



**One Health  
Platform**

**Benefits and Chances  
of One Health Research**

# One Health Symposium

17 – 19 October  
2024

**Book of Abstracts**



Federal Ministry  
of Education  
and Research

Senatsverwaltung  
für Wirtschaft, Energie  
und Betriebe

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**Welcome Note / 185**

## **Welcome Note of the One Health Platform**

**Authors:** Stephan Ludwig; Martin H. Groschup; Fabian Leendertz

Ladies and gentlemen, dear colleagues, dear friends,

We are delighted to welcome you to the first International One Health Symposium hosted by the newly founded One Health Platform.

As most of you know, the One Health Platform grew out of the German Research Platform for Zoonoses. This development, triggered by the experiences of the coronavirus pandemic, was a logical step, as the fight against numerous health challenges of our time can only be realized if we combine the strengths of numerous scientific disciplines.

The Zoonoses Platform founded in 2009 was a true success story. In the end, it united over 1300 members under its roof. More than 150 project applications were assessed via the platform, of which around 45% received funding. In addition, the Research Network for Zoonotic Infectious Diseases was established within the Zoonoses Platform with its 7 network projects and 6 junior research groups. As a consequence, stable research activities across disciplines and institutes could be achieved. A lively interdisciplinary research community was formed, with strong collaborations with the public health sector and a successful support of young scientists. Furthermore, the Zoonoses Platform became as a contact point for science and the public (in Germany).

With this success story behind us and the wealth of experience we have gained, we are now taking the next step and opening up the platform to other disciplines in order to create a powerful One Health research community in Germany. We would like to take this opportunity to thank the supporting ministries for the trust they have placed in us. It is quite unique that 6 federal ministries join forces to advance the One Health approach in research.

We are pleased to welcome not only our successful zoonoses community but also our new colleagues from the environmental sector and, in the future, from the social sciences. By integrating the environmental sector into our expanded One Health Platform, we are creating an even more comprehensive basis for interdisciplinary research and collaboration. This is a crucial step towards better understanding complex interactions and developing more effective strategies for preventing and combating infectious diseases.

Nevertheless, we have a big task ahead of us but we look forward to the opportunities that lie ahead. Over the next few years, it will not only be necessary to incorporate new disciplines without losing the established community. We also need to shift focus from infectious diseases only, reflect the diversity of One Health in our Scientific Advisory Board (SAB, raise public/political awareness for One Health, connect with international initiatives and secure sustainable research funding to live up to the expectations of a true One Health Platform.

Fortunately, we didn't have to start from scratch and we were able to use numerous building blocks and experiences from the Zoonoses Platform for the new project. Therefore, we have already achieved a lot in the past few months. With the SAB, in which environmental research is now also represented, we have created a functioning steering committee and have already set up two project calls, numerous events, a new online presence and a new podcast format, among other things. Furthermore, the promotion of young talents remains an important component of our activities and we are taking the first small steps towards international networking. We were also able to introduce ourselves on the international stage with a presentation at the World One Health Congress 2024 in Cape Town.

Our thanks go to everyone who has helped us, especially our members and their elected representatives, the members of the SAB.

The continued success of the platform stands and falls with the commitment of its members. That's why we hope to continue our close exchange in the future. Together, we have the opportunity to advance the One Health approach in research. We look forward to seizing this opportunity together with you in the coming years!

On behalf of the Office of the One Health Platform

Prof. Dr. Stephan Ludwig, Prof. Dr. Martin H. Groschup, Prof. Dr. Fabian Leendertz

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## Welcome Note of the Federal Government

**Authors:** Federal Ministry of Education and Research (BMBF); Federal Ministry of Health (BMG); Federal Ministry of Defence (BMVg); Federal Ministry of Food and Agriculture (BMEL); Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (BMUV); Federal Ministry for Economic Cooperation and Development (BMZ)

Ladies and Gentlemen,  
esteemed Colleagues,

I am delighted to welcome you to the first International One Health Symposium in Berlin on behalf of the six ministries that jointly promote research through the One Health-approach.

The subject of this symposium, “One Health: connecting infectious diseases with ecological challenges” perfectly highlights the deep interconnection between human, animal, and environmental health. The complexities of health challenges we are facing today, from zoonotic diseases, climate change to environmental degradation, require concerted efforts across various sectors and on all levels.

As we are coming together today, we embrace this shared responsibility to advance the One Health agenda. Not only from a research perspective, but also in our inter-ministerial collaboration. Therefore, the ministries responsible for research and education, health, food and agriculture, environment as well as defence and economic cooperation and development, are working hand in hand to create policies and initiatives that are comprehensive, complementary and effective.

Please allow me to highlight some of our One Health initiatives at the different ministries.

In December 2023 the One Health platform was jointly established by the mentioned six ministries under the lead of the Federal Ministry of Education and Research. It is the direct successor of the zoonoses platform, which was set up in 2009 in order to connect human and veterinary medicine to coordinate and support research in the field of emerging zoonotic diseases. Important elements, especially the environmental aspects and social sciences have now been included in order to form a joint One Health research community with expertise from research and practice. Funding will be provided for annual One Health Symposia such as this one, for networking opportunities for students, early career researchers and scientists. Furthermore, all six ministries seek to provide additional funding for pilot and networking projects. A scientific advisory board will cover a wide range of expert areas essential for the One Health platform. And this is where your expertise is needed - all interested scientists within the One Health community - that is you -, are cordially invited to apply for a position in the scientific advisory board.

Further intersectoral and interministerial initiatives with a pivotal role of the Federal Ministry of Food and Agriculture, the Federal Ministry of Health as well as the Federal Ministry for Environment, Nature Conservation and Nuclear Safety and their departmental research institutions include collaboration on zoonotic diseases; for example the current outbreak of avian influenza virus infections in numerous dairy cow farms in the US, and the SARS-CoV-2 wastewater monitoring in Germany. Wastewater Monitoring for Epidemiological Situation Assessment is nationally and internationally of growing interest in the One Health context and part of pandemic preparedness. This is also reflected in the upcoming EU Urban waste water treatment directive (UWWTD).

In the context of surveillance, Whole Genome Sequencing is increasingly becoming a standard tool across various sectors. Its high-resolution genetic analysis of pathogens enhances our understanding of their spread across human, veterinary, food, and environmental domains providing a solid foundation for cross-sectional collaboration. For example in the context of food-borne diseases, Whole Genome Sequencing helps pinpoint contamination sources more effectively, thereby enhancing food and patient safety. The cross-sectional collaboration in this context is actively promoted by the Federal Ministry of Food and Agriculture and the Federal Ministry of Health.

In the context of AMR, the sectors of human and veterinary medicine as well as the environment each face common as well as rather specialized challenges. To improve the open communication between the sectors and work together to tackle AMR through the One Health approach, an event series was launched to enable practitioners and relevant stakeholders to exchange information. In November 2024, during the World Antibiotic Awareness Week, a workshop including representatives from the sectors of human and veterinary medicine as well as agriculture and environment will take place. This event is jointly organized by the three ministries (BMG, BMEL and BMUV).

Moving on from research towards translation and implementation of One Health measures: The Fed-

eral Ministry of Development Cooperation funds the so-called Rapid Response Expert Group on Health (Schnell Einsetzbare Expertengruppe Gesundheit (SEEG)) that has been active during the outbreaks of avian influenza in several countries and of monkeypox in the Democratic Republic of the Congo. Expert teams from the Bernhard Nocht Institute, the Charité university hospital, the Friedrich Loeffler Institute and the Robert Koch Institute jointly support partner countries in combating and preventing disease outbreaks, including in the veterinary and wildlife sectors, in line with the One Health approach. In addition to teaching basic knowledge, the SEEG also organizes training courses on infection prevention and control. By facilitating rapid implementation of surveillance, prevention, and containment measures, the SEEG enhances collaboration across sectors and strengthens global health security.

Numerous national and international crisis operations have emphasized the link between health and security as well as the importance of Force Health Protection to retain operational force readiness. The German Bundeswehr, like all armed forces, must be able to operate worldwide even when threatened by infectious diseases or in case of biological weapons. The Central Medical Service of the Bundeswehr has proven its specialized expertise time and time again and multidisciplinary teams have been working for decades to advance many aspects of Force Health Protection, the One Health approach being one of the most important cornerstones. For example, a medical knowledge and information management system that takes into account all aspects of One Health is currently being developed and established as part of the Bundeswehr's new tasks on NATO's eastern flank. Targeted research projects based on the One Health approach also support the goal of comprehensive Force Health Protection.

These were just a few examples - further efforts to strengthen One Health are ongoing. You will have seen the BMBF call for research consortia for pandemic prevention and response that recently closed. There are also different European partnerships to strengthen research cooperation around the One Health approach supported by the German government, in particular the partnerships for Animal Health and Welfare, for One Health/AMR and for Pandemic Preparedness.

In conclusion, One Health is more than a concept; it is a way of thinking and a call to action. It requires all of us to reconsider our established approaches on how we communicate, collaborate and conduct research. Although there is still much to do, I am confident that together - from a research perspective and in our inter-ministerial collaboration - we can achieve the ambitious goals incorporated in the One Health approach.

Thank you all for your dedication, your commitment and your attention. I look forward to the productive discussions and innovative ideas that will emerge from this event.

**Keynote I / 174**

## **Agricultural pesticides and the occurrence of zoonoses**

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## Non-invasive surveillance of avian influenza virus and environmental pathogens

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Avian influenza virus (AIV) is causing significant mortality in wild birds, poultry, and mammals, posing a global health threat due to wild bird migration that facilitates AIV transmission and evolution. We developed a genomics-based surveillance protocol using portable nanopore sequencing for rapid AIV profiling from non-invasively collected environmental samples. Using a well-defined AIV strain, we benchmarked portable RNA extraction and detection, compared DNA- and RNA-nanopores, and evaluated computational pipelines for viral sequence creation and analysis. We validated our setup with environmental samples from a duck farm in France, a “vulture restaurant”, and a wetland in Spain. Additionally, we used non-invasive water and air samples to profile the RNA virome and antimicrobial resistome, offering a broader pathogen view. Our results show that converting viral RNA to cDNA for amplification is effective for low-concentration samples. Non-invasive passive and active techniques, including air sampling via liquid impingement and water sampling with torpedo-shaped devices, outperformed traditional methods, detecting AIV in challenging environments and enabling comprehensive RNA virome and antimicrobial resistome characterization. This approach holds promise for monitoring wildlife pathogens, understanding their evolution and transmission, and informing One Health risks at the intersection of human, animal, and environmental health systems.

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## Investigating the epidemiology of mpox in wild, habituated sooty mangabeys (*Cercocebus atys*) in Taï National Park, Côte d'Ivoire

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Mpox is a viral zoonotic disease endemic to parts of Africa whose incidence in the human population has recently surged globally. While our understanding of mpox epidemiology in humans has progressed significantly in the last 2 years, the sylvatic cycle of monkeypox virus (MPXV) remains poorly defined. Since 2012, we have been investigating MPXV outbreaks in non-human primates in Taï National Park (TNP), Côte d'Ivoire, providing clinical and epidemiological insights. Here, we present additional data from a recent outbreak where we also evaluated MPXV detection in the surrounding environment. From January to June 2023 a group of wild, human-habituated sooty mangabeys (*Cercocebus atys*) showed symptoms compatible with MPXV infection. 31 out of ~60 individuals developed maculo-papular skin lesions, and 4 infants died. As part of a longitudinal wildlife health monitoring program in TNP, we performed 3 necropsies and collected non-invasive samples, such as feces (n=1329), urine (n=791) and swabs from food remains (n=157) from the group. To assess potential pathways of indirect viral transmission, we swabbed the vegetation (n=960) and collected flies (n=1204) at the necropsy sites, within the group, and at increasing distance from the group (up to 300m). Laboratory analyses are ongoing. Preliminary results show the presence of MPXV DNA in necropsy and fecal samples, and in flies collected within the group. This study will provide diversified epidemiological data enriching the body of evidence on MPXV sylvatic maintenance in TNP.



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## The MEK1/2 inhibitor ZMN efficiently blocks HPAIV infections

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In 2021 highly pathogenic avian influenza strain H5N1 clade 2.3.4.4b spread in domestic and wild birds, now considered to be a zoonosis. Spillover events in marine mammals, mink farms and dairy cattle farms posing the risk that the virus gains the ability to be transferred between mammals and may also jump to humans. Besides prophylactic vaccinations, antiviral drugs are needed to treat acute infections. Direct acting antivirals (DAA) target viral components, making them highly effective, but prolonged treatment bears the risk of resistance selection. Alternative approaches are host-targeted antivirals (HTA), like the MEK1/2 inhibitor Zaprmetinib (ZMN), interfering with cellular mechanisms misused by viruses. In this study, we evaluated the efficacy of ZMN against HPAIV including H5N1 clade 2.3.4.4b. We could show, that ZMN reduces the production of progeny viral particles of several H5Nx and H7Nx viruses. This antiviral mode of action is not restricted to the commonly used A549 cell line, but could be shown for different cell lines (Calu-3, MDCK II, VeroE6). The inhibitory effect is caused by a nuclear retention of the viral genomes in the later stage of the infection. Additionally, ZMN can act in a synergistic mode of action, with the DAA's Oseltamivir carboxylate (OTC) and Baloxavir acid (BXA) providing an alternative treatment strategy by combining DAAs and HTAs. These results demonstrate that ZMN represents an effective drug against H5N1 and other HPAIV infections.

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## H7 highly pathogenic avian influenza A virus genomes from the first half of the 20th century

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Highly pathogenic avian influenza viruses (HPAIV) cause high mortality in poultry and significant economic losses. Since 1902, H7 and H5 subtypes have been the most prevalent in poultry, posing potential health risks to humans, albeit with only sporadic infections so far.

Despite their importance, the genomic diversity of HPAIV before the advent of large-scale systematic surveillance in the 1980s remains poorly understood. To date, only 15 complete genomes from before 1950 have been reconstructed. Historical pathology collections offer a unique window into the evolutionary history of pathogens and provide a valuable resource for studying ancient HPAIV.

We analyzed 11 formalin-fixed avian specimens from the 1920s to 1950s from Vetmeduni Vienna. High-throughput sequencing yielded IAV reads from five specimens, allowing for the assembly of two high-quality genomes of H7N1 and H7N7 from 1927 and an undated specimen, and lower quality genomes from three specimens collected between 1942 and 1949.

We are currently enhancing the genomic coverage of these last specimens. Subsequent extensive phylogenetic analyses will allow us to explore the evolution of H7 HPAIV during the first half of the 20th century and understand their contribution to the genetic landscape of later IAV lineages.

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## Combining natural products, probiotics and vaccines to decolonize multidrug-resistant *Enterobacterales* from the intestine: An integrative strategy to combat antimicrobial resistance in the One Health context

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The rise of antimicrobial resistance (AMR) in *Enterobacterales* like *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) poses a significant threat to human, animal, and environmental health. This calls for innovative strategies that go beyond the traditional use of antibiotics.

The project *MDR-Dekol* employs a multifaceted approach combining natural products, probiotics and vaccines to effectively decolonize multidrug-resistant (MDR) EC/KP from chicken intestines. To avoid unwanted disruption of the commensal flora, an advanced pangenomic approach was used to identify surface markers of high-risk (HR) EC/KP lineages for targeted vaccine development. In addition, we employ an in vitro model to identify the most promising prebiotic-probiotic combinations that hinder bacterial biofilm formation and adhesion of HR EC/KP strains to epithelial cells but do not affect commensals.

We have already identified 3 promising surface markers each for EC and KP. Suitable vaccine candidates are currently being developed. Several natural products with (so far unknown) biofilm eradication properties and promising effects on decolonization especially of HR EC/KP strains were discovered. The combinatorial effects of natural products and probiotics are still under investigation, with some combinations showing synergistic effects.

In summary, our project offers an alternative strategy to control MDR EC/KP in chickens that is also relevant for other animals, humans and potentially, the environment.

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## ZooNotify - New online portal provides data on the occurrence of zoonotic agents and their antibiotic resistance along the food chain in Germany

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Data on the occurrence of zoonotic agents and related antibiotic resistance in the food chain in Germany are mainly available in reports. This leads to limited accessibility and reusability of this data in science and for risk assessment. ZooNotify makes existing zoonoses data findable, accessible, interoperable and reusable (FAIR).

ZooNotify currently includes data from 2012 to 2022 collected in accordance with the Directive 2003/99/EC and the Regulation 2160/2003/EC. Data is available on prevalence, antibiotic resistance and typing of zoonotic agents and originates from samples taken at various stages of the food chain, such as primary production, slaughterhouses and retail. The majority of the data is related to livestock and foods of animal origin. However, data is also available on feed, wild animals and plant-based foods.

ZooNotify allows customized data searches based on user interests. The selected data can be downloaded for further use in a standardized way. ZooNotify also offers quick and easy data visualisation including time-based evaluations. Each section of ZooNotify features clear and concise explanations, ensuring the tool is user-friendly and facilitates a comprehensive understanding of the data.

The continuous development of ZooNotify supports the EU strategy for open data and forms an important basis for improved risk assessment and the protection of animal and consumer health.

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## **Dangerous hitchhikers! - Cigarette filters promote the spread of high-risk bacterial communities in aquatic ecosystems**

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In addition to direct human health risks of smoking cigarettes, discarded cigarette filters are an important urban pollutant. Around 37% of filters end up in aquatic environments where they provide novel colonizable surfaces for microbes. We here propose that the toxic compounds entrapped on cigarette filters lead to a selective colonization by bacteria thriving under stressful conditions. These might include pathogens and increased levels of antimicrobial resistant genes (ARGs), hence creating additional indirect health hazards.

To test this, used cigarette filters enriched with toxicants and unused control filters were submerged in a sewer. Filter colonizing communities were analyzed for ARGs and mobile genetic elements (MGEs). Further, a live pathogenicity model, *Galleria mellonella* (wax moth larvae) was injected with communities extracted from used and unused filters, with larval survival monitored for 24 hours.

Larvae exposed to used filter communities had significantly higher mortality rates. Thus, filters enriched with toxicants were selectively colonized by communities with higher pathogenicity. Moreover, >30 tested ARGs and MGEs were selectively enriched on used filters.

In conclusion, high-risk communities are colonizing discarded filters and can hitchhike large distances to novel environments, where they can negatively affect environmental and human health. This emphasizes the need for improved waste management strategies to mitigate the impact of cigarette filters.

## Session 2 / 2

## Tradeoffs of increasing temperatures through climate change on the spread of antimicrobial resistance in river biofilms

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River microbial communities regularly act as the first barrier against the spread of antimicrobial resistance genes (ARGs) that enter through wastewater. However, how invasion dynamics of these ARGs into river biofilm communities shift due to climate change remains unknown. Here, we aimed to elucidate effects of increasing temperatures on both, the natural river biofilm resistome, and the invasion success of foreign ARGs entering through wastewater. Natural biofilms were grown in a low-anthropogenic impact river and transferred to laboratory recirculation flume systems operated at 20, 25, and 30°C. After 1 week of temperature acclimatization, significant increases in abundance of naturally occurring ARGs were detected at higher temperatures. After this acclimatization, biofilms were exposed to a single pulse of wastewater, and invasion dynamics of wastewater-borne ARGs were analysed. After 1 day, wastewater-borne ARGs were able to invade the biofilms successfully with no effect of temperature on their relative abundance. Thereafter, ARGs were lost at far increased rates at 30°C, with ARG levels dropping to the initial natural levels after 14 days. Contrary at lower temperatures, ARGs were either lost slower or able to establish in biofilms with stable abundances above natural levels. Hence, higher temperatures come with contrary effects on river biofilm resistomes: naturally occurring ARGs increase in abundance, while foreign, invading ARGs are lost at elevated speed.

