer macrolide, lincosamide and streptogramin B resistance. The most closely related showed 40% amino acid identity, clearly below the threshold of 79% for designating new genes. To test the functionality of the new gene, plasmid pacti_erm3 was constructed consisting of partial vector pJRD215 and the resistance gene including the promoter region. The plasmid was transferred into the susceptible strain 07KM1036 where it led to a clindamycin MIC increase from 0.12 mg/L to 32 mg/L and an erythromycin MIC increase from 0.03 mg/L to 1 mg/L. These results indicate that the discovered gene is indeed responsible for the clindamycin resistance of strain 21MD1404.

AMR - genetic basis / 96

Genetic basis of antimicrobial resistance in Pasteurellaceae of diseased cattle and pigs from Germany

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This study investigated the genetic basis of macrolide resistance and further antimicrobial resistance (AMR) properties in *Mannheimia haemolytica* and *Pasteurella multocida* from diseased cattle and pigs. Seventeen macrolide-resistant isolates from respiratory diseases included in GERM-Vet (*M. haemolytica*, cattle, 2008-2020, n=13/780; *P. multocida*, pigs, 2008-2021, n=4/1115) and eight bovine *P. multocida* from sporadic cases of mastitis (2021-2023) were investigated. Antimicrobial susceptibility testing was done according to CLSI recommendations. Closed whole genome sequences were generated via hybrid assembly of Illumina MiSeq and Oxford Nanopore MinION reads. Among the 25 isolates tested, resistance to several of the antimicrobial agents, including aminoglycosides, phenicols, penicillins, tetracyclines, macrolides and sulfonamides, was detected. In 19 isolates (respiratory disease n=12, mastitis n=7), integrative and conjugative elements (ICEs) were identified that conferred multidrug resistance. These ICEs, some of them novel, harbored the AMR genes *erm*(T), *lnu*(H), *estT*, *mef*(C), *mph*(G), *floR*, *catA3*, *aadA31*, *aad(3")(9)*, *aph(3')-Ia*, *aac(3)-IIa*, *strA*, *strB*, *tet*(H), *tet*(Y), and *sul2* in varying combinations. Four *M. haemolytica* also carried a 4,613-bp plasmid with the β-lactamase gene *blaROB-1*. Resistance-mediating ICEs or plasmids, as found here, can promote the rapid spread of AMR via horizontal gene transfer and co-selection events.

AMR - genetic basis / 111

Antimicrobial resistance profile and mobilome of *Klebsiella pneumoniae* isolates from the reproductive tract of mares and stallions

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Klebsiella pneumoniae is associated with reproductive infections in both mares and stallions, and has great potential for acquiring antimicrobial resistance genes.
Reproductive samples collected from thoroughbred horses in Australia were cultured (2020 and 2022 [inclusive]). Conventional laboratory methods (colony morphology, biochemical, motility tests) were used to presumptively identify *K. pneumoniae*. Antimicrobial susceptibility testing and whole genome sequencing were performed. Genetic diversity and phylogeny were evaluated by MLST and alignments/comparisons of complete sequences. Further analysis was performed to detect resistance genes and mobile genetic elements.
Of 91 *K. pneumoniae* isolates (mare: 76, stallion:15), multidrug-resistance (MDR) was identified in 59% and 33% of isolates from mares and stallions respectively. Thirty-one sequence types were identified, and phylogenetic analysis suggested a significant level of genetic diversity, with isolates grouped into 31 distinct subclades. A high frequency of IncFIB(K) plasmids and integrons was detected among MDR isolates and several novel configurations of resistance genes were identified.
This study revealed a concerning level of antimicrobial resistance and a diverse population of *K. pneumoniae* in the equine reproductive tract. These findings highlight the crucial need for ongoing monitoring and characterization of *K. pneumoniae* for effective disease control and management.

Viral Pathogenicity I / 4

Superinfection exclusion and enhancement of infection in pestiviruses

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Some viruses can suppress superinfections of their host cells by related or different virus species. The phenomenon of superinfection exclusion can be caused by inhibiting virus attachment, receptor binding and entry, by replication interference, or competition for host cell resources. Blocking attachment and entry not only prevents unproductive double infections but also stops newly produced virions from re-entering the cell post-exocytosis. In this study, we investigated the exclusion of superinfections between the different pestivirus species. Bovine and porcine cells pre-infected with non-cytopathogenic pestivirus strains were evaluated for susceptibility to subsequent superinfection using comparative titrations. Our findings revealed significant variation in exclusion potency depending on the virus species as well as the host cells. Despite this variability, all tested classical pestivirus species reduced host cell susceptibility to subsequent infections, indicating a conserved entry mechanism. Unexpectedly, pre-infection with atypical porcine pestivirus (APPV) increased host cell susceptibility to classical pestiviruses. These results indicate that APPV uses different binding and entry mechanisms than the other pestiviruses. The observed increase in susceptibility of cells post-APPV infection warrants further investigation and could aid challenging isolations from diagnostic samples.

Viral Pathogenicity I / 55

Feline Bocaparvovirus in domestic cats with gastrointestinal disease

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Bocaparvoviruses (BoVs) are non-enveloped viruses with linear single-stranded DNA genome and are classified in the genus Bocaparvovirus (subfamily Parvovirinae, family Parvoviridae). BoVs infect the respiratory and gastrointestinal tracts of young animals and humans and have been detected in a wide range of mammalian hosts (i.e. primates, carnivores, ungulates and rodents). In cats, feline BoV (FBoV) has been identified in three genetic types, namely FBoV-1, -2, and -3 (Bocaparvovirus carnivoran 3, 4 and 5, respectively), which have been described in cats with gastrointestinal symptoms. We investigated the prevalence of FBoV in 126 feline rectal swab samples of cats with gastroenteritis from two different regions, Apulia (collection A, 101 samples) and Abruzzi and Molise (collection C, 25 samples) between 2023 and 2025. We used a pan-bocavirus PCR assay with broadly reactive primers able to identify all human and animal BoVs. Overall, 16.7% (21/126) stool samples tested positive for BoVs. Partial NS1 sequences were generated for 9 strains, with nucleotide identities ranging from 95.0 to 100% to FBoV-1 strains. A quantitative PCR assay specific for FBoV-1 was designed and used to re-screen the sample collections, with an infection rate of 19.0% (24/126). Gathering epidemiological data is necessary to improve our understanding of the enteric virome of companion animals.

Viral Pathogenicity I / 99

A defective canine distemper virus strain responsible for CNS disease in a Eurasian Lynx shares key phenotypic traits with measles virus strains associated with SSPE in humans

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Canine distemper virus (CDV) can cause chronic central nervous system (CNS) infections such as old dog encephalitis with parallels to subacute sclerosing panencephalitis (SSPE) in humans. However, it has not been possible to correlate such clinical manifestations to viral molecular determinants. The complete genome sequence of a CDV strain (CDV-lynx) previously identified in the brain of a Eurasian lynx was obtained by next generation sequencing. Sequence analysis showed unique amino acid (aa) changes in all viral proteins, including a premature stop codon in the matrix protein, four aa changes in the F protein and several additional changes in other proteins. A recombinant (r) CDV-lynx-EGFP was rescued, and several gene-swap constructs were generated with a closely related rCDV-Raccoon-EGFP with the resulting recombinant viruses used to study virus infection of immune cells and organotypic ferret brain slices. The CDV-lynx F protein was hyperfusogenic in cell fusion assays as fusion was observed in receptor negative Vero cells. A single aa change (P479A) could abrogate cell fusion in Vero cells. Furthermore, a significant reduction in the entry of VSV pseudotyped with CDV-lynx glycoproteins into Vero-Dog-SLAM cells was observed. Our data show an animal morbillivirus acquired a hyper-fusogenic phenotype and defective matrix protein expression following long-term CNS infection, findings that are strikingly homologous to the phenotype of SSPE strains of measles virus.

Viral Pathogenicity I / 116

Pseudorabies virus-associated encephalitis in hunting dogs in Greece: The role of wild boars as a persistent reservoir in Greece

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Introduction: Pseudorabies, caused by Suid herpesvirus 1 (SuHV-1), primarily affects swine and accidentally other mammals. Although eradicated from domestic pigs in many European countries, SuHV-1 persists in wild boar populations. Hunting dogs are at particular risk due to direct exposure during wild boar hunts.
Methods: Between 2022–2024, seven cases of neurological disease and death in hunting dogs were investigated in the regions of Epirus and Thessaly, Greece. Postmortem brain tissues were tested by PCR targeting a part of the glycoprotein D gene of SuHV-1. Positive samples were subjected to sequencing and phylogenetic analysis.
Results: All seven cases tested PCR-positive for SuHV-1. Phylogenetic analysis of the gD gene sequences revealed genetic divergence among the isolates. The Epirus strains formed a separate clade, suggesting localized viral evolution. The Thessaly isolate showed greater divergence, clustering independently and indicating a potentially unique lineage within the Greek wild boar reservoir.
Conclusion: Our findings confirm the ongoing circulation of SuHV-1 strains in wild boar populations in Greece and demonstrate the fatal risk posed to hunting dogs. These data highlight the need to raise awareness among veterinary practitioners to include pseudorabies in the differential diagnosis of encephalitis in dogs.

Keynote Lecture / 155

Listeria monocytogenes: Survival strategies in food processing environments

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Listeria monocytogenes poses a significant risk, especially in ready-to-eat (RTE) foods, as it can grow at refrigeration temperatures, unlike many other pathogens. This species survives in a variety of harsh environments, including the food processing environment. Long-term survival of Listeria is usually addressed as persistence and the mechanisms are still unelucidated. One trigger of *Listeria* survival is seen in biofilm formation that consists of microbial communities attached to surfaces, embedded in a protective matrix. In food processing facilities, these biofilms can form on equipment, conveyor belts, storage bins, and drains. We found 9-12% of sampling sites carrying a true biofilm in food operations. We have studied the biofilm forming capacity of *L. monocytogenes* in various environments and found *Listeria* being a weak biofilm former. Once in a biofilm, *Listeria* is less susceptible to cleaning agents, disinfectants, and even physical removal, making it extremely difficult to eradicate from the environment. An intriguing question is how Listeria co-colonize biofilms. Data show that other species such as Pseudomonas are drivers of biofilm formation, obviously scarcely in interaction with *Listeria* residing in the same biofilm. We further looked into the genome of persisting clones of *L. monocytogenes* by browsing a database storing more than 17000 *L. monocytogenes* genomes. A thorough bioinformatic analysis revealed that single genetic markers explaining persistence do not exist. Conclusively, survival of *Listeria* in food processing environments is more likely explainable due to failures of hygiene practices than by particular genetic features allowing some clones to persist.

AMR: Transmission - Spread - Envirnoment / 48

Wild coypu (Myocastor coypus) as sentinel of antimicrobial resistance in water ecosystems: preliminary insights using Aeromonas spp. as bioindicator

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This study investigates the potential role of wild coypu (*Myocastor coypus*) as a sentinel of antimicrobial resistance (AMR) in freshwater ecosystems, using *Aeromonas* spp. as bioindicator.
Between December 2024 and May 2025, 51 coypu carcasses and 13 water samples were collected along the Reno River and its tributaries in Italy. *Aeromonas* spp. were isolated and identified by MALDI-TOF. Their AMR profiles were determined using the broth microdilution method.
A total of 74 *Aeromonas* strains isolated from animals (57) and water (17) were detected, belonging to 7 different species. The most common were *A. veronii* (21/74) and *A. media* (19/74).
About 17.54% (10/57) of *Aeromonas* isolates from animal sources were resistant to at least one antimicrobial, most commonly sulphamethoxazole (8/57) and tetracycline (4/57). Only one *A. veronii* strain was multidrug-resistant (MDR) to sulphamethoxazole, tetracycline and gentamicin.
The number of resistant and MDR isolates, as well as the AMR profiles of *Aeromonas* strains of aquatic origin, were comparable to those of animal origin.
The ecological traits of wild coypu (semi-aquatic habits, wide distribution, sedentary behaviour and long lifespan), along with the strong similarity between AMR profiles of *Aeromonas* from animal and aquatic sources, suggest this species as an effective sentinel for AMR monitoring in aquatic environments and its potential use in future surveillance programs targeting freshwater ecosystems.

AMR: Transmission - Spread - Envirnoment / 146

EVIDENCE AND SPREAD OF MULTIDRUG-RESISTANT ACINETOBACTER SPP. IN FARM ANIMALS AND ENVIRONMENT UNDER A ONE HEALTH PERSPECTIVE

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This study aimed to address the knowledge gap on *Acinetobacter calcoaceticus–baumannii* (ACB) and non-ACB complex species in farm animals by: -investigating the occurrence of multidrug-resistant (MDR) strains in animals, operators, and the farm environment; -assessing their potential role in transmission within a One Health framework.

From cattle, horses, sheep, goats, pigs, poultry, human hands, and farm environment, samples were collected. Isolates were identified via culture and MALDI-ToF MS. Antibiotic susceptibility was assessed using E-test and Kirby-Bauer methods.

From 840 samples, 128 *Acinetobacter* strains (ACB: 10.2%, 13/128; and 18 different non-ACB complex: 89.8%, 115/128) were isolated in farm animals (83.6%), humans (13.3%), and environment (3.1%). ACB strains were more frequent in diseased animals (P=0.0028), particularly cattle (P=0.0002), where a high proportion of *A. baumannii* (81.8%, 9/11) was significantly identified. Both ACB (92.3%) and non-ACB strains (46.1%, P=0.0016) showed MDR profile that was significantly associated to carbapenem resistance (3.9%; P=0.029, Cramer's V=0.235, Lambda=0.095±SE 0.074). Non-ACB strains showed polymyxin (1.7%) and aminoglycoside resistance (11.3%). Isolates from animals, humans, and the environment shared identical MDR profiles.

Farm animals and their environments may act as reservoirs for MDR *Acinetobacter* spp., supporting the need for further research on transmission dynamics in a One Health context.

Viral Pathogenicity II / 21

Communicating emerging diseases to animal owners

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Europe has seen continual outbreaks of emerging diseases in both its human and animal populations in recent years. Part of effective response to these disease outbreaks is maintaining public confidence in control measures and the communication of risk, yet many scientists struggle with public communication of specialist knowledge. Veterinarians are trusted figures in animal health and have a key role in communication with animal owners in these outbreaks. This talk will draw on social sciences research that the author has participated in, into owner attitudes, knowledge, impacts (financial and emotional) and compliance and provide recommendations for communication of disease risk and control options using examples from recent UK Avian Influenza, Schmallenberg and Bluetongue communication campaigns.

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The Hunt for Maedi Visna Resistance: Understanding the Current TMEM154 Genetic Situation within the UK National Flock

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MV is a chronic viral disease affecting nearly 10% of the UK national flock. Its long latency period which ultimately ends in fatality in conjunction with the lack of treatment or vaccination options make control efforts difficult. Current strategies rely on voluntary testing and culling, which are costly and not widely effective. Selective breeding for genetic resistance therefore offers an alternative. Studies have identified a mutation in Transmembrane protein 154 (TMEM154) to be strongly associated with decreased risk of MV. Prior to implementing a selective breeding programme, there are several questions requiring answers such as do the resistant genetics have adverse effects on animal welfare and what resistant genetics are currently present within the UK flock. The latter of these is addressed in the current work.

Animals from major UK sheep breeds were genotyped to assess allele frequency and determine the prevalence of MV resistance. A minimum of 20 animals per breed from diverse bloodlines were tested to ensure accurate breed representation at a national level.

Data from 35 UK sheep breeds showed a wide range (3-90%) of animals homozygous for MV resistance. No breed lacked resistance, supporting selective breeding for this trait in UK breeds. The variation in prevalence suggests breed-specific approaches would be necessary to avoid genetic bottlenecks and loss of valuable genetics.

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WGS challenges and opportunities in bacteriological diagnostics

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Whole-genome sequencing (WGS) has attracted the attention of experts in various fields for decades. Due to the development of new and highly efficient sequencing technologies, sequencing costs per genome have drastically decreased in the last decade, making WGS a powerful and widely used application for diverse scientific questions. In modern bacterial diagnostics there is also a methodological shift from classical detection and typing techniques towards WGS-based approaches, utilizing different Next Generation Sequencing (NGS) technologies. WGS allows for comprehensive analysis and possesses high discriminatory power for bacterial characterization, thus enabling a wide range of applications in routine diagnostics and fundamental research. Whereas some promising approaches using NGS to facilitate bacterial detection and characterization from complex samples are still primarily applied and optimized in research projects, WGS-based bacterial isolate characterization has already replaced traditional standard methods in microbiological laboratories worldwide. Also, at the NRL for Salmonella in Germany, WGS is now used on a routine basis and is the gold standard approach for Salmonella characterization. However, new developments are accompanied by new challenges that affect decision making, risk management strategies and even national or international regulations.

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Rustrela virus (RusV) – a newly discovered cause of fatal encephalomyelitis in domestic, wild and zoo animals.

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Rustrela virus (RusV; species Rubivirus strelense) is a recently discovered relative of the human rubella virus and causes usually fatal non-suppurative meningoencephalomyelitis in a broad range of mammals, including felids, canids, mustelids, rodents and even marsupials. The virus was first identified in zoo animals from northeastern Germany, in 2019. Meanwhile, it has been found also in domestic, wild and zoo animals in Germany, Austria, Sweden and the USA. Meanwhile, RusV has been demonstrated to be the causative agent of 'staggering disease' in domestic cats as well as 'lion encephalitis' in lions, two neurological disorder that had remained of unknown aetiology for almost five decades. The clinical course is characterized by a broad range of neurological signs, with hind leg ataxia being the most prominent. Based on its broad range of susceptible hosts, a zoonotic potential of RusV cannot be excluded.
While encephalitic individuals appear to act as dead ends and do not spread the virus after spill-over transmission, apparently healthy yellow-necked field mice (Apodemus flavicollis) and wood mice (Apodemus sylvaticus) were identified as potential wild reservoir hosts of RusV. Experimental studies have confirmed the susceptibility of wood mice to RusV infection via intracerebral and oculonasal inoculation, but not via subcutaneous and intramuscular route and demonstrated shedding of viral RNA. The phylogeographic pattern of RusV sequences, with different sequence clusters occurring in separated, non-overlapping parts of the known dispersal areas, further suggests the virus to be bound to a rather non-mobile reservoir host, such as small mammals. However, many questions regarding the biology and epidemiology of RusV in reservoir and spill-over hosts remain elusive, such as course of infection, pathogenesis, and transmission routes.

AMR - Epidemiology & Surveillance: "ESGVM Session" / 144

Towards harmonised methods for surveillance of antimicrobial resistance in clinical infections from companion animals in the UK (VetCLIN AMR)

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Introduction: Understanding antimicrobial resistance (AMR) transmission between different sectors requires robust surveillance systems aligned with One Health principles. However, the lack of consensus for performing and interpreting antimicrobial susceptibility testing (AST) on veterinary clinical isolates, is hampering the usefulness of these data.
Aim: to evaluate the current methodological approaches used by veterinary diagnostic laboratories in the UK, for AST and clinically relevant AMR phenotypes in companion animals bacterial isolates.
Methods: New minimum inhibitory concentration (MIC) plates were designed. Target bacterial isolates (Staphylococcus aureus, n=189; S. pseudintermedius n=641; Escherichia coli, n=669) were collected from collaborating laboratories and tested with a standardised AST method. Results were interpreted according to CLSI VET01 7th Ed. and compared with results generated by laboratories. A free proficiency testing (PT) assay was established to identify variability in AST methodology.
Results: Initial AST data comparison showed important discrepancies in the clinical interpretation of AST for some bacterial pathogen/antibiotic combinations. PT assays revealed that 92% and 85% of laboratories correctly detected methicillin resistance in S. aureus and ESBL-production in E. coli, respectively.
Conclusions: Further training and guidance for AST methodology must be addressed to harmonise methods and improve data comparability for AMR surveillance.

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First Epidemiological Surveillance of Methicillin-Resistant Staphylococci in a Veterinary Teaching Hospital Using IR Biotyper®

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In a global view on Antimicrobial Resistance (AMR), Methicillin-resistant Staphylococci (MRS) are one of the most threatening pathogens in both human and veterinary medicine. The aim of this work was to assess the impact of MRS within a Small Animal Veterinary Teaching Hospital (VTH) in Italy, through a multilevel data collection on clinical, commensal and environmental isolates and a subsequent analysis through Fourier-transform infrared (FTIR) spectroscopy by IR Biotyper®. From May 2021 to May 2023, a total of 81 MRS clinical isolates was recorded, mainly MR *S. pseudintermedius* (MRSP, 81.1%). High resistance rates towards most of the antimicrobials tested were recorded, such as 87.8% for tetracycline and 85.6% for enrofloxacin. MRS prevalence in hospitalized patients' oral flora was 22% (33/150) at admission, while in-hospital acquisition was 19.7% (23/117). The environmental analysis showed a high frequency of MRS detection in the Intensive Care Unit area (29.4%), and in the personnel' shoe soles (85.7%) and the floor (71.4%). Strains typing using IR Biotyper® on 96 selected MRSP isolated showed the presence of three main clusters, one of them detected at all levels, suggesting its endemic presence within the hospital. These findings confirm the importance of MRS in small animal practice, highlighting as a multilevel surveillance program can consent to achieve an exhaustive overview that could lead to tailored measures of infection control.

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Methicillin-resistant Staphylococcus pseudintermedius and Staphylococcus aureus in dogs and cats: isolation rates from different clinical conditions

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Methicillin-resistant Staphylococcus pseudintermedius (MRSP) and Staphylococcus aureus (MRSA) are important pathogens in companion animals with implications for both animal and human health due to their zoonotic potential. This retrospective study evaluated MRSP and MRSA presence in samples from animals with various clinical conditions. Bacterial identification was performed by MALDI-TOF, and antimicrobial resistance was assessed via MICs and PCR targeting the mecA gene. Clinical conditions were classified using anamnestic data, cytology, or histopathology. Methicillin resistance was detected in 48/128 (37%) S. pseudintermedius and 2/9 (22%) S. aureus, corresponding to 50/137 (36%) of the total isolates. Particularly, MRS was found in 26/69 (38%) of suspected urinary infections, 8/34 (23%) otitis externa, 8/18 (44%) pyodermitis, and 8/16 (50%) surgical site infections. No statistical association was found between clinical condition and MRS presence. The highest resistance rates were against penicillin (84%), erythromycin (55%), clindamycin (46%), enrofloxacin (37%), marbofloxacin (37%), doxycycline (37%), pradofloxacin (36%), oxacillin (36%), trimethoprim/sulfamethoxazole (34%), chloramphenicol (31%), gentamicin (25%), and 1% for amikacin, nitrofurantoin, and florfenicol. Our findings indicate a high prevalence of MRS across clinical conditions, suggesting that methicillin resistance should always be suspected in clinical practice.