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## **Building Knowledge in Urban Agriculture Water Supply in São Paulo - The Context of Irrigation, Foodborne Pathogens and Antimicrobial Resistance**

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The serious increase of antimicrobial resistance (AMR) in the urban food sectors threatens global health. Urbanization in many developing countries is linked to rising poverty, polluted environments, and growing food insecurity. The World Health Organization estimates unsafe food causes 600 million cases of foodborne diseases and 420,000 deaths annually, mostly due to inadequate sanitation, poor hygiene, and unclean water. Urban agriculture improves local economies, health conditions, and food supply. São Paulo, as one of the world's largest metropolitan areas, is a reference for research on whether irrigation water management can diminish foodborne diseases and reduce the occurrence of AMR. From June 2023 to February 2024, a comparative case-control study on 150 urban farms was conducted in São Paulo. We interviewed face to face 200 lead farmers using quantitative questionnaire to analyze socio-demographic, health, and environmental data related to their agricultural practices. Water, food and fecal samples were collected, for micro- and molecular-biological (quantitative polymerase chain reaction - qPCR) evaluation of pathogens along the food chain. Preliminary results indicate irrigation water as a contamination source with pathogenic presence varying by sources. Inequalities in technical assistance and water access affect farming decision. Understanding contamination mechanisms in the urban water system, through food into the human microbiome, aims to develop evidence-based guidelines and hygiene recommendations for disease prevention.

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## **Intensive land usage induces shift in the bacterial communities and subsequent enrichment of antibiotic resistance genes at different trophic levels of grassland ecosystems**

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The intensive use of fertilizers significantly impacts grassland ecosystems, inducing selective enrichment of microbial taxa, which can reduce biodiversity and promote the spread of antibiotic resistance, posing serious health concerns. This study examines the effects of various fertilization regimes on the diversity and function of microbiomes across different trophic levels in grassland ecosystems using metabarcoding. Additionally, the functional potential of these bacterial communities was inferred through imputed metagenomics. Our analysis revealed significant differences in bacterial diversity among grasslands fertilized with biogas, cow manure, and pig slurry, as measured by the Shannon index. These differences were consistent across target organisms, including earthworms, voles, and bumblebees, representing various trophic levels. Metagenomic imputation indicated that gene families related to streptomycin and tetracycline biosynthesis were commonly present and particularly abundant in voles, suggesting an enrichment of antibiotic-resistance genes at higher trophic levels. This study highlights the detrimental effects of intensive land use on microbial communities across multiple trophic levels in grassland ecosystems. The proliferation of allochthonous microbial taxa due to fertilization regimes and the subsequent enrichment of antibiotic-resistance genes at higher trophic levels have serious implications for animal and public health, warranting detailed investigation.



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## Pesticide pollution and *Schistosoma mansoni* tolerance: implications for Schistosomiasis risk in Western Kenya

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Schistosomiasis is a neglected tropical disease caused by trematodes of the genus *Schistosoma*. The pathogen is transmitted via freshwater snails, which are highly tolerant to agricultural pesticides and indirectly benefit from pesticide exposure due to adverse effects on their less tolerant competitors. Our field studies show that pesticide concentrations deemed “safe” in environmental risk assessments can indirectly affect human health by increasing populations of *Schistosoma*-host snails. Pesticides in surface waters may thus elevate the risk of schistosomiasis transmission unless they also affect the pathogen. To address this concern, we also tested the tolerance of the free-swimming life stages (miracidia and cercariae) of *Schistosoma mansoni* to insecticides diazinon and imidacloprid, which were frequently detected in inland waters of Kenya. Additionally, we investigated whether these pesticides impair the ability of miracidia to infect and develop as sporocysts within the host snail *Biomphalaria pfeifferi*. We showed that these insecticides did not affect the performance of *S. mansoni* at environmentally relevant concentrations. Particularly within its host snail, the pathogen can escape exposure peaks that have been shown to affect other sensitive invertebrates and their biological control of host snails. Our findings suggest that freshwater pollution with agricultural pesticides increases the risk of schistosomiasis, illustrating the need to integrate environmental and public health risk assessment and management.

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## **Agroecology as pathway to achieve environmental, animal and human health**

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## Significance of the hatchery in the introduction of antibiotic-resistant *Enterobacterales* into an organic broiler farm

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Despite efforts to minimize antibiotics in conventional broiler husbandry, usage remains high, resulting in high prevalence of antibiotic-resistant bacteria in the animals and meat thereof. With growing dietary awareness, the demand for organic broiler meat and the number of organic broiler farms are increasing. To address knowledge gaps on the occurrence and transmission of antibiotic-resistant *Enterobacterales*, we conducted a longitudinal study in several organic broiler farms in Germany, monitoring four consecutive fattening periods with five sampling points each. We identified one farm from which ESBL-producing *K. pneumoniae* and quinolone-, ampicillin-, and/or tetracycline-resistant *E. coli* were continuously isolated. Resistant isolates were already collected from chick papers of 1-day-old chicks and at later stages of three fattening periods. Transmission dynamics differed between *K. pneumoniae* and *E. coli*. *K. pneumoniae* were clonally related and carried a conjugative IncFIB/FII plasmid harboring genes for resistances against four antibiotic classes. *E. coli* isolates were genetically highly diverse and carried different resistance plasmids, whose transmissibility is currently being investigated. For the fourth fattening period, chicks were purchased from a different hatchery and predominantly susceptible *Enterobacterales* were isolated highlighting the role of organic certified hatcheries in introducing antibiotic-resistant bacteria into the organic broiler food chain.

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## MetagenomeWatch: Harnessing Big Data for Pathogen Surveillance and Genome Discovery

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With the rapid advancement in Next-Generation Sequencing technology, the amount of publicly available metagenome sequencing data has increased exponentially. The Sequence Read Archive (SRA) comprises over 9 million genomic sequencing datasets from a variety of environments around the globe, from sources such as human, animal, wastewater, and others. This data provides a unique opportunity to better understand microbial communities and their impact on public health. The ability to search the SRA would improve the surveillance, and characterisation of pathogens, facilitating outbreak detection, and disease spread prevention. Analyzing petabyte-scale datasets such as the SRA has been challenging due to both storage as well as computational requirements. However, with recent advances in compression techniques such as sketching, which uses only a fraction of the original data, being stochastically sufficient to determine whether a given sequence is likely to be contained in a dataset, this is now feasible within a reasonable amount of time and storage space. We introduce MetagenomeWatch, a web interface to the state-of-the-art computational methods implemented by the tool “sourmash”. It offers pathogen experts the ability to search publicly available metagenome databases in real-time for viruses, bacteria, and eukaryotic pathogens. With the option to set up automated watches that report on database updates, it will be possible to monitor and track outbreaks on a global scale.

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## **RIBAP: A comprehensive bacterial core genome annotation pipeline for pangenome calculation beyond species level and applications in environmental and wastewater metagenomics**

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Microbial pangenome analysis reveals gene presence and absence across prokaryotic genomes. Traditional tools struggle with species exhibiting high sequence diversity or in genus/family-level analyses. RIBAP (Roary ILP Bacterial Core Annotation Pipeline) addresses these issues with an integer linear programming (ILP) approach to refine gene clusters and accurately identify core genes, even in diverse genomes and bacterial communities.

RIBAP can annotate genomes, calculate pangenomes with Roary, refine gene clusters using ILPs, and visualize results in interactive formats. We tested RIBAP on diverse datasets (*Brucella*, *Chlamydia*, *Enterococcus*, *Klebsiella*) and metagenome-assembled genomes from wastewater communities. We consistently identified more comprehensive core genomes when analyzing bacterial isolates. In a metagenomic and wastewater context, RIBAP's performance is challenged by the heavy ILP calculations but can yield core gene connections not detectable by other tools. Our results highlight RIBAP's ability to handle high sequence diversity and accurately predict core genes.

Thus, RIBAP provides an advanced framework for pangenome analysis, especially for diverse or genus-level datasets, offering a more accurate view of core genetic components such as ARGs. Additionally, RIBAP shows promise for analyzing core genes in microbial communities from environmental samples, such as wastewater metagenomics data.

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## Pan-Proteomics for a Better Understanding of Host Adaptation and Antibiotic Resistance in Public Health-Relevant Bacteria

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The rise of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a significant threat to public health. The understanding of AMR-driven dynamics is of utmost importance to tackle this challenge. However, current genomic approaches are limited due to predetermined loci in the form of MLST schemes. Pangenomics solves this by extending beyond traditional genomics analyzing the entire gene pool of a species, providing novel information that a single genome cannot. This approach allows us to identify different subclades based on genetic differences and gain insights into horizontal gene transfer, which plays a crucial role in bacterial adaptation and antibiotic resistance. Recent development in the field of bacterial proteomics enabled fast and precise detection of AMR. In this study, we are using state-of-the-art proteomics workflows to gain complementary protein expression and function that can provide additional information about the genes and their clusters beyond their mere presence. Pangenomics and proteomics, each of which provides unique insights into microbial behavior and evolution, are both important fields in the study of bacterial dynamics. By integrating both datasets into Pan-Proteomics, the goal is to uncover mechanisms of host adaptation and resistance that are not apparent when these fields are studied in isolation. This could potentially lead to improved strategies for managing infectious diseases - a significant step in the fight against AMR.

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## Decoding Bacterial Secrets: Unraveling DNA Modifications through Nanopore Sequencing for Public Health Insights

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Investigating bacterial methylation profiles is critical for assessing the potential influence that DNA modifications have on virulence, antibiotic resistance, and bacteria's ability to evade the immune system. Real-time Nanopore sequencing and recent advances in basecalling algorithms allow the accurate identification of modified bases from the raw signal data. However, decoding methylation signals for various bacteria remains challenging and needs species-specific investigations.

Here, we focus on public health-relevant, multi-drug-resistant strains to analyze methylation profiles and extract methylation motifs. Our dataset includes samples from *S. aureus*, *L. monocytogenes*, *E. faecium*, and *K. pneumoniae*, which were sequenced using the Nanopore GridION platform with recent advances in flow cell chemistry (R10.4.1) and modification basecalling (Dorado).

Our investigation aims to reveal distinct methylation profiles among the bacterial strains and species, highlighting species-specific modification patterns and shedding light on the most heavily modified genes. Ongoing efforts aim to connect active methyltransferases with methylated motifs, enhancing our understanding of bacterial DNA modifications. Additionally, we evaluate error rates and improve assembly accuracy with the latest basecalling models.

The primary goal of our study is to explore the potential of recent advanced tools for modification detection and make them accessible to the scientific community.

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## First comprehensive analysis of Wūhàn sharpbelly bornavirus in grass carp cell lines: A Multi-method Approach

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The family Bornaviridae, within the order Mononegavirales, contains three genera: Orthobornavirus, Carbovirus, and Cultervirus. Orthobornaviruses have the broadest host range, infecting avian, reptilian, and mammalian species, whereas carboviruses and culterviruses have been found in reptiles and fish, respectively.

Our recent study using in silico data mining identified novel fish culterviruses, including Wūhàn sharpbelly bornavirus (WhSBV), which was discovered in grass carp kidney and liver cell lines. Further next-generation sequencing of fish cell lines from the cell culture bank of the Friedrich Loeffler Institute revealed nearly identical WhSBV genomes in two grass carp swim bladder cell lines.

We developed RT-qPCR assays to detect all WhSBV genes and determined the 5' and 3' ends of the WhSBV genome using RACE. In addition, we obtained electron microscopic image of WhSBV in grass carp cell lines. Infection experiments indicated persistent WhSBV infection in cell lines from Cyprinidae and Leuciscidae. Mass spectrometry detected peptides corresponding to the predicted N, X, P, G and M proteins of WhSBV. Northern blot analysis revealed that the X, P, and G genes are expressed from polycistronic mRNA, whereas the N gene is transcribed from monocistronic mRNA.

Our multi-method approach provides the first molecular characterization of WhSBV and provides critical data for a better understanding of the genetic structure, replication mechanism, and host spectrum of culterviruses.



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## **Increasing preparedness by networking: 20-year network “Rodent-borne pathogens”**

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The network was established as a platform for an interdisciplinary collaboration of scientists in mammalogy, ecology, genetics, immunology, toxicology, epidemiology, virology, microbiology, parasitology and human and veterinary medicine.

Over the last 20 years, about 30,000 rodents and other small mammals were collected by network collaborators from forests, grasslands, zoological gardens, urban landscapes and pet rat breedings and other husbandries. A variety of pathogen-specific, generic and open view molecular methods were used to detect and identify known, and yet undiscovered, pathogens.

The network was involved in the discovery of numerous rodent- and shrew-specific herpes- and polyomaviruses. Based on clinical cases in humans, domestic, wild and zoo animals, novel zoonotic and animal pathogens were identified, e.g. variegated squirrel bornavirus 1, squirrel adenovirus and rustrela virus. The network enables the detection of several viruses of unknown zoonotic potential, e.g. novel hepeviruses in rats, common voles and shrews, and the identification of the rodent reservoirs of rustrela virus and lymphocytic choriomeningitis virus. In addition to hantaviruses in wildlife, the zoonotic Seoul hantavirus was recently identified in pet rats in Germany.

In conclusion, the network provides an important infrastructure for interdisciplinary scientific work in a One Health perspective and allows a holistic view on environment-host-pathogen interactions.

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**Molecular-based profiling of the gastrointestinal resistome in wild mice**

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Antimicrobial resistance represents a global challenge, primarily driven by antibiotic misuse, leading to the spread of bacteria carrying antimicrobial resistance genes in various environments. However, the dynamics and maintenance of antimicrobial resistance genes within host-derived microbiomes remain to be fully understood, especially in hosts with close interactions with the environment and humans. Here, we conducted a molecular profiling of the collection of all antimicrobial resistance genes (resistome) along the gastrointestinal tract of house mice from their natural environment. Utilizing metagenomic sequencing of intestinal content, we assessed the richness and composition of the microbiome and resistome. Microbiome composition accounted for approximately 23% of resistome composition, with gastrointestinal and geographical distance as additional significant predictors. We traced antimicrobial resistance genes associated with transmission risk and anthropogenic impact in the different GI segments. Notably, we identified a high prevalence of genes associated with resistance to glycopeptides, specially the vancomycin resistance genes *vanA* and *vanR*, with abundance that steadily increased along the gastrointestinal tract. Our findings highlight the impact of the microbial composition of intestinal niches on antimicrobial resistance gene content. The results suggest a crucial role of interspecies bacterial interactions in maintaining pre-existing resistance independent of direct antibiotic exposure. Microbiomes of mice from their natural environment are a reservoir for resistant bacteria linked to human exposure and an experimentally traceable model.

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## Applied One Health research on Borna disease virus 1 (BoDV-1) – Introducing the ZooBoFo project

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Borna disease virus 1 (BoDV-1) is known as the causative agent of Borna disease in animals for many decades. However, it was only proven in 2018 that BoDV-1 is also transmissible to humans, causing severe, usually fatal encephalitis. While the reservoir host for BoDV-1, the bicolored white-toothed shrew (*Crocidura leucodon*), is evidenced, transmission routes remain unknown. After investigating the first detected local cluster of this rare disease in Bavaria in 2022, we continued our research using an applied One Health approach. Our interdisciplinary project “Zoonotic Bornavirus Focalpoint Bavaria” (ZooBoFo) aims to gain further insights into BoDV-1 in order to be able to make more targeted prevention recommendations in the future. Further objectives are to narrow down transmission routes, to gain further insights into the reservoir host and BoDV-1 environmental stability, to precisely describe the regional occurrence of the virus in Bavaria and to further determine clinical symptoms of human BoDV-1 infections. Among other things, a shrew model was established to carry out infection and tenacity tests for this purpose. Moreover, shrews sent in by concerned individuals are continuously tested and if a BoDV-1-positive shrew is detected, an environmental investigation is triggered and we have been able to detect BoDV-1-RNA at several locations. Another aim of ZooBoFo is to raise awareness of BoDV-1 among physicians/veterinarians and to create a platform for informing the public.

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## Education for Sustainable Development as a relevant field for One-Health: an interdisciplinary approach

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Climate change and environmental degradation alter ecosystems. This generates health risks for human and non-human life. These risks are unevenly distributed across geographical space, human populations, and plant, animal, bacterial, and viral species (Githeko et al. 2000). Global health approaches based on One Health principles thus must focus jointly on human and more-than-human health (cf. Gruetzmacher et al., 2021). Education in regions with high transmission risks for infectious diseases are often characterized by colonial histories and subsequent imbalances of power and knowledge (Tuhebwe et al., 2023). Moreover, knowledge transmitted through schools and colleges is human-centered. It perpetuates non-systemic, dualistic models of thinking about health risks associated with climate and environmental change (Ress et al., 2022). The proposed paper introduces education for sustainable development (ESD) in postcolonial constellations. ESD plays a key role in tackling OneHealth challenges globally. Based on general insights about learning, it can help shape interdisciplinary collaborations (e.g., human and veterinary medicine) by conceptualizing such collaborations as spaces of learning in the context of uncertainty (cf. Taube & Ress, forthcoming). The proposed paper reflects a model for interdisciplinary learning. It constitutes itself an interdisciplinary collaboration between scholars in epidemiology and education sciences.

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## Development of a standardized One Health surveillance of antimicrobial resistance and heavy metal tolerance in wild birds and surface waters of the Baltic Sea

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The spread of antimicrobial resistance (AMR) and heavy metal tolerance (HMT) in microorganisms of wildlife and aquatic environments is an often underestimated but detrimental threat to human, animal, and environmental health. Therefore standardized and feasible strategies are needed for comprehensive monitoring of AMR/HMT bacteria and related genes.

The project BALTIC-AMR employs a longitudinal surveillance approach combining molecular (e.g. genomics) and phenotyping (e.g. culturomics) techniques to characterize AMR/HMT *Enterobacterales* and *Pseudomonadales* from the Baltic Sea from four littoral states. In addition, the occurrence of other waterborne pathogens such as *Vibrio* spp. will also be investigated. To study migratory birds as a connected reservoir in the Baltic region, we also examine fecal samples of selected gull and duck species and include movement data in our comprehensive analysis.

A protocol for reliable AMR/HMT monitoring has been established by the interdisciplinary consortium. Although the longitudinal sampling is ongoing, we have already isolated a large number of AMR/HMT strains from surface waters and wild bird droppings, many exhibit resistance to at least three clinically used antibiotic classes and thus exhibit a multidrug-resistant phenotype.

In summary, this is a proof-of-concept study that demonstrates how a standardized and reliable AMR surveillance strategy can be established within the One Health context.

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## Building up One Health Surveillance systems in the African tropics and in Germany

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The Helmholtz Institute for One Health (HIOH) is dedicated to addressing the interdependence of human, animal, and ecosystem health and how they are affected by climate change.

The One Health Surveillance (OHS) Core Unit is at the heart of the institute. Similar to weather stations that record various parameters over many years, the OHS systematically collects and analyzes a broad spectrum of samples and data – from climate and environment to health and social data – at high resolution in defined indicator regions. The integration of this data from multiple disciplines will contribute to pandemic prevention and preparedness. In addition to the indicator regions in sub-Saharan Africa (Côte d'Ivoire (CIV) and Central African Republic (CAR)), the OHS will be active at the doorstep of the HIOH in northeast Germany.

**Clinical Surveillance:** To determine which pathogens are relevant to the populations in the indicator regions, we are establishing clinical surveillance in collaboration with local hospitals and health centers.

**Wildlife Disease Monitoring:** We are running veterinary programs to detect wildlife diseases in CIV and CAR using non-invasive methods such as the analysis of urine and fecal samples, or necropsies of wild animals found dead.

**One Health Cohorts:** Currently we are setting up cohort studies with human volunteers whose health we will follow over the course of many years. In addition, we systematically survey associated livestock and small mammal vectors, biodiversity and micro - and macro climate.

**Snacks & Poster Viewing 1 / 132****Scrapie eradication in Iceland – Progress and challenges**

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Scrapie is a fatal disease of the brain (encephalopathy) in sheep caused by a prion protein. It was introduced into Iceland by an infected ram in 1878. The massive increase in scrapie outbreaks in the 1970s, led to the start of a control programme in 1986. The aim of the control programme was to reduce outbreaks and eradicate the disease by killing all animals on outbreak farms, the destruction of the facilities and the cleaning and disinfection of the buildings. In addition, biosecurity measures and subsequent waiting periods before restocking and trade restrictions were implemented. In 2023, a further component was included, breeding for resistant genotypes.