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The burden and drivers of antimicrobial resistance in commensal E.coli from shelter dogs in North Macedonia

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Dog shelters, with their dynamic populations from varied backgrounds, serve as critical environments for antimicrobial resistance (AMR). This study aimed to determine the prevalence of AMR in commensal E. coli in shelter dogs in North Macedonia and pinpoint contributing factors within shelter management. A total of 112 E. coli isolates were recovered from 119 fecal samples across six shelters. Antimicrobial susceptibility profiles were established via broth microdilution and resistance genes were identified by PCR. Shelter practices were assessed through a questionnaire.

High resistance rates were observed for sulfamethoxazole (68.8%) and ampicillin (52.7%). Multidrug resistance (MDR) was detected in 50% of isolates. Notably, 15.1% of isolates were confirmed as ESBL producers, carrying the blaCTX-M and blaTEM genes. The plasmid-mediated AmpC gene blaCMY-2 was detected in 14.3% of all isolates, indicating potential for horizontal gene transfer. A strong statistical association was found between intensive antimicrobial use (AMU) and ESBL prevalence. All ESBL-producing isolates came from shelters with high AMU. Longer shelter stays also correlated significantly with increased AMR.

Shelter dogs in North Macedonia are reservoirs of MDR and ESBL-producing E. coli. The study highlights that specific shelter practices, especially intensive AMU, are critical drivers of AMR. These findings underscore the urgent need for antimicrobial stewardship programs in shelters.

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Detection of Antimicrobial Resistance and ESBL-Producing E. coli from Mammals at UK Petting Zoos

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The role of petting zoos in bi-directional zoonotic disease transmission is well documented, however their potential role as reservoirs of antimicrobial resistance (AMR) is un-explored within the United Kingdom (UK). This study investigated AMR in *Escherichia coli* and coagulase-positive staphylococci (CoPS) isolated from mammals at eight UK centres. Faecal and skin samples were collected from 166 animals to recover *E. coli* and CoPS. Samples underwent enrichment culture, followed by plating on non-AMR-selective media (tryptone bile-x agar, mannitol salt agar) and selective media (ESBL ChromID, mannitol salt agar with 6 mg/L oxacillin). Susceptibility to eight antimicrobial classes was assessed using Kirby-Bauer disc diffusion. Antimicrobial usage data from the last 12 months were obtained from 7/8 centres. A total of 145/166 faecal samples yielded *E. coli*, with an overall AMR prevalence of 42.4%, and 8.5% classified as multidrug-resistant. ESBL-producing *E. coli* were detected in five animals. CoPS were recovered from 54 skin swabs: *Staphylococcus aureus* (n=70), *Staphylococcus intermedius* group (SIG) (n=13), *S. hyicus* (n=1), with an AMR prevalence of 25.3% and a single MDR-SIG. No MRSA/MRSP were identified. Antimicrobial usage was positively correlated with AMR for *E. coli* (r=0.81, P=0.03) and CoPS (r=0.87, P=0.05). This study demonstrates for the first time the presence of AMR within bacteria isolated from UK petting zoo animals.

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Abortion caused by Coxiella burnetii in captive Finnish Forrest reindeer in a zoo in the Netherlands

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Coxiella burnetii (Cb) is a Gram-negative intracellular bacterium causing coxiellosis in animals and Q fever in humans. Cb causes reproductive disorders in ruminants. Abortions are observed mainly in goats, sheep and less in cattle. The largest Q-fever outbreak was reported in 2007-2010 in the Netherlands caused by Cb shedding dairy goats. Since, there is a mandatory vaccination and monitoring program in the Netherlands for dairy goat and sheep.
In a zoo in the Netherlands, four Finnish forest reindeer (Rangifer tarandus fennicus) in a herd of 13, of which six were pregnant, suffered from abortions in April and May 2025. Diagnostic sampling showed positive antigen detection with qPCR from vaginal swabs and positive serology (ELISA) in all cases of abortion (4/4) and in one animal after normal parturition. Placental tissue (n=2) and fetal liver tissue (n=1) retrieved after two abortions indicated positive for Cb infection by qPCR. Necropsy of one female showed positive Cb qPCR results for spleen, liver and vaginal swab.
Direct sequencing from placental tissue resulted in a full genome and plasmid sequence. Initial strain typing with in silico Multi Locus Variable Analysis (MLVA) resulted in a Cb strain predominantly associated with cattle.
This is the first case of coxiellosis reported in Finnish forest reindeer. Due to the public function of the zoo, immediate preventive measures were taken for animal care takers and visitors according to the Dutch One Health approach.

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Comparison of conventional urine culture and BACT/ALERT® PF PLUS bottles for monitoring urinary tract infections in companion animals under different clinical and therapeutic conditions

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Urinary tract infections (UTIs) are common in companion animals. According to current guidelines, sporadic UTIs can typically be managed with a short course of antibiotics. However, recurrent UTIs are common and warrants closer follow-up. Assessing treatment efficacy typically requires a temporary suspension for culture to prevent false negative results, which may pose risks of infection worsening or recurrence. This study compares conventional urine culture with BACT/ALERT® PF PLUS bottles, which neutralize antimicrobials and may allow accurate microbiological monitoring during treatment. A total of 814 urine samples, mostly from animals under antibiotic therapy, were collected via cystocentesis. Each sample was tested with both conventional culture on CHROMID® CPS® ELITE and BACT/ALERT® PF PLUS and incubated with the BACT/ALERT® 3D system. Positive samples were plated for identification via MALDI-TOF MS and underwent antibiotic susceptibility testing. Results were evaluated considering clinical history and urinalysis. BACT/ALERT® PF PLUS demonstrated greater sensitivity, detecting pathogens in samples negative by conventional culture and identifying additional bacterial species. This method was especially useful in follow-up of recurrent or complicated UTIs. It supports informed decision-making on antimicrobial therapy discontinuation, thereby promoting targeted treatment and contributing to antimicrobial resistance prevention.

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Culture or PCR? Benchmarking Brucellosis Diagnosis Without a Gold Standard

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Brucellosis is a zoonosis of major public health concern. However, its near-eradication in high-income countries has limited recent research, and the performance of diagnostic tests in livestock remains unclear due to the lack of a gold-standard.
This study evaluated the performance of bacteriological culture and qPCR for diagnosing brucellosis in buffaloes and cattle.
A total of 5,149 animals, slaughtered in 2022 from confirmed or suspect infected herds in Campania (Italy), were tested according to the protocols provided by the European Union Reference Laboratory. Tissue samples from both seropositive and seronegative animals underwent culture and qPCR. Results were analysed using Bayesian latent class analysis, which estimates test performance without prior knowledge of true infection status.
Overall, 35.9% of animals tested positive to at least one method. Culture sensitivity ranged from 43.9% to 59.2% (median = 51.7%), and qPCR from 65.8% to 82.2% (median = 74.4%). Culture specificity ranged from 89.8% to 99.4% (median = 94.3%), and qPCR from 84.2% to 94.0% (median = 89.3%). Despite qPCR showing higher sensitivity overall, culture yielded better positive predictive values in seronegative animals.
These large-scale results provide robust benchmarking of brucellosis diagnostic and confirm that qPCR cannot fully replace culture, especially in low-prevalence settings. Moreover, the enhanced culture protocol adopted here showed promising results warranting further evaluation.

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Serovar detector: a bioinformatic tool for serotyping Actinobacillus pleuropneumoniae

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Serovar detector is a new bioinformatic tool for determining the serovar of *Actinobacillus pleuropneumoniae* using whole genome sequencing. The composition of capsular polysaccharide (*cps*) genes of isolates is compared to those of the serovar reference strains and the serovar is determined both by the number of common genes as well as the similarities between the homologous genes. A validation of the bioinformatic tool was performed using 732 genomes representing all described serovars. The isolates included had been characterized by conventional serotyping and PCR tests. Out of the 732 isolates included in the investigation, only 36 isolates (4.9%) could not be allocated into the 19 recognized serovars. The method could discriminate between most serovars, except for serovar 9 and 11. Phylogenetic analyses showed that although most serovars are genetically homogeneous, there is a degree of genetic variation that cannot be explained by the *cps* genes alone. This indicates that SNP-based phylogeny or wgMLST might be valuable supplements for assessing the variation of *A. pleuropneumoniae* in a given region. Serovar detector is a robust method for determining the serovar of isolates and a valuable tool for further characterization of the genetic heterogeneity within *A. pleuropneumoniae*

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Development and evaluation of a Pan-Borrelia TaqMan qPCR for detection of Borrelia spp. in ticks collected from cattle in Tanzania

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Bacteria of the genus Borrelia are tick-borne pathogens divided into two groups: Lyme disease (LD) group and Relapsing Fever (RF) group. Both include numerous species and new ones are still being identified. Recent studies have also reported the presence of Borrelia spp. in different hosts and across different geographical areas, highlighting the existing gaps in knowledge regarding the epidemiology of this pathogen. Continued surveys are needed to understand the prevalence and the host range of each species in order to safeguard the health of wildlife, domestic animals and humans. For this purpose, ticks collected from cattle in Tanzania in 2023, were tested for the presence of Borrelia spp. DNA. Molecular screening was performed using a new validated TaqMan real-time qPCR targeting the 16S rRNA gene able to detect all Borrelia species. A fragment of the 16SrRNA gene of the identified pathogens was sequenced and analysed for typing. One tick of 62 (1.6%) tested positive for bacteria belonging to the RF group, potentially Borrelia theileri, the main causative agent of bovine borreliosis. This species was already reported in cows in Africa, but never in Tanzania. This result confirms the importance of monitoring the spread of this pathogen in order to control the disease. Supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

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Accelerating blood culture diagnostics in veterinary medicine: pathogen identification and antimicrobial susceptibility testing

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Background and objectives: Blood cultures (BCs) are the gold standard for the diagnosis of sepsis, with rapid diagnostics being crucial for treatment. The current standard in veterinary medicine includes pathogen identification (ID) and antimicrobial susceptibility testing (AST). We aimed to accelerate BC diagnostics and compare its performance to the currently applied methodologies.
Methods: A manual BC system (Oxoid) was inoculated with frequently detected clinical pathogens. ID and AST were determined before positive signal of the BC system without cultivation steps or after short-term incubation of five hours on agar plates. ID was performed using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). AST was performed by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) standards.
Results: Short-term incubation allows determination of ID and preparation of the inoculum for AST of relevant clinical pathogens. The ID- and AST inoculum preparation of negative BCs without a cultivation step yielded less reliable results. Gram-positive species such as staphylococci posed limitations.
Conclusions: Shortening BC diagnostic steps in the microbiological laboratory is possible, but limitations exist presumably due to the species-specific growth rates. Together with improved management, short-term incubation of negative BCs can reduce the time to ID and AST communication to the clinician.

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Oxytetracycline 30µg agar disk diffusion results for four QC strains

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Antimicrobial susceptibility testing is important to predict the outcome of antimicrobial therapy. Therefore, the aim was to compare oxytetracycline 30µg disk and media lots for four reference strains as a pre-requisite to establish QC ranges. Eight laboratories tested *Escherichia coli* ATCC® 25922, *Staphylococcus aureus* ATCC® 25923, *S. aureus* ATCC® 29213, and *Streptococcus pneumoniae* ATCC® 49619 with 2 lots oxytetracycline 30µg disks 10 times on 3 lots agar according to CLSI and EUCAST. As quality control, one lot tetracycline 30µg disks was tested on one medium lot. The data was analyzed using the RangeFinder software. All tetracycline values were in range. For *S. aureus* ATCC® 25923 a range of 21-35mm (98.96% values included), mean values were 25.74mm, 30.63mm and 26.53mm for media und 28.12.mm and 27.15mm for disks. For *S. aureus* ATCC® 29213 the calculated. range was 20-33mm (96.88%; mean 24.45/28.22/25.54mm [media], 26.68/25.46mm [disks]). The *E. coli* ATCC® 25922 range is 17-29mm (99.79%; mean 21.47/25.76/21.78mm [media], 23.85/22.16mm [disks]). For *S. pneumoniae* ATCC® 49619 (CLSI) a range of 24-35mm (98.96 %; mean 28.57/30.49/28.87mm [media], 29.90/28.72mm [disks]) was determined. EUCAST method revealed a range of 24-37mm (100%; mean 29.19/31.60/29.85mm [media], 30.74/29.69mm [disks]). In conclusion, for media lot B the zone diameters seemed larger. When quality control data is out of range, the use of an alternative medium/disks lots might be an option.

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Diversity of Mycobacterium avium virulence illustrated by mouse infection models

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Mycobacterium avium is the most important mycobacterial species with medical relevance besides M. tuberculosis and M. bovis. It is a slow-growing non-tuberculous mycobacterium divided into four subspecies (ssp.): M. avium ssp. avium (MAA), M. avium ssp. silvaticum (MAS), M. avium ssp. paratuberculosis (MAP) and M. avium ssp. hominissuis (MAH). Despite high genetic identity, they differ in growth, genome structure, pathogenicity and host preference.
MAA, MAS and MAP are obligate animal pathogens. MAA causes avian tuberculosis, mainly in poultry under extensive husbandry or in zoo enclosures. After oral infection, the disease manifests systemically, especially in liver, spleen and intestine. Rare infections occur in cattle, pigs and humans. MAS has been isolated from avian tuberculosis-like lesions in wild pigeons. MAP causes paratuberculosis, a fatal chronic enteritis of ruminants, typically in the distal ileum and ileocecal valve. It can also infect other species and humans, often subclinically. Its potential role in Crohn's disease remains debated. MAH, in contrast, is an opportunistic pathogen occurring in the environment with a broad host range. Rising incidence in pigs and humans suggests certain host preference. In pigs, oral infection leads mainly to regional lymphadenitis affecting intestinal, head and cervical lymph nodes. Infections in cattle, poultry and other animals are usually subclinical. In humans, MAH causes local to systemic infections, primarily in immunocompromised but occasionally also in immunocompetent individuals.
Relatively little is known about the pathogenicity and host preference of the subspecies. Despite many studies in mice, immunopathology remains insufficiently investigated. Examining immunopathology of M. avium subspecies in mice will provide insights into pathogenicity and contribute to understanding virulence in natural hosts.

Keynote Lecture / 164

Harnessing phages to combat bacterial pathogens in animal production: From research to application

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The accelerating emergence of antimicrobial resistance in animal production poses a growing threat to both veterinary medicine and public health worldwide, demanding innovative and sustainable alternatives to antibiotics.
Bacteriophages are viruses that specifically infect and lyse bacteria and are increasingly recognized as a natural and highly promising solution against pathogenic bacteria. Their unique biological features make them particularly well-suited for veterinary applications: they precisely target pathogenic bacteria, self-amplify at the site of infection, and spare the beneficial microbiota. Phages can be applied in multiple complementary ways: therapeutically to treat infections where antibiotics fail; preventively through biocontrol strategies that reduce bacterial load in herds and flocks; for biosanitation of farm environments and processing facilities; and in biopreservation to enhance the microbial safety and shelf life of animal-derived products.
Research and experimental studies have demonstrated phage efficacy against key bacterial pathogens in veterinary microbiology, including Escherichia coli, Salmonella enterica, Campylobacter spp., Clostridium perfringens, Pseudomonas aeruginosa, and Staphylococcus aureus, in both livestock and companion animals.
Phage-based approaches therefore represent a versatile, environmentally friendly alternative to antibiotics, with the potential to improve animal health, enhance food safety, and mitigate the public health risks associated with antimicrobial resistance.

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Characterization of Enterococcal Groups Present in Hospital Environments

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Enterococci are one of the most frequent bacteria associated to hospital-acquired infections, so their antibiotic resistance and virulence characterization is important to prevent and treat these infections.
This study focusses on bacteria from four different sources: 34 environmental enterococci from the surfaces of a veterinary Biological Isolation and Containment Unit, 10 clinical enterococci from urinary tract infections of dogs, 10 commensal enterococci from the oral cavity of dogs and 10 clinical enterococci from human diabetic foot ulcers. Susceptibility testing by disk diffusion for thirteen antibiotics and phenotypic virulence factor production using six selective mediums were performed. Multiple Antibiotic Resistance (MAR) and Virulence (VIR) Indexes were calculated by dividing the number of resistances or positive expression of virulence factors, by the total number tested.
The group that presented the highest MAR index was the environmental enterococci, mainly composed of Enterococcus faecium, known for its high antibiotic resistance. The commensal isolates presented the highest VIR index, probably because 5 of the 10 representative isolates were identified as Enterococcus faecalis, known for their high virulence. When comparing both indexes, human clinical isolates were the ones with the highest pathogenic potential.
This study is important in showing that different environments may compile bacteria with different characteristics, even within a single genus.

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Identification and phylogenetic analysis of Mycobacterium avium subsp. avium strains isolated from three Abyssinian cats from Northern Italy with disseminated mycobacteriosis

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Nontuberculous mycobacteria, including those in the *Mycobacterium avium* complex (MAC), are emerging pathogens of humans and animals. An outbreak of fatal disseminated mycobacteriosis caused by MAC was observed in eight Abyssinian cats in Italy in autumn 2024 and isolation of MAC strains was successful in three cats. This study focused on the genomic characterization of these strains. Whole-genome sequencing was obtained with Miniseq Illumina platform. PanX and MTBseq tools were used to characterize the strains. Phylogenetic analysis was performed both on core genome using PanX and whole genome with maximum likelihood method, implemented in iqtree2. All three samples were identified as *Mycobacterium avium* (MA) and were further phylogenetically identified as *Mycobacterium avium* subsp. *avium* (MAA) showing 100% genome sequence identity. Their genome size was 4,855,006 - 4,860,371 bp with a GC content of 69.33-69.34%. Phylogeny showed that the three strains clustered with a MAA strain from Germany (Assembly GCF_020735405). Major virulence factors such as SigE, SigF, and Phop were detected in the three strains, together with antibiotic resistance associated genes. Disseminated mycobacteriosis caused by MAA in Abyssinian cats confirms previous reports showing a breed predisposition to MAC infections, highlighting the need for MAC surveillance and suggesting further studies to define MAA ecology, evolution and pathogenesis.

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Mycobacterium bovis infected domestic cats in an officially bovine tuberculosis free country resulting in human infection

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Despite the official bovine tuberculosis free status, Mycobacterium bovis sporadically causes tuberculosis (TB) in non-bovine mammals in the Netherlands. In early 2023, two domestic cats from unrelated households were diagnosed with M. bovis following euthanasia due to severe respiratory symptoms. In one household, three additional cats were euthanized, with post-mortem confirmation of M. bovis infection. An epidemiological link was hypothesized but not supported by genetic analysis, as the isolates from the two households differed in spoligotype and by at least 500 single nucleotide polymorphisms (SNPs). Commercial raw pet food was suspected as the probable source, but this could not be confirmed. Given the zoonotic potential of M. bovis, human contacts were screened using the Tuberculin Skin Test (TST) and Interferon-Gamma Release Assay (IGRA). Lung lesions were detected by computed tomography in a TST-positive, IGRA-negative contact and M. bovis DNA was isolated from a lung biopsy. This DNA contained specific SNPs also identified in the feline M. bovis isolates from the respective household, supporting the hypothesis of intra-species M. bovis transmission. All TST-positive contacts received antibiotic therapy.
These cases indicate that TB should be considered in the differential diagnosis of respiratory conditions in companion animals and highlight the need for One Health vigilance to prevent M. bovis transmission among humans, companion animals, wildlife, and livestock.

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Tracing the Introduction and Rise of a Single Leptospira Pomona Clone in Animals in Israel

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Leptospirosis is a globally important animal infection and zoonosis caused by pathogenic Leptospira species. In Israel, Leptospira serovar Pomona emerged over the past two decades from undetected to endemic status, becoming the dominant cause of bovine leptospirosis. Incidence in cattle culminated in large-scale outbreaks in 2018, coinciding with an exceptional human outbreak. These events prompted enhanced wildlife surveillance. From 2015–2024, >3,400 wildlife sera and ~400 kidney samples were tested by MAT and PCR. PCR-positive samples underwent 7-locus MLST, serovar-specific PCR, and high-resolution AmpSeq (42 genes, ~10,000 bp); isolates from cattle and wild boars were whole-genome sequenced. The highest seroprevalence was found in wild boars (20–43%), with high titers and PCR confirming actual infection with L. interrogans serovar Pomona. MLST identified ST52 in all typed samples from cattle and wildlife. AmpSeq and WGS confirmed complete identity of ST52 profiles across samples from 2011 to 2024. Notably, archival DNA from a 2011 outbreak related to imported cattle—among the first Pomona cases in Israel—matched all later samples, providing genomic evidence of a single introduction event. These findings identify wild boars as key reservoirs and demonstrate the establishment of a single Leptospira Pomona clone in Israel following introduction. Continued surveillance and coordinated response across the wildlife–livestock–human interface are essential.

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Usefulness of Metagenome-Assembled Genomes (MAGs) for the study of pathogen–microbiota interaction in swine dysetenry model

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This study aims to deepen the mapping of bacteria that may participate in infectious processes as pathobionts, using information obtained from short-read sequencing and the subsequent assembly of genomes from these reads, a method known as metagenome-assembled genomes (MAGs) in an experimental infection model of swine dysentery by Brachyspira hyodysenteriae. Shotgun metagenomic sequencing with Illumina NovaSeq technologywas performed in 112 samples which enabled the reconstruction of 3,735 MAGs. These MAGS were filter by quality and GTDB database annotation, identifying 576 MAGs at the species level. We further explored MAGs from species of interest based on metataxonomic analyses Prevotella pectinovora (9 MAGs), Acetivibrio ethanolgingens (7 MAGs), and Campylobacter hyointestinalis (4 MAGs) Roseburia inulinivorans (1 MAG), all exclusively reconstructed from samples of animals affected by the disease, and in all cases, at least one of the sequences was of high quality. No major virulence genes were detected in the MAGs of these species although several minor factors and resisntace genes were identified. In addition, we observed discrepancies in the taxonomic classification of the specie A. ethanolgignens which in the GTDB database is annotated as “Velocimicrobium ethanolgignens” based on gene markers. In conclusion, the study results reveal the usefulness and discriminatory power of metagenomic assemblies to characterise novel species in disease pathogenesis.

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Genetic Diversity and Lewis Antigen Status Shape Intestinal Mucus O-Glycome: A Network Perspective in TLR5-Deficient Pig Model

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Dissecting intestinal homeostasis requires understanding subtle interactions between host genetics, innate immunity, and the microbiota before overt disease occurs. Using genetically diverse pigs, we mapped O-glycan structures in intestinal mucus under non-induced conditions, integrating these with transcriptomic and microbiota data. Porous graphitized carbon LC-MS/MS identified ~140 distinct O-glycans across gut regions, revealing that Lewis antigen status—particularly $Le^{a/x}$ vs. $Le^{b/y}$—dominates glycan diversity, often outweighing TLR5 deficiency. Transcriptomic data showed Lewis antigen profiles and TLR5 functionality jointly shape glycosylation enzyme expression and immune signatures, indicating a bidirectional interplay between immune sensing and epithelial glycan remodeling. Microbiota changes were subtle but genotype-dependent, with specific taxa enriched in $Le^{a/x}$ animals. Combined analysis of FUT2/3 expression, a MUC13 SNP, and TLR5 deficiency revealed complex, location-specific microbial shifts, especially in the colon. These findings demonstrate that glycomics can be scaled to network-level resolution, underscoring the importance of host genetic variation in maintaining mucus barrier integrity and microbial balance, a key to preventing chronic gut disorders.

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Genomic and virulence insights of Western European Aeromonas salmonicida subsp. salmonicida and development of Galleria mellonella infection assay

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Aeromonas salmonicida subsp. salmonicida is the etiological agent of furunculosis, a fish disease highly aggressive for salmonids and responsible for significant economic losses in aquaculture worldwide. This study aimed to explore genomic and antimicrobial resistance traits of Western European A. salmonicida subsp. salmonicida strains and to develop an adapted Galleria mellonella infection model to assess the pathogenic potential of this psychrophilic subspecies. Three strains isolated from salmonids displaying symptoms of furunculosis were tested against a panel of antibiotics and sequenced to characterize their genome. Virulence was evaluated in G. mellonella larvae using bacterial doses ranging from 101 to 106 CFU/larva. Two isolates exhibited multidrug resistance to antibiotics commonly used against furunculosis. Although closely related to the reference strain A449, genomic analyses revealed multiple plasmids known to encode antibiotic resistance genes. Virulence assays demonstrated that this subspecies is lethal at doses as low as 101 CFU/larva, and that a fully functional Type III Secretion System (T3SS) is not essential for the infection of G. mellonella, likely due to the presence of other virulence factors in T3SS-deficient strains. These findings enhance the genomic characterization of European A. salmonicida subsp. salmonicida and validate the use of Galleria mellonella larvae as a relevant alternative infection model for studying this psychrophilic subspecies.

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PRELIMINARY METABOLOMICS DATA ON VIRUS INFECTION: THE CASE OF BOVINE CORONAVIRUS (BCoV)

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Objectives: The mechanisms regulating the interconnections between viruses and cell hosts are not yet fully understood and this is necessary for early diagnosis and an effective operational response to counter infections caused by emerging and/or re-emerging viruses. Metabolomics gives a comprehensive representation of metabolites providing further information on mechanisms involved in cell responses during infectious diseases. This study is focused on bovine coronavirus (BCoV), a betacoronavirus, like SARS-COV-2, causing enteric diarrhea in calves, winter dysentery, as well as Bovine Respiratory Disease. Hence, we developed an in vitro strategy, based on both virology and metabolomics techniques to provide insights into virus-host interactions. In addition, this strategy could be useful in the search for new antiviral compounds.
Methods: GC-MS, NMR, cytomorphological analysis, immunofluorescence assay.
Results: We developed a full strategy for the evaluation of intracellular metabolites to obtain an insight into the variations caused in bovine cells (MDBK) during BCoV (strain 282/23) infection. The dataset comprises over 50 metabolites belonging to different classes of natural products.
Conclusions: In conclusion, in this work we offer a snapshot of the physiological state of the cell before and after BCoV infection. Moreover, the workflow employed by us could be suitable to gather valuable information on the mechanism of action of potential antiviral candidates.

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Characterizing the Pathogenic Potential of Vibrio parahaemolyticus: Phenotypic and Genotypic Analysis of Biofilm Formation and Virulence Gene Expression in Clinical and Environmental Strains on Mussel Shells

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Vibrio parahaemolyticus is a major food-borne pathogen associated with contaminated seafood and capable of causing varying degrees of gastroenteritis in humans. Its pathogenicity is mediated by multiple virulence factors, including flagella and adhesion factors, and is further enhanced by its ability to form biofilms, increasing its resistance to environmental stress. However, the mechanisms underlying its pathogenicity remain incompletely understood.

The main objective of this study is to examine the biofilm formation ability and virulence gene profiles of *V. parahaemolyticus* isolates on mussel shells, comparing genotypic and phenotypic traits of clinical reference and environmental strains at 25℃, 30℃ and 37℃. A total of 25 strains were examined. Motility was assessed by swimming and swarming assays, while biofilm formation was determined by crystal violet staining. The presence of 32 associated genes was analyzed using real-time qPCR.

While the reference strain *V. parahaemolyticus* RIMD 2210633 contained all target genes, some strains lacked key adhesion genes. Swarming motility appeared inversely regulated with biofilm formation. Gene expression analyses following biofilm formation on mussel shells will be conducted to further elucidate regulatory mechanisms.

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PSEUDOMONAS AERUGINOSA: ONE HEALTH APPROACH TO DECIPHERING HIDDEN RELATIONSHIPS IN NORTHERN PORTUGAL

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This study aimed to investigate antimicrobial resistance, the presence of virulence genes, and the genetic diversity of Pseudomonas aeruginosa isolates from various sources within a One Health framework. Antimicrobial resistance in P. aeruginosa represents a significant global challenge for public and veterinary health. A total of 737 P. aeruginosa isolates were collected from humans, domestic animals, and aquatic environments in northern Portugal. Antimicrobial resistance profiles were assessed through susceptibility testing, while genomic analysis was used to detect resistance and virulence genes and to perform multilocus sequence typing. The results revealed a high prevalence of multidrug-resistant isolates, including high-risk clones such as ST244 and ST446, particularly in hospital sources and wastewater treatment plants. Key genes associated with resistance and virulence, including efflux pumps (MexA and MexB) and secretion systems (T3SS and T6SS), were identified. This work highlights the complex dynamics of multidrug-resistant P. aeruginosa across interconnected ecosystems in northern Portugal, emphasizing the importance of genomic studies in understanding resistance and virulence mechanisms. It contributes to a broader understanding of resistance dynamics and supports the development of future mitigation strategies.

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Multidrug-resistant ESBL-producing Klebsiella pneumoniae isolated from healthy meat rabbits: a potential public health concern

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Klebsiella pneumoniae is a multidrug-resistant pathogen increasingly found in animals, but data on meat rabbits are limited. This study isolated and characterized strains from healthy rabbits in Portugal, assessing resistance and genetic lineages.A total of 295 fecal samples were collected from healthy rabbits across 20 commercial farms in northern Portugal. Bacterial isolation was performed using selective culture media and confirmation via MALDI-TOF MS. Antimicrobial susceptibility testing was conducted. Whole-genome sequencing was carried out on all isolates to identify resistance genes, plasmid replicons, virulence determinants, and sequence types.

Six (2%) *K. pneumoniae* isolates were recovered, displaying a high level of multidrug resistance. All isolates harbored multiple antibiotic resistance genes, including *blaCTX-M-15, blaSHV* variants, *tet*(A), *sul1/sul2*, *fosA*, and various aminoglycoside-modifying enzymes. Plasmid analysis revealed the presence of IncFII, IncN, and IncR types. Efflux pump genes (*acrA, acrB, mdtB, mdtC)*, metal and biocide resistance determinants were also detected. MLST analysis identified diverse lineages including ST307, ST45, ST193, and ST2026.

The presence of multidrug-resistant ESBL-producing *K. pneumoniae* in healthy meat rabbits suggests a potential reservoir of resistance and virulence genes. These results reinforce the need for surveillance in rabbit production to mitigate public health risks within a One Health framework.

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Antimicrobial Resistance of Staphylococcus aureus in Rabbits for Consumption: Food Safety and Public Health Implications

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Staphylococcus aureus, a bacterium colonizing humans and animals, causes diverse infections across hosts. Antibiotic resistant strains in food animals, notably rabbits, are a food safety and public health concern. This study investigated methicillin sensitive (MSSA) and methicillin resistant (MRSA) *S. aureus* in rabbits for human consumption and their resistance profiles.

A total of 65 samples from northern Portuguese farmed rabbits were analyzed. Eight MRSA isolates were randomly selected for susceptibility testing against 14 antimicrobials (penicillin, cephalosporins, fluoroquinolones, aminoglycosides, macrolides, tetracyclines, oxazolidinones, and others) via agar disc diffusion (EUCAST/CLSI guidelines).

MRSA prevalence was 16.92%, and MSSA was 9.23%. All isolates resisted penicillin and ciprofloxacin. No resistance to linezolid or chloramphenicol was detected. Resistance to gentamicin was 37.5%; cefoxitin, tobramycin, kanamycin, clindamycin, fusidic acid, and mupirocin were 25%; and erythromycin, tetracycline, and trimethoprim-sulfamethoxazole were 12.5%. Multidrug resistance was observed in 25% of MRSA isolates.

The findings highlight the prevalence and antimicrobial resistance of S. aureus in rabbits for consumption, signifying potential food safety and public health risks. Mitigating *S. aureus* contamination in rabbit meat is crucial for targeted interventions to limit resistance dissemination and protect the food chain.

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Occurrence and antimicrobial susceptibility of Gram-negative bacteria isolated from bovine mastitis cases: evidence from three dairy farms in Serbia

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Bovine mastitis remains one of the most significant diseases in the dairy industry, with Gram-negative bacteria increasingly implicated as causative agents and antimicrobial resistance complicating effective treatment. This study aimed to determine the occurrence of Gram-negative bacteria in clinical and subclinical cases of bovine mastitis on dairy farms in Serbia and to assess their antimicrobial susceptibility patterns. Milk samples from three Holstein-Friesian farms, collected between May and July 2022, were analyzed using standard microbiological methods and antimicrobial susceptibility was assessed following CLSI disk diffusion standards. Out of 90 milk samples, bacterial pathogens were isolated in 69 (76.66%). Gram-negative bacteria were identified in 22 samples (24.44%), including *Escherichia coli* (17.77%), *Klebsiella oxytoca* (2.22%), *Enterobacter sakazakii* (2.22%), *Proteus mirabilis* (1.11%), and *Serratia marcescens* (1.11%). All isolates showed 100% resistance to ampicillin, erythromycin, lincomycin, penicillin, novobiocin, and cloxacillin. High resistance was also observed to amoxicillin (85%) and amoxicillin-clavulanic acid (70%), while all isolates remained susceptible to enrofloxacin, gentamicin, streptomycin, and trimethoprim-sulfamethoxazole. These results highlight the growing concern of antimicrobial resistance in bovine mastitis and emphasize the importance of routine bacteriological testing and susceptibility profiling in guiding effective therapy.

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Development of combination therapy against biofilm-forming uropathogenic E. coli from companion animals

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Uropathogenic Escherichia coli (UPEC) are the principal cause of recurrent urinary tract infections in dogs and cats. In some cases, multi-drug resistance and biofilm formation can complicate treatment. This study aimed to assess the efficacy of bacteriophage and antimicrobial peptide (AMP) combinations against biofilm-forming UPEC from dogs and cats. Newly discovered AMPs produced by Staphylococcus felis C4 were evaluated for antibacterial activity against multiple strains of UPEC. Four lytic bacteriophages targeting diverse UPEC strains from dogs, cats, and humans were combined into phage cocktails. A checkerboard assay was employed to determine the dose-dependent effects of phage cocktails, S. felis C4 AMPs, and their combination against key canine and feline UPEC strains. Additionally, a standard crystal violet assay was used to assess UPEC isolates' ability to form biofilm in rich media and urine. To better characterize biofilm development, an in vitro assay was developed to model UPEC biofilm formation on urinary catheters in rich media and urine. There was strain-specific variation in the degree of biofilm formation by UPEC in canine and feline urine. The phage cocktail and AMPs suppressed UPEC growth independently and demonstrated synergistic, dose-dependent suppression of UPEC growth. Preliminary findings suggest potential synergistic inhibitory effects of bacteriophage and AMPs on urinary catheter-associated biofilms.

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ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS FROM CROTON MUBANGO, TEPHROSIA VOGELII, IPOMOEA BATATAS, AND PRUNUS PERSICA AGAINST STAPHYLOCOCCAL ISOLATES FROM DOGS.

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In Africa, plant extracts are used to treat human and animal ailments. We tested the antimicrobial activity of Croton mubango, Tephrosia vogelii, Ipomoea batatas and Prunus persica from Huambo (Angola) against 56 staphylococci from dogs. After producing aqueous concentrated extracts from leaves, activity was tested by spot-on-lawn on Mueller-Hinton agar, using 10 µL of extract on bacterial cultures (10^8 CFU/mL). Inhibition was checked after 24, 48 and 72 h at 37 ℃. Staphylococcus aureus ATCC 25923 and Ampicillin were used as controls. Results were analysed through a generalized linear mixed model (PROC GLIMMIX). Differences with $p \leq 0.05$ were significant. After 24 h, bacterial inhibition ranged from 4.8% for T. vogelii to 97% for P. persica. C. mubango and I. batatas showed 20.8% and 23.8% inhibition. At 48 h, inhibition increased to 6.8%, 27.4%, 23.8% and 98.8% for T. vogelii, C. mubango, I. batatas and P. persica. After 72 h, inhibition reached 8.9% for T. vogelii, 29.2% for C. mubango, 32.1% for I. batatas and 100% for P. persica. Statistical analysis revealed significant differences between 24 h and 48 h ($p=0.006$). P. persica showed the highest antimicrobial activity, being significantly more effective than C. mubango ($p=0.038$), I. batatas ($p=0.044$) and T. vogelii ($p=0.022$). Antimicrobial action increased up to 48 h and then stabilised. P. persica showed greater efficacy, standing out as promising natural alternatives for controlling staphylococcal infections in dogs.

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Antimicrobial resistance in gut commensals, a potential AMR reservoir and its link to antimcirobial use

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The promotion of antimicrobial resistance (AMR) by use of antimicrobials (AMU) in commensal bacteria is poorly studied, even more so in livestock, where they can act as potential reservoirs and may compromise the effectiveness of veterinary treatments through horizontal gene transfer (HGT) of these resistances. Our study aims to analyze the dynamics of AMR in pig farming, focusing on the presence of resistance to broad-spectrum antibiotics classified as critically important antibiotics (CIAs) in gut commensal bacteria.
Samples were collected from three local production farms two with frequent AMU and one with low AMU. For general isolation of resistant commensal LAB, Brain Heart Infusion broth enriched with 0.5% yeast extract and supplemented with cefotaxime (2 mg/L) was used, with incubation for 24–48 hours at 37℃ under anaerobic conditions.
A total of 148 cefotaxime-resistant isolates were obtained. Of these, 82 (55.41%) belonged to the lactobacilli group, including different species in this group. Additionally, 41 isolates (27.70%) were from 9 species of the genus Enterococcus; 11 isolates (7.43%) were from one species of the genus Megasphaera; 8 isolates (5.41%) were from 4 species of the genus Streptococcus; and 6 isolates (4.05%) were from the genus Pediococcus.
The next step is to thoroughly characterize the genetic determinants of these resistances, their interaction with the microbiota, and their possible association with the antimicrobial use.

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Monitoring of antimicrobial resistance in clinical isolates from bovine mastitis in Denmark, 2024

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On initiative of the Danish Veterinary and Food Administration, a new monitoring of antimicrobial resistance in clinical cattle mastitis started in 2024. 487 milk samples were obtained from 15 participating veterinary practices and examined by culture methods at Statens Serum Institut. Clinically-relevant colonies were sub-cultured and identified with MALDI-TOF. Selected pathogens were tested for antimicrobial susceptibility using broth microdilution, and MIC results were interpreted using ECOFFs or clinical breakpoints when ECOFFs were unavailable.
Among the 487 samples, 402 (83%) were found positive for one or more pathogens, while the remaining 85 samples (17%) were interpreted as negative, because of lack of growth or unspecific growth.
In total, 462 isolates were obtained, including 397 (86%) relevant mastitis pathogens such as *Streptococcus uberis* (27%), *Escherichia coli* (20%), *Staphylococcus aureus* (13%), *Streptococcus dysgalactiae* (11%), coagulase-negative staphylococci (5%), *Trueperella pyogenes* (3%), *Streptococcus agalactiae* (3%), and *Klebsiella pneumoniae* (3%). Additionally, other bacterial species and yeasts were identified in less than 1% of the samples. Susceptibility testing of 376 isolates was performed.
Overall, the resistance levels were low although reduced susceptibility to penicillin was observed in 20% of the *S. uberis* isolates. A single ESBL-producing *E. coli* was identified,while resistance to colistin or carbapenems was not found.

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Use of a visual simulation tool - Veterinary Infection Prevention through Visualisation (VIPVis), to minimise environmental contamination throughout a veterinary practice

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Innovative interventions are required to tackle antimicrobial resistance (AMR), a critical One Health issue. A pilot study was undertaken to assess whether using the Veterinary Infection Prevention through Visualisation (VIPVis) simulation app could aid in lowering bacterial burden in a veterinary practice. VIPVis visually simulates contextual interactions between humans, animals, and pathogens in the veterinary environment. To determine the impact of using VIPVis, swabs (n=28 per time point) were taken from different surfaces in rooms throughout the veterinary practice, before and after introducing VIPVis to staff. Swabs were plated onto solid growth media to detect five clinically relevant bacterial species and evaluate overall contamination throughout the practice. Representative isolates were tested biochemically to establish a presumptive identity. The pharmacy and preparation room experienced significant ($P<0.05$) reductions in bacterial numbers (CFU/mL) following the use of VIPVis by staff. Additionally, the most commonly isolated organism was *S. pseudintermedius*, which was predominantly found on the pharmacy door, stethoscope, keyboard, and prep mat. Molecular analysis is ongoing to confirm the presumptive identity of these isolates and investigate the spread of these organisms throughout the veterinary practice. The pilot study has shown potential for the VIPVis app to contribute to reductions in bacterial contamination in the veterinary practice environment.

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Resistance and virulence profiles of Escherichia coli from Bubulcus ibis, Ciconia ciconia and Erinaceus europaeus from a wildlife recovery center

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Antimicrobial resistance (AMR) is a serious threat to public health, with wildlife being a potential reservoir and disseminator of resistant and virulent bacteria.
We characterized the phenotypic resistance and virulence profiles of fecal Escherichia coli isolates from Ciconia ciconia, Bubulcus ibis and Erinaceus europaeus recovering at Wildlife Recovery and Research Centre (RIAS), Portugal. The Multiple Antibiotic Resistance (MAR) and Virulence (Vir) indexes were determined for all isolates.
It was possible to obtain 79 isolates, 75 of which were selected for characterization. Approximately 64% were resistant to at least one antibiotic and 8% were classified as multidrug-resistant (MDR). Resistance to ampicillin (36%), tetracycline (12%) and chloramphenicol (8%) were observed. All isolates were susceptible to meropenem, aztreonam and third-generation cephalosporins, and most did not express virulence factors (81%). Only 11% produced proteases, 7% formed biofilm, and 4% exhibited beta-haemolysis. The mean MAR index was 0.09 and the mean Vir index 0.04. No significant association was observed between biofilm production, the MAR index, and the isolates MDR profile ($p>0.05$); between biofilm production and the other virulence factors ($p>0.05$); and between the MAR and Vir indexes ($p=0.48$).
The presence of resistant and virulent E. coli in the wildlife of southern Portugal highlights the importance of AMR monitoring and of the One Health approach to mitigate associated risks.

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Detection and Characterization of Acinetobacter Species isolated from fecal material of pet Lizards

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Lizards are increasingly popular exotic pets, leading to greater human-reptile contact and zoonotic concerns. This study aimed to evaluate the One Health risk posed by *Acinetobacter* spp. in lizards, focusing on potential zoonotic transmission. Fecal samples from 35 lizards were collected from exotic pet shops in Hong Kong (n=11) and reptile breeders in Mainland China (n=24). Samples were cultured on CHROMagar with and without meropenem. Bacterial identification was done by MALDI-TOF. Whole genome sequencing (WGS) was performed by Nanopore technology. *Acinetobacter* spp. were isolated from 17 samples: *A. baumannii*(4), *A. pittii*(5), *A. bereziniae*(3), *A. radioresistens*(2) and *A. nosocomialis*(3). One *A. baumannii* isolate showed phenotypic carbapenem resistance and harboured *blaOXA*-180. WGS revealed diverse *blaOXA*-like genes, and chromosomal *blaADC*-25 was present in all except in *A. bereziniae* and *A. radioresistens*. The tetracycline resistance gene *tet*(39) was found on non-mobilizable plasmids in two *A. baumannii* strains. Virulence genes related to biofilm formation, adherence, iron acquisition, toxin secretion, and immune evasion were common in *A. baumannii* complex strains, supporting pathogenic potential. *A. radioresistens* and *A. bereziniae* carried fewer resistance and virulence genes. These findings highlight the need for proper hygienic precautions and monitoring pet lizards as part of One Health approach.[1]: https://tinyurl.com/crkrus6d

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Aeromonas spp. as candidate indicator for antimicrobial resistance surveillance in Belgian aquaculture.

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In Belgium, aquaculture is primarily semi-intensive, with salmonids raised in river-fed ponds. The absence of authorized fish-specific vaccines or antibiotics leads to the use of antimicrobials approved for other animal species. Unlike other food-producing animals, aquaculture lacks systematic monitoring of antimicrobial resistance (AMR). This study aimed to evaluate Aeromonas spp. as a potential AMR surveillance indicator by isolating them from fish skin mucus, water, and sediment across 22 Belgian fish farms during winter. Mucus swabs from fish skin (n = 88), water samples (n = 88), and sediment (n = 29) were cultured on Glutamate Starch Phenol-red (GSP) Agar and Columbia Blood Agar at 18℃ and 30℃. Yellow GSP colonies were subcultured and stored. One colony per sample type per farm (total n = 58) from GSP at 18℃ was selected for identification using API® 20 NE. Among 35 isolates identified to date: Aeromonas hydrophila/caviae (n = 19), Aeromonas sobria (n = 2), Vibrio alginolyticus (n = 3), Mannheimia haemolytica (n = 4), Brevundimonas vesicularis (n = 2), Burkholderia cepacia (n = 2), Pseudomonas putida (n = 1), Pseudomonas fluorescens (n = 1), and Ralstonia pickettii (n = 1) were found. Further identification using MALDI-TOF MS is ongoing to refine isolate classification and determine the most suitable sample type for Aeromonas recovery. Confirmed Aeromonas isolates will undergo susceptibility testing and whole-genome sequencing to assess AMR profiles.