This study examines systematically the control measures implemented over time, and the specific challenges in Iceland, e.g. risk factors, or the genetic pool.

For this purpose, regulations were analysed, interviews were performed with sheep farmers affected by the disease and the measures were discussed with the relevant authorities. This was compared to the number of outbreaks.

The control measures led to a reduction in outbreaks and the eradication of scrapie in several regions. Nevertheless, the disease could not be eradicated so far. Particularly in the north-west a baseline of affected farms can be observed. Some risk factors were identified, e.g. on-site burial of cadavers. The breeding options in the control programme leads to new challenges, especially on outbreak farms.

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## Extracellular Vesicles as Vectors for the Spread of Antibiotic Resistance Genes

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Antimicrobial resistance (AMR) has posed a serious global health threat over the last few decades. Extracellular vesicles (EVs), nano-particles released from the microbiome, and are newly recognized as important vectors for carrying and spreading antibiotic resistance genes (ARGs). However, the presence and transfer potential of ARGs harbored by EVs in various environments remain unclear.

In previous research, we demonstrated the ubiquitous presence of EVs in different environmental habitats, including dust, soil, and wastewater. Our results showed that EVs serve as vectors for a large number of ARGs, with the highest number found in dust (241), followed by wastewater (128) and soil (19). In our current study, we aim to determine the ARG profiles in EVs from agricultural soils receiving livestock waste as fertilizer. We will investigate whether manure application increases the soil resistome and the abundance of EVs, and whether there is an enrichment of ARGs in these EVs. As agriculture is a direct One Health interface, it is important to investigate soil EVs as a potential vectors for the transmission of ARGs from environmental reservoirs is crucial. EVs are more likely than whole-cell bacteria to cross biological barriers and simultaneously stabilize extracellular DNA, highlighting their potential role in the context of AMR spread across compartments.



**Snacks & Poster Viewing 1 / 147****Brno loanvirus (BRNV) in bats inhabiting urban area of Brno, Czech Republic**

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Bats, known reservoirs of various emerging pathogens have recently been found to host a novel hantavirus named Brno loanvirus (BRNV) within the Mammantavirinae subfamily (family Hantaviridae, order Bunyvirales). This research reports the detection of BRNV in bats from the urban area of Brno, Czech Republic. The results of this study confirm that *Nyctalus noctula* can serve as a reservoir host for BRNV even in urban areas. Moreover, the study utilizes RT qPCR to quantify BRNV RNA levels in various bat organs. The analysis reveals positive results for viral RNA in organs such as kidneys, heart, spleen, brain, liver, lung, gut, and body cavity fluid. Notably, the liver exhibits the highest levels of viral RNA among all the tested organs.

The study was supported by the Ministry of Health of the Czech Republic (NU21-05-00143).

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## **Molecular Interactions During Flavivirus Coinfections**

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The genus Orthoflavivirus (formerly Flavivirus) contains multiple vector-transmitted viruses that can have detrimental effects on human and animal health. Two of these, the West Nile virus (WNV) and the Usutu virus (USUV), co-circulate in Germany as well as other countries. Furthermore, they can coinfect and subsequently be co-transmitted by the same vectors, e.g., mosquitoes of the *Culex pipiens* species complex. Multiple studies have focused on the interaction in the vector and have shown that both viruses can interact with each other and influence their coinfection and co-transmission efficiencies; however, little to nothing is known about the underlying molecular mechanisms of how these viruses interact with one another, especially when coinfecting the same cells. To shed light on this, we tested virus-specific anti E antibodies for their specificity and cross-reactivity. Afterwards, we infected cells simultaneously with both viruses under different conditions, with the goal of either detecting coinfecting cells or gaining evidence for superinfection exclusion.

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## **Borna Disease Virus 1 infection kinetics in organotypic hippocampal slice cultures of adult Lewis rats**

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Borna Disease Virus 1 (BoDV-1) is the causative agent of a fatal neurological disease in numerous mammals including humans called Borna disease. To better understand the spread of Borna Disease Virus-1 in the brain, the kinetic of viral spread and infection in hippocampal organotypic slice cultures (OHCs) of Lewis rats was investigated. Viral spread was analyzed at 3, 7, 10, 14, 21, 28 and 42 days post infection (p.i.), respectively. Virus infection was visualized by immunofluorescence applying a BoDV 1- specific antibody recognizing the viral nucleoprotein and viral RNA was amplified by real time RT-PCR. Virus infection was successful and was confirmed first day p.i. and was present until the last time point of infection indicating viral persistence in the rat OHCs. Viral nucleoprotein was mainly found in neurons.

The time points with the most remarkable morphological alterations and/or changes in viral load will then be selected for further transcriptome investigations.

This poster shows additionally that manufacturing organotypic hippocampal slice cultures even of adult rats is a promising technique to replace life animal testing, respecting the 3R concept.

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## **Identification and characterization of Flavivirus Host Factors**

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Endemic and emerging arboviruses are a rising global health threat. Among arboviruses, flaviviruses contain a plethora of viruses with sometimes severe effects on human and animal health. Of these West Nile virus, Usutu virus, and tick-borne encephalitis virus are endemic in Germany and are thus of particular interest to us. Flaviviruses are small, enveloped, positive-stranded RNA viruses with a broad host spectrum. WNV and USUV are transmitted in a bird-mosquito-bird life cycle with humans and other mammals as dead-end hosts. TBEV is transmitted via ticks and rodents serve as the reservoir host. Both TBEV and WNV can infect the central nervous system and can cause in rare cases severe neurological symptoms, whereas USUV rarely causes symptomatic infections in humans. For any viral infection cellular host factors are essential. Several such host factors have been described for different flaviviruses in recent years. We set out to test if those host factors only played a role during the infection with one flavivirus or if they had an effect on several flaviviruses. Towards this aim gene-specific knockout cell lines were generated via CRISPR/Cas9 genome editing. We compared infection with different flaviviruses in these knockout cells to their parental cell lines. Here we summarize insights gained into studying host dependency factors suspected, identified, or excluded as flavivirus host factors.

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**High immunogenicity of CCHFV vaccine candidates in ruminants**

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Crimean-Congo haemorrhagic fever orthonairovirus (CCHFV) belongs to the family Nairoviridae and causes Crimean-Congo haemorrhagic fever in humans. Since the virus coincides with the distribution of its primary vector and reservoir, Hyalomma ticks, it is present in Asia, Africa, the Middle East and Southern Europe. So far, there is no internationally approved CCHFV vaccine available for humans. Moreover, despite usually subclinical infections, viraemic livestock can play a concealed role in the CCHFV life cycle by transmitting the virus to ticks and humans. Therefore, vaccinating animals can reduce the infection pressure for humans. In this study, ruminants were vaccinated with different vaccine candidates based either on the nucleoprotein (N) or the glycoprotein precursor (GPC) of CCHFV. All candidates triggered an antibody response after vaccination. However, the mRNA vaccine encoding CCHFV-N showed highest antibody response which was detected for at least 9 months following a simple prime boost regime. Since not only the antibody response but also the T-cell response is important for protection in a CCHFV infection, IFN $\gamma$ -ELISpots were performed using a peptide library of CCHFV-N. All animals immunized with the mRNA CCHFV-N vaccine showed T-cell responses against peptides. Overall, this vaccine candidate for ruminants showed most promising results and will be further evaluated in a CCHFV challenge in sheep.

**Snacks & Poster Viewing 1 / 30****Investigation of regional differences in the diagnostics of tick-borne encephalitis (TBE)****Authors:** Jennifer Leifheit<sup>1</sup>; Kathrin Lehmann<sup>2</sup>; Claudius Malerczyk<sup>3</sup>; Christof von Eiff<sup>3</sup>; Karsten Becker<sup>2</sup><sup>1</sup> *Pfizer Pharma GmbH; Friedrich Loeffler-Institut für Medizinische Mikrobiologie, Universitätsmedizin Greifswald*<sup>2</sup> *Friedrich Loeffler-Institut für Medizinische Mikrobiologie, Universitätsmedizin Greifswald*<sup>3</sup> *Pfizer Pharma GmbH***Corresponding Author:**

Tick-borne encephalitis (TBE) is a viral disease of the central nervous system (CNS) that can lead to neurological complications. Most German cases occur in the southern endemic areas but are also reported from the north. It is assumed that TBE occurrence in non-endemic areas is underestimated. This study aimed to investigate whether TBE diagnostic rate and the proportion of positive samples are consistent nationwide or whether regional differences exist.

This multicenter study collected data over three years from ten laboratories. Samples included all cerebrospinal fluid (CSF) samples with suspected CNS infection and all sera for which Lyme disease and/or TBE diagnostics were requested. The TBE diagnostic rates were evaluated relative to general CNS infection diagnostics and suspected Lyme disease, regionally stratified.

Preliminary evaluations were conducted for two laboratories each within and outside TBE risk areas. The TBE diagnostic rate (CSF/serum samples tested for TBE relative to CSF samples with suspected CNS infection) was significantly lower outside risk areas (2.8%) compared to risk areas (9.9%). The proportion of positive TBE samples was 3.5% outside risk areas, 1.6 times lower than in risk areas (8%).

The lower TBE diagnostic rates in non-risk areas suggest underdiagnosis. Specific TBE diagnostics should be performed even in non-risk areas. Expanding the risk area definition to include regional TBE virus prevalence could raise awareness among physicians and patients.

**Snacks & Poster Viewing 1 / 118****Insight into the diversity of *Yersinia ruckeri* isolates from fish farms**

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*Yersinia ruckeri*, the causative agent of enteric redmouth disease, causes acute/chronic diseases in fish (especially salmonids) and can lead to economic losses in fish farms. In addition to specific diagnostics for pathogen detection, information on mobile genetic elements, pathogen modification and control options are of interest. A *Y. ruckeri* collection was characterized by classical and sequence-based methods. Isolates were collected from infected fish between 2008 and 2023. They were typed and analyzed for genetic diversity and plasmid content by PFGE. In addition, agar diffusion tests were performed to determine the antimicrobial susceptibility (AST) of the isolates. The results show a high diversity among the isolates. A total of ten macro-resistance profiles were identified, some of which belong to different subclusters and to the two biotypes 1 and 2. AST show resistance to different classes of antibiotics, which could be traced to specific, mostly plasmid-encoded genes by WGS. In addition, similar virulence factors were reported for the isolates, exclusively associated with motility, immune regulation and effector supply. Different prophage sequences were detected in the chromosome of the isolates, but their activation has not been possible experimentally. Phenotypic and genotypic data will be presented and interpreted comparatively and statements on the significance of the isolates will be made.

**Snacks & Poster Viewing 1 / 83****Swine Influenza in Humans – Which Factors Drive Interspecies Transmission?**

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**Background:** From a one health-perspective, epidemiological and laboratory-based assessment of zoonotic risks posed by Influenza A Viruses (IAV) remains a significant challenge. As evidenced by the quadruple reassortment event leading to the 2009 A(H1N1) pandemic, the swine population serves as significant reservoir of pre-pandemic strains. Here, two isolates from pig to human-spillover events are compared with porcine, human pandemic, and seasonal IAV strains using human respiratory tract organoids to assess presence of adaptation to humans.

**Methods:** Human adult stem cell-derived nasal and bronchial organoids were cultured in 3D or differentiated at the air liquid-interface and subsequently infected with porcine, zoonotic, human pandemic, and seasonal IAV strains. Replication kinetics, interferon response, receptor affinity-profiles and transepithelial electric resistance were analyzed in comparison to conventional cell lines.

**Results:** Human-adapted virus replicated to higher titers than a closely related porcine isolate in both nasal and bronchial organoids, indicating susceptibility of the model to wildtype isolates without cell culture adaptation and recapitulation of cellular host tropism. Zoonotic isolates behaved differently in terms of replication and interferon responses, drawing attention to interferon antagonism as potential host switch-mechanism.

**Conclusion:** Human adult stem cell-derived organoids hold promise as invaluable tool for laboratory-based emerging virus risk-assessment.



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## Hepatitis E virus infection in porcine permanent cell lines

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The hepatitis E virus (HEV) is the causative agent for over 3.3 million symptomatic infections in humans annually. Domestic pigs and wild boar are known to be the main reservoir hosts for the zoonotic HEV genotypes within the species *Paslahepevirus balayani*. However, all established cell culture systems to study HEV are based on permanent human cell lines. So far, there are only a few approaches to use porcine cells for HEV infection research. In order to develop robust porcine cell culture systems, porcine permanent cell lines IPEC/J2, 3D4/21, Riebe255 and SK6 were selected and infected with different human as well as porcine HEV strains belonging to different genotype 3 subtypes. For comparison of infection efficiency, the well-established human hepatoma cell line PLC/PRF/5 was also included.

All of the porcine cell lines were susceptible for HEV infection, with the exception of IPEC/J2 cells. Several approaches showed that simultaneous infection was more efficient than infection of confluent cells. No significant differences in infectivity were observed for the different porcine and human HEV strains. Furthermore, virus replication was detected in all five cell lines after transfection with synthetic full-length HEV RNA. These cell culture systems can be used to determine molecular factors for viral entry that are largely unknown for HEV. In addition, the differences in viral host interactions between the human and porcine host can be investigated.

**Snacks & Poster Viewing 1 / 94****An integrated approach to mitigate health risks in wildlife trade**

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Wildlife trade and consumption are implicated in the emergence of zoonotic pathogens and biodiversity loss. Hundreds of thousands of unknown viruses with zoonotic potential are estimated to exist in wild mammals and birds, however, biodiversity and risky practices regarding wildlife vary greatly among regions. In most countries, health risks across the entire wildlife trade are not sufficiently recognized or addressed.

To bridge these gaps, the International Alliance against Health Risks in Wildlife Trade was launched in 2021 as a multi-stakeholder platform that, in 2024, has over 400 members from NGOs, IGOs, and academic institutions to individual experts. The Alliance funds 18 projects in 23 countries addressing and interlinking fields of action, such as behavioral and social sciences, pathogen discovery and assessment, legislation, and regulation.

The Alliance's work highlights the urgent need to address wildlife trade for primary pandemic prevention and confirms that risks and opportunities for intervention are highly context specific, while cross-sectoral governance on managing health risks from wildlife trade remains patchy. This global and multifaceted problem provides the opportunity to address health risks and biodiversity loss in an integrative fashion, protecting vulnerable human and wildlife populations while further improving our understanding of the ecological determinants of health.

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**Orthoflavivirus in vitro Neuroinvasion & Neuropathology is Independent of the Breakdown of the Blood-Brain-Barrier**

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The genus *Orthoflaviviruses* includes several neurotropic viruses capable of causing severe central nervous system (CNS) disease in humans. Mosquito-borne West-Nile Virus (WNV) and Tick-borne Encephalitis Virus (TBEV) are well-studied examples. However, the mechanisms by which these viruses enter the CNS remain poorly understood. The blood-brain-barrier (BBB) appears to be a potential entry site of these viruses. Following CNS entry, neuronal cells are the major targets; while resident glial cells (e.g., astrocytes and microglia) possibly contribute towards the neuroinflammatory response triggered by infection. Our hypothesis is that virus entry through the BBB and ensuing neuroinflammation are critical factors in the pathogenesis of these viruses. Hence, we established an *in vitro* model, using primary mouse brain endothelial cells and neonatal mouse brain glial cells and exposed them to pathogenic and non-pathogenic *Orthoflaviviruses*. The preliminary findings indicate that infection *per se* did not affect the integrity of the compact endothelial barrier. Interestingly certain *Orthoflaviviruses* crossed the intact endothelial barrier most likely through transcellular routes. Further, we could show that neurotropic TBEV is capable of infecting primary endothelial cells, astrocytes and microglia and trigger inflammatory responses in these cell types. The knowledge obtained from our model will help towards a better understanding of the neuropathogenesis of *Orthoflaviviruses*.

**Snacks & Poster Viewing 1 / 25****Differentiation of zoonotic flaviviruses in birds and horses using mutated recombinant envelope proteins in serological assays**

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West Nile virus (WNV), Usutu virus (USUV) and tick-borne encephalitis virus (TBEV) are zoonotic flaviviruses. WNV and USUV are transmitted to mammals by Culex mosquitoes with birds as reservoir hosts, whereas TBEV is transmitted from mammalian hosts by ticks. WNV is increasingly widespread with no human vaccine available. In Europe more than 700 human WNV infections were recorded in 2023 highlighting the importance of WNV as a major zoonosis. Survey of resident birds for WNV antibodies can be used to monitor WNV circulation.

Current commercial ELISAs show high cross-reactive binding among flavivirus antibodies, requiring extensive virus neutralization tests for differentiation, especially between the closely related viruses WNV and USUV. Flavivirus envelope (E-)proteins with four point mutations in the fusion loop of domain II (E<sub>2</sub> proteins) of WNV, USUV, and TBEV were used in indirect ELISAs to detect flavivirus IgG antibodies in horse and poultry sera. E<sub>2</sub> proteins have been shown to reduce cross-reactivity among related flaviviruses. The aim is to develop a rapid and reliable WNV screening method for several animal species, including resident and wild birds as sentinels to monitor flavivirus circulation.

**Snacks & Poster Viewing 1 / 103****Report of Dengue Virus 1 in an encephalitis patient from Ghana**

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**Background:**

Dengue fever is a common arboviral infection caused by dengue virus (DENV) and transmitted to humans via the mosquito species *Aedes aegypti* and *Aedes albopictus*. There are 4 different but closely related serotypes, endemic in about 100 tropical or sub-tropical countries. Ghana is considered non-endemic for Dengue virus with limited prevalence data.

**Methods:**

A panel of 86 human cerebrospinal fluid (CSF) was available from hospitalized patients with symptoms of encephalitis. PCR screening was performed for typical aetiological agents. All PCR-negative samples were further assessed by high throughput sequencing.

**Results:**

Bioinformatic analysis identified reads for DENV in one CSF sample from a seven-year-old female without travel history, admitted to the pediatric emergency unit. The serotype was identified as DENV-1, which was only reported once before in a traveler returning to Europe from Ghana. Comparative genome analyses of the virus polymerase revealed a high identity and phylogenetic clustering with DENV-1 strains from neighboring countries.

**Conclusions:**

Although DENV is not considered endemic in Ghana, cases of mainly DENV-2 and DENV-3 were reported occasionally in the past. Occurrence of DENV-1 suggests that the *Aedes* mosquito vector generally hosts all DENV serotypes in the West African region. Future studies on the mosquito vector as well as the population in this area can provide insight to the actual prevalence and clinical impact of DENV serotypes.

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## Quantification and localization of PERV in newly infected pig cells

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Xenotransplantation (XTx) using pigs as organ donors has experienced a significant progress in the recent years. However, XTx still poses a zoonotic risk, and the potential transmission of porcine endogenous retroviruses (PERVs) and its consequences need to be further investigated. In contrast to the majority of porcine pathogens, PERVs cannot be easily eradicated from donor pigs, as they are integrated in the pig genome. To evaluate their risk, in case of transmission, it is essential to determine the number of integration events, preferred sites and its impact on host cells expression profile.

In this context, we used PERV-C as a model organism to infect PERV-C negative ST-IOWA cells analyzing the number and sites of viral integration. Detailed information on the proviral copy numbers were obtained by droplet digital PCR (ddPCR) using *env* and *pol* specific primer sets comparing native and infected cells. The results revealed a total PERV copy number of 98-127 copies per set of chromosomes, with 2-13 newly integrated proviruses. We then identified potential integration sites of PERV by targeted locus amplification (TLA analysis) assigning it to sequential pattern like transcriptional start sites (TSS), genes, intronic and exonic regions as well as its genomic distribution. The highly variable integration of PERV was then compared to known C-Type retroviruses to identify potential effects on the host cell expression in view of oncogenes and cell signature modulation.