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From Farm to Glass: A Cross-Sectional Study of Antibiotic-Resistant Bacteria in Raw Milk in Israel

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Antibiotic-resistant bacteria (ARB) in food-producing animals are a public health concern. This cross-sectional country-wide study investigated key resistance indicators—methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE), and vancomycin-resistant enterococci (VRE)—in raw bulk milk from cattle (n=70) and small ruminant farms (n=20) in Israel, and examined associations with farm-level factors. Tanks were sampled in triplicate to assess consistency. Raw milk samples were enriched in selective broths and plated on selective media for each target ARB. Colonies were identified by MALDI-TOF MS. Resistance phenotypes were confirmed by disk diffusion and profiled by MIC; resistance genes were detected by PCR. ESBL-PE were confirmed in 25% of farms, mainly E. coli (51%) and Klebsiella pneumoniae (44%) carrying CTX-M and TEM genes; all multidrug-resistant. VRE, initially detected in 80% of farms by selective culture, were unconfirmed, raising concerns about reliance on selective media alone. MRSA was not detected. Higher herd turnover and total tank bacterial count, avoiding milk powder, and use of preventive antibiotics for calf diarrhea were associated with increased odds for ESBL-PE detection. Though pasteurization reduces bacterial load, resistance genes may persist and transfer to gut microbiota. Further research is warranted to investigate the impact of these findings.

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Genetic relationship and antimicrobial resistance among Staphylococcus aureus CC1 and CC1660 from humans and horses

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The aim of this study was to investigate horse-associated *Staphylococcus aureus* of the clonal complexes (CCs) CC1 and CC1660 for their genetic relationships and antimicrobial resistance properties. In total, 91 *S. aureus* isolates (64 human, 27 equine) were investigated by whole-genome sequencing (WGS), multilocus sequence typing (MLST), core genome MLST (cgMLST), *spa* typing and for the antimicrobial resistance and virulence properties. Antimicrobial susceptibility testing was performed for 31 antimicrobials. WGS confirmed 75 CC1 isolates, including nine STs and 16 CC1660 isolates with four STs. The difference between the CCs was 1398/1492 alleles with differences of 0 to 288 alleles in CC1 and 0 to 249 in CC1660. The isolates of CC1 and CC1660 belonged to ten and five *spa* types. Antimicrobial susceptibility testing identified 17 pheno- and 19 genotypic patterns. Penicillin resistance (*blaZ*) was present in 72 isolates and ten were oxacillin-resistant (*mecA* n=7). Resistance to gentamicin (n=32, *aacA-aphD*), tetracycline (n=30, *tet*(L)/*tet*(K)) and neomycin (n=30, *aadD* or *aphA3*) was quite common. The equine leucocidin genes *lukP/Q* were found in 22 equine and 38 human isolates. No Panton-Valentine leucocidin genes *lukF-PV* and *lukS-PV* were found. Three human CC1660 isolates carried the toxic shock syndrome toxin-1 gene *tst*. In conclusion, the investigation of the 91 isolates by cgMLST revealed two clearly separated CCs and various resistance patterns.

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Investigation of Staphylococcus aureus CC1 and CC1660 for their biocide susceptibility

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The aim of the study was to investigate *Staphylococcus aureus* of the clonal complexes (CCs) CC1 (n=75) and CC1660 (n=16) for their phenotypic biocide susceptibility and the respective resistance genes and mechanisms. Susceptibility testing by broth microdilution for benzalkonium chloride (BAC), octenidine, polyhexanide and chlorhexidine was performed for the 91 *S. aureus* isolates. The presence of *qac* genes was confirmed by WGS analysis and increased efflux activity was detected indirectly by the evaluation of the fluorescence emitted by ethidium bromide accumulated inside the cells, through cartwheel assays and for eight selected isolates by real time fluorometry. For BAC, minimum inhibitory concentrations (MICs) of 0.000125% (n= 50), 0.00025% (n=17) and 0.0005% (n=24) were detected. Among the isolates with MICs of 0.0005%, the gene *qacA* was present in 19 and *qacC* in five isolates, including one *qacC* with a S99L mutation. Unimodal distributions were observed for the remaining biocides. The efflux activity of the *qac*-positive isolates was confirmed via real-time fluorometry for the selected isolates. The isolate with the *qacC* mutation S99L showed compared to the other *qacC*-positive isolates a lower level of efflux activity by both methods. The efflux activity of *qac*-positive *S. aureus* isolates can decrease their susceptibility to BAC. To further investigate this effect, susceptibility testing for BAC in the presence of efflux inhibitors will be performed.

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Emergence of Plasmid-Mediated Colistin Resistance in Wastewater ESBL-Producing E. coli Isolates in Thessaloniki- Greece

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Antimicrobial resistance (AMR) is a growing global threat, with Greece among the highest antibiotic consumers in Europe. The overuse of β-lactam antibiotics has contributed to the emergence of resistant bacteria. Wastewater serves as a reservoir and potential transmission route for AMR organisms.
This study investigates the presence and phenotypic and genotypic characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-Ec) in wastewater from three treatment plants in Thessaloniki—two urban and one industrial—sampled monthly over a 2-year period (2023–2024). In total, 80 ESBL-Ec strains were isolated before treatment and 20 after. Isolates were analyzed for antimicrobial susceptibility, resistance genes, biofilm formation, and phylogenetic grouping. Urban wastewater strains frequently carried *bla*_{CTX-M-1} and *bla*_{TEM} group genes and belonged to phylogroup B2, while industrial strains showed higher resistance, increased prevalence of *bla*_{OXA}, and were mostly classified as B1. The *mcr-2*, *mcr-3*, and *mcr-4* genes, conferring colistin resistance, were detected in 24 plasmid-bearing isolates, six of which were recovered post-treatment. These findings highlight the persistence of multidrug-resistant bacteria in treated effluents and underline the importance of environmental surveillance. A One Health approach is essential for managing AMR risks across human, animal, and environmental interfaces.

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Recommendation of a method for harmonized antimicrobial susceptibility testing of Mycoplasma ('Mycoplasmoides') gallisepticum using broth microdilution

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As there is currently no standardized method for antimicrobial susceptibility testing of *Mycoplasma (Mycoplasmoides) gallisepticum*, this study aimed at developing a harmonized broth microdilution susceptibility testing method. Sixteen *M. gallisepticum* isolates and the type strain were collected. Growth experiments using four different media showed that only in SP4 broth all isolates showed visible growth after three days of incubation at 37℃ and had high CFU/ml counts. After incubation times of 72, 96 and 168h, a good readability of MICs was observed and essential and exact MIC agreements ranged between 92-100% and 68-100%, respectively. However,72±2h appeared to be the most suitable time for reading microtiter plates. Homogeneous MIC values were also achieved with media components from different manufacturers. The MIC values of three CLSI-approved quality control (QC) strains were within the official ranges for twelve of the tested 24 antimicrobial agents. In addition, *M. bovis* DSM22781T MICs were homogeneous under the developed test conditions, indicating suitability as QC strain. Field isolates were tested and showed elevated MIC values for fluoroquinolones, macrolides and aminoglycosides. For harmonized broth microdilution susceptibility testing of *M. gallisepticum*, SP4 broth with an incubation period of 72±2h proved to be suitable. CLSI-approved QC strains can be used temporarily, but further validation of type strain DSM22781T as a new QC strain is recommended.

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In vitro antimicrobial susceptibility testing of Bordetella avium: Comparison of results obtained with the harmonized broth microdilution method and disk diffusion testing

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A method for standardized broth microdilution susceptibility testing for *B. avium* was recently developed. The present study aimed to determine whether the disk diffusion method is also suitable for susceptibility testing of the pathogen. *B. avium* type strain ATCC35086T and 48 field isolates were used. After obtaining MIC values for these isolates, zone diameters were determined for 22 antimicrobial agents, using the disk diffusion method according to CLSI performance standards for bacteria isolated from animals. As there are currently no approved breakpoints, it was not possible to classify the isolates as resistant or susceptible. However, the results showed a good correlation between measured zone diameters and MICs. Five isolates with MICs of ≥64 µg/ml tetracycline and harboring the resistance gene *tet*(A) showed small zone diameters of ≤12 mm diameter. In addition, a small zone diameter of 12 mm was observed for an isolate with florfenicol MIC of 32 µg/ml and carrying the resistance gene *floR*. However, deviations in the results were observed for some antimicrobial agents (e.g. penicillin, tiamulin, trimethoprim/sulfamethoxazole). In some cases, isolates with the same zone diameter showed differences in MICs of up to 5 dilution steps. Overall, the results revealed a good correlation between zone diameters and MICs. Thus, the agar diffusion method appears to be generally suitable for B. avium, although the reasons for occasional deviations still need to be clarified.

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Urinary Tract Infections in Dogs- a Survey on occurring Bacteria Species and on Resistency Patterns

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Urinary tract infections (UTI) are common in dogs. Optimization of antibiotic treatment is a crucial therapeutic goal for which ISCAID guidelines are routinely used.

Materials and methods

Results of bacteriological analyses from 2021 and 2023 were compared retrospectively. All analyses included culture, identity test (MALDI TOF, Bruker, Germany) and antimicrobial susceptibility tests (AST; break point method, Sifin, Germany) according to CLSI guidelines.

Results

A total of 2111 and 5429 samples were evaluated in 2021 and 2023 respectively. Approx. 60% of samples showed pathogenic growth (1543 in 2021, 4926 in 2023), with Escherichia (E.). coli being the most common isolate (37.9% and 31.9%), followed by Staphyloccus (S.) pseudintermedius (15.0% and 14.5%).

In 2021, E. coli (n=585) showed the most resistance to amoxicillin (26.7%), cephalexin (15.7%) and amoxicillin/clavulanic acid (11.7%). S. pseudintermedius (n=230) showed resistance to amoxicillin in 67.6% of the cases, followed by chloramphenicol (15.1%) and trimethoprim/sulfamethoxazole (9.3%). Resistance profiles were similar in 2023.

Discussion

ISCAID guidelines recommend amoxicillin and trimethorim/sulfamethoxazole as first-line treatment in canine UTI. Our data support the use of trimethoprim/sulfamethoxazole over amoxicillin, indicating that guidelines should be modified regionally. There were no significant differences between the years, indicating relative stability of resistance profiles over time.

Antimictobial Resistance / 97

Identification of the novel multiresistance transposon Tn7731 in bovine Pasteurella multocida from Germany

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Mobile genetic elements are commonly involved in the dissemination of antimicrobial resistance (AMR) in *Pasteurella multocida*. Here, we characterized a novel multiresistance transposon among 179 bovine *P. multocida* obtained in the German resistance monitoring GERM-Vet 2010-2022. These isolates were subjected to whole-genome sequencing via Illumina MiSeq and Oxford Nanopore MinION. Hybrid assembly followed by sequence analysis revealed that isolate 200011 carried two copies of a novel transposon, designated Tn7731. Tn7731 was 9,701 bp in size and harbored the IS10 insertion sequences IS10L and IS10R in opposite orientations at its termini. Moreover, Tn7731 carried the four complete AMR genes *sul2* (sulfonamides), *catA3* (chloramphenicol), *strA* (streptomycin) and *tet*(B) (tetracycline), as well as a truncated *strB* gene (streptomycin). A 13,216-bp transposon-like element from a bovine respiratory disease-associated *Gallibacterium anatis* strain closely resembled Tn7731 in the initial 3,172 bp and in the final part 3,837–9,701 bp. Between these sections, *catA3* in Tn7731 replaced the AMR gene cluster *floR-IS15DII-aadB-aphA1* present in *G. anatis*. Two recombination sites were identified that might have served for this replacement. The identification of chromosomally located multiresistance transposons, such as Tn7731, in bovine respiratory tract pathogens might limit therapeutic options in the control of economically highly relevant respiratory diseases in cattle.

Antimictobial Resistance / 98

Identification of two novel phenicol resistance plasmids in bovine Pasteurella multocida from Germany

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Antimicrobial resistance (AMR) in *Pasteurella multocida* is of growing concern and plasmids have been identified to be involved in the dissemination of AMR genes in this organism. In this study, we characterized two novel phenicol resistance plasmids among 179 bovine *P. multocida* from the German national resistance monitoring GERM-Vet 2010-2022 which were subjected to whole-genome sequencing on Oxford Nanopore MinION and Illumina MiSeq platforms, followed by hybrid assembly and sequence analysis. Plasmid DNA was extracted and visualized by gel electrophoresis. Antimicrobial susceptibility testing was performed according to CLSI recommendations. The two novel phenicol resistance plasmids, designated pHKH170411 (15,822 bp) and pHKH190067 (5,727 bp), respectively, carried the *floR* gene, coding for a phenicol exporter. The novel plasmids shared similarities with previously described plasmids from *P. multocida* and *Bibersteinia trehalosi* associated with bovine respiratory disease (BRD), highlighting the importance of interplasmid recombination processes possibly facilitating the spread of AMR determinants. Although recent data from GERM-Vet indicate a comparatively low and stable florfenicol resistance rate of < 5% for *P. multocida* from cattle in Germany, our findings highlight the risk of limited therapeutic options in the control of BRD due to the further dissemination of phenicol resistance via horizontal gene transfer of mobile genetic elements, such as plasmids.

Antimictobial Resistance / 105

Recommendation of a method for harmonized antimicrobial susceptibility testing of Mycoplasma (‘Mycoplasmopsis‘) bovis via bouillon microdilution

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Currently, there is no method for standardized antimicrobial susceptibility testing (AST) of *Mycoplasma bovis*, an important bovine pathogen. Therefore, this study aimed at developing a suitable method for harmonized broth microdilution susceptibility testing of *M. bovis*.
For this study, a total of 120 *M. bovis* isolates were collected. Macrorestriction analyses and cluster analyses revealed a low clonality of isolates, which allowed the selection of epidemiologically unrelated test isolates. Growth experiments with 5 media were performed, but only the CLSI-approved SP4 broth enabled visible growth and sufficient cfu/ml of *M. bovis*. Susceptibility testing revealed easily readable MICs after 72 h incubation at 37 °C. At this time point, essential MIC agreements between 92-100 % were observed for all 16 tested antimicrobials, exceeding the requirement of ≥90% for a new AST method and indicating good homogeneity of MICs. Testing of the remaining field isolates revealed high MIC90 values for tilmicosin (≥256 µg/ml) and tulathromycin (≥64 µg/ml), while single isolates showed elevated MICs of fluoroquinolones (16-≥32 µg/ml, n=1) or gentamicin (128 µg/ml, n=2). While the MICs of three CLSI-approved QC strains partially deviated from their official QC ranges, *M. bovis* DSM 22781T fulfilled the requirements for a new QC strain, as shown after 20-fold repeated tests. In summary, the SP4 broth seems to be suitable for harmonized AST of *M. bovis* using an incubation time of 72 h.

Antimictobial Resistance / 107

Molecular characterization of ESBL-producing Klebsiella pneumoniae isolates from dogs in Cape Verde and São Tomé and Príncipe

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The dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae is an emerging concern in the One Health context, mostly in low-resource settings where surveillance is limited. This study aimed to assess the molecular epidemiology of ESBL-producing *Klebsiella pneumoniae* isolated from rectal swabs of dogs from Cape Verde and São Tomé and Príncipe. ESBL production was confirmed phenotypically, and the presence of blaCTX-M, blaTEM, and blaSHV genes was investigated by PCR. ERIC-PCR fingerprinting was performed to evaluate the genetic relatedness among isolates. blaCTX-M, blaSHV, and blaTEM were detected in 96.9%, 65.6%, and 56.3% of the 32 ESBL-producing *K. pneumoniae* isolates, respectively. Most of the isolates harbored two or more resistance genes, with the most frequent pattern being the co-occurrence of all three genes (40.6%). All blaCTX-M-positive isolates belonged to group 1, which includes CTX-M-15—one of the most widespread ESBLs. ERIC-PCR analysis revealed seven clusters with varying degrees of similarity, suggesting both clonal expansion and geographic dissemination. These findings underscore the importance of molecular surveillance tools in tracking the dissemination of multidrug-resistant *K. pneumoniae* in animal populations. The detection of CTX-M group 1 enzymes in all positive isolates further highlights the circulation of globally relevant ESBL variants in data-scarce regions, reinforcing the need for integrated One Health approaches.

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Human Health to Animal Health: Interim Results of an Analytical Validation of Astratus Rapid Antimicrobial Susceptibility Testing for Gram-positive Pathogens

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Background
Fast, high-throughput, digital automated AST methods are urgently required for veterinary and human health. Astratus Limited, a University of Reading spinout, has developed a novel testing platform to meet this need. We previously validated this with gram-negative pathogens. Rapid gram-positive AST is especially critical in a One Health context. We present here for the first time an analytical validation with gram-positive bacteria.

Methods
Staphylococcus isolates collected from canine urine, pus and wounds (Batt Laboratories, UK) were tested alongside *Staphylococcus* plus *Enterococcus* isolates from human urine (Hampshire Hospitals, UK NHS). The Astratus rapid AST platform was benchmarked against reference microplate broth microdilution. The Astratus system tests up to 18 antibiotics simultaneously per isolate, with continuous growth monitoring.

Results
Interim results showed 95% concordance for gram-positive isolates with Ciprofloxacin, Nitrofurantoin and Amoxicillin-Clavulanate (138 antibiotic/isolate combinations). For *Staphylococcus* the average time to result was 3.4 h.

Conclusions
This interim data confirms rapid AST for gram-positive pathogens is feasible. The unique patented properties of our rapid AST technology enables cost-effective, rapid, and accurate AST for One Health. The scalable instrument configuration offers high throughput capacity in an innovative platform that can improve productivity, with digital results.

Antimictobial Resistance / 113

Circulation of carbapenemase producing Enterobacterales in the Portuguese veterinary healthcare

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Carbapenemase-producing Enterobacterales (CPE) are WHO-prioritized pathogens, requiring veterinary studies to assess their impact.
In this study, clinical CPE from companion animals (CA) (2020-2023) underwent WGS (Illumina Novaseq) for antimicrobial resistance/virulence/plasmid genotyping and in silico MLST. SNP distance and core genome phylogenies were constructed for species with >1 isolate.
Detected CPE included *Escherichia coli* (n=7, ST372 n=1, ST410 n=3, ST457 n=1 and ST4981 n=2) and *Klebsiella pneumoniae* (n=10, ST11=2, ST147 n=4, ST273 n=2, ST307 n=1 and ST382 n=1). Carbapenemase genes were blaOXA-181 (n=10), blaKPC-3 (n=5), blaOXA-244 (n=2) and blaNDM-5 (n=1). Four *E. coli* were pathotyped: 1 UPEC, 1 APEC, and 2 ETEC. All *K. pneumoniae* lacked hypervirulence-associated genes. Plasmid replicons associated with carbapenemase carriage were ColKP3 (blaOXA-181), IncFIA (blaKPC-3), IncN (blaKPC-3), IncX3 (blaOXA-181), and undetermined for 6 isolates. CPE clustered by ST, with 4 isolate pairs <10 SNPs apart. One pair originated from the same veterinary hospital within one month (different animals/infections).
CPE in CA primarily originated from human-associated nosocomial lineages. However, the UPEC ST372 isolate shows that veterinary lineages can also acquire carbapenemases. Closely related isolates suggest transmission routes involving veterinary healthcare units, highlighting the need for enhanced infection prevention and control practices in veterinary medicine.

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Extensively Drug-Resistant and Extended Spectrum β-Lactamase-Producing *Raoultella terrigena* from the reproductive tract of a mare

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Raoultella terrigena is a Gram-negative bacterium primarily isolated from environmental sources, with sporadic reports of isolation from clinical samples.
Raoultella terrigena was isolated from the uterine lavage of a thoroughbred mare. Antimicrobial susceptibility testing (broth microdilution) and whole genome sequencing was performed. Pan-genome analysis was used to compare this isolate with other *R. terrigena*.
Phenotypic resistance was detected to cefazolin, ceftiofur, cefotaxime, ticarcillin/clavulanic acid, tetracycline, doxycycline, gentamicin, sulfamethoxazole/trimethoprim and chloramphenicol. The genome included two plasmids (IncFII/rep_cluster_2078 and potentially conjugative IncQ1/IncU). Genes conferring resistance to aminoglycosides, fluoroquinolones, rifamycin, phenicols, cephalosporins, diaminopyrimidines, sulfonamides and tetracycline were carried by the IncQ1/IncU plasmid. Novel arrangements of resistance genes in two insertion sequence clusters/transposons and one integron gene cassette array within the IncQ1/IncU backbone were identified. Genes conferring resistance to antiseptics, biocides and heavy metals were also detected.
The detection of mobile genetic elements carrying multiple antimicrobial resistance genes in rare opportunistic pathogens, such as *R. terrigena*, highlights the importance of continuous monitoring of such bacteria and emphasizing the need for developing antimicrobial stewardship programs in veterinary settings.

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From Wild to Wards: Antimicrobial Resistance Profiles of Escherichia coli from Hospitalized Hedgehogs (Erinaceus europaeus)

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The European hedgehog (*Erinaceus europeaus*) is distributed across Europe, having adapted to anthropogenic environments, where it finds shelter, food, and few predators. Synanthropic habits likely promote the acquisition of antimicrobial resistance (AMR).
This study aimed to assess AMR and the effect of hospitalization in hedgehogs admitted to a wildlife rescue centre (WRC), using enteric *E. coli* as a marker.
E. coli isolates were obtained from rectal swabs collected upon admission of hedgehogs to a WRC (Turin University, IT) during 2022-23. A second swab was collected between five and 15 days after admission. Minimum inhibitory concentrations (MICs) of 19 antibiotics were interpreted using epidemiological cutoffs. MICs at admission and after hospitalization were compared using generalized linear mixed models.
At admission, hedgehogs carried *E. coli* resistant to several antibiotics, most commonly cefazolin (41.5%), ampicillin (37.7%), and enrofloxacin (22.6%). Hospitalization was associated with increased MICs of almost all antibiotics, leading to higher resistance to doxycycline, enrofloxacin, and trimethoprim-sulfamethoxazole. The proportion of hedgehogs carrying extended-spectrum beta-lactamase-producing *E. coli* rose from 5.7% to 20.8%.
We confirmed that wild hedgehogs carry AMR strains and that hospitalization at WRC increases the risk of harbouring them, supporting the hypothesis that hedgehogs can spread AMR and that WRCs may act as AMR amplifiers.

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Antibiotic Resistance Genes and Virulence Factors in Enterococcus spp. Isolated from Fecal Samples of Apparently Healthy Broilers at Farm Level

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Enterococcus spp. are common commensals in the gastrointestinal tract of poultry but may act as opportunistic pathogens. Their ability to acquire and disseminate antimicrobial resistance (AMR) genes poses a risk to animal and public health. Characterizing the resistome of Enterococcus in poultry is essential for surveillance and mitigation strategies. Fecal samples were collected from 40 apparently healthy broilers at a poultry farm in Portugal. Thirty-three Enterococcus spp. were isolated using conventional microbiological methods. Molecular characterization was performed by PCR to detect antibiotic resistance genes (vanA, vanB, erm(A), erm(B), erm(C), catA, vatE, vatD, tet(M), tet(O)) and virulence genes (gelE, cpd, fsr). All isolates were identified as E. faecalis. PCR detected tet(M) in 48.5% (n = 16) and tet(O) in 3% (n = 1). Among the virulence genes screened, only gelE was detected, being present in nearly all isolates (n = 32). The predominance of E. faecalis among fecal isolates highlights its persistence in healthy poultry. The presence of tet(M) suggests selective pressure for tetracycline resistance, while the high prevalence of gelE indicates the circulation of virulence traits. These results reinforce the importance of monitoring commensal E. faecalis within a One Health framework.

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Antibiotic Resistance and Virulence Markers in Enterococcus spp. Isolated from Bursitis Lesions in Broilers

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Sternal bursitis is an increasingly reported lesion in broiler chickens, yet its microbial aetiology remains underexplored. Enterococcus spp., known for intrinsic resistance and the ability to acquire virulence and resistance determinants, may contribute to such infections, with implications for animal and public health. This study aimed to characterise Enterococcus spp. isolated from sternal bursitis lesions in broilers intended for human consumption in Portugal, focusing on antimicrobial resistance and virulence/resistance genes. Forty-eight samples were collected from lesions of broilers on a single farm. Isolates were identified using conventional methods. Antimicrobial susceptibility was assessed by disk diffusion (CLSI). PCR was used to identify species and detect resistance genes (vanA, vanB, erm(A), erm(B), erm(C), catA, vatE, vatD, tet(M), tet(O)) and virulence genes (gelE, cpd, fsr). Forty-four isolates were recovered, all identified as E. faecalis. High resistance was seen to quinupristin–dalfopristin (100%), tetracycline (63.6%) and erythromycin (40.9%). One isolate was resistant to ciprofloxacin. tet(O) and tet(M) were detected in 36.4% and 29.5% of isolates, respectively. gelE was present in 97.7%. The exclusive presence of E. faecalis suggests a species-specific link to bursitis. Findings stress the need for surveillance of E. faecalis in poultry within a One Health framework.

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Development of a Method for Horizontal Gene Transfer into Avibacterium paragallinarum and Functional Characterization of the MdtK Exporter

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No established method currently enables broadly applicable horizontal gene transfer into *Avibacterium paragallinarum*, the causative agent of infectious coryza in poultry. This hampers functional analysis of genes. An example is the *mdtK* gene, which encodes a putative efflux transporter for antimicrobial compounds. The aim of this study was to develop a conjugative transfer system and to investigate the function of the MdtK transporter. MdtK was cloned into vector pUC19 (ampR, ori ColE1) and the broad-host-range vector pVZ322 (genR, kanR, ori RSF1010). pUC19:mdtK was transformed into chemically competent *E. coli* TOP10. To transfer pVZ322:*mdtK* into *A. paragallinarum*, a conjugation system was established. For this, *E. coli* SM10 λpir:pVZ322/mdtK was used as the donor strain, and two *mdtK*-negative *A. paragallinarum* strains, a field isolate and type strain ATCC 29545T, served as recipients. Selection of *A. paragallinarum* transconjugants were based on streptomycin resistance of the recipient strains and the selection marker of the pVZ322 construct. *E. coli* pUC19:mdtK transformants showed a twofold increase in minimum inhibitory concentrations (MICs) for tetracycline, nalidixic acid, ciprofloxacin, and benzalkonium chloride. MICs of the *A. paragallinarum* transconjugants revealed a twofold increase for quinolones, tetracyclines, and benzalkonium chloride, indicating that the MdtK exporter contributes to antibiotic and biocide resistance in *A. paragallinarum*.

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Biofilm-related genes and antimicrobial resistance in wild boar Escherichia coli: An initial investigation

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The wild boar (*Sus scrofa*) is considered one of the most widely distributed mammal species in the world, capable of colonizing and thriving in a wide range of environments. Today, its significance lies in its role as a bioindicator for monitoring various zoonotic and non-zoonotic diseases. In the present study, *Escherichia coli* strains were isolated from nasal swabs of healthy wild boars, collected at the time of capture during the 2024 hunting season in the Campania Region (Southern Italy). A total of 164 *E. coli* strains were isolated on Mac Conkey agar plates and the identification by MALDI-TOF MS yielded log(score) values ≥ 2.2 for all isolates, indicating reliable identification. Phenotypic and genotypic antimicrobial resistance profiles of the isolates, as well as the presence of genes associated with biofilm production, were investigated. The study revealed that genes related to biofilm formation were detected in 43 out of 164 (26.2%) *E. coli* strains. Surprisingly, however, no more than one of the tested genes was found in any recovered strain. Furthermore, among biofilm-associated genes *fliC* was the highest (12/43, 27.9%) followed by *fimH* (8/43, 18.6%). A statistically significant difference in antimicrobial profiles was observed between the *E. coli* group carrying biofilm-associated genes and the group lacking these genes. These findings support the association between biofilm production capacity and elevated antimicrobial resistance profiles.

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Monitoring trends in antimicrobial susceptibility in Staphylococcus pseudintermedius from the University College Dublin veterinary laboratory

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Antimicrobial resistance (AMR) surveillance is critical to guide therapy and monitor for emerging resistance. *Staphylococcus pseudintermedius* is an important veterinary pathogen in which methicillin resistance and multi-drug resistance are a particular concern.
This study evaluated temporal trends in antimicrobial susceptibility test results for 9 antimicrobial compounds among 1072 *S. pseudintermedius* isolates (recovered from 1037 dogs and 35 cats) from skin and ear samples submitted to the UCD veterinary microbiology laboratory between 2016 and 2024.
MIC data from the first isolate per patient per calendar year was exported to RStudio from the bioMérieux Vitek2 system. The Jonckheere-Terpstra test was used to test for trends in MIC category distributions over time.
A significant increasing trend in MIC category distributions was detected for oxacillin (P=0.002), enrofloxacin (P=0.043), gentamicin (P=0.002) and trimethoprim/sulfamethoxazole (P=0.027). A significant decreasing trend in MIC category distribution was detected for erythromycin (P=0.001). Significant trends in MIC category distribution were not detected for fusidic acid, doxycycline, clindamycin or chloramphenicol.
Resistance to some antimicrobials in *S. pseudintermedius* has increased over time which raises concerns about the future efficacy of these drugs in clinical practice and highlights the need for appropriate antimicrobial use and antimicrobial susceptibility testing.

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Molecular characterization of antibiotic resistance and virulence genes in Enterococcus spp. from cloacal samples of healthy broiler chickens

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Enterococcus spp. are commensal bacteria from the gastrointestinal tract of poultry but can act as opportunistic pathogens. Their role as reservoirs of antibiotic resistance and virulence genes poses a risk for animal and public health. Understanding the genetic profiles of Enterococcus in healthy broilers is essential for surveillance and control measures. Cloacal swabs were collected from 170 apparently healthy broilers at a single poultry farm in Portugal. Eighty-six Enterococcus spp. were isolated using conventional microbiological techniques. Molecular characterization was performed by PCR to detect antibiotic resistance genes (vanA, vanB, erm(A), erm(B), erm(C), catA, vatE, vatD, tet(M), tet(O)) and virulence genes (gelE, cpd, fsr). Five isolates were identified as E. casseliflavus, while 81 were E. gallinarum. PCR revealed the presence of resistance genes: tet(M) in 64% (n=55), tet(O) in 25.6% (n=22), and erm(A) in 2.3% (n=2). No virulence genes were detected. The predominance of E. gallinarum among Enterococcus spp. isolated from healthy broilers, along with the detection of tetracycline resistance genes, particularly tet(M), highlights the circulation of resistant commensal strains within poultry flocks. The low prevalence of macrolide resistance and absence of virulence genes suggest a limited pathogenic potential. Nonetheless, their role as reservoirs of antimicrobial resistance reinforces the importance of continued monitoring within a One Health framework.

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Growth inhibition of pathogenic Prototheca algae by the shikimate pathway inhibitor herbicide glyphosate

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Prototheca are algae lacking chloroplasts that cause rare but often lethal infections. Current treatments are limited to antifungals such as amphotericin B, which targets both algal and animal cell membranes and results in high toxicity. Shikimate pathway is absent from animals but is present in plants. The objective of this study was to the investigate whether the growth of pathogenic Prototheca spp can be supressed by the selective shikimate pathway inhibitor herbicide glyphosate.
Growth of five Prototheca species (P. blaschkeae, P. wickerhamii, P. cutis, P. bovis and P. ciferrii) was followed in a plate-reader for 72 hours in the presence of two-fold dilution solutions of glyphosate (12.5 – 1600 µg/mL) and amphotericin B (1 -32 µg/mL) in Sabouraud medium. Glyphosate efficiently inhibited growth of all five Prototheca species at 50 – 100 µg/mL, and demonstrated a more consistent inhibition and no regrowth when compared to amphotericin B. IC50 values were particularly low for P. blaschkeae, P. bovis and P. ciferrii (18.9 µg/mL, 19.4 µg/mL and 20.7 µg/mL, respectively), while IC50 of P. cutis and P. wickerhamii were somewhat higher (35.2 µg/mL and 60.8 µg/mL, respectively).
Our results demonstrate that targeting the shikimate pathway may be a promising approach to anti-algal drug development with a lower potential for toxicity for animal cells.

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Prevalence and molecular typing of Salmonella enterica subsp. diarizonae in sheep intended for slaughter in Germany

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Salmonellae are one of the most important zoonotic pathogens and are of great importance as causative agents of foodborne infections in Europe. The serovars 61:k:1,5,(7) and 61:-:1,5,(7) are known as sheep-associated *Salmonella enterica* subspecies *diarizonae* (SASd) and commonly found in this animals. In our study, the tonsils of 287 sheep and lambs were removed after slaughter and examined according to the official collection of test methods of the German Food and Feed Code. Obtained isolates were sent to the National Reference Laboratory for Salmonella (Federal Institute for Risk Assessment) in Berlin for final confirmation, serotyping and susceptibility testing. Confirmed isolates were further characterized by pulsed-field gel electrophoresis (PFGE). Our results, in witch 15% of lambs and 76% of sheep were positive, showed that SASd are present in German sheep populations and that especially adult animals represent a reservoir for these organisms. All isolates were susceptible to the twelve antibiotics tested and characterization by PFGE showed similarities in the fragment patterns of isolates of the same geographical origin as well as deviating patterns of isolates from individual sheep flocks. In addition, it was shown that different selective enrichment media had a major influence on the results. The obtained isolates should be further characterised with regard to their virulence potential to better assess public health risks associated with SASd.

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Dumpster diving: Microbiological and sensory quality of food collected from dumpsters

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Dumpster diving is the term used to describe the practice of mostly young adults to rummage through the garbage cans of grocery stores in order to find products that are still edible. These “rescued” products are intended not only to cover their own food consumption, but are also given to others. However, little is known about the microbiological hazards and potential public health risks associated with the consumption of dumped foods. For this reason, various meat products, fresh beef and chicken, milk and various dairy products were examined in an experimental setup that mimics the procedure used for disposing food in grocery stores. After expiry of the best-before or use-by dates, the food products were stored in a bin and microbiological and sensory analyses were carried out at various time points according to DIN EN ISO standards and methods from the official collection of test methods of the German Food and Feed Code. Foods from almost all product categories were assessed as unsatisfactory after evaluation of perishability parameters. In addition, coagulase-positive staphylococci and *Clostridium perfringens* were isolated from beef and chicken, and *Campylobacter* spp. were also detected in the latter group of products. The results of this study show that the overall quality of processed meat and dairy products as well as fresh meat deteriorates during storage in a garbage can, although pathogenic bacteria were only detected in some of the samples.

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Bacteriological Contamination in Livestock: Association with Animal Factors and Hygiene Status

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This study aimed to evaluate the prevalence of mesophilic bacteria, Enterobacteriaceae, Escherichia coli, and E. coli O157:H7 in animal and environmental samples from four different lots. A total of 244 samples were analyzed, with 220 from animals and 24 from environmental sources. Mesophilic bacteria were found in over 95% of all samples, regardless of origin, sex, age, or breed. Enterobacteriaceae were significantly more prevalent in animal samples (67.7%) than in environmental ones (41.7%) ($p = 0.011$). E. coli was detected in 30.9% of animal and 16.7% of environmental samples, while E. coli O157:H7 showed similar frequencies in both (approximately 29%). Among the lots, significant differences were observed in the prevalence of all bacterial groups, especially E. coli O157:H7, which was absent in lot 4 but highly prevalent in lots 1 and 2 ($p < 0.001$). Regarding animal demographics, Enterobacteriaceae and E. coli were significantly more prevalent in older animals and non-native breeds. The cleanliness of the animals had a strong association with bacterial isolation, particularly for E. coli and E. coli O157:H7, which were rarely found in clean animals ($p < 0.05$). The findings highlight the role of hygiene, animal characteristics, and sample source in the microbial contamination of animals and environments, reinforcing the need for improved control measures in livestock production.

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Contamination of broiler meat and offal by moulds: prevalence and safety implications

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This study aimed to detect and identify moulds in various tissues of broilers, namely the breast, liver, heart, gizzard, neck, skin, feet, and also in feed. A total of sixty-two samples were collected from hypermarkets and local markets, including liver, heart, gizzard, neck, feet, and carcasses (skin and breast). The samples were analysed according to ISO methods for the enumeration and isolation of yeasts and moulds. Ten grams of each sample were aseptically cut and homogenized in 90 mL of 0.1% peptone water, followed by decimal dilutions and inoculation of 0.1 mL on Chloramphenicol Glucose Agar. Fungal isolation and identification were performed by macroscopic examination of colony features (colour, shape, size, and hyphal characteristics). Moulds were most prevalent in the skin, followed by the neck, liver, feet, gizzard, and heart. In total, six different genera of moulds were identified: Acremonium spp., Cladosporium spp., Verticillium spp., Fusarium spp., Penicillium spp., and Aspergillus spp. The breast muscle, once the skin was removed, appeared to be free of moulds, suggesting that the skin may act as a protective barrier against contamination. Further studies are needed to clarify the implications of mould presence and potential mycotoxin production in broiler meat and offal, particularly regarding their impact on food safety.

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Molecular and Metagenomic Characterization of Avian Orthoavulavirus 1 in Columbiform Birds Presenting Neurological Signs

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Several *Columba livia* and *Streptotelia decaocto* housed in a wildlife rehabilitation center in Northern Portugal exhibited acute neurological signs culminating in death. Post-mortem examinations were conducted on all specimens, revealing consistent cerebral hemorrhage and congestion, along with splenic abnormalities. Histopathology demonstrated lesions suggestive of viral infection, including perivascular cuffing, neuronal necrosis, and eosinophilic intracytoplasmic inclusions.

Initial molecular testing using WHOA and USDA recommended primers targeting the matrix (M) gene of *Avian orthoavulavirus 1* (AOAV-1; Newcastle disease virus) was negative. However, metagenomic sequencing of brain tissue using third-generation sequencing identified a viral genome taxonomically classified as AOAV-1. BLAST analysis revealed a highest 94.29% identity to known sequences. Phylogenetic analysis placed the partial genome within Class II, Group 6, typically associated with pigeon paramyxovirus. Further *in silico* analysis revealed mismatches in the standard primer binding sites, explaining the initial false-negative PCR results.

These findings underscore the circulation of a divergent, highly pathogenic AOAV-1 strain capable of evading standard molecular detection, emphasizing the need to update diagnostic tools for both surveillance and clinical diagnosis of Newcastle disease.

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Metagenomic Characterisation of Migratory Bird Microbiota and Their Role in Disseminating Antimicrobial Resistance Genes

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Migratory birds are recognised as carriers and disseminators in the global spread of antimicrobial resistance (AMR) due to the ability to travel intercontinentally. Recent advances in metagenomic sequencing have enabled characterisation of the gut microbiota. Despite their recognised role as reservoirs of AMR, research has largely focused on birds of economic importance, while the potential threat posed by migratory wild birds as carriers of AMR remains understudied.

In this study, metagenomes from 60 migratory bird fecal samples from Japan and China were analysed. Our preliminary results show that, AMR genes were detected in 55 samples, indicating widespread prevalence in the study population. Using AMRfinderplus, 325 unique AMR genes were identified. AMR accounted for approximately 45% of genes detected, with stress resistance genes and virulence factors comprising the majority of the remainder. The dominant AMR classes included beta-lactams (21.1%), aminoglycosides (15.9%) and tetracyclines (11.0%), reflecting a broad spectrum of resistance mechanisms. To our knowledge, this is the first study to combine metagenomic data from migratory birds across these geographic regions to comprehensively characterise their resistome. These findings underscore the potential role of migratory birds as environmental dissemination of AMR; emphasizes the need for integrated One Health surveillance to monitor and mitigate the spread of AMR across wildlife, environmental, and human domains.

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A global genomic perspective on Streptococcus equi subsp. zooepidemicus

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Streptococcus equi sp. *zooepidemicus* (SEZ) is a zoonotic pathogen causing severe disease in many mammals [1, 2], with significant welfare and economic costs, notably in horse and pig industries [3, 4]. Currently, no vaccine is available, and antibiotics are primary treatment of SEZ infections, driving antimicrobial resistance (AMR) [5]. To guide treatment and prevention, we analyzed 442 SEZ genomes (1993–2024) from 6 countries. Four phylogenetic clades with distinct signatures could be defined. There was no geographical association, except that the Icelandic strains clustered in Clade 2. We found 27 core and 40 accessory virulence associated genes (VAGs). Clade 1 lacked adherence factor genes *srtC.2/3/4* and immune modulation genes *zag/se18.9*; Clade 4 and 2 were enriched for invasion gene *virD4* (88% vs. 59% on average) and exoenzyme gene ideZ (62% vs. 19% on average), respectively. There were 50 intact prophages and 21 VAGs carried by prophages (mostly exotoxin genes), while ICEs carried only 4 VAGs (mainly *virD4*). Only 79 strains harbored AMR genes, with multidrug resistance in one strain from Clade 4. No penicillin-binding protein genes detected. Most AMR genes (69%) were ICE-associated, mainly on *ICEsz1*. Our study highlights clade-specific VAGs like *virD4/ideZ*, and shows AMR in SEZ is mainly driven by ICEs, providing insights for targeted prevention and treatment strategies for SEZ infections.1

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Distribution of Small Ruminant Lentivirus Genotypes A and B in Sheep and Goat flocks in Portugal

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Small ruminant lentiviruses (SRLV) affect ovine and caprine species, causing a chronic and progressive disease. Currently, there are 5 known genotypes (A – E) and more than 30 subgroups. In Portugal, diverse production systems contribute to the profitability and social value of small ruminant production. This work aimed to investigate which SRLV genotypes are present in Portugal. The study was conducted in flocks previously involved in a seroepidemiological survey. Two hundred and five SRLV ELISA-positive samples were randomly selected from 105 farms. Each was subjected to a commercial ELISA genotyping test (In3diagnostics) to determine circulating genotypes. Of the 205 samples analysed, 124 tested positive: 54 for genotype B, 32 for genotype A, and 38 unclassified, suggesting coinfection or cross reactions. Upon further analysis by species, 20 genotype A samples were ovine and 12 caprine; 24 genotype B samples were ovine and 30 caprine. We conclude that both genotypes A and B are present in ovine and caprine flocks in Portugal. Genotype A predominates in sheep, while B is more common in goats. However, these genotypes are not exclusive and can occur in either species. We emphasize that coinfection with both genotypes can be an important issue at both the flock and individual levels.
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Genomic Analysis of Escherichia coli from Canine and Feline Urinary Tract Infections in the UK - A VetCLIN AMR Project Insight

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Introduction: The VetClin AMR Project aims to understand the variability in antimicrobial resistance (AMR) data generated by veterinary diagnostic laboratories for the most clinically relevant companion animal pathogens and clinical infections1.
Aim: To investigate the molecular epidemiology and AMR of clinical E. coli from diagnostic isolations of companion animal urinary tract infections (UTIs).
Methods: Three hundred and eight (n=308, ~ 2/3 canine and 1/3 feline) E. coli UTI isolates collected from 11 veterinary diagnostic laboratories over 1 year underwent Illumina WGS for AMR detection and genotyping.
Results: Preliminary findings have shown the molecular background of companion animal UTI-E. coli circulating in the UK is heterogeneous. Extraintestinal pathogenic E. coli (ExPEC) phylogroup B2, including human ST73 and canine ST372 lineages, were the most prevalent uropathogenic clones. Furthermore, dominant ExPEC lineages in the community and hospitals in the UK (ST12, ST127 and ST131) were present. Acquired AMR determinants were overall uncommon for critically important antimicrobials; prevalence of extended-spectrum beta-lactamases (ESBLs) was 3%, whilst no carbapenem-resistance was identified.
Conclusions: Both human and canine-specific uropathogenic clonal lineages are associated with companion animal clinical UTIs in the UK, suggesting a potential for bi-directional transmission, without evidence for concerning resistome in companion animal clinical isolates

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Outbreak of Streptococcus canis Infection in a Mink Farm in Northwestern Greece

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Introduction:
S. canis is a Gram-positive, multi-host bacterium isolated from a broad range of mammals, including humans. In the spring of 2024, approximately 8% of the animals in a mink farm located in northwestern Greece developed purulent lesions, characterized primarily by abscesses on the head. Around 1% of the affected animals exhibited neurological symptoms consistent with encephalitis and succumbed to the disease.
Methods:
Deceased mink were submitted to the Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, for diagnostic investigation. Brain tissue and oropharyngeal swab samples were analyzed using conventional microbiological techniques and Oxford Nanopore Technologies (ONT) sequencing.
Results:
S. canis was identified in brain samples using both methodologies. Antimicrobial susceptibility testing (Kirby–Bauer disk diffusion method) showed that the isolated strain was sensitive to ampicillin. Therapeutic administration of ampicillin to the remaining affected animals led to a marked clinical improvement and a significant reduction in new cases.
Conclusion:
This outbreak highlights the pathogenic potential of S. canis in farmed mink and underscores the importance of prompt diagnosis and targeted antimicrobial treatment. Enhanced hygiene practices and strict biosafety measures are essential to reduce the occurrence of skin wounds, which may serve as portals of entry for the bacterium.