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**Detection of *Coxiella burnetii* in dust samples from ruminant farms and new perspectives for a Q fever vaccine**

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Q fever is caused by *Coxiella burnetii* (*Cb*), which is shed by ruminants during abortion or parturition. Contaminated facilities pose a risk for humans through the inhalation of dust particles. A vaccine is available for ruminants but can cause side effects and does not completely prevent shedding. Dust swabs have gained popularity for identifying *Cb*-positive farms. This tool was applied on one dairy cattle farm (calving barn) and 18 sheep farms (e.g., shearing and lambing sheds) in Australia. The dust swabs were analysed by a three-gene multiplex PCR (*IS1111*, *Com1*, *htpAB*). Swabs from the calving barn tested positive (Cq 19-35), indicating *Cb* shedding by the cattle. All swabs from the sheep farms tested negative, likely due to outdoor lambing and extensive farming practices. Thus, dust analysis for identifying *Cb* may be more suitable for cattle than for sheep farms under Australian conditions. Vaccination is the only option to protect animals. The COX-SAVE consortium will develop a new vaccine candidate against *Cb* based on the novel LEEI-(Low-Energy Electron Irradiation)-technology. LEEI destroys the nucleic acids of pathogens but causes less damage to structural components like proteins. Moreover, the optimal irradiation dose for inactivating pathogens (safety) can be determined while maintaining metabolic activity (quality). The immune responses after the vaccination of sheep and cattle will be investigated to confirm the protective effect and efficacy of the *Cb*-LEEI-vaccine.

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## Mosquito community composition and virus prevalence along a disturbance gradient in a Savannah ecosystem

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Mosquitoes pose a significant threat to human and animal health by functioning as vectors for various pathogens. Mosquito-borne infections include a considerable number of viruses such as West Nile virus and Zika virus. Investigating the link between man-made alterations of ecosystems, vector abundance and virus prevalence is crucial for understanding zoonotic health risks. Previous studies focused mainly on ecosystem alterations in the tropics, whereas our approach concentrates on a Savannah woodland ecosystem in the Kavango Zambezi Transfrontier Conservation Area in north-eastern Namibia.

In 2023, a total of 7727 mosquitoes was caught in areas categorized as national park, communal conservation areas, rangeland, agriculture, village and town. BG Sentinel traps, CDC light traps and Gravid traps were set up for 5 consecutive trap nights with four replicates per land-use type. In total, 52 mosquito species were identified. Mosquito abundance and community composition varied between the different land-use types. PCR screening for a broad spectrum of virus genera is ongoing. Interestingly, the urban area showed the highest mosquito density. Out of the 3766 mosquitoes caught in Katima Mulilo, ca. 90% are anthropophilic species, with *Culex Culex quinquefasciatus* as the most prevalent one. The latter is a vector for several arboviruses, such as West Nile virus and Rift Valley fever virus. Risk of acquiring an arbovirus infection increased near human settlements.



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## The Role of Veterinarians in Risk Communication of Zoonoses and AMR - How do Exotic Pet Owners perceive the Communication with their Veterinarians?

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Exotic animals may carry various pathogens. When traded and kept as pets, they can potentially transmit a variety of diseases to humans, other animals and vice versa. Therefore, it is essential for pet owners, particularly vulnerable groups, to be informed about the associated risks. As veterinarians play a crucial role in informing pet owners, they should not only have expertise but also good communication skills. Therefore, a survey was conducted among exotic pet owners (n=344) in Germany to gain insights into animal husbandry, veterinary consultations, and risk communication on zoonoses and antimicrobial resistance (AMR). Furthermore, exotic pet owners' perception of veterinarian communication was assessed. Our findings revealed the presence of numerous risks connected to exotic pet ownership. Fortunately, the perception of veterinarian communication received a high average score showing a high level of satisfaction. The duration of the veterinarian-client relationship was associated with better communication perception. The frequency of communication on zoonoses and AMR was associated with the presence of a permanent veterinarian. However, only 20% and 17% of the participants stated to have been informed about zoonoses and AMR, respectively. At the same time, 66% and 64% of participants indicated that they would like to receive veterinary advice on zoonoses and AMR. Therefore, more educational material on zoonoses and AMR should be made available, and the awareness concerning risk communication should be increased by further education and training at universities. This study highlights the important role of veterinarians in risk communication to strengthen global health at the human-animal interface.

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## Human and animal leptospirosis in Kenya: A comprehensive review and meta-analysis

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Leptospirosis is a priority zoonotic disease in Kenya, but an in-depth review of its presence in humans, animals, and the environment is lacking. We conducted this systematic review and meta-analysis to understand the epidemiological situation to date.

We searched for literature in African journals online, AGRIS, Embase, the *Leptospira* WOA reference laboratory library, ProMED-mail, PubMed, Scopus, Web of Science, and the institutional repositories of 33 academic institutions, and included 66 publications on leptospirosis in Kenya from 1951 to 2022.

Most investigations were conducted in rural and urban areas in western, southern, central, and coastal Kenya, with the largely pastoral eastern and north-eastern areas being under-represented. A wide host range of domestic animals and wildlife was revealed, and occupational exposure was an important risk factor for humans. The microscopic agglutination test (MAT) was the most prominent test, especially in studies from the 1980s and 1990s, although varying MAT panels and cut-off titres were observed. Molecular tests were seldom used to determine species and illustrate strain diversity. There was a lack of awareness of leptospirosis among farmers and health practitioners.

The widespread presence of leptospires and inadequate diagnostic capacity demonstrate that leptospirosis is a common but underreported disease in Kenya. Raising awareness and boosting the country's diagnostic capacity is crucial for timely detection and disease control.

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## Improved molecular surveillance and assessment of host adaptation and virulence of *Coxiella burnetii* in Europe

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*Coxiella (C.) burnetii* is the causative agent of the zoonotic disease Q fever. The bacteria display a broad host range with diverse clinical manifestations in ruminants. Human Q fever outbreaks mostly originate from infected sheep. We hypothesize that the zoonotic potential and clinical relevance of an actual *C. burnetii* isolate can be deduced from yet undisclosed differences in genomic traits. First, we aimed at increasing the availability of isolates currently circulating in Europe. To this end, an improved isolation protocol using homogenization and sequential filtration was established with a bacterial loss of 1 to 1.5 log. Treatment with ampicillin, gentamycin and vancomycin as post isolation option was found to be applicable to limit contaminations. Archived *C. burnetii* isolates were sequenced for phylogenetic analysis and identification of genomic traits. Core genome SNP analysis of 34 achieved *C. burnetii* isolates revealed two major clusters that were assigned to Genomic Groups II and III. The later consists mostly of cattle isolates. For phenotypic characterization, an ex vivo whole blood model was adapted. Whole blood from ruminants was inoculated with *C. burnetii* isolates from different hosts and disease manifestations. Host-pathogen interaction was assessed using transcription analysis of immune-related genes. Preliminary data suggest that the whole blood model provides reliable results and is suitable for virulence testing of isolates. Testing with field isolates is ongoing.

**Snacks & Poster Viewing 1 / 34****Outbreak of leptospirosis in Germany linked to the keeping of fancy rats as pets****Authors:** Kaya Stollberg<sup>1</sup>; Martin Richter<sup>2</sup><sup>1</sup> *German Federal Institute for Risk Assessment*<sup>2</sup> *German Federal Institute for Risk Assessment***Corresponding Author:** martin.richter@bfr.bund.de

Leptospirosis is a bacterial zoonosis of global importance that is caused by pathogenic *Leptospira* species. Transmission can occur through direct or indirect contact with the urine of infected animals through small skin lesions or mucous membranes.

In 2023 an outbreak of leptospirosis in Germany was linked to the keeping of fancy rats (*Rattus norvegicus forma domestica*) as pets.

The consultant laboratory for leptospirosis tested serum samples of humans who had been in contact with potentially infected rats. Serum samples were tested using the microscopic agglutination test (MAT) and an in-house enzyme-linked immunosorbent assay (ELISA). In addition, rat urine samples were investigated by a PCR targeting the *LipL32* gene to detect the presence of pathogenic *Leptospira*.

A total of 33 human serum samples were tested using the MAT. Three of the 33 serum samples showed positive results. When investigated by ELISA, four serum samples showed positive results for IgG and IgM and three serum samples for IgM only. Both MAT and ELISA were negative in 21 samples. Of the 56 rat urine samples that were investigated, nine positive samples were identified by PCR (*LipL32*).

Contact with rats should not be underestimated as a source of infection in the future and owners should be advised of the potential risk of infection.

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**Combination of microfluidic cell culture infection with Transposon Directed Insertion Site Sequencing (TraDIS) identifies putative new adherence factors of *Streptococcus canis***

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*Streptococcus canis* (*S. canis*) is a zoonotic pathogen which causes systemic infections such as septicemias in animals and in humans. Little is known about factors directly contributing to cell adherence and bacterial invasion into various host niches. Therefore, the main aim of this project is the identification of new bacterial adherence factors contributing to colonization of vascular endothelium under blood flow conditions.

For *in vitro* simulation of the physical parameter mediated by the blood flow, infection analyses of a *S. canis* transposon-library and human primary endothelial cells (EC) were performed using a microfluidic system. This system enables the application of defined shear stress throughout infection. Bacterial adherence to vascular ECs was visualized and quantified microscopically after immunofluorescence staining, indicating that *S. canis* effectively adheres to EC even at high shear force levels during flow infection.

After bacterial infection of ECs, adherent and non-adherent transposant pools were separately recovered. Bacterial genomic DNA was processed for comparative Transposon Directed Insertion Site Sequencing (TraDIS). Bioinformatical evaluation of sequence pools resulted in identification of several putative factors contributing to bacterial adherence.

The combination of microfluidic infection of endothelial cells with the TraDIS technique proved to be ideally suited for the pathomechanistic analysis of *S. canis* infections in the vascular system.

**Snacks & Poster Viewing 1 / 12****Environmental Pollution by Antibiotic Mixtures Impacts Soil Microbiome Activity and Resistance Gene Abundance****Authors:** Anja Worrich<sup>1</sup>; Chika Ejikeugwu<sup>1</sup>; Maja Hinkel<sup>1</sup><sup>1</sup> *Helmholtz Centre for Environmental Research - UFZ, Leipzig***Corresponding Author:** [chika-ebiye-peter.ejikeugwu@ufz.de](mailto:chika-ebiye-peter.ejikeugwu@ufz.de)

Antibiotic resistance (AR) in the environment poses a potential threat to human and animal health, as resistance can be transmitted back from environmental reservoirs. Manure is a primary source of AR entering the environment, but the drivers of its persistence remain largely unexplored. Animals are often treated with mixtures, and the repeated application of manure introduces various compounds. These mixtures may have additive or synergistic effects on the selection and spread of AR in soil bacterial communities. We investigated the effects of binary antibiotic mixtures on soil bacterial activity and the abundance of antibiotic resistance genes (ARGs) in 3 different soils to account for the influence of soil physicochemical properties. Soils were spiked with tetracyclines, sulfonamides, and fluoroquinolones at low (1.2 mg kg<sup>-1</sup>), medium (12 mg kg<sup>-1</sup>) and high (120 mg kg<sup>-1</sup>) concentrations of single compounds and mixtures. Bacterial activity was assessed via respiration measurements and the incorporation of deuterium from deuterated water as a general activity marker to trace the effects of antibiotics down to the level of single cells. We observed a strong dependency on concentration and antibiotic identity, with initial results indicating that the effects of antibiotic mixture differed markedly from those of single compounds. In the next step, we will analyze the effects on ARG abundances in the different soils to link mixture effects to antibiotic resistance prevalence. e compounds. In the next step, we will analyze the effects on ARG abundances in the different soils to link mixture effects to antibiotic resistance prevalence.

**Snacks & Poster Viewing 1 / 15****Long-term circulation of rat hepatitis E virus in wild rat populations from Berlin**

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Cases of human rat hepatitis E virus (ratHEV) infections have recently been described in several patients with acute and chronic hepatitis in Hongkong, Canada, Spain and France. RatHEV has also been frequently detected in rats worldwide, which are suspected to represent the main animal reservoir of this zoonotic virus. However, long-term studies demonstrating the persistence of ratHEV in wild rat populations are missing so far.

Here, wild Norway rats (*Rattus norvegicus*) from Berlin, Germany, were collected in 2023 and their liver tissue samples analyzed for the presence of ratHEV RNA using qRT-PCR. The results were then compared to that of a similar study performed between 2009 and 2010 in Berlin. Positive samples from both studies were further characterized.

A total of 11/131 (8.3%) rat samples from 2023 were positive by qRT-PCR, which is similar to the findings in 2009-2010 with 7/61 (11.5%) ratHEV-positive rats. Partial sequences of the ORF1, encoding the viral polymerase, were nearly identical to those from 2009-2010, and formed a monophyletic clade, clustering closely with other sequences from Europe. Whole genomes could be obtained for 8 and 3 ratHEV strains from 2023 and 2009-2010, respectively; sequence analysis is still ongoing.

The results of the study indicate a continued circulation of ratHEV for more than 10 years in the wild rat population of Berlin, supporting the role of wild rats as an animal reservoir for ratHEV and a potential source of human infections.

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## Human kidney cells revealed differences in replication kinetics for pathogenic and non-/low- pathogenic orthohantaviruses

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Zoonotic infections caused by pathogenic Eurasian hantaviruses manifest as hemorrhagic fever with renal syndrome (HFRS) characterized by acute kidney injury and a broad range of severity dependent on the causing virus species. Direct and immune-mediated effects may play a role in pathogenicity. Therefore, we analyzed the permissiveness of human tubular and glomerular (podocytes, endothelial, mesangial) kidney cells for hantaviruses with different virulence. We used primary cells, and we examined cell lines for their suitability as cell culture model. The replication competence of Hantaan virus (HTNV) as highly pathogenic member was compared with Tula virus (TULV) as non-/low-pathogenic virus. HTNV infected tubular cells, podocytes, and glomerular endothelial cells and replicated efficiently with more than 2/3 of cells being infected. However, in mesangial cells, infection is abortive despite initially high infection rates and release of infectious particles. In contrast, TULV showed very low infection rates (< 10%) and no increase of infection in all tested cell types. Infection of the corresponding renal cell lines with HTNV and TULV revealed replication kinetics that are similar to the results obtained in primary cells.

Our findings revealed differences in the replication competence of hantaviruses with different pathogenicity, which may be directly linked to the broad range of HFRS severity. In addition, we identified renal cell lines as suitable in vitro infection model.



**Snacks & Poster Viewing 1 / 41****Human stem cell-derived neurospheres to explore the consequences of *Listeria* infection on brain development**

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Zoonotic pathogens infecting pregnant women can result in central nervous system (CNS) infections in their offspring. The underlying mechanisms are not well understood, especially regarding long-term consequences of pre- and perinatal CNS infections.

To address this, we test the suitability of human induced pluripotent stem cell (iPSC)-derived neurospheres, which mimic the developing brain as a model to study fetal CNS infections. Our initial focus is on infection with *Listeria monocytogenes*, a pathogen that causes lasting neurological sequelae in offspring.

We hypothesize that infection depletes primary neural stem cells (NSC) and accelerates the maturation of surviving NSCs, resulting in impaired differentiation, aberrant migration, and altered network activity.

To test this, we generated NSCs that can be stably cryopreserved to produce neurospheres from the same batch for reproducible follow-up infection experiments. Neurosphere differentiation is established and characterized via RT-qPCR and immunocytochemistry.

We have also developed cryopreservation protocols to freeze neurospheres for transfer to biosafety level facilities for infection experiments. Preliminary results show that different freezing media do not affect post-cryopreservation recovery, though long-term cell survival rates did not meet our requirements for further experiments, necessitating optimization of the protocol. Next, we aim to identify an appropriate infection dose in initial experiments.

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## Tularemia in Germany – Human Cases and Genotyping of Isolates

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*Francisella tularensis* is the causative agent of tularemia, a zoonotic disease with a wide host range. *F. tularensis* ssp. *holarctica* (*Fth*) is of clinical relevance for European countries, including Germany.

Germany is a country with a low incidence of tularemia in humans. However, surveillance of human cases shows an increase in reports over the last 10 years. Climate change, but also more frequent testing and increased awareness of the disease may also have contributed to the observed increase in reported tularemia cases. According to Germany's Infection Protection Act, tularemia is classified as notifiable disease since 2001. A mean annual incidence of approx. 0.1 cases per 100,000 population with more than 65 cases annually reported since 2019. However, tularemia is assumed to be significantly underdiagnosed and underreported in Germany. Among the notified cases, the most frequent clinical presentations of tularemia were the glandular and ulcero-glandular form, the pneumonic form, and the oropharyngeal form. Regarding the genotypic diversity and geographical segregation of *Fth* two clades are most frequently found in Germany: the basal clade B.6 (Biovar I, erythromycin-sensitive) and basal clade B.12 (Biovar II, erythromycin-resistant). Whereas *Fth* strains of clade B.6 are more prevalent in south-western, clade B.12 isolates of *Fth* are more prevalent in north-eastern Germany.

Here, we describe some particularly interesting cases of human tularemia caused by *Fth* and the genotyping of *Fth* isolates in Germany.

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## Mycobacterium avium subsp. paratuberculosis (MAP) in Sudanese Livestock and Humans: a Public Health Threat

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Mycobacterium avium subsp. paratuberculosis (MAP) is a causative agent of paratuberculosis (PTB). In the Sudan, MAP infection in animals is neglected, and we have recently reported data on its presence in humans. The present study aimed to investigate the presence of viable MAP in animals and humans and to identify the phylogenetic relationships between Sudanese MAP isolates and other MAP strains worldwide. Faecal and tissue samples (N=73) collected from animals, also faecal and tissue samples of 107 patients with gastrointestinal diseases were subjected to the culture. The IS1311 of the MAP isolates was amplified and sequenced. MAP was isolated from cattle, sheep, goats and camels, respectively, as 17.6% (3/17), 14.2 % (2/14), 5.8 % (1/17) and 20% (1/5) faecal samples and tissue samples of 8.3% (1/12) sheep, 12.5% (1/8) goats. In humans, it was isolated as 22.2% (10/45) and 25.8% (16/62) from faecal and tissues samples, respectively. The phylogenetic tree showed close relatedness between Sudanese MAP isolates and MAP strains from different continents. The close relationship between Sudanese MAP isolates from animals and humans supports the zoonotic potential of MAP. Also, viable MAP in animals represents a source of contamination for the food chain and environment, thus a potential hazard to humans. Therefore, developing effective vaccines from local isolates and applying biosecurity practices along the food chain are essential approaches for reducing MAP in the environment.

**Snacks & Poster Viewing 1 / 51****Rapid test during outbreak: lesson learned from the Sudan deployment**

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In the Sudan the capacity of PCR tests for infections remains limited, indeed in low resource settings, which necessitates looking for alternatives. This study aimed to evaluate a novel isothermal amplification assay integrated in a mobile suitcase laboratory for rapid detection of SARS-CoV-2 and to highlight the factors that affect its' implementation and deployment in field conditions. Archived nasal samples (368) from patients who tested positive for SARS-CoV-2 and from patients who were suspected having COVID-19 and tested negative were retrieved and used in the study. The extracted RNA, using QIAmp Viral RNA Mini Kit, was tested for SARS-CoV-2 Nucleocapsid (N) and RNA-dependent RNA polymerase (RdRP) gene by reverse transcription Recombinase-Aided Amplification (RT-RAA) assays, and by real-time PCR. The sensitivity of both RT-RAA assays varied with the viral load; samples with Ct <30 showed sensitivity of 60.4% and 55.3% for the RdRP and N genes, respectively. The agreement between real-time PCR and the two RT-RAA assays was fair ( $k = 0.21 - 0.40$ ). Rapid and affordable molecular diagnostics are required at the point of need in low resource settings; albeit, continuous in- person training would improve the overall laboratory workflow, and ensure successful diagnostic testing. Along with having good quality kits and devices, quality control would also be critical for test performance. Such learned lessons are valuable for planning and performing diagnostic clinical trials.

**Snacks & Poster Viewing 1 / 54****Microbial diversity and antimicrobial resistance in rodents and livestock: Insight from the Hainich-Dün Biodiversity exploratory**

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The gut microbiome impacts host health and vitality, is itself influenced by internal and external factors and may affect pathogen epidemiology. Gut microbes can harbor antimicrobial resistance (AMR) genes and virulence factors, transmittable to the environment and its inhabitants (wildlife, livestock, humans). Wild rodents are assumed sentinels for many (zoonotic) pathogens. We assessed the fungal and bacterial microbiome diversity and composition of *Myodes glareolus* and *Microtus arvalis*, using 16S rRNA and ITS sequencing.