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EFFECT OF DELAYED PROCESSING OF HEMOCULTURES ON DIRECT IDENTIFICATION MALDI-TOF SCORE VALUES

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Veterinary diagnostic work flows for handling hemocultures show varying incubation times or storage episodes before identification with MALDI-TOF MS. We quantified the effect of delayed processing of positive hemocultures on the MALDI-TOF score values for 4 bacterial species associated with bacteremia in companion animals.

Hemoculture flasks, spiked with E. coli, S. pseudintermedius, S. equi subsp. zooepidemicus or A. equuli were incubated overnight at 35℃ (2 independent repeats), subsequently divided in 15 aliquots of 1ml per bacterial species, of which 5 were incubated at 4℃, 20℃ or 35℃. After 0, 1, 4, 7 and 14 days of incubation, an extraction was performed (MBT Sepsityper Kit, Bruker Daltonik). Per extraction, 3 replicates of 1 µl were spotted on a target, covered with 1µl of HCCA matrix and spectra were analysed with the MBT Compass software (Bruker Daltonik), providing score values of the best hit.

Surprisingly, prolonged exposure of positive hemocultures to 4℃, 20℃ or 35℃ only marginally influenced the MALDI-TOF score values for E. coli, S. pseudintermedius and S. equi subsp. zooepidemicus, with mean score values ≥2.00 until the end of the experiment. For A. equuli, a significant decrease of mean MALDI-TOF score values was observed after 7 and 14 days of incubation at 20℃ and after 4, 7 and 14 days of incubation at 35℃.

Except for some fastidious species, short-term delayed processing of hemocultures will have limited effect on MALDI-TOF score values.

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Mapping Methicillin-Resistant Staphylococcus aureus (MRSA) Transmission in an Equine Referral Hospital: A Pilot Study Using FT-IR Spectroscopy

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Introduction: Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) ST398 is a concern for equine nosocomial infections and outbreaks. Contaminated hospital surfaces and staff members can contribute to the spread of LA-MRSA in equine settings1,2.
Aim: To retrospectively map MRSA transmission at an equine referral hospital and investigate its molecular epidemiology.
Methods: Eighty-four (n=84) oxacillin-resistant S. aureus from equine clinical (n=22), environmental surveillance (n=47) and staff hand-plates (n=15) samples were typed using Fourier-Transform InfraRed (FT-IR) spectroscopy (IR Biotyper, Bruker Daltonics) for tracking possible hospital dissemination. Gold-standard WGS was performed on a subset of 39 isolates for in-depth genotyping.
Results: MRSA clusters of clinical, environmental and hand-plate samples were common. However, the ability of FT-IR spectroscopy to accurately predict WGS cluster composition was poor (Adjusted Rand Index 0.185-0.360) using FT-IR spectroscopy cutoffs between 0.120-0.125 and the Euclidean algorithm to construct dendograms.
Seventy-two percent of isolates were of ST398, spa type t011 and carried the SCCmec IVa(2B).
Conclusions: Continued surveillance is required to contain MRSA transmission within equine facilities. Further trials employing FT-IR spectroscopy are needed to enhance the potential of this rapid technology in the control of equine hospital-associated MRSA, e.g. by its application in outbreak scenarios.

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Survey on Water, Sanitation, and Hygiene (WaSH) in Uganda's Karamoja Sub-Region, using a KAP Questionnaire within a One Health framework

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The Karamoja sub-region of Uganda addresses major challenges in water, sanitation, and hygiene (WaSH), deeply linked to public and environmental health and regional development. This study, conducted in the Moroto and Napak districts, utilised a Knowledge, Attitudes, and Practices (KAP) survey to evaluate hygiene behaviours, water access, and infectious disease prevention from a One Health perspective. A total of 195 respondents were surveyed, providing insights into socio-demographic factors, hygiene practices, livestock management, and disease prevention. Findings highlighted gender disparities: women were less likely to have adequate knowledge, potentially due to limited access to information in male-focused community settings, and their role in water collection. Age influenced WaSH knowledge, with older individuals demonstrating greater awareness, probably due to their role in knowledge transmission. Proximity to water sources shaped behaviours: greater distances were associated with increased awareness but reduced hygienic practices. Livestock ownership, particularly of small ruminants, correlated with better hygiene knowledge, probably due to interactions with veterinarians and authorities during vaccination campaigns. Integrated communication strategies (community meetings, home visits, and radio outreach) proved effective in addressing these gaps. These data represent a baseline for future targeted training and diagnostic activities in human and animal infections.

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DEVELOPMENT AND VALIDATION OF TWO COMPETITIVE ELISAs FOR THE DETECTION OF LEPTOSPIRA SEROVAR GRIPPOTYPHOSA AND AUSTRALIS ANTIBODIES IN CATTLE SERA

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Bovine leptospirosis in Belgium has been associated with outbreaks caused by *Leptospira* Grippotyphosa and Australis serovars. Due to shortcomings of modern-day diagnostic tools, we developed and validated two separate *Leptospira* serovar-specific competitive (c) ELISA assays for detection of leptospiral antibodies against *L.* sv Grippotyphosa and *L.* sv Australis.

Animal sera were pre-absorbed with inactivated *Leptospira* of the relevant serovar/strain and then challenged to a sandwich ELISA using identical monoclonal antibodies for both capture and detection—the latter being horseradish peroxidase (HRP)-conjugated. After incubation with the HRP-substrate, the reaction was halted and optical density measured. Results were expressed as a percentage of inhibition relative to a negative control.

The developed *L.* sv Grippotyphosa and *L.* sv Australis cELISA assays (here named "G" and "A", respectively) were validated based on following validation parameters: robustness, limits of quantification, dilutional linearity ("G": R^2=0,98; "A": R^2=0,99), repeatability (Coefficient of variation: "G": 3,65%; "A": 7,30%), diagnostic specificity ("G": 94%; "A": 100%; (both n=52)) and diagnostic sensitivity on retrospective samples ("G": >85% (n=134); "A": 88% (n=45)).

These sensitive and specific platforms are intended for use on large-scale screenings of cattle sera. As the conception is multi-species, validation of the cELISAs could be extended to use in other animal species.

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Detection of Tusavirus in wastewater samples, Spain

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been recently detected in domestic small ruminants [3]. The epidemiology and genetic diversity of TuVs, however, remain largely unexplored. We utilized wastewater-based epidemiology (WBE) as a tool to survey for TuV circulation in two distinct Spanish locations, Palma de Mallorca and Don Benito, representative of an urban and rural area, respectively. All samples were collected bi-weekly and screened for TuV DNA using a real-time PCR (qPCR) (VP2 gene) and a nested PCR protocol (NS1 gene). Overall, TuV DNA was detected in 68.51% (37/54) of wastewater samples, with similar prevalence in both locations. On sequence analysis of the NS1 diagnostic region, the sequences shared 94.3-100% nucleotide identity (nt id). Two complete genomes were assembled using an Artic-like PCR strategy combined with Oxford Nanopore Technologies sequencing. Genome-wise, the two viruses shared nt id of 93.18%. On phylogenetic analysis of the NS1 and VP1/VP2 genes, inconsistencies in the segregation patterns with human and animal TuV sequences were observed in the rural-derived strain ESP/168DB/2023. The high prevalence detected in this WBE survey, and the mosaic (human/animal-like) TuV genome identified in the rural area, highlight the importance of investigating viral ecology using a holistic approach.

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AVIAN FLU OUTBREAKS IN ROMANIA IN THE LAST DECADE

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Avian influenza is an infectious, highly contagious disease caused by RNA viruses belonging to the genus Influenzavirus, part of the Orthomyxoviridae family. According to the WOAH, the avian influenza viruses that pose an epidemic and zoonotic risk are those belonging to the H5 and H7 types-HPAI. The aim of these researcher was to resume the outbreaks of the Avian Flu in the last decade. In Romania, after a period of epidemiological silence over six decades, avian influenza has re-emerged, with H5N1 strain. In 2015, 118 pelicans in the Danube Delta were death because of H5N1 infection. Following the evolution of avian influenza on the territory of Romania, mortality losses within the domestic poultry population reached 34,090 heads (WOAH). In domestic birds, the recorded mortality was 224,713 heads. In 2024, Romania reported 36 outbreaks of avian influenza with the H5N1 viral subtype. WOAH show that the prevalent strains of avian influenza virus on the territory of Romania belong to the H5N1 serotype. The wild reservoir is intensely active in the epidemiology of avian influenza and is a major factor of pressure on domestic populations. This epidemiological reality, to which is added scientific information that demonstrates the spill-over of the H5N1 strain to other mammalian species of economic interest - cattle - requiring monitoring of the wild reservoir and investigation of other animal species in relation to the disease outbreaks discovered in domestic birds.

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DISTRIBUTION OF OUTBREAKS OF AFRICAN SWINE SWINE FEVER IN ROMANIA DURING 2017-2024

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ASFv first emerged in Europe in the last century, being eradicated in Europe (except Sardinia) in the ‘90s. Since 2007, the disease emerged in Georgia, and in 2014 the first outbreaks were noticed in the European Union.
The first ASF outbreak in Romania emerged in 2017 in Satu Mare county, in traditional backyards (GP) where 4 outbreaks were recorded, incriminating the unofficial introduction of pork products purchased from Ukraine.
During january to june 2018, ASFv has been identified in wild boar populations of the area. Overall, in 2018, 1339 new outbreaks of ASF were declared, in domestic pigs (GP and commercial) as in wild boars. Next years the prevalence of identified outbreaks decreased (2019-2020), but a new peak arise in 2021, with 2618 outbreaks, 1628 in domestic and 990 in wild. Until september 2024 the prevalence slope decreased, the domestic outbreaks being under the control mesures, in force in commercial and adapted to the GP. In wild boars the pasive and active surveillance revealed a quite high and undulant prevalence, relatated to the wild population dinamic, in number and areal.
Considering the high outbreaks prevalence of ASF on the Romanian territory in the last 7 years, and the endemicity of the infection, monitoring of pigs and wild boars, very rigorous control of their movement are the only tools available for disease control.

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Pseudomonas aeruginosa: a challenge clinical case

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Pseudomonas aeruginosa, known for its antimicrobial resistance, is a significant pathogen in cats and dogs in the European Union. In cats, it is more commonly linked to respiratory than urinary infections. This case involves a 2-year-old, indoor, non-neutered male Persian cat presenting in shock due to urethral obstruction, with a history of food allergy, asthma, and low body condition (BCS 2/5). Blood tests showed anemia, azotemia, hyperphosphatemia, and hyperkalemia; ultrasound revealed bladder sediment. Urine was collected before catheterization, and enrofloxacin therapy was started. Culture identified multidrug-resistant *P. aeruginosa.* The cat was hospitalized and treated with IV gentamicin (2 mg/kg SID) and fluids. A second culture showed *P. aeruginosa* with intermediate gentamicin sensitivity. Treatment continued, and a week later the culture was negative, with no clinical signs. A follow-up urine culture was advised but not performed due to financial constraints. *P. aeruginosa*'s ability to form biofilms raises concerns about false-negative cultures. This case highlights the challenges of treating multidrug-resistant infections and emphasizes the importance of bacterial culture and responsible antibiotic use in veterinary practice.

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Hunting-training dogs and companion dogs in the Netherlands are frequently exposed to highly pathogenic avian influenza (HPAI H5) and human H1N1 virus.

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Dogs are susceptible to the currently circulating highly pathogenic avian influenza (HPAI) H5 and human H1N1pdm2009 (pandemic H1N1) viruses, yet little is known about the extent to which dogs are exposed to both these viruses. Here we investigated HPAI H5 and human H1N1pdm2009 virus exposure in domestic dogs–including dogs that participated in hunting-training–and investigated lifestyle factors associated with HPAI H5 virus exposure. We screened sera from 538 dogs, sampled between 2021 and 2023, for influenza A virus antibodies, using ELISA and hemagglutination inhibition assays (HAIs). We analyzed lung tissue and (naso)pharyngeal swabs for influenza A viruses using RT-qPCR. Seropositivity to HPAI H5 virus was more frequent (13.3%) in hunting-training dogs than in companion dogs with unknown bird contact (3.7%). In contrast, seropositivity to H1N1pdm2009 was more frequent in companion dogs (7.1%) than in hunting-training dogs (0.7%). Based on owner questionnaires, seropositivity to HPAI H5 by ELISA in hunting-training dogs was significantly associated with recent bird contact in/near water (odds ratio 6.9). No influenza A viruses were detected in 207 necropsy dogs and 180 (hunting) dogs. Our findings suggest that dogs are frequently exposed to zoonotic influenza A viruses, and we recommend dog owners to avoid dog contact with sick/dead birds.

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Spontaneously Resolving Alopecia Resulting in Dermatophytic Pseudomycetoma in an Exotic Shorthair Cat

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Feline dermatophytic pseudomycetoma (DPM) is a rare deep infection caused by *Microsporum canis*, with a known predisposition in Persian cats. We describe a 12-year-old male Exotic Shorthair with a history of spontaneously resolving alopecia three years earlier. The cat presented with two chronic, non-ulcerated, enlarging dorsal masses (7×5 cm and 3×2 cm). Laboratory tests showed mild eosinophilia and hypergammaglobulinemia; FIV and FeLV were negative. Cytology from fine-needle aspiration revealed necrotic debris and hyaline hyphae. *M. canis* was subsequently isolated from both lesions and haircoat, with identification confirmed by MALDI-TOF mass spectrometry. The masses were surgically excised, followed by oral terbinafine (26 mg/kg SID) for 90 days. Histopathology confirmed DPM, with PAS-positive fungal colonies surrounded by prominent homogeneous eosinophilic material consistent with the Splendore–Hoeppli phenomenon. Intralesional hyphae showed a distorted, non-branching morphology, 2–3 μm in diameter, with irregular septation and cylindrical wall dilations. Thick-walled vesicular elements (2–18 μm) were also observed. This case highlights the typical clinical and pathological features of DPM in an Exotic Shorthair and underscores the importance of a multimodal diagnostic approach in uncommon fungal presentations.

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Severe aspergillosis caused by a projectile path: a case report

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A free-living short-toed eagle was admitted to a wildlife rehabilitation centre, victim of illegal shooting. Physical examination and blood analysis revealed a highly regenerative haemolytic anaemia. Radiographic assessment showed various lead pellets, and an unicortical fracture of the left humerus. Supportive fluid therapy and feeding, prednisolone 0.5mg/kg PO BID and doxycycline 50 mg/kg PO BID were started. Regurgitation, marked leucocytosis and hyperglobulinaemia were detected a few days later. Marbofloxacin 10mg/kg IM SID was started and the bird's general condition improved. After one month, the animal was found depressed and dyspnoeic. X-rays and an exploratory coelioscopy were carried out and a severe air sacculitis and many scattered granulomas were detected. On the right side, in the abdominal air sac, a particularly large, grey to black mold granuloma was found, forming a sort of pathway into the cavity, compatible with the trajectory of a projectile. As many plaques as possible were removed by biopsy, both for diagnostic and treatment purposes. *Aspergillus fumigatus* were isolated from the fungal culture. Itraconazole 10 mg/kg PO BID and F10™ nebulisations (1:125 dilution) twice a day were initiated. Despite all efforts, the individual was euthanised. This case reports a possible aspergillosis infection caused by skin puncture and aims to draw attention to other possible routes of infection besides inhalation of fungal spores.

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Fungal Foes under Exotic Scales: Managing Disseminated Fungal Granulomas in a Green Iguana (Iguana iguana)

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Fungal infections are increasingly reported in reptiles. This case describes a chronic granulomatous mycosis of the dewlap in a green iguana (*Iguana iguana*), with evidence of systemic spread. An adult male iguana presented with two firm, slow-growing granulomas (10–15 mm) on the proximal dewlap. After ulceration, empirical treatment with topical disinfectants (chlorhexidine, F10 SC®) and antibiotics (amoxicillin-clavulanic acid) yielded no improvement. Surgical biopsy revealed a vascular, adherent lesion; histopathology confirmed granulomatous dermatitis with caseous necrosis of probable fungal origin. Bacterial cultures identified *Staphylococcus* spp. and *Enterobacter cloacae*, while fungal culture isolated *Nannizziopsis* spp. Lesions enlarged (up to 25 mm), and new granulomas developed. Oral itraconazole and environmental management led to remission of marginal lesions after four months. However, relapse occurred after treatment cessation. CT revealed two enlarging submandibular masses (up to 84 mm) and pulmonary nodules (≤8 mm). A multimodal antifungal protocol with oral itraconazole, nebulized and intralesional amphotericin B was initiated. Serum itraconazole levels were therapeutic. Five years after onset, pulmonary lesions had resolved, dewlap granulomas regressed, and cultures remained negative. This case illustrates the aggressive and systemic nature of *Nannizziopsis* infections in reptiles and emphasizes the need for long-term, multimodal antifungal therapy.

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Pityriasis versicolor in a southern ground hornbill (Bucorvus leadbeateri)

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A 20-year-old southern ground hornbill (*Bucorvus leadbeateri*) developed several round, non-scaly, hyperpigmented macules on its gular pouch (day 0). Physical exam and bloodwork were unremarkable, and no previous skin disease was reported. Cytology revealed yeast overgrowth morphologically consistent with *Malassezia* spp. (>100 yeasts/OIF), whereas healthy skin showed minimal yeast presence. Fungal culture yielded rare, smooth, cream-colored colonies, and PCR identified *Malassezia slooffiae*. Histopathology revealed lymphocytic perivascular dermatitis with PAS-positive yeasts. Lesions were regularly monitored via visual and cytological assessment. Remarkably, macules resolved spontaneously within four months without treatment (day 131). However, by day 309, similar lesions recurred, with cytology showing 20 yeasts/OIF. *M. slooffiae*, known to cause pityriasis versicolor in humans, may contribute to skin dyschromia in birds under altered cutaneous or immune conditions. While *Malassezia* spp. are part of avian skin microbiota, this case highlights their potential pathogenicity. To the authors' knowledge, this is the first report implicating *Malassezia* spp. as a cause of pityriasis versicolor-like lesions in a bird, reinforcing the need for thorough dermatological assessment in exotic species with poorly understood microbiomes.

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Fatal Fungal Pneumonia Caused by Beauveria bassiana in a Rarely Sighted Kemp's Ridley Sea Turtle (Lepidochelys kempii) in Portugal

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The Kemp's ridley turtle (*Lepidochelys kempii*), the most endangered sea turtle species, has a natural range mainly in the Gulf of Mexico. Juveniles may drift to the northwestern Atlantic, but strandings in Portugal are rare. In May 2024, a juvenile was found off Sines, Portugal and admitted to Porto d'Abrigo (Zoomarine). Despite intensive care, its condition worsened, and it died after 11 days. Necropsy revealed multiple pulmonary lesions (up to 2 cm), with a powdery texture. Histopathology showed severe heterophilic interstitial pneumonia involving bronchi, with PAS-positive fungal hyphae. Fungal culture and molecular analysis identified *Beauveria bassiana*. PCR ruled out herpesvirus, adenovirus, and paramyxovirus. Fungal pathogens are increasingly relevant under One Health, especially with climate-driven shifts in fungal ecology. This case underscores the need to consider fungal pneumonia in stranded sea turtles, especially when clinical signs worsen despite antibiotics. Opportunistic fungi like *B. bassiana*, often overlooked, may threaten rehabilitation and conservation of endangered marine reptiles, particularly when found outside their native habitats.

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Listeriosis in cats – an emerging disease?

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Listeria monocytogenes is a gram positive, facultative intracellular saprophytic bacterium that can grow at a temperature range of 3-45℃ and at a pH range of 5.6-9.6 and is widespread in the environment. Ingestion of contaminated feed is the primary route of transmission. *L. monocytogenes* is an important zoonotic pathogen causing septicemia and meningoencephalitis in immunocompromised or elderly persons and fetal-placental infection in pregnant women. Although many species are thought to be susceptible, infections are rarely documented in cats. There are reports of systemic infection and mesenteric lymphadenitis in cats.

We queried our database and found eight cases of listeriosis in cats in Switzerland between 2016-2025. All isolates were successfully recovered and submitted for whole genome sequencing using the PacBio HiFi system. Six cases were associated with abscesses or masses in the abdomen and three cases showed changes in the mesenterial lymph nodes. A neighbor joining tree with the isolates and additional genomes obtained from GenBank revealed that the cat isolates did not form a dedicated cluster but rather were associated with different clusters including isolates from food and clinical isolates from humans. Three isolates belonged to the serotype 4b, 4d, 4e and the other five isolates belonged to the serotype 1/2a, 3a. They had typical virulence factors including different internalins, listeriolysin O, phospholipases and the bacterial surface protein ActA.

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Coxiella burnetii detected in Rhipicephalus sanguineus ticks collected in Portuguese autochthonous livestock

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Coxiella burnetii is the etiological agent of coxiellosis in animals and Q fever in humans, with ruminants recognized as the primary reservoirs. However, the epidemiological role of ticks in the transmission dynamics of this pathogen remains insufficiently characterized. This study investigated the presence of *C. burnetii* in ticks parasitizing two indigenous Portuguese livestock breeds namely, Churra Galega Mirandesa sheep and Garrano horses. A total of 555 ticks were collected and tested using molecular methods. This included 100 *Rhipicephalus sanguineus sensu stricto* from sheep and 455 *Rhipicephalus bursa* from horses. *C. burnetii* DNA was detected in 7% of *R. sanguineus* s.s. specimens, while none of the *R. bursa* ticks tested positive. Subsequent phylogenetic analyses confirmed the identity of the detected strains, reinforcing the molecular findings. These results indicate a potential involvement of *R. sanguineus* s.s. in the ecology of *C. burnetii* in livestock environments. Although the absence of detection in *R. bursa* suggests possible species-specific vector competence or exposure differences, further research is required to elucidate the actual role of ticks in the pathogen's transmission cycle in Portugal. These findings underscore the need to integrate vector surveillance into national strategies for Q fever risk assessment and control.

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Detection of PCV2d in Pig Industry of the Iberian Peninsula Suggests Silent Circulation in Portugal

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Porcine circovirus type 2 (PCV2) is a globally significant swine virus, causing substantial economic impact namely, €500 million annually in Europe, due to its link with porcine circovirus-associated diseases. While widespread use of vaccines targeting PCV2a and PCV2b has reduced clinical signs, the virus remains endemic, with the PCV2d genotype becoming increasingly prevalent in various regions. To date, and based on available indexed literature, there have been no confirmed reports of PCV2d in domestic pigs in Portugal. This study conducted molecular screening of fecal samples collected in a slaughterhouse in the Iberian Peninsula. Among the 400 samples analyzed, 8.5% (17/200; 95% CI: 5.03–13.26) from Portugal tested positive for PCV2d, while none of the 200 Spanish samples showed evidence of the virus. These results mark the first documented detection of PCV2d in Portugal's domestic pig population and point to undetected, subclinical circulation. The absence of PCV2d in Spanish pigs may reflect differences in biosecurity practices. The findings emphasize the importance of enhancing molecular surveillance and farm-level monitoring, as well as re-evaluating current vaccination approaches in light of shifting genotypes. Additionally, the study underscores the need for integrated disease management strategies that include consideration of wildlife reservoirs and environmental transmission routes in efforts to control PCV2 dynamics within the domestic swine population.

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Mapping PRNP Polymorphisms in Portuguese Ovine Populations: Insights into Scrapie Susceptibility and Disease Surveillance

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Scrapie is a fatal neurodegenerative disease affecting sheep, with susceptibility largely determined by polymorphisms in the prion protein gene (PRNP). Understanding the distribution of PRNP variants in local ovine populations is critical for effective prion disease surveillance and for the development of resistance-based breeding programmes. This study aimed to investigate the genetic variation of the PRNP gene in Portuguese sheep, particularly within autochthonous breeds, to assess their susceptibility to classical scrapie. Brain tissue samples were collected from slaughterhouses, and blood samples were obtained from multiple farms across the country. Genetic analysis focused on the identification and distribution of key PRNP haplotypes, including polymorphisms in codons traditionally associated with resistance or susceptibility to scrapie. The results revealed novel polymorphic patterns, with several deviations from the canonical codons 136, 154, and 171. Additionally, we identified several single nucleotide polymorphisms (SNPs) with putative protective or prion-promoting effects. A particularly noteworthy finding was the consistent presence of a conserved SNP in all sampled individuals of a specific Portuguese autochthonous breed, which was associated with increased aggregation propensity of the prion protein. This variant may mark breed-specific susceptibility to scrapie, supporting genetic strategies to enhance resistance in Portuguese sheep.

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Study of the Yeast Population in the Middle/Outer Ear of Dogs and Cats in Portugal

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Otitis is a common condition in companion animals, with yeast species playing a significant role in its pathogenesis. While *Malassezia pachydermatis* is well-established as the leading yeast associated with otitis externa, the broader diversity of otic yeasts remains underexplored, particularly in the middle ear. This study aimed to identify and characterize the yeast populations present in the external and middle ears of dogs and cats, exploring their potential clinical and zoonotic relevance. Samples were collected from 13 animals (9 dogs and 4 cats) in veterinary clinics across three Portuguese regions. Yeasts were isolated on Potato Dextrose Agar, followed by morphological and molecular identification based on D1/D2 26S rDNA sequencing. A total of 20 yeast colonies were cultured and subsequently studied, resulting in 19 successfully sequenced isolates. *Malassezia pachydermatis* was the most frequently detected species (57.9%), followed by *Meyerozyma guilliermondii* (15.8%), *Candida lusitaniae* (10.5%), and one isolate each of *Candida parapsilosis*, *Pichia norvegensis*, and *Saccharomyces cerevisiae* (5.3% each). Notably, non-*Malassezia* yeasts were predominantly isolated from cats, whereas *M. pachydermatis* was mostly found in dogs. These findings confirm *M. pachydermatis* dominance in dogs and greater yeast diversity in cats, highlighting the need to study otic fungi for diagnosis, treatment, and zoonotic risks.

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Are We Missing Brucella canis? A Comprehensive 13-Year Seroepidemiological Retrospective Study (2013–2025)

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Brucella canis is a neglected zoonotic pathogen causing canine reproductive disorders and posing risks to human health. Despite its importance, it remains underdiagnosed and excluded from routine surveillance. This 13-year retrospective seroepidemiological study (2013–2025) analysed 132 dog serum samples from mainland Portugal and Madeira, revealing an overall seroprevalence of 23.5%. The highest value was in the North (27.5%), though regional differences (NUTS 2) were not statistically significant ($p = 0.177$). Seropositivity peaked in spring, especially in May ($p < 0.001$). A significant association was found between breed and seropositivity ($p = 0.001$), with Setters, Pointers, Pugs, and German Shepherds at higher risk compared to Labrador Retrievers. Although sex and age were not significant, higher rates were noted in males and young adults. Municipality-level analysis showed marked heterogeneity; Trofa recorded the highest seropositivity (58.8%) and a pooled odds ratio of 11.28 (95% CI: 2.90–43.94, $p < 0.001$), using Lagos (0%) as reference. These findings highlight the need for targeted surveillance and the integration of *B. canis* in differential diagnoses. The results support a One Health approach, urging greater recognition of *B. canis* in both veterinary and public health frameworks.

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Antimicrobial resistance in E. coli isolated from the faeces of hedgehogs at a rescue station

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In 2020, faecal samples were taken from 23 hedgehogs (Erinaceus europaeus and Erinaceus roumanicus) housed at a wildlife rescue centre. These were either injured animals or juveniles with low body weights that would not have allowed them to survive the winter. The hedgehogs were examined by veterinarians, who determined the therapeutic protocol based on their condition. This protocol commonly included conservative or surgical intervention, accompanied by systemic antimicrobial therapy. Our study aimed to culture E. coli from the feces of hedgehogs (three E. coli isolates per individual) that had been treated with antibiotics, and to identify their antimicrobial resistance phenotypes and genotypes. Multidrug-resistance via disc diffusion test was demonstrated in at least one strain isolated from 14 hedgehogs (14/23; 61%) specifically in 37 of the 69 isolates (54%). Most isolates were resistant to ampicillin (50/69; 72%) and nalidixic acid (37/69; 54%). Genomic analysis via WGS confirmed the presence of genes encoding resistance to beta-lactams (blaTEM-1B, blaTEM-1D), aminoglycosides [aac(3)-IIa, aadA1, aadA2, aadA5, aph(3')-Ia, aph(3')-Ic, strA, strB], phenicols (cmlA1, catA2, floR), sulphonamides (sul1, sul2, sul3), trimetoprime (dfrA1, dfrA12, dfrA14, dfrA17, dfrA25), tetracyclines [tet(A), tet(B)] and quinolones (qnrB2). Our findings indicate that hedgehogs could potentially act as a source of multidrug-resistant E. coli when they are released back into the wild.

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European hedhehogs in a rescue station sharing multidrug-resistant phylogroup B1 and F E. coli strains

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Twenty-one Escherichia coli isolates from fecal samples of hedgehogs hospitalized in a rescue station during the year 2020 were analyzed by WGS to evaluate the prevalence of STs, serotypes, replicon types, resistance and virulence genes and the occurrence of potentially risk lineages. The isolates were sequenced by Illumina HiSeq. The acquired data were analyzed by online typing tools and maximum-likehood phylogenetic tree was constructed. Despite the intense AM treatment, no ESBL-producing strain was detected, although most (17/21) isolates showed multidrug resistance and all except 2 carried blaTEM-1 gene. Replicons IncF, IncI1, IncX1 and/or IncX4 were carried by the isolates with the widest resistance profiles, suggesting their responsibility for AMR spread between strains colonizing different animals in the station. Most animals carried strains from both B1 and F phylogenetic group, represented by several STs: ST457, ST624, ST224, ST212, ST162 and ST58. The phylogenetic analysis demonstrated sharing of closely related strains between animals. In conclusion, our data show that wildlife animals kept in a rescue station for medical treatment, although not in a direct contact, are colonized by the same E. coli strains. The intensive AM therapy keeps high selective pressure facilitating the emergence and transmission of MDR strains. The findings remind the need of standardized biosafety rules for such facilities, which may be another source of AMR for both humans and wildlife.

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"One Health" perspective on distribution of ESKAPE pathogens in companion animals: potential risk for human health

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ESKAPE bacteria (*Enterococcus faecium* - EC, *Staphylococcus aureus* - SA, *Klebsiella pneumoniae* - KP, *Acinetobacter baumannii* - AB, *Pseudomonas aeruginosa* - PA, *Enterobacter* spp. - ES) are major opportunistic pathogens, primarily involved in nosocomial infections. This study evaluates the potential role of dogs and cats as reservoirs of ESKAPE bacteria for humans.
Between January 2024 and May 2025, 516 clinical samples from pets were analysed at IZSLER laboratories in Emilia-Romagna, Italy. Sample types included urine, organs from carcasses, auricular, cutaneous and "Other" (vaginal, ocular, etc.) swabs. ESKAPE pathogens were isolated in 115/516 samples (22.29%). PA was the most frequent (85/115, 73.91%), followed by ES (11/115, 9.57%), SA (10/115, 8.70%), EC (5/115, 4.35%) and KP (4/115, 3.48%). AB was not detected. PA was mainly found in auricular and cutaneous swabs, while ES in urine and internal organs. EC, SA and KP were distributed across different sample types.
Our findings highlight that ESKAPE pathogens were present in approximately 20% of clinical samples from pets. Notably, PA was the most prevalent ESKAPE, well known for its concerning antimicrobial resistance profiles, often associated with treatment failures. Infections involving exposed anatomical sites (e.g., skin, ears and eyes), along with bacterial shedding through bodily fluids (e.g., urine), may facilitate the transmission of ESKAPE pathogens to owners, especially if immunocompromised.

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CLINICAL, PATHOLOGICAL AND VIROLOGICAL DESCRIPTION OF A VIRULENT SYSTEMIC FELINE CALICIVIRUS INFECTION IN ONE KITTEN WITH FOOTPADS OEDEMA

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Feline calicivirus (FCV) is widespread in multi-cat environments causing acute upper respiratory tract disease (FCV-URTD) in cats. FCV also causes clinically distinct outbreaks of virulent systemic disease (FCV-VSD), mainly in adults, involving multiple organs with vasculitis, dermal oedema, multifocal ulceration of the skin and footpads, jaundice and pneumonia. This study reports a fatal case in a 20-days old female Maine Coon kitten from an Italian breeding, in which a previous outbreak of FCV-URTD occurred. The kitten showed dyspnoea and swollen footpads. After spontaneous death, necropsy revealed histopathologic findings as severe pneumonia with diffuse alveolar damage and dermal oedema of the footpads. Immunohistochemistry detected FCV antigens in pulmonary macrophages. Viral RNA was identified using a real-time RT-qPCR in oropharyngeal swab, trachea, larynx, lung, skin, heart, brain, kidney, spleen and liver samples, with the highest amount detected in the lung (9×10^5 copies of viral RNA/μL). The nucleotide sequences of the viral ORF2 gene amplified from all positive samples were identical and phylogeny showed no clustering based on clinical, geographical and temporal basis. In conclusion the case presented showed typical symptom of FCV-VSD in a kitten and no mutations that clearly distinguished FCV-URTD from FCV-VSD phenotypes were identified. Funded by the EU - NextGenerationEU – NRRP_PRIN 2022 PNRR COVDC P2022FR49N (CUP N. J53D23014340001).

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Loss of hemagglutinating activity in a canine parvovirus 2b strain

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Canine parvovirus type 2 (CPV-2) is a member of the *Parvoviridae* family, genus *Protoparvovirus*, and species *Carnivore protoparvovirus 1*. Hemagglutination (HA) is a critical biological feature of CPV-2, serving as the basis for the hemagglutination inhibition (HI) assay, which quantitatively measures serum antibody levels and is widely used to evaluate the immune status of dogs following vaccination or natural infection. Additionally, HA has been used for direct diagnostics for a long time, before the introduction of molecular assays. The loss of hemagglutinating activity due to amino acid substitutions (such as at position 377) and/or deletions in the N-terminus of the VP2 protein has been reported in the literature. In this study, a non-hemagglutinating (non-HA) CPV-2b strain is described. Although the strain exhibited high-titer replication in A72 cells, as demonstrated by the presence of viral antigens detected by immunofluorescence assay and high titers of viral DNA, quantified by a TaqMan assay, the virus was unable to agglutinate porcine erythrocytes under the standard laboratory conditions for HA. Viral genome sequencing revealed unique mutations in VP1 and VP2, which have not been associated with the loss of HA phenotype.

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Detection of Helicobacter spp. in oropharyngeal swabs of stray cats

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Helicobacter spp. comprises Gram-negative, microaerophilic, curved to spiral-shaped bacteria belonging to the Helicobacteraceae family and is regarded as the most significant bacterium in the digestive system of humans and animals. Helicobacter species can be found not only in the stomach but also in the oral cavity of cats, suggesting a potential role in transmission of the infection to other animals or to humans. Helicobacter spp. has been identified in the oral cavity, stomach, intestines, liver, and pancreas of human patients suffering from gastric disorders. The present study aimed at assessing the prevalence of Helicobacter spp. in oropharyngeal swabs of cats. A total of 122 stray cats, aged between 1 and 5 years and presented at the hospital of the Department of Veterinary Medicine, University of Bari, were sampled between March and November 2024. The samples were screened using a PCR targeted to 16S rRNA gene of Helicobacter spp. Overall, 78 out of 122 samples (63.93%) tested positive for Helicobacter spp. On sequencing, 13 (16.7%) samples displayed the highest nt identity (98.5-100%) for H. winghamensis, a bacterial species originally identified in humans and subsequently in chicken and dogs. Considering the potential zoonotic transmission, surveillance of Helicobacter spp. in cats is necessary to thoroughly assess this risk. Moreover, investigating the role of Helicobacter spp. in gastrointestinal disorders of cats is required

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AIRBORNE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) IN A NATURAL SHEEP FARMING ENVIRONMENT IN SLOVENIA

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Mycobacterium avium subsp. paratuberculosis (MAP), the causative agent of paratuberculosis, remains a persistent issue in ruminant farming, with implications for both animal health and possible zoonotic transmission. We conducted air sampling and qPCR-based detection of MAP at a Slovenian sheep farm rearing the indigenous Istrian Pramenka breed, known for organic milk and cheese production (550 lambs, 25,000 L milk, 6,500 kg cheese annually).

Air samples were collected using the Coriolis+ Microbial Air Sampler (Bertin Technologies) at a flow rate of 300 L/min for 30, 60 and 90 min at two locations (A, B) within a sheep barn housing animals and lacking active ventilation. Samples were collected into PBS with Triton X. DNA was extracted using two commercial kits: iHelix Complex (IMMT, Slovenia) and DNeasy Blood & Tissue Kit (Qiagen). qPCR was performed for MAP, but also for Brucella sp., Listeria sp., Salmonella sp. and Coxiella burnetii. One sample (A-90) was positive for C. burnetii and four (A-30, A-60, A-90 and B-90) for MAP. Only iHelix-based extractions yielded detectable DNA, indicating the need for combined mechanical and enzymatic lysis.

Our results demonstrate the feasibility of airborne MAP detection in livestock environments and the importance of optimized DNA extraction. The Coriolis+ sampler was effective but limited by battery capacity. This work sets the stage for extended temporal monitoring of MAP and other pathogens in correlation with animal health status.

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Establishment of a co-culture system with primary porcine respiratory epithelial cells and porcine alveolar macrophages under air-liquid interface conditions

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Respiratory infections in pigs pose a major health concern for animals and entail high economic losses. The respiratory epithelial barrier represents the first line of defense against respiratory pathogens by removing harmful particles using the mucociliary clearing mechanism and initiating an immune response.
Air-liquid interface (ALI) cultures with primary porcine respiratory epithelial cells (PREC) provide a versatile *in vitro* model to study host-pathogen interactions. Under ALI conditions, PREC build a pseudostratified epithelial barrier with mucus-producing and ciliated cells within a few weeks. However, this model disregards the influence of resident immune cells. Thus, our aim was to establish a co-culture system consisting of PREC and porcine alveolar macrophages (PAM).
PAM were isolated from bronchoalveolar lavage fluid and were sown on well-differentiated PREC under ALI conditions in a trans-well system. After attachment of the PAM for two hours, the co-culture was treated with lipopolysaccharide or infected with *Streptococcus suis*, a common bacterial pathobiont in the porcine respiratory tract. The presence of PAM was verified by fluorescence microscopy and the immunological capability of the system was assessed by analyzing cytokine expression and secretion.
This co-culture system greatly expands the investigation of host-pathogen interactions and can be used to study bacterial as well as viral respiratory infections in all possible species.

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Fatal tick-borne encephalitis (TBE) in alpacas (Vicugna pacos) infected with a European TBE virus subtype in Austria

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Tick-borne encephalitis (TBE) is caused by infection of the central nervous system with TBE virus (TBEV), an arbovirus in the *Flaviviridae* family. The TBEV transmission cycle includes hard ticks as vectors and mammals and birds as hosts. The TBEV transmission cycle includes hard ticks as vectors and mammals and birds as hosts. In 2023, 22 of 29 European union countries reported TBE cases in humans, according to the ECDC. In Austria, TBEV has been considered endemic for decades.

In spring and early summer 2022, two fatal cases of TBEV-infection in Huacaya alpacas with neurological signs were diagnosed independently in the south-eastern federal states of Austria by virological and pathological analyses. Sequencing, BLAST-nucleotide comparison (NCBI) and phylogenetic analyses of partial NS4b and NS5 genes revealed closest relationship to the Western (European) (TBEV-Eu) subtype, and a nucleotide identity of 95.9% and 97.9%, respectively, between both cases. The phylogenetic analyses indicated that the two TBEV strains are closely related, but are reassortants of TBEV-Eu strains from different countries. Differential diagnostic examination for other neurological pathogens yielded negative results.

To date, the prevalence of TBE and TBEV-infection in camelids remains unknown. Our findings highlight that TBEV should be considered and investigated as potential cause of neurological signs in alpacas and other camelids in the future.

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Surgical site infection in clean and clean contaminated procedures in veterinary practice – a multicentre study of perioperative practices in Portugal

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Surgical site infection (SSI) increases morbidity and antimicrobial use. It is essential to assess antimicrobial practices, especially in clean and clean-contaminated surgeries (C/CCS), where their necessity is often debated. This study assessed perioperative practices and antimicrobial use in eight Portuguese veterinary facilities, alongside SSI monitoring.

Three clinics and five hospitals were asked to complete a questionnaire on 20 consecutive surgeries performed in ASA I–II patients undergoing C/CCS. Patients were monitored for 30 days for SSI.

A total of 149 surgeries were included (64.4% dogs, 35.6% cats), mostly elective gonadectomy procedures (81%). Trichotomy (TRI) was mostly done with reusable clippers/blades (96.6%), often without prior disinfection (61.1%). TRI to incision time was 10–19 min in 61.7%, with antiseptic contact of 5–9 min in 71.1% of patients. One hospital routinely performed TRI in the surgical room. Pre/intra- postoperative antimicrobials were used in 48.3% and 24.8% of patients, including in elective gonadectomies, in five and three practices, respectively. Two out of four suspected SSI were confirmed by culture. Postoperative antimicrobial use was not associated with a significant reduction in SSI frequency ($p > 0.05$, Fisher's exact test).

While confirming low SSI incidence in C/CCS, this study reveals ongoing needs to improve antimicrobial stewardship and standardize surgical protocols in veterinary practice.

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From waste to safe fertilizer: Vermicomposting as a strategy for microbial risk mitigation

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Despite their rich nutrient content, animal manure, sewage sludge and agricultural by-products raise concerns over soil contamination and microbial safety. Vermicomposting (VC) is a sustainable option for managing organic residues that may reduce pathogens such as *Escherichia coli* and *Salmonella* sp. This study evaluated their presence during VC using a mixture of vine prunings, sewage sludge, cattle slurry solid fraction, grape pomace and grape stalks. Samples were collected at the beginning (T0) and at the end, after 90 days (TF). For *E. coli*, samples were cultured on CHROMagar™CC and incubated at 37℃ for 24 h and quantified as CFU/g. For *Salmonella* sp., samples were pre-enriched in buffered peptone water and incubated in Modified Semi-Solid Rappaport-Vassiliadis medium at 42℃, followed by confirmation on selective media. Results showed a reduction in *E. coli* from 1.21×10^{2} CFU/g (T0) to absence at TF. *Salmonella* sp. was not detected at both time points. These results highlight the potential of VC as an efficient strategy to reduce microbial risk and enhance the safety of organic amendments. Acknowledgments: Vine&Wine PT is funded by NextGeneration UE programme, through PRR Plan (project no. C644866286-00000011). This work was supported by FCT: UID/04033/2023 and LA/P/0126/2020 (DOI:10.54499/LA/P/0126/2020). ENG and CM thank PRR and European Funds NextGeneration EU for their fellowship grant (BI/UTAD/15/2023 and BI/UTAD/30/2024).

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Cat Owners' Knowledge, Attitudes, and Practices on Feline Vaccination in the Lisbon Metropolitan Area, Portugal

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Vaccination is a cornerstone of feline preventive healthcare, but its success depends on owner compliance with recommended protocols. This study evaluated the knowledge, attitudes, and practices(KAP) of cat owners regarding feline vaccination in the Lisbon Metropolitan Area, Portugal.
A structured questionnaire was made available (April to October 2024) online and in paper format at veterinary clinics, covering owner/cat demographics and feline infetious diseases(FID) vaccination KAP.
Of 114 valid respondents, most were female (84.2%) and held higher education degrees (75.4%). The majority (88.6%) reported vaccinating their cats and recognised its importance for feline health. Annual boosters were the most commonly reported schedule (60.5%). Perceived risk and severity of FID were key drivers of vaccination. Although 98.2% claimed to understand the term "vaccine", only 43.3% correctly identified its use in preventing viral infections. Fewer than 15% of owners reported knowledge of FID, and 24% were unaware of which diseases their cats were vaccinated against. Veterinarians were identified as the most trusted source of information, with 71.9% rating them as "extremely reliable", highlighting their key role in owner education.
Despite high adherence to feline vaccination, this study revealed important knowledge gaps that may affect vaccination practices and contribute to misconceptions, reinforcing the need for improved veterinary communication and targeted cat owner education.

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Antimicrobial use patterns in Spheniscus penguins: A multi-Institutional analysis using the Species 360 Zoological Information Management Software

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Understanding antimicrobial use and stewardship in Spheniscus penguins is key for their health management and supports One Health, as these species bridge wildlife, ecosystem and public health. This study aimed to describe the antimicrobial use in Spheniscus penguins under human care.
Global data was extracted from the Species 360 ZIMS database in April 2025. Treatment estimates were based on the 'number of records' variable, and thus were subject to the accuracy and completeness of data entry.
Of the 28,361 records, 45.8% (n=12,979) were of antifungals, 30.8% (n=8,742) of antibiotics, and 20.7% (n=5,875) of antimalarials. Antifungals included azoles (76.2%, mainly itraconazole [77.7%] and voriconazole [17.3%]), terbinafine (an allylamine, 19.9%), amphotericin B (a polyene, 2.7%), and flucytosine (a pyrimidine analogue, 1.2%). Prescribed antibiotics included fluoroquinolones (54.7%, mainly enrofloxacin [82%] and marbofloxacin [12%]), beta-lactams (19.0%, mostly amoxicillin-clavulanate [59%] and third-generation cephalosporins [17%]), and tetracyclines (13.9%, mainly doxycycline [93%]). Primaquine (75.9%) and chloroquine (23.4%) were the most common antimalarials.
This study reveals the use of medically important antimicrobials (AMEG-B) in Spheniscus penguin healthcare, highlighting the need for targeted stewardship. Monitoring use in this setting is vital to identify research gaps and protect endangered species while safeguarding antimicrobials within a One Health framework.