Additionally, metagenomic and plate-genomic sequencing of cattle, sheep, and *M. arvalis* feces was conducted to detect AMR genes, virulence factors, and transmission between wildlife and livestock. Samples were collected in the DFG-biodiversity exploratory Hainich-Dün in Thuringia, Germany. Significant differences in alpha and beta diversities of the microbiomes were found between the two rodent species, with season (sampling month) having a significant influence on microbiota diversity and composition. Sheep and cattle samples showed higher bacterial diversity than rodent samples. Gassner and MacConkey agar plates represent the One Health relevant bacterial family of Enterobacteriaceae best, whereas blood and TSB agar plates show a higher diversity of bacteria, AMR, and virulence factors. Our results give insight into the microbiomes of important vector and reservoir species and the transmission of AMR and virulence factors between wildlife and livestock.

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## Analysis of *Staphylococcus aureus* CC1 and CC1660 from humans and horses

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Equine and human *Staphylococcus aureus* were investigated for their genetic relationships and biocide susceptibility.

In total, 93 *S. aureus* from 60 human and 28 equine samples were subjected to whole-genome sequencing (WGS) using Illumina MiSeq, antimicrobial and biocide susceptibility testing.

WGS assigned 77 isolates to clonal complex (CC) 1 and 16 to CC1660. Ten *spa* types (t114, t127, t273, t559, t922, t1383, t1491, t1508, t14504, t18599) were detected among CC1 and five *spa* types (t549, t3043, t11926, t12047, t15977) among CC1660 isolates. cgMLST revealed differences in at least 1329/1430 alleles between CC1 and CC1660 isolates. The alleles differences within the CC1 isolates ranged from 0 to 269 and from 0 to 233 between the CC1660 isolates. All isolates were negative for the Pantone-Valentine leukocidin genes and only two harboured the toxic shock syndrome toxin gene *tst1*. Moreover, 71 isolates harboured a *blaZ* gene (penicillin resistance), whereas only seven carried the methicillin-resistance gene *mecA*. The biocide MICs ranges from 0.00013-0.0005% for benzalkonium chloride, from 0.00013-0.001% for polyhexanide, and from 0.00006-0.003% for chlorhexidine and octenidine. However, *qacA/B* or *qacC* genes for decreased benzalkonium chloride susceptibility were detected in 19 and six isolates, respectively.

In conclusion, related *S. aureus* CC1 and CC1660 were present among humans and horses. The presence of the major virulence gene *tst1* gene and the *qac* genes should be monitored.

**Snacks & Poster Viewing 1 / 149****Tick-borne encephalitis seroprevalence in sheep, goats, and dogs in the Czech Republic**

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Tick-borne encephalitis virus (TBEV) is the causing severe neuroinfections in humans in Europe and Asia. Humans are typically infected after a tick bite; however, alimentary infection can occur after consumption of unpasteurized dairy products. In addition, sheep and goats are often used as suitable sentinels for surveillance of TBEV-associated risks in endemic areas. We conducted a serological survey describing TBEV exposure in these animals in the Czech Republic. We collected sera samples from sheep and goats in 11 of the 14 administrative districts of the country. The seroprevalence was significantly higher among sheep (32.5%) than goats (19.7%) Positive samples were identified in 56.7% of goat farms, and 82.4% of sheep farms, and in 9 of the 11 examined administrative districts. Also dogs are frequently infected with TBEV (rarely also with clinical manifestation) and can be used as sentinel animals. In the case of dogs, total of 453 sera were collected. The sera were collected from either healthy dogs (130 animals) or dogs that had been investigated for various health problems (323 animals). Serological analysis showed similar seroprevalence in healthy dogs and dogs with health impairments, 13.1 % and 12.1%, respectively. The ages examined dogs ranged from 0.5 to 15 years and we identified positive correlation between age and seropositivity.

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## Pathogenicity of *Arcobacter cryaerophilus*

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The gram-negative bacterium *Arcobacter cryaerophilus* is associated with gastrointestinal illnesses in infected humans. Due to its occurrence in not only various environmental habitats but also different kinds of food, it is considered an emerging foodborne pathogen. Nevertheless, the pathogenicity mechanisms responsible for symptoms such as watery diarrhea and abdominal cramps remain largely unknown. Therefore, we tested twelve *Arcobacter cryaerophilus* strains of different origins for their pathogenic potential in the two human intestinal cell lines HT-29/B6 and T84.

We observed that all twelve tested strains were able to adhere to and invade both cell lines on a similar level. Moreover, in a cytotoxicity assay, we measured the decrease in the metabolic activity of infected cells. We observed two of the twelve strains to decrease the metabolic activity to less than 50% residual metabolic activity in both cell lines.

In addition, to detect a decrease in the permeability of the epithelial barrier, we measured the reduction of the transepithelial resistance in T84 cells. Four of five tested strains reduced the transepithelial resistance of the cells to 50% or less.

The various observed pathogenicity mechanisms of *A. cryaerophilus* in two human colonic cell lines support the suggestion that this bacterium poses a possible hazard to human health and underline the importance of further investigations of the bacterium.



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## T-cell Responses to Middle East Respiratory Syndrome Coronavirus among High-Risk Exposure Camel Abattoir Workers in Kenya

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**Background:** Despite comparable MERS-Coronavirus seroprevalence in camels on the Arabian Peninsula and the African continent, only 6 of the >2600 human MERS cases were notified in African countries. Previous studies showed discrepant results between MERS-CoV Spike-specific IgG and T-cell responses in MERS-CoV-exposed individuals suggesting a possible underestimation of MERS cases.

**Objectives:** Here, we compared anti-MERS-CoV IgG levels with MERS-CoV Spike-specific T-cell responses in a high-risk exposure group of camel abattoir workers.

**Methods:** We adapted a SARS-CoV-2 Interferon-gamma release assay (IGRA) for the detection of MERS-CoV-specific T-cell responses. We assayed responses to peptide pools (15-mers offset by 4 amino acids) of the SARS-CoV-2 and MERS-CoV Spikes. The assay validation was done using MERS-CoV-vaccinated (n=35; previously SARS-CoV-2-exposed) and SARS-CoV-2-vaccinated (n=16) individuals. The study cohort comprised camel abattoir workers (n=48) from Isiolo, Kenya, and unexposed controls.

**Results:** 31/35 (89%) of the MERS-CoV vaccinees showed MERS-CoV-reactive T-cells with significantly higher IFN- $\gamma$  levels compared to the SARS-CoV-2-vaccinated group. 3/48 (6%) camel workers had elevated MERS-CoV- IFN- $\gamma$  levels (>200 mIU/ml), 2 of the 3 also being SARS-CoV-2 reactive. All 3/48 IGRA-positive camel workers were however anti-MERS-CoV IgG negative.

**Conclusion:** A combination of antibody and T-cell response assays might elucidate the true rate of MERS-CoV transmission.

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## How studies on zoonotic risks in wildlife implement the One Health approach – A Systematic Review

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The emergence of infectious diseases underscores the urgent need for holistic prevention strategies. Mitigating zoonotic risks represents a global challenge for human, animal and environmental health and draws attention to the One Health approach. A systematic literature review was carried out to evaluate study design, interdisciplinary collaboration, and participatory approaches of recent One Health research on zoonotic diseases and wildlife. PubMed and Web of Science were searched for primary research papers from 2018 to 2023. A total of 105 out of 228 retrieved studies were eligible for inclusion. Only a small number of studies integrated human, animal, and environmental domains simultaneously in data collection (4.8%) and knowledge generation (29.5%). While extensive knowledge was generated for animal health (97.1%) and human health (84.8%), environmental health (34.3%) remained underrepresented. Laboratory methods predominated (82.9%), while social science methodologies were limited (19%). The majority were epidemiological studies (86.7%). The participation of non-academic stakeholders was described in 36.2% of studies, while 3.8% employed participative approaches. It can be concluded, that greater inter- and transdisciplinary collaboration, along with participatory approaches, are still needed for advancing One Health research in the field of zoonotic risk and wildlife in order to integrate competence, understand disease dynamics and effectively prevent zoonoses.

**Snacks & Poster Viewing 1 / 162****Colonization of healthy horses and their owners with Methicillin-resistant *Staphylococcus aureus* (MRSA) in the Community and the question of interhost transmission.**

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Methicillin-resistant *S. aureus* (MRSA) exhibiting multiple resistance phenotypes have globally emerged as nosocomial pathogens in hospitals for humans and also those for horses. Nasal MRSA colonization bears the risk of subsequent infections for both. The study reported here, is based on nasal swabs collected from 335 horses and their 188 owners in contact with them in 118 equestrian facilities located in several German federal countries. Isolates were subjected to antibiotic susceptibility testing and to molecular typing. Overall rates of nasal *S. aureus* colonization were 6,3% (1,8% MRSA) among horses and 37,2% (1,1% MRSA) among humans. The MRSA isolates from horses as well as those from humans were attributed to the horse clinic associated subpopulation of LA-MRSA CC398. For these and also for methicillin susceptible isolates (MSSA), typing suggested transmission between horses and humans. Interestingly, particular clonal lineages among MSSA (ST816, ST1640, ST1660) from horses seem to be specifically associated with this host. They are not registered in the database of the German National Reference Centre for Staphylococci and Enterococci among isolates from humans and other animal species collected during the past 30 years. In conclusion, our results confirm previous observations that nasal colonization with *S. aureus* in horses is infrequent. Introduction of horse clinic associated MRSA into the community is obviously rare.

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**Influence of climate change-driven adaptation on biofilm formation of *Acinetobacter baumannii***

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*Acinetobacter baumannii* has emerged as a red-hot nosocomial pathogen globally due to its proven ability to acquire resistance and ability to withstand harsh environments. Climate change, together with environmental exposure to residues of antibiotics, disinfectants and other pollutants, play a role in modulating the adaptation of *A. baumannii*. The relationship between the antibiotic resistance and ecological conditions is not clearly understood. The present study was aimed at investigating the influence of varying temperature (4°C, 20°C, 27°C, 37°C and 42°C) and varying pH (6.5, 7.5, and 8.0) on biofilm formation (16, 24, 48, and 72 hours) of *A. baumannii* isolates of environment and animals. Two each of environmental origin (multilocus sequence typing sequence type 52; ST52) and animal origin (ST2) isolates were utilized in this study. Isolates exhibited comparable antimicrobial resistance pattern but displayed phenotype difference in biofilm formation and variations in response to temperature and pH. Optimal biofilm production was observed at 27°C for environmental isolates and at 37°C for animal-origin isolates, with peak formation occurring within 24 to 48 hours. Significant biofilm production was noted at pH 6.5, while temperature variations influenced biofilm formation at pH 7.5 and 8.0. Surface adherence increased with rising temperatures, with enhanced biofilm attachment observed at 42°C. The isolates displayed better adaptability to the temperature and pH stress.

**Snacks & Poster Viewing 1 / 4****Novel 3D-co-cultivation system reveals adherence of *Streptococcus canis* to vascular smooth muscle cells under microfluidic conditions**

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During systemic streptococcal infections, bacteria adhere to activated vascular surfaces thereby inducing inflammatory responses of the endothelial cells.

We aim to decipher bacterial and cellular factors mediating adherence, cell internalization and transmigration of the blood vessel wall by the zoonotic pathogen *S. canis* using our recently established 3D-co-cultivation system.

The inner lining of the vessel wall is formed by endothelial cells (EC) followed by smooth muscle cells (SMC) embedded in a collagen-rich extracellular matrix. After monitoring adherence and internalization of *S. canis* into both single cell types, EC and SMC, we applied the 3D-co-cultivation system comprising EC and SMC embedded in polymerized collagen for *in vitro* infection analyses. Furthermore, we connected the 3D-co-cultivation system with a microfluidic pump to simulate physiological blood flow parameters during bacterial infection.

Differential immunofluorescent staining followed by confocal microscopic visualization demonstrated effective adherence of *S. canis* to EC and to SMC even under high shear stress conditions. Furthermore, various imaging techniques provided strong evidence for a transcellular migration of the bacteria through all cell layers of the reconstructed blood vessel wall.

Our data provide first evidence that transcellular migration might represent an effective pathomechanism determining the severity of bacterial blood stream infections.

**Snacks & Poster Viewing 1 / 19****Luminex-based multiplex serology for detection of antibodies to zoonotic arboviruses for One Health surveillance**

**Authors:** Lydia Kasper<sup>1</sup>; Fee Zimmermann<sup>2</sup>; Anne Balkema-Buschmann<sup>1</sup>; Balal Sadeghi<sup>1</sup>; Franziska Stoek<sup>2</sup>; Anca Dorhoi<sup>1</sup>; Fabian Leendertz<sup>2</sup>; Martin H. Groschup<sup>1</sup>; Martin Eiden<sup>1</sup>

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Zoonotic diseases pose a serious problem to human and animal health. To monitor the dynamics of pathogen transmission within and among host species, the Helmholtz Institute for One Health (HIOH) and the Friedrich-Loeffler-Institut (FLI) are establishing One Health Surveillance Platforms, for sample acquisition at human/domestic/wildlife interfaces as well as the implementation of multifunctional diagnostic assays. In this context, the InnoDia project aims to develop serological Luminex-based multiplex assays, enabling large-scale screening for antibodies against different pathogens.

Our current assays focus on hepatitis E (HEV), Rift Valley fever (RVFV), Crimean-Congo hemorrhagic fever (CCHFV), West Nile (WNV), Usutu (USUV) and tick-borne encephalitis (TBEV) viruses. Recombinant proteins representing viral capsid protein fragment p239 (HEV), nucleoproteins (RVFV, CCHFV) and NS1 protein (WNV, USUV, TBEV) were coupled to magnetic, carboxylated beads, and antigen-binding serum IgG antibodies were detected. Assay performances were evaluated using defined positive and negative sera from susceptible animal species, including pigs, wild boar, cattle, goat and sheep. Cut-off levels and diagnostic sensitivity and specificity were calculated using Receiver Operating Characteristic (ROC) curves.

In future, Luminex-based serology will be extended to other pathogens, to allow for screening against multiple infectious agents in One Health surveillance studies.

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## Detection and Characterization of Alongshan Virus in Ticks and Tick Saliva from Lower Saxony, Germany with Serological Evidence for Viral Transmission to Game and Domestic Animals

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Alongshan virus (ALSV) is a segmented, flavi-like virus assigned to the Jingmen virus group, which was first discovered in a blood sample of a human case associated with febrile illness in China in 2017. Since then, there have been numerous virus findings in Asia and Europe predominantly in ticks, but there also have been further detections in vertebrates including wild animals and livestock. The genetic close relationship to other tick-borne Orthoflaviviruses raises concerns regarding its impact on public health. During a tick survey in Lower Saxony, Germany, Ebert *et al.* (2023) discovered ALSV in ticks and tick saliva samples. In addition, ALSV RNA was also detected in a serum sample of a red deer. Furthermore, serological investigations led to the detection of ALSV-specific antibodies in domestic and wild animals. In a vector competence study, it was demonstrated that ALSV is able to replicate in *Ixodes ricinus* and *Dermacentor reticulatus* tick. In an artificial blood feeding system, transmission of viral RNA during the tick feeding process was proven. The results of this study shed light on the distribution of ALSV in tick populations from Lower Saxony and gives an insight on the host range of ALSV as well as ALSV-specific antibodies in vertebrate hosts.

To evaluate the extent of health significance of ALSV for humans and animals requires further investigations.

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## Semantic Enrichment of the Animal Necropsy Data of the Helmholtz Institute One Health

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The implementation of common terminologies enables the harmonisation of data elements within and across data-driven research projects and thereby further enhances data interoperability and queryability.<sup>1,2</sup> FAIR data has rich metadata, clear information about licences that stipulate data reuse and is better findable.<sup>2,3</sup> It has the potential for broader incorporation in follow-up of One Health studies, higher data quality and linkage to other existing data sources.<sup>3</sup> FAIR data can be particularly beneficial in One Health settings, where the data stewardship is often highly complex, costly, interdisciplinary and distributed across countries.

Semantic enrichment is a key FAIRification step.<sup>2</sup> Studies show that semantic enrichment of medical data is more commonly practised in human medicine than in veterinary medicine.<sup>4,5</sup> Among the contributing factors to this is that unlike human medicine, there is no external or widely used uniform veterinary coding system, the resources required to annotate patient records with standard medical diagnostic codes are limited and there are no incentives directed towards this venture.<sup>4,6</sup> Most veterinary visits are therefore captured in free-text notes which makes data retrieval and reuse challenging.<sup>6</sup> The quality of the semantic enrichments is heavily influenced by the selection of the appropriate target for the source data.<sup>7</sup> This work promotes a structured FAIR assessment to analyse the current situation in the veterinary medicine in the context of One Health research and it suggests the next steps to be taken by the One Health community to provide FAIRer data sets. As a specific example for FAIRification, we show how a coding system was systematically chosen for the semantic enrichment of the animal necropsy data of the Helmholtz Institute for One Health. To achieve this, we will show how we employ the recommendations of the European Union's Task Force on Evaluation and Enrichment on selecting target datasets for semantic enrichment. The steps in this methodology include; source data analysis, identification of requirements that the selected target terminology should address, selection of target terminology and testing of the selected target terminology. We anticipate that the application of this recommendations will lead to the establishment of a protocol for high-quality semantic enrichment of veterinary medical data.



**Snacks & Poster Viewing 1 / 35****Flavivirus Vaccine strategies**

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West Nile Virus (WNV), transmitted by mosquitoes, is a zoonotic flavivirus, which can cause severe neurological infections in humans. Recently, increasing numbers of cases have been reported in Southern Europe. To date, a vaccine for human use is not available.

It has been reported, that neutralizing antibodies targeting the envelope protein (E) of flaviviruses provide complete protection against the disease, therefore, using the E protein as antigen is a central vaccine strategy. The conserved fusion loop (FL) domain within the E protein elicits antibodies, that can cross-react and enhance infections with related flaviviruses.

Through modification of the FL sequence within the WNV E protein we aimed to reduce the risk of induction of a cross-reactive immune response. We found that recombinantly expressed WNV E proteins with modified FL partially protected mice from a lethal WNV infection and improved specificity of serum antibodies. mRNA packed lipid nanoparticles encoding variants of WNV E wildtype or FL mutants will be further examined.

Additional mutations were identified using computational protein design focusing on dimer and interface stabilization. Additionally, an evolutionary based design was applied to identify dimer stabilizing mutations of other flaviviruses, which were incorporated into the WNV E protein dimer to enhance stability.

Transfecting different cell types, protein expression and stability of these mRNAs and a reporter construct are currently assessed.

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## The Raccoon (*Procyon lotor*) as an emerging neozoon and potential reservoir for tick-borne pathogens in Germany

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The raccoon (*Procyon lotor*) is an omnivore belonging to the family of small bears (Procyonidae) that originally belongs to Central and Northern America. Nowadays, it has colonized different parts of the world due to deliberate or accidental releases and is listed as invasive neozoon in Germany. The recent rise in population densities is likely to increase the risk of pathogen transmission to humans, wildlife and domestic animals. Many zoonotic pathogens are found in raccoons worldwide, but there is a lack of epidemiological data for most of Germany's raccoon populations concerning tick-borne pathogens. Tissue samples of 485 free-ranging raccoons obtained as hunting bag in Germany between 2017-2021 were examined for the presence of six vector-borne pathogens (*Rickettsia* spp., *Borrelia* spp., *Anaplasma phagocytophilum*, *Bartonella* spp., *Babesia* spp., *Neoehrlichia mikurensis*). *Borrelia* spp. was detected in 21 (6.3%) raccoons, identified as *Borrelia afzelii* and *Borrelia burgdorferi* s.l. *Rickettsia* spp. was found in 26 (7.8%) individuals. *Anaplasma phagocytophilum* was confirmed in 51 (10.7%) raccoons. *Neoehrlichia mikurensis* was not detected. The results for *Bartonella* spp. and *Babesia* spp. are still pending. Future studies should monitor these invasive omnivore populations. However, raccoons may act as reservoir for pathogens, especially for *Anaplasma* spp. and *Rickettsia* spp., between humans, domestic animals and wildlife, with a risk of infection due to their invasive behaviour and synanthropic habitat.

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## Initial Immune Response Against *Orthobunyavirus bataiense* and *Orthobunyavirus Reassortants*

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Orthobunyaviruses (OBV) constitute the largest genus of arboviruses, with many species traversing the world as unwelcome passengers carried by ticks and mosquitoes. Most species within this genus cause diseases in ruminants, yet it is noteworthy that at least 30 members also affect humans.

While human infections with OBV are generally mild, severe cases like encephalitis and hemorrhagic fever can still occur. The Ngari virus, for instance, underscored the seriousness of OBV infections with a significant outbreak of hemorrhagic fever in East Africa.

Though our understanding of how OBV interact with their hosts remains limited, grasping these dynamics is vital for predicting clinical outcomes. Addressing this knowledge gap, we concentrate on unravelling the initial immune response provoked by the undercharacterized *Orthobunyavirus bataiense* (BATV) and the two reassortants of BATV and *Orthobunyavirus bunyamweraense*, namely Ngari-like virus and Batunya virus.

RNA-sequencing revealed significant differential expression of multiple antiviral genes in infected murine dendritic cells. Moreover, comparison of gene expression profiles across murine, ovine, and human immune cells indicated that viruses with similar S-segments exhibited similar expression patterns.

In conclusion, our study highlights the cross-species importance of interferon signalling in response to OBV infections and supports current knowledge that NSs is the major host interferon antagonist in the Bunyavirales order.

**Snacks & Poster Viewing 1 / 53****Characterisation of innate immune responses induced by the Rift Valley Fever Virus (RVFV) in humans**

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Rift Valley fever virus (RVFV) is a phlebovirus causing febrile or haemorrhagic illness in ruminants and humans. Among ruminants, the disease is transmitted by mosquitoes, while humans acquire the disease mainly from infected ruminants through contact with blood, carcasses or consumption of raw milk. The virus possesses a negative-sense tripartite RNA genome encoding several structural and non-structural proteins. The non-structural protein NSs acts as the major virulence factor of the virus by e.g. suppressing the IFN- $\beta$  response in host cells. Virus variants with mutated NSs genes have been shown to be highly attenuated *in vivo*. We here hypothesise that the innate immune response to RVFV infection is an important factor in inducing protective adaptive immunity, which in turn determines disease outcome. Preliminary flow cytometric analysis of infected human PBMCs has revealed monocytes to become infected by the virus. Analysis of culture supernatants sampled at different time points post infection revealed no productive virus replication and possible efficient viral clearance. Instead, infection of primary monocytes led to their activation, as indicated by the upregulation of activation markers. Further studies aim at characterizing the innate immune responses to RVFV infection in monocytes using RNA-seq and Luminex assays. The knowledge obtained from these studies will contribute to a better understanding of immunity and pathogenesis of RVFV infections, especially in humans.

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**Shiga toxin-encoding *Escherichia coli* from South American Camelids in Germany – prevalence and *stx* gene subtype distribution**

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South American camelids (SAC) are frequently kept with other livestock and in close contact with humans in Europe. As they represent a potential reservoir for transmission of epizootic and zoonotic bacteria, SAC are considered in the revised European Animal Health Law. However, knowledge on bacterial pathogens in SAC is too sparse for drafting appropriate monitoring and preventive medicine programs. To investigate the presence of Shiga toxin-encoding *Escherichia coli* (STEC), 20 SAC each were sampled at four different time-points in ten and then nine flocks. The herd prevalence was 90% in the first sampling round and 100% in rounds two to four. Of a total of 708 samples from individual animals, 31 (4.2%) were PCR-positive for *stx1*, 139 for *stx2* (19.6%) and 54 for both genes (7.6%). The intra-flock prevalence fluctuated widely (0-95%) between rounds. The *stx*-gene subtypes identified were predominantly *stx1c* (95%; 81/85) and *stx2b* (85%; 165/193), which are considered as being of low risk for causing severe human disease. A few animals were transiently PCR-positive for *stx1a*, *stx2c*, *stx2d*, *stx2e*, *stx2f* and *stx2g*. Multiple *stx2*-subtype signals were detected in 37.8% of the samples (73/193). In 19 *stx2*-positive samples, a subtype could not be assigned. Current efforts focus on identifying these *stx2* subtypes and on obtaining isolates. The data show that, similar to ruminants, SAC represent STEC reservoir animals but intermitted shedding patterns hamper detection of the pathogens.

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## Evaluating a novel surveillance approach for AMR prevalence in veterinary medicine using data of *Escherichia coli* from fattening pigs

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Antimicrobial resistance (AMR) surveillance data for livestock is aggregated at EU member state level and published with considerable delay, preventing timely and regionally adapted interventions. An alternative approach to classify AMR prevalence as above or below a threshold could facilitate AMR surveillance by reducing sample size, but still deliver sufficient information for actions.

'Lot Quality Assurance Sampling' (LQAS) has been used successfully to classify AMR prevalence in human urinary tract infections. To evaluate LQAS in the veterinary field, a conventional AMR prevalence survey tested 992 *E. coli* strains, obtained from composite fecal samples of fattening pigs, with a panel of 14 antibiotics. Minimum inhibitory concentrations were classified using EUCAST ECOFFs.

Our results show a range of very low (0.1%; amikacin; piperacillin/tazobactam) to medium high (38.8%; sulfamethoxazole) non-wild type prevalence of 'microbiological' resistance, suitable for validation of the LQAS method and matching recent national EFSA data, where comparable. Regionalized analysis unveils significant differences for several federal states/antibiotic combinations.

*In-silico* LQAS simulation with different thresholds points to good agreement between the LQAS classifications and the underlying AMR prevalence when numbers of only 44-76 samples were considered. Based on the high sensitivity and specificity values obtained here, LQAS can complement existing AMR surveillance in livestock.

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## Thermophilic *Campylobacter* species in New World camelids from Germany

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Little is known about the zoonotic risk associated with New World Camelids (NWC), which are becoming increasingly popular as pet animals. Therefore, the emergence of thermophilic *Campylobacter* (*C.*) spp., which cause the most common notifiable bacterial zoonosis in Germany, was investigated. Fecal swabs were taken from up to 20 NWC on 10 farms in each of 4 semi-annual samplings. *Campylobacter* spp. were isolated from 23 of 717 samples, with *C. jejuni* detected in 16 samples from 7 farms and *C. coli* in 7 samples from 2 farms.

Sequencing mostly revealed heterogeneity among strains from different farms. Repeated detection of sequence types in one farm/animal indicated prolonged intestinal colonization. Overall, nine STs were detected among *C. jejuni* and two STs among *C. coli* strains. Several virulence genes were detected, including those involved in adhesion, invasion, and motility. All strains possessed the *cdtABC* genes, coding for the major *Campylobacter* toxin. Antimicrobial susceptibility testing revealed uncritical resistance profiles (epidemiological cut-off values). Two *C. jejuni* and one *C. coli* were resistant to ciprofloxacin due to the most common point mutation in *gyrA* (T86I). One *C. jejuni* isolate possessed the tetracycline resistance gene *tet(O)*.

Our data suggest that thermophilic *Campylobacter* spp. may be widespread in German NWC farms. A source attribution analysis is currently performed to infer the contribution of NWC to the burden of human campylobacteriosis.

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**Ascaris suum and hepatitis E virus coinfections in fattening pigs at slaughter****Author:** Alexandra Laubschat<sup>1</sup>; Josephine Schlosser-Brandenburg<sup>1</sup>; Larissa Oser<sup>1</sup>; Susanne Hartmann<sup>1</sup><sup>1</sup> *Department of Veterinary Medicine, Institute of Immunology, Centre for Infection Medicine, Freie Universität Berlin, Berlin, Germany***Corresponding Author:** a.laubschat@fu-berlin.de

*Ascaris suum* and hepatitis E virus (HEV) are widespread zoonotic pathogens in pig production and therefore of great public health interest. The development of protective immunity to *Ascaris* is slow, with reinfection being common, resulting in repeated inflammatory liver lesions that are macroscopically visible at slaughter ("milk spots"). Despite the frequent occurrence of both pathogens in pigs, there are no studies on the coinfection prevalence and whether concurrent *Ascaris* infection impairs hepatic antiviral immunity and thus HEV transmission. Our hypothesis is that repeated *Ascaris* exposure leads to a dominant hepatic type 2 immune response that counteracts type 1 immune activity, resulting in increased HEV replication and ultimately increased virus shedding. Therefore, we first investigated the prevalence of coinfection in fattening pigs and its impact on HEV serology and virus detection. For this purpose, fattening pigs (n=547) were sampled at the abattoir to collect meat juice and serum for the detection of anti-*Ascaris* and anti-HEV antibodies by ELISA as well as bile and liver tissue for the quantification of HEV RNA by qRT-PCR. The initial data analyses revealed that 7.13 % of the fattening pigs had an acute *Ascaris* infection (milk spot positive), while 12.39% of the fattening pigs were infected with HEV (HEV RNA positive). Moreover, antibodies against *Ascaris* L3 larvae antigens were detected in all herds. Additionally, 68% of the herds had at least one animal that tested positive for anti-HEV IgG. Collectively, these data demonstrate that both pathogens were present in approximately 70% of the herds investigated, and that 51% of the fattening pigs had been exposed to both pathogens at least once during their lifetime. This study confirms a high prevalence of HEV and *A. suum* in pig farms. However, the data do not suggest that *Ascaris*-infected animals are at a higher risk of concurrent HEV infection. Nevertheless, the high prevalence of HEV RNA-positive animals at slaughter again emphasises the high risk of zoonotic transmission to humans, either through direct contact or through the consumption of contaminated food.



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**Are you really a *V. cholerae* and how to handle *V. cholerae*-like species in food regarding their (potential) pathogenic potential?**

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As bacteria of aquatic ecosystems, vibrios can be important for consumer protection. *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* are responsible for the majority of human (extra)intestinal diseases worldwide. The genus consists of >150 species, the human pathogenicity of most of which has not yet been clarified. The seasonal occurrence of these climate-associated indicator bacteria is expected to increase the importance of pathogenic vibrios in the future.

*Vibrio* spp. have been recovered from Louisiana crawfish during surveillance and have been classified as *V. mimicus* and *V. cholerae* by routine methods. However, WGS of the *V. cholerae* isolates suggests that some belong to the species *V. tarriae*.

To date, limited data are available on the pathogenicity of *V. tarriae*. Bioinformatic analysis of the species from Louisiana crayfish revealed differences in virulotyping and phylogenetic relationship. The *V. tarriae* genomes were further used to derive a species-specific pangenome (~3,100 CDS). In addition, in silico characteristics of *V. tarriae* were compared with phenotypic characteristics (i.e. AST). The data generated provide a detailed insight into the genetic diversity and possible human pathogenic potential of *V. tarriae* and highlight limitations in the detection of new *Vibrio* species in routine diagnostics.

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## **Detection and quantification of porcine cytomegalovirus (PCMV) glycoprotein B specific antibodies in early and latent infected pig**

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The early detection of porcine cytomegalovirus (PCMV) in donor pigs intended for xenotransplantation (XTx) is immanent as PCMV may contribute to early graft failure.

Since PCMV is endemic in the pig population and highly transmissible at early age, the monitoring of breeder animals, sentinel pigs and donors is an important step to secure the transplant and thereof the later recipient. As a member of the roseolovirus family PCMV resides in latency with recurring viremic events being detectable by PCR. For PCMV that undergoes latency the determination of serum antibodies directed against viral antigens is the method of choice.

Here we focus on PCMV glycoprotein B (gB) using full length protein and selected epitopes to test on varieties in the pig immune response against gB differentiating early recognized, highly immunogenic epitopes from lesser immunogenic epitopes that may direct to long term infection. Besides pig IgG the pig IgM serum level was additionally determined and compared. Focus was given to the differentiation from maternal antibodies that decreases without further infection of the piglet and piglet derived antibody response. The differentiation and assignment of the response in view of the timely development and gB epitope specificity gives insight to early infection in order to distinguish none infected, first time infected from latent infected pigs.

**Snacks & Poster Viewing 1 / 140****Dominance of mecC gene among methicillin- resistant Staphylococcus aureus Sudanese clinical isolates**

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This study aimed at investigating the presence of the methicillin- resistance genes (*mecA* and *mecC*) in phenotypically methicillin resistant *Staphylococcus aureus* (MRSA) Sudanese clinical isolates. The subject was 45 MRSA isolates representing two main sets: set-I was isolates (N=34) obtained in 2017 from skin and soft tissue (SST) infections (N=27) and ear- nose and throat infections (N=7), while set-II was isolates (N=11) obtained in 2021 from SST infections only. The isolates were identified as MRSA by the disc diffusion test. The DNA of the isolates was tested by PCR for detection of *mecA* and *mecC* in addition to three virulence genes: Pantone- Valentine leukocidin (PVL), haemolysin G (*hlg*) and toxic shock syndrome toxin (*tsst*). In total, 15 (33.3%) of the isolates were positive for *mecA*, *mecC* or both: 4 (8.9%) were positive for *mecA* alone, 6 (13.3%) for *mecC* alone and 5 (11.1%) for both genes. In set-I, only 23.5% of the isolates were positive to either of the *mec* genes, while in set-II his positivity reached 63.6%, among which none was *mecA*- positive alone. *hlg* and PVL genes were detected in total as 33.3% and 22.2%, representing 38.2% and 18.2% in set-I, and 26.5% and 9.1% in set-II, respectively. The *tsst* gene was not detected in any isolate. These results show dominance of *mecC* gene with increasing trend in MRSA Sudanese clinical isolates. The genetic basis for methicillin resistance of phenotypically MRSA but genotypically MSSA is yet to be elucidated.

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## **Transport, fate, and transmission of resistance genes during sub-surface passage and drinking water treatment**

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The occurrence, persistence and spread of antibiotic-resistant bacteria (ARB) and the transfer of resistance through gene transfer is of significant importance to human health, making this issue one of the greatest global threats to the health of humans, animals, plants, and ecosystems. For this reason, antibiotic resistance has been recognized as a critical One Health problem. However, the role of the environment, particularly the introduction of antibiotic-resistant bacteria and antibiotic resistance genes (ARG) into surface waters, is not yet fully understood within the One Health framework, necessitating further research in this area. While effective retention of ARB through natural processes in drinking water treatment and groundwater recharge via the soil-groundwater pathway is highly probable, little is known about the fate of ARG and potential resistance transfers (e.g., in biofilms). Therefore, it is imperative to establish an assessment framework to understand to what extent potential contamination of raw waters could endanger drinking water supplies. To achieve this, appropriate, standardized, and valid cultural and molecular biological methods must be developed to detect ARB and ARG. These insights are necessary to constrain the ABR spread cycle and to enhance awareness and education on the use of antibiotics.

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## Impact of Fusarium Fungi on Environmental and Wildlife Health

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Emerging infectious diseases pose a significant and increasingly prevalent global threat. While research largely focuses on bacterial and viral pathogens, fungal pathogens, despite their critical impact on health, are often overlooked. *Fusarium*, a trans-kingdom pathogen included in the World Health Organization's High Priority Group of harmful fungi, merits particular attention due to its extensive impact on human, animal, and plant health. It can cause fatal infections in humans, jeopardize global food security by affecting agricultural crops, and threaten species like sea turtles by reducing hatching success. Our three-year study at Donau-Auen National Park in Austria investigated the presence and impact of *Fusarium* on the endangered European pond turtle. We aimed to identify prevalent *Fusarium* strains and link infections to changes in the microbiome. Preliminary results indicate a reduction in bacterial diversity in nests harbouring *Fusarium*, correlating with decreased hatching success. Our findings highlight the fundamental role of soil as a reservoir for fungal pathogens, beneficial microorganisms, and overall microbial diversity, affecting wildlife health. Demonstrating that fungi, such as *Fusarium*, can significantly alter the soil microbiome, thereby influencing plant, animal, and human health, we emphasize the urgent need for strategies to mitigate *Fusarium* spread, preserve biodiversity and prevent pathogen spillover in vulnerable wildlife populations.

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**Zoonotic infections of *Streptococcus equi* ssp. *zooepidemicus* using large-scale comparative genomics to gain insights into population dynamics and surface-associated antigen vaccination candidates**

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*Streptococcus equi* ssp. *zooepidemicus* (SEZ) is a zoonotic pathogen, impacting both human and animal health. Phylogroup A Streptococci, predominantly affecting children and young adults, contribute significantly to disease burden, accounting for millions of cases worldwide. To address zoonotic infections from phylogroup A, we conducted research to computationally characterize antigen candidates for potential vaccinations against SEZ.

Utilizing extensive clinical data and comparative genomics for 121 in-house sequenced SEZ strains, encompassing human and animal isolates, we employed bioinformatics methods to design a novel computational workflow (BacPop) for studying bacterial population structures, including functions for SzP- and antigen typing.

Pan-genomic analysis revealed factors facilitating horizontal transmission of genetic elements, including virulence determinants and AMR genes linked to SEZ's pathogenicity and host specificity, highlighting unique genomic variations within zoonotic strains. Our association study pinpointed promising antigen candidates for SEZ vaccines. These candidates demonstrated consistent presence across zoonotic strains with minimal variation, strengthening their suitability for vaccine development.

Coupled with the novel BacPop pipeline, the proposed vaccination candidates establish a groundwork for subsequent clinical investigations. This comprehensive data set contributes to addressing the health-care challenge posed by SEZ infections.

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## **Interplay between anthropogenic stressors, gut microbiomes and wildlife health**

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## **Macaques, humans & oil palm: an integrative approach to foster 'One Health' in Malaysian plantations**

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One major factor contributing to biodiversity loss is the conversion of rainforest into oil palm plantation. In Malaysia, pig-tailed macaques (*Macaca nemestrina*) have adapted to this new environment, daily entering plantations adjacent to their remaining forest habitat. There, macaques feed on palm fruits but also provide an ecosystem service by reducing plantation rats, the main pest species of oil palm crops. Our research uncovered significant impacts on macaque fitness in this landscape matrix. Recently, we reported a high mean infant mortality rate (~60% over 10 years), with prolonged plantation visits increasing risks of infant death. Our results suggest that the exposure to agricultural chemicals may play a significant role. Some herbicides gradually accumulate in mammals, increasing pesticide exposure for developing offspring, especially those born to first-time mothers and after prolonged interbirth intervals. Indeed, our data show changes in macaques' chemical profiles following herbicide exposure. Further, human-primate interactions pose risks to humans through the potential transmission of zoonotic diseases. Gut microbiome data of macaques revealed links between frequent human interactions and the diversity and richness of potentially pathogenic bacteria. Our future work aims at developing an 'One Health' project to assess the impact of anthropogenic activities on the health of wildlife, humans, and the environment, exemplified by the shared oil palm landscape.



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## The ecology of species-specific and ubiquitous rodent-borne pathogens in the network of mammalian biodiversity and seasonality

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Many rodent species are reservoirs for zoonotic pathogens. Biodiversity could be closely related to pathogen prevalence through direct and indirect effects and all actors are probably affected by seasonality (vegetative period, food, shelter, competition, predation etc.). Increased biodiversity should modulate transmission of pathogens in the small mammal host species. It can minimize transmission of species-specific pathogens (dilution) and enhance the transmission of ubiquitous pathogens (amplification).

We investigated the relative impact of population-level regulation of the prevalence of species-specific Puumala orthohantavirus (PUUV) in bank voles and Tula orthohantavirus (TULV) in common voles and the ubiquitous *Leptospira* in the small mammal community. Rodents were trapped in grasslands and forests, necropsied and tissue and blood samples analyzed for pathogen infections. The biodiversity of large terrestrial mammals was monitored with wildlife cameras.

There was a strong influence of seasonality on vole host abundance and pathogen prevalence. For instance, in some seasons, shrew abundance was negatively correlated to bank vole abundance. Host abundance promoted PUUV seroprevalence. Increased abundance of non-host small mammals reduced PUUV seroprevalence in the host. Vole abundance in summer was a strong driver of TULV prevalence. In summer, predator abundance decreased and mammal biodiversity increased *Leptospira* prevalence in the community. The results indicate the expected dilution and amplification effects and suggest a complex network that considerably depends on season.