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Coronavirus strains circulating in dogs in Italy in the period 2022-2024

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In recent years, new hypervirulent coronavirus (CoV) strains, highly pathogenic to humans (e.g. MERS-CoV, SARS-CoV-2) have emerged. Also, canine/feline-like CoVs have been reported repeatedly in the respiratory tract of children with respiratory disease, highlighting the importance of CoV spillovers from animal sources to humans. Information about the CoV strains circulating in dogs is still limited. For this purpose, the presence of canine CoV (CCov) was investigated in samples collected from 916 dogs during the period 2022-2024. Extraction, detection, and molecular characterisation of CCoV strains was done using standardized molecular assays (Gouilh et al. 2011; Decaro et al. 2004). A subset of positive samples was sequenced using the ONTTM platform (Oxford Nanopore Technology). Overall, CCoV was detected in 330/916 (36%) samples. Based on the virus titer and RNA quality, the genome of four CCoV strains was reconstructed using a sequence-independent approach for enrichment of viral RNA. Two strains were similar to type I CCoV, while 1 strain clustered with type 2a CCoV, and another strain showed inconsistencies in the phylogenetic analysis, suggesting a chimeric genome make-up.
Further investigations and increased surveillance are required to assess the repertoire of CoV genetic diversity in animals and track the origin of zoonotic CoVs.

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Sporothrix schenckii and Candida parapsilosis cutaneous co-infection in a dog imported from Brazil to Portugal

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Emerging fungal pathogens and global animal travel pose increasing challenges in veterinary diagnosis. We report a six-year-old male Shih-Tzu, brought to Portugal from Brazil, exhibiting chronic ulcerative and nodular skin lesions on the left thigh and inguinal region. The animal had previously received multiple empirical antibiotics with minimal response. Prior histopathology and bacterial culture revealed nodular granulomatous dermatitis and Streptococcus canis. Despite temporary healing, lesions recurred after surgical excision, and lymphadenopathy persisted. At referral, skin cytology showed pyogranulomatous inflammation, rare intracellular fungal elements, and secondary pyoderma. Histopathology confirmed chronic granulomatous dermatitis, however failed to identify microorganisms, with negative PAS/Grocott stains. Deep skin fungal culture (Sabouraud agar,25ºC/37ºC) and ITS gene sequencing identified Candida parapsilosis and Sporothrix schenckii.

The dog exhibited severe side effects to itraconazole and developed multiple skin nodules and respiratory symptoms, raising suspicion of fungal dissemination. Cytology and culture of new lesions confirmed the persistence and dissemination of Sporothrix. Due to disease progression, euthanasia was elected.

To our knowledge, this is the first documented case of co-infection by Sporothrix and Candida in a dog. Both are uncommon pathogens in Europe, highlighting the need for awareness of imported zoonoses within a One-health framework.

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Animals as Potential Carriers of Candida auris: A Preliminary Survey

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Candida auris is an emerging fungal pathogen that poses a growing threat to global public health. Due to the ongoing rise in infections, the World Health Organization classified it in 2022 as a fungal pathogen of critical priority. *C. auris* exhibits resistance to multiple antifungal classes, efficiently colonizes skin, mucous membranes, and inanimate surfaces, and tolerates extreme conditions, including temperatures up to 42 ℃ and high salinity. These traits support its persistence in healthcare settings and suggest a potential for adaptation to new environments and hosts, including animals. This preliminary study aimed to investigate the potential role of animals as reservoirs of *C. auris*. A total of 205 oro-fecal samples from mammals, birds, and reptiles submitted for routine diagnostics were analyzed. Samples underwent selective enrichment in CABroth™, followed by plating on Selective auris Medium (SAM), and incubation at 41 ℃ for 2 and 7 days, respectively. Colonies were Gram-stained and identified using MALDI-TOF mass spectrometry. From five samples (2.4%) — from 2 cats, 2 chickens, and 1 rabbit— C. auris was confirmed with high-confidence MALDI-TOF identification. Molecular characterization based on ITS1/2 sequencing and antifungal susceptibility testing are ongoing. These preliminary findings underscore the need for a One Health approach to better understand the ecology and spread of this still partly unknown pathogen.

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Button-like Pulmonary Lesions in a Hooded Crow co-infected with Aspergillus fumigatus and West Nile Virus

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In August 2024, a hooded crow (*Corvus cornix*) was found dead in an urban park in Pavia, Northern Italy, and submitted for necropsy within a regional avian wildlife surveillance program. Gross examination revealed multiple firm, pale, button-like nodules in the lungs, without other macroscopic lesions. Cytology showed necrotic debris, macrophages, and occasional septate fungal hyphae. Histopathology confirmed multifocal granulomatous inflammation with Grocott-positive, septate, dichotomously branching hyphae. Mycological culture and MALDI-TOF mass spectrometry identified *Aspergillus fumigatus*. PCR testing was negative for Influenza A and Usutu virus but detected West Nile virus (WNV) Lineage 2 in the brain, heart, spleen, and kidney.

Clinical signs and predisposing factors could not be assessed prior to death, making it impossible to determine the sequence of infection or a direct relationship between the two identified pathogens. However, the chronicity of the fungal lesions suggests a prolonged disease process that may have preceded, and potentially predisposed the bird to fatal WNV infection.

This case underscores the potential synergism and complexity of virus–fungus interactions in free-living birds and highlights the importance of applying integrated diagnostic approaches to wildlife health surveillance as well.

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Isolation and Characterization of Bacteriophages against Aeromonas salmonicida

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*Aeromonas salmonicid*a subsp. *salmonicida*, is the causative agent of furunculosis, an infectious disease of fish. Antibiotics are commonly used to treat the disease. Hence, the aim of the project was to isolate and characterize phages that are able to lyse isolates and offer a potential alternative treatment method. For the study, 24 water samples were collected from aquacultures in Germany and France and investigated for the occurrence of phages using two different isolation methods. In total, 90 phages targeting *A. salmonicida* were isolated and five of them were analyzed in more detail. Illumina NGS sequencing and electron microscopy revealed that three had genome sizes between 170 and 174 kb, with a GC-content of 44% and a high sequence identity to each other. The fourth phage was 237 kb in size and had a GC-content of 37%. The fifth phage (46.5 kb, GC-content 56%), was regarded as temperate based on sequence analysis results, while the other four phages belong to the Straboviridae family and were classified as lytic. Host range analysis confirmed that these four phages cause bacterial lysis in a broad range of *A. salmonicida*-isolates, as well as in some other *Aeromonas* species. In summary, four different phages belonging to the family Straboviridae with a lytic potential against *A. salmonicida* were isolated in the course of the study, but further studies are still required to characterize their potential suitability for the treatment of furunculosis.

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Distribution and historical occurrence of Ophidiomyces ophidiicola across wild Italian snakes

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Ophidiomycosis, caused by the fungus *Ophidiomyces ophidiicola* (Oo), poses a growing threat to snake conservation, but still remains poorly documented across Europe. To clarify and characterise its distribution in Italy, we performed molecular screening and histology for Oo on both contemporary samples from wild populations and historical museum specimens. Our study analysed 423 snakes across 17 species from diverse Italian regions, detecting Oo in 32 individuals spanning five species. Detection and infection predominantly involved semi-aquatic snakes, especially *Natrix tessellata*. Additional molecular screening for *Paranannizziopsis* spp. in 13 Oo-negative snakes with clinical lesions yielded negative results. Interestingly, Oo Clade I was primarily detected in older museum samples, whereas contemporary specimens predominantly harboured Oo Clade II, indicating a shift or evolution in epidemiological patterns. Despite the opportunistic nature of the sampling, our data document a long-standing presence of Oo and a distribution mostly confined to northern Italy and its great lakes. Continuous monitoring and standardised surveillance protocols are essential for better understanding the distribution and ecological impact of snake fungal diseases.

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Serological and molecular investigation of Scutavirus testudinidalpha3 in free-ranging Sardinian Testudo graeca

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Testudinid alphaherpesviruses comprise four distinct but related chelonian viruses. Scutavirus testudinidalpha3 (TeHV3 – formerly Testudinid alphaherpesvirus 3) is studied due to its association with stomatitis and glossitis along with neurological signs, often fatal in Testudo hermanni. Current knowledge primarily comes from captivity, with limited data on free-ranging tortoises. Investigating these populations is crucial to understand TeHV3's disease ecology and its clinical significance under natural conditions. Between 2019 and 2023, oral swabs for PCR and blood samples for ELISA serology were collected from 157 free-ranging T. graeca in Sardinia, Italy. Animals were immediately released after sampling, although not all provided both types of samples. Twenty individuals were captured more than once. PCR detected TeHV3 in 4 tortoises while 153 were negative (prevalence 2.6%). Serologically, 48 were positive, 68 negative, and 15 doubtful (36.6% of positives). Only one PCR-positive tortoise was also seropositive. Among recaptured individuals, 12 remained negative, seven positive; one seroconverted with its PCR results changing from negative to positive. The absence of significant mortality or clinical signs indicates TeHV3 may not cause primary disease naturally in this species, potentially occurring secondarily to immunosuppressive noxae. Serology was crucial for accurately assessing the epidemiology, demonstrating a prevalence not evident through molecular testing alone.

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Field spectrum of genotype II african swine fever: clinical and pathological insights from 30 Romanian outbreaks

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ASF threatens pig production, yet European field data are sparse. We examined 30 Romanian outbreaks confirmed in 2020—commercial, type A and backyard—to characterise genotype II disease. In nine holdings pigs were still alive, allowing direct observation and necropsy of 13 freshly culled animals.
Clinical signs: lethargy, recumbency, dyspnoea/cough, cyanosis; abortions and cooling behaviour in large herds. Still, three backyard sites and ≈25 % of pigs in one commercial unit appeared healthy, showing marked variability.
Gross lesions: thoracic effusions, pulmonary oedema/congestion (sometimes hydropericardium); "blood-clot" lymph nodes; enlarged friable spleen; renal petechiae; serosal haemorrhages of gall- and urinary bladder; intestinal necrosis. Two pigs had hepatomegaly with haemorrhagic colon. Lesions reflected overlapping acute and sub-acute courses.
Epidemiology: veterinarians reported 40–50 % mortality in backyard units and up to 70 % in type A farms before culling—figures typical of sub-acute ASF. The coexistence of healthy and lesion-free pigs in infected pens questions uniform lethality and hints at lower contagiousness at low doses.
This first cross-system Romanian field study broadens the recognised lesion spectrum of genotype II ASFV and shows that surveillance must look beyond sudden high mortality or hallmark signs. A clearer grasp of host-virus interaction is vital for risk-based control and training.

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PREVALENCE OF AVIAN INFLUEZA AND THE DISTRIBUTION OF VIRAL STRAINS IN ROMANIA IN THE PERIOD 2000-2024

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At the intersection of three Eurasian–African flyways, Romania is a key sentinel for avian influenza (AI). Collating WOAH, ADIS and national data (2000-2024) we logged 428 highly pathogenic (HPAI) and 77 low-pathogenic events, 88 % in wild birds. Three waves dominated:
• 2005-06 H5N1 (clade 2.2): emerged in the Danube Delta, spread to >150 villages; >60 000 poultry culled in Tulcea, Brăila, Constanţa.
• 2016-17 H5N8 (clade 2.3.4.4b): 115 detections in 11 counties; swift stamping-out kept farm losses low.
• 2020-24 endemic H5N1 (clade 2.3.4.4b): began in farms (18 000 & 180 000 birds) but since 2021 centers on waterfowl. Dec 2023–Mar 2024 brought 25 swan cases along the Black-Sea/Danube coast; backyard spill-over persists (47 birds, Constanţa, Oct 2024).
H5Nx made 63 % of isolates (H5N1 46 %, H5N8 17 %); sporadic H7N7 and LPAI H9N2 came from backyard flocks. Clade 2.2 was replaced by 2.3.4.4b via repeated migratory introductions, with little spread in commercial systems. Hotspots linger in the Danube Delta and Black-Sea littoral; large-farm outbreaks cluster on the Transylvanian plateau.
Conclusion: Two decades of surveillance reveal a shift from explosive farm epidemics to sporadic wildlife-driven incursions. Persistent H5N1 in swans/backyards and the plasticity of clade 2.3.4.4b demand integrated wild-bird–poultry monitoring, vaccine trials in high-risk counties and firm data sharing along the Lower Danube.

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Diverse Genetic Profiles and Environmental Transmission of Escherichia coli on a dairy farm in Northen Italy: Insights from RAPD-PCR Analysis

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To investigate the genetic relatedness of *Escherichia coli* isolated from bovine milk and environmental sources on a dairy farm in northern Italy, we employed RAPD-PCR technique . We collected 15 milk samples, from clinical mastitis, and 40 environmental samples from bedding, water, milking parlour and feces from cows and calves, which were plated onto MacConkey agar. A total of 33 *E. coli* isolates were identified using MALDI-TOF-MS. RAPD-PCR analysis, using the ERIC1 primer, was performed on 26 representative strains, and their banding patterns were visually compared to assess genetic similarity. Fifteen distinct genetic profiles (A–P) were identified. Interestingly, four profiles included isolates from both milk and environmental sources, suggesting clonal lineages circulating between the mammary gland and farm environment. The largest cluster (A) comprised four milk isolates and strains from bedding and calf feces, indicating a possible environmental reservoir contributing to mastitis cases. No water isolates matched milk strains, implying water was not a significant source of infection in this setting. These findings enhance our understanding of *E. coli* diversity and epidemiology on dairy farms and support improved control strategies. RAPD-PCR with ERIC1 proved to be a rapid, practical, and cost-effective method for tracking strain distribution in dairy herd studies.

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Occurrence and genomic characterisation of Streptococcus equi subsp. zooepidemicus in Martina Franca (Italy) and Andalusian (Spain) donkey populations

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In the context of the European Green Deal, donkey breeding has been garnering renewed attention due to their suitability for new forms of production, including meat, milk, and tourism, thereby allowing for the growth of small rural businesses (Regulation (EU) 2021/1119). Our study investigated the occurrence of Streptococcus equi subsp. zooepidemicus (SEZ), in genital samples from healthy animals of two endangered donkey breeds - Martina Franca (Italy) and Andalusian (Spain). An overall SEZ prevalence of 16.67% (95% CI: 10.41-22.92) was recorded, with animals from farms not adopting biosecurity measures being at a significantly higher risk of infection (p-value = 0.006, OR = 10.69). Through multilocus sequence typing (MLST) analysis of 10 representative SEZ isolates, six novel sequence types (ST) (ST-N1 to ST-N6) were identified in animals from both countries. Additionally, two strains from an Italian donkey farm matched the already known ST-30, which has been previously associated with respiratory disease in mares from the UK and with mastitis in small ruminants from Spain. This finding indicates that ST-30 has the potential to circulate among different animal species and within various tissues. This is the first report of SEZ infection occurring in the genital tract of healthy donkeys possibly acting as reservoirs of SEZ. The discovery of SEZ, which is also zoonotic, in donkeys employed as food producers and onoturism requires attention from a “One Health” perspective.

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SPHAEROPSIDIN A AND B AS POTENTIAL ANTIVIRALS AGAINST FELINE CORONAVIRUS

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Objectives: Feline coronavirus (FCoV) commonly provokes mild infection in cats, but by genomic mutations, FCoV infects macrophages causing feline infectious peritonitis. Hence, to control FCoV infection, antiviral therapies involving original mechanisms of action were developed. During coronaviruses (CoVs) infection, including FCoV, the activation of aryl hydrocarbon receptor (AhR), a transcription factor stimulated by various substrates, modulates microbial defence, by releasing cytokines and regulating immune response. Interestingly, fungal secondary metabolites (SMs) revealed their potential antiviral activity against CoVs. Herein, the antiviral activity of pimarane diterpenes, like sphaeropsidin A (SphA) and its analogue sphaeropsidin B (SphB), produced by fungi belonging to the genus Diplodia, was evaluated during FCoV infection.
Methods: Chromatographic techniques, bioscreen in vitro, immunofluorescence assay, molecular docking.
Results: Following FCoV (FECV) infection in feline (CRFK) and in canine (A72) cells, non-toxic doses of SphA and SphB increased cell viability, and reduced virus yield as well as the expression of viral nucleocapsid N protein. Moreover, during infection, SphA and SphB downregulated AhR and CYP1A1, an AhR downstream target protein. Notably, a high sequence identity of the 3D structural models of human and feline AhRs has been emerged by bioinformatics analysis.
Conclusions: Both SMs showed a potential anti-FCoV activity, by modulating AhR pathway

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AHR AND FPR2 AS NEW ANTIVIRAL TARGETS FOR CANINE CORONAVIRUS INFECTION

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Traditional antiviral drugs mainly act targeting viral replication, but to obtain a wide-spectrum application, and a diminished attitude for promoting the acquisition of drug resistance, host-targeted compounds have been developed. Canine coronavirus (CCoV) causes enteritis, but recombination may generate new lethal strains as those found in puppies. Furthermore, new alphacoronaviruses were detected from humans, highlighting the ability of CCoV to overcome the species barrier. Hence, antiviral compounds based on new mechanisms of action has been studied against CCoV. The aryl hydrocarbon receptor (AhR) and formyl peptide receptors (FPRs) have recently emerged for their activity in control of the immune response in viral infections. Here, the involvement of AhR and FPR2 during CCoV infection in A72 cells was investigated by in vitro and in silico approaches.
During infection, in the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a typical AhR activator, an intensified CCoV replication was observed, while CH223191, a selective AhR inhibitor, provoked an opposite modulation. WRW4, a specific FPR2 inhibitor, increased CCoV replication. Whereas, a reverse trend was induced by HP2-20, an agonist of FPR2. In silico studies supported these results.
Conclusions: Overall, CCoV replication is related to AhR as well as to FPR2, suggesting them as interesting targets to develop new drugs to fight CoVs infection.

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Neglected Hepatotropic Viruses in Carnivores: Evidence from Northern Italy

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Emerging infectious diseases in humans are often of zoonotic origin, urging the study of animal viruses essential to identify potential reservoirs of human pathogens. Recently, novel hepatotropic viruses such as Hepadnaviridae, Hepeviridae and Hepatoviruses have been identified in various animal species. The aim of this study was to investigate the presence of novel and emerging hepatotropic viruses in carnivores from the Lombardy region. Between 2024 and 2025, we analyzed 264 liver samples collected from 117 domestic and 147 wild carnivore carcasses submitted for routine diagnostics, and tested for HEV, Hepadnaviruses, and Hepatoviruses using broadly reactive primers. One liver sample (red fox) tested positive for HEV. Six samples (5 cats, 1 beech marten) tested positive for Hepadnaviruses, and a DCH-specific qPCR was used for confirmation. All samples tested negative for Hepatoviruses. These preliminary data indicate a low prevalence of HEV in the region. By using an Artic-like PCR strategy the full genome of 3 DCH strains was obtained by Oxford Nanopore Technologies (ONT) platform, falling within Clade A2. The detection of Hepadnavirus in cats (5,41%) is consistent with the circulation of DCH in the literature; Sequencing of the detected viruses and histopathological analysis of the positive samples are currently ongoing. Understanding the dynamics of these viruses in carnivore populations is crucial for assessing potential zoonotic risks and their impact on public health.

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Exploring the presence of Staphylococcus spp. in dogs and their owners: A study from two Italian Veterinary Hospitals

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Staphylococcus spp., common skin and mucosal commensals in humans and animals, can act as opportunistic pathogens. This preliminary study aimed to detect and compare nasal *Staphylococcus* spp. in dogs and owners from two Italian Veterinary Teaching Hospitals (Turin and Naples), using nasal swabs collected during dog surgeries.
Approximately 100 pairs were sampled per hospital. Isolated colonies were identified by MALDI–TOF MS. A low concordance (8%) of the same *Staphylococcus* species between owners and dogs was observed in Naples, while a higher concordance (27%) was found in Turin, with a statistically significant difference ($p < 0.05$). In Naples, the most common Staphylococcus species from pets was *S. pseudintermedius* (n=24), followed by *S. aureus* (n=11). Among owners, *S. epidermidis* (n=52) and *S. aureus* (n=24) were most frequent. Similarly, in Turin, *S. pseudintermedius* (n=19) was most common in dogs, followed by *S. aureus* (n=17), while in owners, *S. aureus* (n=36) and *S. epidermidis* (n=32) predominated. Matching species were found in eight and twenty-four dog-owner pairs in Naples and Turin, respectively, with *S. aureus* being the most frequently identified species in the pairs of both veterinary hospitals. In conclusion, this study emphasizes the importance of *S. aureus* and *S. epidermidis*, primary colonizers of the human nasal microbiota, in dog-owner pairs, suggesting a higher likelihood of human-to-animal transmission.

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Parvovirus strains circulating in dogs in Italy, 2023-2024

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Canine parvovirus (CPV-2) is an important pathogen associated with severe gastroenteritis in young dogs. Shortly after the emergence of CPV-2 in the 1970s, variants (CPV-2a, -2b, and -2c) with increased adaptation to the canine host emerged, replacing the original type-2. The variants show different antigenic and biological phenotypes. For instance, they have extended their host species range to cats. The presence of CPV-2 was investigated in the organs of 204 dogs, collected during the years 2023-2024. The samples were subjected to nucleic acid extraction, detection, and characterisation of CPV-2 variants using standardized molecular assays.
Overall, CPV-2 was detected in 121 (59.3%) dogs. The animals with single infection were 97 (80.2%), whilst the animals with coinfection by two CPV-2 variants were 24 (19.8%). In detail, the variant CPV-2a was detected in 19 (15.7%) dogs, variant CPV-2b in 57 (47.1%), and the variant CPV-2c in 18 (14.9%). In 3 animals (2.5%), the CPV-2 variant was not typed. Fourteen (11.6%) dogs were co-infected with CPV-2a and CPV-2b, nine (7.4%) with CPV-2b and CPV-2c, and only one dog (0.8%) with the variants CPV-2a and CPV-2c. Continual surveillance for CPV-2 is necessary for the timely detection of emerging novel variants. Also, due to the complex ecology of CPV-2, surveillance should be extended to other susceptible pets and wild carnivores.

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