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## Extensive diversity of viruses identified in mosquitoes from South-western Uganda

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Mosquitoes are vectors for pathogenic viruses, such as Dengue or Zika viruses. Although over 3,700 mosquito species exist, only about 200 have been tested for viral infections, mostly anthropophilic mosquitoes as *Aedes aegypti* or *Ae. albopictus*. In addition, knowledge about viruses emerging from enzootic to epidemic transmission cycles remains limited. Our study aimed to assess the genetic diversity of viruses circulating in mosquitoes in primary and adjacent rural ecosystems in Uganda and to analyse emergence patterns.

In 2019 and 2020, we collected 39,834 mosquitoes from lowland rainforest, savannah gallery forest, montane rainforest, and adjacent rural areas in southwestern Uganda. An extraordinary richness of 139 mosquito species was found, including many species that have never been tested for viruses before. We observed that mosquito communities significantly differed between the three ecosystems and for each ecosystem between undisturbed and disturbed areas. Testing for viral infections revealed 709 viruses belonging to 158 species. Most of these showed less than 90% nucleotide identity to known viruses, suggesting the detection of numerous previously unknown viruses belonging to the families Toga-, Flavi-, Mesoni-, Peribunya-, Reo-, Rhabdo- and Phenuiviridae.

Intriguingly, diversity of mosquitoes and viruses was found to be temperature-dependent and varied significantly among ecosystems with highest species richness found in the warmest undisturbed primary ecosystems.

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## **CiFly – a fly-based Citizen Science project with pupils to monitor mammal biodiversity and anti-microbial resistance (AMR)**

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In this project, we develop and evaluate an educational measure to integrate One Health and hands-on science into the curriculum of a local high school in the Vorpommern region. The measure is designed as a sustainable citizen science project with pupils to investigate the impact of urbanisation and agriculture on wild mammal biodiversity and antimicrobial resistance in the environment. Together with scientists, pupils develop a fly-based monitoring of wildlife and AMR in Vorpommern-Greifswald based on state-of-the-art methods in the field of environmental DNA and bacteriology. As flies thrive in many environments and feed, among others, on mammal faeces or carcasses, ingesting DNA from different sources, we use them as samplers of DNA from wildlife and bacteria. The project aims to improve the pupils' general understanding of science, convey the basics of the One Health concept and generate scientific data. The first group of pupils started the project in March 2024, caught 222 flies and will complete 12 lessons of lectures and experimental sessions with a final lesson, presenting their results to a panel of scientists and their teacher. To assess the project's impact on the pupils' attitudes and confidence in science, they completed a questionnaire before the project began, which will be repeated at the end of the project.

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## Effects of tree diversity on human health – results from the Dr.Forest project

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Forest risks and benefits to human health are widely recognised. Yet, the role of forest characteristics – such as tree species diversity or canopy density – driving health effects remains underexplored. Based on data from a European forest network (Dr.Forest), we quantified causal pathways relating different forest types to physical and mental health. Specifically, we determined i) mental wellbeing via visual cues and ii) via auditory cues; iii) thermal comfort; iv) polyphenol content of medicinal plants; v) nutritive qualities of mushrooms, vi) air quality, and vii) ticks and Lyme disease.

Results show that forests generate net health benefits regardless of their ecological characteristics, except for the high tick prevalence compared to other ecosystems. Canopy density and tree species diversity emerge as key drivers, but their effect size and directionality are strongly pathway-dependent. Changes in canopy density can generate trade-offs; e.g., forests optimised for heat buffering may enhance Lyme disease prevalence. Tree diversity effects were weaker but more consistently positive.

Forest management may enhance health benefits and mitigate risks. Even when modulation effects are small, this can translate to considerable impact at the public health level. Importantly, management should account for trade-offs to tailor forest biodiversity and functioning to local public health needs of priority.

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## Urban Distribution and Abundance of *Aedes albopictus* in Response to Climate Change: A Model Incorporating Temperature and Land-Cover

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Mosquitoes, particularly *Aedes albopictus*, pose health and economic risks. Recently, this invasive species has spread to central Europe, including Germany, raising concerns for uninhabited areas like Bavaria due to climate change. The mosquito's survival and reproduction can fluctuate across fine spatial resolutions, often overlooked in studies. Urban areas show this variation in habitat suitability. With *Ae. albopictus* favoring urban habitats, understanding their urban distribution is crucial for public health.

Eight Bavarian cities with similar climates, helping to isolate the effect of land-cover, and high populations were selected for study: Munich, Nuremberg, Augsburg, Regensburg, Ingolstadt, Fürth, Bayreuth, and Würzburg. All are along a highway. The modeling was based on the mechanistic model dynamAedes (Da Re et al., 2022). Random Forest machine learning approaches were employed to model air surface temperature based on MODIS Land surface temperature (Moderate Resolution Imaging Spectroradiometer) for the study areas as well as producing novel land-cover maps (grids 30 x 30 m, validated by confusion matrix and an out of bag score) as input data. A weighting of the land-cover classes based on an expert survey was applied.

The study revealed a positive correlation between the probability of *Ae. albopictus* establishment and the proportion of urban green spaces within city boundaries. Climate change was found to intensify this correlation, further favoring urban green spaces over built-up areas for *Ae. albopictus* establishment. Moreover, climate change was observed to increase the overall abundance of *Ae. albopictus* across all study areas. The disparity in both abundance and establishment probabilities between the climate scenario RCP 2.6 and RCP 8.5 is significant. In fact, transitioning from RCP 8.5 to RCP 2.6 could potentially result in a more than five-fold reduction in mosquito abundance numbers.

The current absence of *Ae. albopictus* from these cities despite their suitability for the species highlights the importance of continued monitoring programs to prevent populations from establishing or reduce their abundance.

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## Analyzing the Complexity: interactions of arboviruses and insect-specific viruses in vector competence studies with two invasive *Aedes* species

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The global spread of invasive mosquito species increases the risk of arbovirus infections in humans and livestock. Besides *Aedes albopictus*; *Aedes koreicus* and *Aedes japonicus* have recently spread within Central Europe. Extensive information on the vector competence of the latter two species is missing. Therefore, we evaluated the vector competence of field caught *Ae. koreicus* and *Ae. japonicus* mosquitoes from Germany for different arboviruses. Additionally, the virome of the examined mosquitoes was investigated, and the impact of co-infection with arbovirus and insect-specific virus (ISV) on vector competence was evaluated.

Mosquitoes were orally infected and incubated under different temperature conditions ( $27 \pm 5^\circ\text{C}$ ;  $24 \pm 5^\circ\text{C}$ ,  $21 \pm 5^\circ\text{C}$ ), to evaluate the influence of temperature on transmission dynamics. A salivation assay was performed to investigate the vector competence. Using a whole virome analysis via NGS, novel insect-specific viruses (ISVs) were identified.

*Ae. koreicus* had the potential to transmit Chikungunya virus (CHIKV) and Zika virus (ZIKV) but not West Nile virus (WNV). Transmission was only observed at the higher temperature with low transmission efficiency. *Ae. japonicus* was able to transmit CHIKV, Sindbis virus (SINV) and western equine encephalitis virus (WEEV). The risk of CHIKV transmission by *Ae. japonicus* was also rather low and exclusively observed at the higher temperature. Transmission of SINV and WEEV was observed at all investigated temperature profiles with high transmission efficiency.

While we detected only one ISV in *Ae. koreicus*, we could identify seven ISVs in *Ae. japonicus*. Correlating the ISV infection status of individual specimens with their transmission capability for specific arboviruses revealed a potential interference of ISVs on arbovirus infection, characterized by highly complex dynamics.

Jansen S, Cadar D, Lühken R, et al. Vector Competence of the Invasive Mosquito Species *Aedes koreicus* for Arboviruses and Interference with a Novel Insect Specific Virus. *Viruses*. 2021;13(12):2507. Published 2021 Dec 14. doi:10.3390/v13122507

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## Recent and future ecological niche changes of major Anopheles species in the European and Mediterranean areas

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Anopheles species are the main vectors of malaria, and changes in their habitat due to climate or land-use also alter the disease risk. Especially in the Mediterranean, a climate hotspot, changes in temperature and precipitation could substantially modify habitat suitability in the coming decades. In addition, ongoing land-use changes such as urbanization, deforestation and irrigation have also significant impacts e.g., on host availability and breeding sites.

38 climate and 14 land-use predictors were considered to model the recent and future ecological niches of *An. atroparvus*, *An. labranchiae*, *An. messeae*, *An. sacharovi*, *An. sergentii*, and *An. superpictus* using Boosted Regression Trees (BRT). For each species we established 16 different models based on four different sets of predictors and four different background selection methods. Changes in the ecological niches are assessed by a model ensemble and the best model in terms of model skill and model transferability. Furthermore, the most important predictors for vector occurrences and the predictors that are responsible for changes in the ecological niches were identified for each species.

Results show that, on a large scale, climate predictors are generally more important than land-use predictors for the establishment of the species. The expected changes will help some species to expand, while leaving others unable to cope. Depending on the species, habitat suitability depends on different subgroups of climatic predictors.

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## Genotypic and Phylogeographic Insights into a Pre-Epidemic Variant of Wesselsbron Virus detected in sylvatic *Aedes mcintoshi* from Semuliki Forest, Uganda

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Wesselsbron virus (WSLV) is a neglected mosquito-borne virus within the yellow fever sub-group of the Orthoflavivirus genus in the Flaviviridae family. Primarily a veterinary pathogen causing stillbirths, congenital malformations, and mortality in ruminants, WSLV also infects humans, causing a self-limiting febrile illness with rare neurological complications. WSLV causes sporadic outbreaks in Southern Africa but findings in other African countries suggest a wider distribution. We detected and isolated WSLV from an *Aedes mcintoshi* mosquito in Semuliki National Park, Uganda. The novel strain showed impaired infectivity across various host cell lines compared to an epidemic reference strain from South Africa. Complete genome sequencing and phylogenetic analysis revealed that the virus grouped with strains from South Africa and Senegal. Phylogeographic reconstruction of WSLV's spatial and temporal spread combined with displacement of the origin of the newly detected strain suggest that WSLV may be widely distributed across Africa. Our data show that the geographic distribution of WSLV and its impact on human and animal health is likely underestimated.



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## Effects of rodent control in sewer systems on zoonotic pathogen composition and prevalence in Norway rats (*Rattus norvegicus*) - a pilot study

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Several rodent species can be problematic in cities and agricultural landscapes and they are carriers of zoonotic pathogens. Some of these pathogens can cause serious disease in humans, livestock and pet animals. Commensal rodents in urban areas pose a considerable threat to human health by transmitting zoonotic pathogens. Rodent management – often using anticoagulant rodenticides – is often applied in such situations to reduce their abundance. However, management itself may disturb rodent population structure and cause influx of new individuals, potentially with new suits of pathogens. We monitored pathogen occurrence in Norway rats (*Rattus norvegicus*) caught in the Berlin sewer systems and above ground before and after regular rodent management action. We included viruses [hepatitis E virus (HEV), Seoul hantavirus, intestinal viruses, Mpox virus], bacteria [*Leptospira* spp., *Bartonella* spp., *Salmonella* spp., *Campylobacter* spp., pathogenic *Escherichia coli*] and endoparasites [*Babesia* spp., *Toxoplasma gondii*]. Prevalences were low for HEV (5%), *Toxoplasma* (2%) and Mpox (1%). Seoul virus, Shigatoxin-producing *E. coli* and *Salmonella* were absent, enteropathogenic *E. coli* prevalence was 10% and *Campylobacter jejuni* prevalence was about 20%. More results including habitat effects will be presented. Our study contributes to a better understanding of the presence and transmission risk of rodent-borne pathogens in urban areas in the context of rodent management actions.

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## Bluetongue virus serotype 3 (BTV-3) and Schmallenberg virus in *Culicoides* biting midges, collected in western Germany during the BTV-3 outbreak in neighbouring Netherlands, autumn 2023

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After the outbreak of bluetongue virus serotype 8 (BTV-8) from 2006-2009, bluetongue disease occurred in central Europe only sporadically. By contrast, Schmallenberg virus (SBV) has become enzootic since its first detection in 2011. Both viruses are transmitted by *Culicoides* biting midges (Diptera: Ceratopogonidae). In September 2023, BTV-3 emerged for the first time in continental Europe. From an initially affected four farms in the Netherlands, it rapidly spread, soon reaching Belgium, the UK and Germany. During September 24-26, i.e. before any of BTV-3 cases had been reported from Germany, we installed UV-light traps in cattle, sheep and goat stables on 18 farms (1 trap per farm) in the federal states of Lower Saxony and North Rhine-Westphalia to collect putative BTV-3 vectors and test them for virus RNA. Captured biting midges were morphologically identified as *C. obsoletus* group, *C. pulicaris* complex, and other *Culicoides*. Quantitative real-time PCRs were used to screen pools of ≤50 individuals for BTV, SBV and epizootic hemorrhagic disease virus (EHDV). BTV-3 was detected in a pool of *C. obsoletus* group biting midges collected on October 12, 2023, in the same district where the first infected sheep was observed. SBV-RNA was found in numerous *Culicoides* pools, reflecting extensive regional circulation with substantial virus loads. All pools tested negative for EHDV. BTV-3 circulation must be expected to pick up speed during spring and summer 2024 in Germany.

**Lunch & Poster Viewing 2 / 27****Corona-, paramyxo-, and filoviruses in rodents and shrews in Guinea**

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Micromammals (rodents and shrews) are known for carrying a high diversity of pathogens and are widely distributed in varied habitats. Thus, when coming in close contact to humans, livestock or other wildlife, the risk for pathogens such as e.g. corona-, paramyxo-, and filoviruses crossing species-barriers and human transmission could be increased. To prevent the spread and spillover of the above mentioned viruses, it is important to have a better understanding of their presence, distribution, and characteristics. Therefore, we sampled about 200 micromammals in 3 different regions of Guinea (Forest, Maritime and Middle Guinea), a hotspot of emerging infectious diseases in Sub-Saharan Africa. The samples were tested for the presence of corona-, paramyxo-, and filoviruses using virus family specific pan-RT-PCRs. While 22/194 (11,34%) of the tested micromammals were found to be positive for paramyxoviruses and none for coronaviruses, further analyses are required for concluding filovirus results. Although more in-depth analyses through whole genome sequencing (e.g., nanopore-based MinION sequencing) are necessary for further characterization, these results already highlight the broad distribution of paramyxoviruses in micromammals. Overall, it is of great importance to perform screening studies in relevant wildlife, which could play a role in pathogen transmission to humans as potential reservoir species.

**Lunch & Poster Viewing 2 / 11****Modifying epicPCR for the surveillance of antibiotic resistances in wastewater**

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The monitoring of antibiotic resistances (ABRs) in wastewater (WW) often involves only the quantitative amplification of present ABR sequences. However, linking information on ABR with taxonomic information is crucial. EpicPCR (Emulsion, Paired isolation and Concatenation PCR), developed at MIT in 2016 and used for WW surveillance in 2018, enables linking ABR genes and taxonomy via a combination of PCR and DNA sequencing. We have significantly revised the method to enable longer sequence fragments and sequencing with Oxford Nanopore. We present the revised in vitro method, the results of our initial studies and a software suite for designing primers and evaluating results. In our initial experiments, we found thousands of sequences containing both the 16S rDNA gene and a subset of ABR genes that we provided primers for. We are now working on extending the method and applying it to additional real-world data. Our overall goal with epicPCR is to improve environmental monitoring systems, potentially leading to a small-scale warning system. Such a warning system could also support public health prevention strategies.

**Lunch & Poster Viewing 2 / 71****The role of health promotion in the One Health approach****Authors:** Katharina Brugger<sup>1</sup>; Ilonka Horváth<sup>None</sup>; Andrea E. Schmidt<sup>None</sup><sup>1</sup> *Gesundheit Österreich GmbH***Corresponding Author:** [katharina.brugger@goeg.at](mailto:katharina.brugger@goeg.at)

Humanity is facing multiple man-made pressure points such as climate change, biodiversity loss, environmental pollution, and growing social inequalities. These leads to increasing and intertwined economic, social, and health problems. A multidimensional and interconnected approach is needed to address and deal with the adverse impacts appropriately and sustain future health and resilience. The One Health approach aims to sustainably balance and optimize the health of people, animals and ecosystems. And the health promotion approach gives us an understanding what determines health. Both concepts are regularly used in politics, science, and health professions. The question is to what extent the two concepts fit together or complete each other. Further, where health promotion aspects can be found in the health concept and vice versa. In this presentation an overview on the basic of health promotion and One Health will be given. Synergies and further development on this topic will be presented to define the potential role of health promotion within the One Health approach.

## Lunch &amp; Poster Viewing 2 / 9

## Occurrence and characterization of antimicrobial-resistant coagulase-negative staphylococci in wild ungulates in Brandenburg, Germany

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Data regarding the occurrence of antimicrobial-resistant coagulase-negative staphylococci (AMR-CoNS) in wild living animals is rare. The aim of this study was to provide insights into AMR-CoNS from wild ungulates in Brandenburg, Germany.

From 2019 to 2023, a total of 450 nasal swab samples from wild boars, fallow, roe and red deer were collected during the respective hunting seasons in Brandenburg, Germany. Staphylococci were isolated by selective enrichment with a beta-lactam antibiotic. Species were identified by MALDI-TOF-MS. Antimicrobial susceptibility testing was carried out and AMR genes were analyzed by whole-genome sequencing.

The occurrence of AMR-CoNS was low (6/450; 1%). Strains of *Staphylococcus (S.) saprophyticus* (n=3), *S. epidermidis* (n=2) and *S. casei* (n=1) were isolated from nostrils of fallow (n=2), red (n=2) and roe deer (n=2). The strains exhibited resistance to penicillin (6/6), ceftiofur (4/6), mupirocin (2/6), erythromycin (2/6), rifampicin (1/6), tetracycline (1/6), tiamulin (1/6) and fusidic acid (1/6). Corresponding AMR genes such as *bla<sub>Z</sub>*, *mecA*, *mph(C)*, *msr(A)* or *fusD* were detected in the respective isolates.

Using a beta-lactam antibiotic enrichment approach, it was shown that wild ungulates occasionally carry AMR-CoNS in their nostrils. Although CoNS are regarded as less virulent than coagulase-positive staphylococci such as *S. aureus*, they may act as AMR gene reservoir, potentially transmitting genes to more pathogenic staphylococcal species.

**Lunch & Poster Viewing 2 / 126****Using a One Health Lens to investigate inter-sectoral collaboration between human, animal and wildlife zoonotic disease surveillance and control systems in the Greater Accra Metropolitan Area of Ghana****Authors:** Sherry Ama Mawuko Johnson<sup>1</sup>; Sandul Yasobant<sup>2</sup>; Walter Bruchhausen<sup>3</sup>; Joannishka Dsani<sup>3</sup><sup>1</sup> *School of Veterinary Medicine, College of Basic and Applied Sciences (CBAS), University of Ghana, Accra, Ghana*<sup>2</sup> *Institute for Hygiene and Public Health (IHPH), Global Health, University Hospital Bonn, Bonn, Germany / One Health Graduate School, Center for Development Research (ZEF), University of Bonn, Bonn, Germany / Centre for One Health Education, Research and Development (COHERD), Indian Institute of Public Health Gandhinagar (IIPHG), Gujarat, India / School of Epidemiology and Public Health, Datta Meghe Institute of Medical Sciences (DMIMS), Wardha, India*<sup>3</sup> *Institute for Hygiene and Public Health (IHPH), Global Health, University Hospital Bonn, Bonn, Germany / One Health Graduate School, Center for Development Research (ZEF), University of Bonn, Bonn, Germany***Corresponding Author:** jdsani@uni-bonn.de**Introduction**

Zoonotic diseases cause millions of deaths annually and pose a significant threat to human and animal health, livelihoods and economies. The One Health approach, which focuses on integrated surveillance response, is proposed as a viable means of controlling zoonoses. However, there is inadequate evidence on the essence of intersectoral collaborations in optimizing existing surveillance systems in a more integrated way. Ghana has made innovative changes by creating a Technical Working Group tasked with drafting policies for One Health implementation and many stakeholder educational attempts have been made but anecdotal evidence suggests these efforts have not been impactful in reality. Thus, this study explores the current human, animal and wildlife zoonotic disease surveillance systems and their potential convergence points for optimizing intersectoral collaborations.

**Methods**

Focusing on the core technical functions, data sharing and communication channels, the study examines Ghana's zoonotic disease surveillance and response systems as well as the system knobs for maximizing intersectoral collaborations at the district level. Using a qualitative approach (key informant interview) through a semi-structured interview guide, this study interviewed 67 officers from the surveillance systems across the three sectors and representing 16 districts in the Greater Accra Metropolitan Area (GAMA). The ECOSUR, a semi-quantitative tool was used to evaluate the degree/quality of intersectoral collaboration. Data was coded and analyzed through the MAXQDA Analytics Pro 2022 software.

**Results**

Preliminary results from the study show limited intersectoral collaboration between the human and animal health sectors in about 60% of GAMA districts. Collaborative activities were mainly driven by the human health sector while the wildlife sector was not visible at the district/subdistrict levels. Rabies, Avian Influenza, Lassa Fever and Covid 19 outbreaks were the main triggers and drivers of intersectoral collaboration. Inter/multisectoral efforts were observed in response and control efforts whereas disease detection, confirmation and data analysis activities were handled in a siloed manner. The results also show that existing intersectoral relationships made it easier to obtain information and resources and enabled timely interventions in response to disease threats. Thematic analysis of key stakeholder interviews reveals that factors influencing collaboration include resource availability, surveillance system structures, workforce capacities, individual motivation and public health knowledge. Participant discourse analysis reveals that the existence of some informal collaborations and adequate human resource of the human health sector could be leveraged as opportunities for one health integration in the surveillance system. A lack of resources, differing health system structures, lack of one health focused policies and a lack of leadership on collaboration would hamper the successful integration of the approach in the current systems.

**Conclusion**

These preliminary findings on current intersectoral collaborations of the studied disease surveillance

systems are highly relevant as they provide a pillar to guide One Health policy and action implementation efforts in Ghana. This study highlights the need for a better understanding of how a One Health surveillance system can be operationalized practically and sustainably, emphasizing the significance of adequate financing and technology-driven integrated communication and data sharing mechanisms. The study sheds light on the need to develop One health implementation strategies that streamline collaborative efforts in multisectoral zoonotic disease surveillance and control systems.



## Lunch &amp; Poster Viewing 2 / 56

## Investigations of the interactions of zoonotic pathogens in humans and animals (SHIP-NEXT Module One Health): Analysis of viral pathogens in dogs, cats and poultry in households of Mecklenburg-Western Pomerania

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One of the most challenging aspects of controlling zoonoses is the complex interaction between humans, animals and environmental factors. Notably, the frequency of infections with zoonotic pathogens among humans, farm animals and pets in private households, as well as the transmission dynamics between these hosts, have not yet been adequately researched. To address this gap, the Modul One Health was introduced as part of the population-based project Study of Health in Pomerania (third cohort SHIP-NEXT). This module involves studying animal contacts of participants to investigate husbandry and environmental conditions. For this purpose, serum samples from dogs, cats, poultry and pigeons have been provided by study veterinarians and were tested for infection with viral pathogens including Hepatitis E virus (HEV), Tick-borne encephalitis virus (TBEV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The study comprises analysis of antibodies against TBEV, HEV and SARS-Cov-2 by ELISA and molecular determination of viral RNA by PCR assays. The results along with data from project partners in human health can be used to investigate the impact of zoonoses on human health in association with animal contact and husbandry conditions and to analyse the potential influence of human lifestyle on animal health. This will finally generate new quantitative and qualitative parameters for risk assessments and the planning for targeted surveillance activities in future.

**Lunch & Poster Viewing 2 / 75****Immunological effects and safety of COVID-19 mRNA-vaccine**

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During the SARS-CoV-2 pandemic, innovative mRNA-vaccines were approved with unprecedented speed. The COVID-19 mRNA-vaccines consist of mRNA coding for SARS-CoV-2 spike-glycoprotein, enclosed by lipid nanoparticles (LNPs). The vaccines reliably protect from severe disease and allowed for the transition from a pandemic to an endemic phase. The risk-benefit assessment was in favor of the vaccines, but rare adverse reactions such as myocarditis have been reported, of which the pathogenesis is still elusive.

The goal of our study is to understand how mRNA vaccines modulate the immune system, which may allow to decouple safety related effects from efficacy-defining mechanisms. We aim to understand the increased risk of myocarditis. Potential explanations could be an aberrant expression of spike-protein in the myocardium leading to apoptosis, a direct negative impact by LNPs on cardiomyocytes or a generalized inflammatory response.

An animal trial was conducted, in which guinea pigs were immunized with mRNA-vaccine or empty LNPs. Two and seven days after each immunization animals were sacrificed to perform necropsies and to collect organ-samples. Samples were analysed by qRT-PCR and histopathology for cytokine and SARS-CoV-2 spike expression and for inflammatory processes in the tissue.

Preliminary data show elevated levels of the inflammatory chemokines, CCL3 and CXCL10, at the injection site and the heart. High amounts of IL-1, CCL3 and CXCL10 were found in spleen and liver. Vaccine spike-mRNA was detected in large quantities at the injection site, spleen and liver but only at low levels in the heart. Immunohistopathology indicates inflammatory processes at injection site, heart, spleen and liver shortly after immunization. We aim to find out, if these inflammations can be correlated with the presence of mRNA vaccine particles.

In view of their versatility and excellent immunogenicity mRNA vaccines have become an indispensable part of our pandemic-preparedness-armamentarium. However, any potential safety issue has to be elucidated and resolved. Our efforts will contribute to this process and thereby enhance general vaccine acceptance.

**Lunch & Poster Viewing 2 / 13****Implication of local elected officials for the implementation of One Health in Mali**

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**Background:**

During the last thirty years, populations have been increasingly attentive to environmental and health issues. Health in all its components is today of great importance and is at the heart of many citizen concerns. However, the links between human health, animal health and environmental health remain less understood. Therefore, it is fundamental that information and awareness on One health approach gain more attention. The objective of the study is to contribute to the dissemination of the “One Health” approach in the District of Bamako for its appropriation by local elected officials, technical officers and communities.

**Methods:**

Two days’ workshop was organized on the “One Health” approach from December 7 to 8, 2023 at the General Directory of Territorial Communities, Bamako. The District of Bamako has six community municipalities. In each municipality four participants have been chosen as well for the Municipality of the District. A total of twenty eight persons participated to the workshop. Participants were divided in four working groups (Human health, animal health, environment and agriculture). The method SWOT has been used to know their perceptions on the One Health approach implementation in the District of Bamako. The results have been synthetized with a road map.

**Results:**

The elected officials of Bamako have been informed about One Health. For a better implementation of One Health in the District of Bamako , strength, weakness, opportunities, and threat have been identify and solutions proposed by the participants. From the recommendations a road map has been elaborated.

**Conclusions:**

The local elected officials and technical officers of the District and municipalities of Bamako acquired knowledge about the “One Health” approach.. This knowledge will enable them to better understand and integrate the “One Health” approach into their practices and decisions. Consequently, local elected officials will be able to implement policies and actions that promote public health, animal health and the environmental health in an integrated manner.

## Lunch &amp; Poster Viewing 2 / 95

## Improvements of weaned pigs barn hygiene to reduce the spread of antimicrobial resistance

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The spread of antimicrobial resistance (AMR) in animal husbandry is usually attributed to antibiotics, poor hygiene, and biosecurity. We therefore conducted experimental trials to improve hygiene management in weaned pig houses and assessed the impact on the spread of AMR. Two groups were examined, the experimental group (EG) and the control group (CG). In the flat decks of the experimental groups, the hygiene conditions were improved, while regular hygiene measures were carried out in the control groups. The occurrence and spread of AMR were determined in *Escherichia coli* (*E. coli*; resistance indicator) using cultivation-dependent (CFU) and -independent (qPCR) methods as well as whole genome sequencing of isolates in samples of various origins. Surprisingly, there were no significant differences ( $p > 0.05$ ) in the prevalence of resistant *E. coli* between the flat decks managed with conventional techniques and those managed with improved techniques. Selective cultivation delivered ampicillin- and sulfonamide-resistant *E. coli* proportions of up to 100% and 1.2%, respectively. There was a significant difference ( $p < 0.01$ ) in the abundance of the *bla*<sub>TEM-1</sub> gene in fecal samples between EG and CG groups. Managing hygiene alone was insufficient for reducing antimicrobial resistance in piglet rearing. We conclude that the complex factors contributing to the presence and distribution of AMR in piglet barns underscore the necessity for a comprehensive management strategy.

**Lunch & Poster Viewing 2 / 100****De-novo genome assembly and quality assessment of bacterial NGS data within the scope of integrated genomic surveillance**

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Genome assemblies are an essential resource for in-depth genetic characterization of microbes based on NGS data. Reconstructed genomes are widely utilized to characterize specific samples, to identify resistance and virulence elements, and to investigate potential outbreaks.

Despite the common practice of performing de-novo reconstruction, assessing the quality of such assemblies is crucial yet often neglected. Quality control of short-read based genomes (typically considered as “draft assemblies”) is especially important, as they can suffer from various issues such as contamination, high fragmentation, and regions with low coverage or completeness. Downstream analyses of such low-quality genomes can lead to mischaracterization and wrongful inferences about relationships between samples.

To address these challenges, we developed the Generic Assembly and Reconstruction pipeline (GARi). GARi takes as input short-read bacterial NGS data and performs genome assembly and quality control. This Nextflow-based pipeline incorporates pathogen-specific models, thresholds, and quality metrics to assess the contiguity, consistency, and genetic completeness of an assembly. Based on various quality metrics the pipeline can automatically classify reconstructed genomes as passed, flagged or failed ensuring that low-quality assemblies are identified and can be excluded from downstream analyses such as cgMLST, thereby improving the accuracy and reliability of public-health studies.

**Lunch & Poster Viewing 2 / 135****Urban-rural differentiation and zoonotic risk assessment of *Aedes*, *Anopheles* and *Culex* vector dispersal****Author:** Christian Voll<sup>1</sup> ; Mandy Schäfer<sup>2</sup>; Elke Hertig<sup>1</sup><sup>1</sup> *Universität Augsburg*<sup>2</sup> *Friedrich-Loeffler-Institut***Corresponding Author:** christian.voll@uni-a.de

The incidence of mosquito-borne diseases in Europe is expected to increase with climate change. Changes in temperature, precipitation, humidity, and land use are projected to cause potential disease vectors to expand. Cities are implementing climate change adaptation measures such as creating green spaces and water bodies, which may inadvertently affect vector dispersal. This interdisciplinary research project investigates mosquito distribution in Augsburg, Southern Germany, and its surrounding areas to conduct a spatially differentiated risk assessment of potential vector occurrence along an urban-rural gradient. The study emphasizes small-scale spatial differences in vector distribution, abundance and seasonal activity of mosquito vectors through field studies. For this purpose, CO<sub>2</sub>-baited traps and ovitraps are implemented at selected sites, and local climatic and environmental conditions are measured and recorded. Based on the results of the field surveys, the habitats of the studied species will be characterized by including meteorological, climate and land use data. Suitable or unsuitable urban and rural habitats are identified. A spatially differentiated statistical analysis using the results of the field investigations and other spatial climate and environmental datasets as well as non-climatic influencing variables allows an estimation of the risk potential for the entire study region. The project receives funding by the Federal Ministry of Health.

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## Genetic determinants of *Burkholderia pseudomallei* persistence and spread

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*Burkholderia pseudomallei* is the causative agent of melioidosis, an often-fatal neglected tropical disease with high mortality rate, relapse and persistent infections. The disease has been increasingly concerned, yet the understanding of the disease-causing bacterial lineages, their dissemination, and adaptive mechanisms remains limited. To address this, we first conduct a comprehensive genomic analysis of 1,391 clinical and environmental *B. pseudomallei* isolates collected from nine hospitals in northeast Thailand between 2015 and 2018, and contemporaneous isolates from neighbouring countries, representing the most densely sampled collection to date. Our study identifies three dominant lineages, each with unique gene sets potentially enhancing bacterial fitness in the environment. Transcriptome analyses of representative clinical isolates from each dominant lineage reveal increased expression of lineage-specific genes under environmental conditions in two out of three lineages. This underscores the potential importance of environmental persistence for these dominant lineages. We then performed the comparative genomic analysis of additional 20 pair isolates collected from 20 relapse and persistent melioidosis patients (two isolates per patients). We found that during persisting in human host, the mutations are occurred in *tssB* and *amrR* genes. The mutations have been tested to be associated with the increased virulence and antibiotic resistance. Collectively, our findings suggest that environmental persistence using the lineage-specific genes may play a role in facilitating the spread of *B. pseudomallei*, and as a prerequisite for exposure and infection. After infecting into human, *B. pseudomallei* can also persist in human host and the mutations that are associated with virulence and antibiotic resistance are occurred. Our study provides useful insights for informing melioidosis prevention and control strategies.

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## Reemergence of Oropouche Virus in Brazil (2023-2024): Molecular and Serological Characterization of a Novel Reassortant Strain and Its Impact on Public Health

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Oropouche virus (OROV; species *Orthobunyavirus oropoucheense*) is an arthropod-borne virus primarily transmitted to humans by *Culicoides paraensis* and has caused outbreaks of Oropouche fever in Central and South America since the 1950s. In 2023-2024, significant OROV outbreaks were reported in Brazil, with cases spreading to previously non-endemic regions. This study investigates the virological factors contributing to the reemergence of Oropouche fever in Brazil between 2023 and 2024 using comprehensive genomic, molecular, and serological data collected from January 2015 to June 2024.

We screened 203 patients with febrile illness from Amazonas State between December 2023 and May 2024. Among those tested, 10.75% (20/186) were positive for OROV RNA by RT-PCR. Genomic analysis of two OROV sequences from patients identified a novel reassortant strain linked to the 2023-2024 epidemic, which demonstrated a 199-fold increase in incidence. In 2024, OROV infections were detected in all five regions of Brazil, with high positivity rates among febrile patients in Rondônia (45%), Mato Grosso (40.7%), Acre (39.6%), and Amazonas (24%). The novel reassortant strain replicated approximately 100-fold higher titers in mammalian cells compared to the prototype strain and evaded pre-existing immunity, showing a 32-fold reduction in neutralizing capacity against serum from individuals infected with earlier OROV strains.

Clinical symptoms in infected individuals, including fever, myalgia, and back pain, overlapped with those caused by other endemic arboviruses such as DENV, ZIKV, MAYV, and CHIKV. Our findings suggest that the recent increase in OROV cases may be driven by the higher replication efficiency and immune evasion capabilities of the novel reassortant strain. Enhanced febrile disease surveillance and further studies are essential to evaluate the role of climate change and environmental factors in the spread of this emergent OROV variant and to guide public health responses in affected regions.



**Lunch & Poster Viewing 2 / 183****One Health Threats on Poland's Militarized Border in Białowieża Forest**

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Linear infrastructure and militarization are increasingly features of Europe's borderlands. Białowieża Forest (BF) is a transboundary World Heritage site recently militarized and split by a state border barrier. We preliminarily summarize threats based on available evidence and own observations from 2021-2024 using the three One Health targets: human, animal and environmental. From an environmental perspective, threats include landscape fragmentation, pollution, and increase of human disturbance by facilitated access into remote areas. From human health angle: burden on local health systems to address emerging clinical conditions (including border infrastructure-related injuries), exposure to vector-borne diseases, border community's stress associated with militarization (presence of uniformed services, exclusion zones) and economic hardship from lack of attractiveness of region for tourists. From animal health perspective: roadkill from higher vehicle traffic, human habituation and food-conditioning of some wildlife species, attraction of domestic animals to military outposts changing the human-wild-domestic animal interface and disease transmission risk. A mandate to develop and implement a One Health approach (aligned with the ECDC Foresight Programme) is needed with engagement of public health sector, ecologists, forest users, veterinarians, sociologists, first responders and activists, to understand interconnections and mitigate risks, prior to additional planned border fortification, forest disturbance and changes in the human-forest interface.

**Lunch & Poster Viewing 2 / 49****Diagnostic stewardship in the one health context: The first online survey in German speaking countries in humane and veterinary medicine**

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**Background:** Diagnostic stewardship aims to provide patients with timely and appropriate tests to optimize results, improve antibiotic use and reduce unnecessary diagnostics and costs. In the context of the global antimicrobial resistance rise requiring a “One Health” approach, we surveyed German-speaking healthcare worker (HCWs) and veterinary professionals, and lab staff to identify clinician-laboratory interface challenges.

**Materials and Methods:** An open online survey was conducted via Lime Survey. We created a questionnaire to evaluate knowledge, practices, and perceived diagnostic procedure improvement needs in infectious diseases. The survey, with 166 questions across 16 domains, was active from October 2022 to July 2023.

**Results:** We analyzed 1,742 responses, predominantly from physicians (49.8%) followed by veterinarians (18.8%) and nurses (13.5%) with more than a decade of experience. The need for improved communication between clinicians and microbiology laboratories was evident, with veterinarians expressing fewer improvement needs than human healthcare workers.

**Conclusion:** Our study underscores the necessity for better communication between clinicians and microbiology laboratories. Due to heightened awareness of infectious diseases, especially zoonoses, Veterinarians expressed fewer improvement needs than staff members in human medicine.

**Acknowledgements:** We are grateful to the Young Infection Medicine Network (jUNITE e.V.) and all survey participants for their support.

**Lunch & Poster Viewing 2 / 46****Use of organ cultures of the reproductive tract of different animal species to determine the impact of host factors on coronavirus infections**

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For the investigation of virus-host- interaction the 3R-principle has to be considered. Furthermore, to compare between different animal species, organ cultures may provide a suitable model, as infection studies can be conducted under well controlled conditions. Besides the respiratory tract, coronaviruses may target also the reproductive tract. While reproductive organ cultures models have been described as suitable for host-pathogen-interaction studies in avian species, comparable systems for the investigation of mammalian host are lacking, but may be of specific interest especially for research on the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). The aim of our study was to establish comparable reproductive organ culture systems for different host species. The chicken oviduct organ cultures were used as a well established model to identify host parameters involved in coronavirus-host-interaction, and applied to rodent reproductive organ cultures. Our results indicate that organ cultures provide the possibility of a repeatable in vitro system that allows a deeper insight into host responses compared to cell culture systems.

**Lunch & Poster Viewing 2 / 18****Stability of hepatitis E virus during the production of pork liver sausage and raw sausage**

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The zoonotic hepatitis E virus (HEV) can cause acute and chronic hepatitis in humans. Meat from domestic pigs, a major animal reservoir of HEV, plays a key role in HEV transmission. Although pork meat products can contain HEV-RNA, it is unknown whether infectious HEV is still present after their manufacturing process. Therefore, this study investigated the HEV inactivation during production of spreadable liver sausage and salami-like raw sausage.

Cell culture-adapted HEV-3 strain 47832c was used to contaminate meat preparations for liver and raw sausage. Canned liver sausage was heated in a 70°C water bath and sampled up to 18 min after reaching a core temperature of 70°C (corresponding to usually applied heating regimes). The produced raw sausages were cured at 18°C and 80 % relative humidity, and sampled up to day 21. Samples were analyzed for infectious HEV and viral RNA using cell culture and RT-qPCR, respectively.

In liver sausage, infectious HEV was completely inactivated (4.6 log<sub>10</sub> decrease) after holding a core temperature of 70°C for 2 min, while the HEV RNA titer decreased only marginally. In raw sausage, infectious HEV decreased by 1.3 log<sub>10</sub> over three weeks, while the HEV RNA titer remained unchanged. In conclusion, properly manufactured liver sausage does not pose an HEV infection risk, while raw sausage can contain infectious HEV if starting material with a high HEV amount was used. Viral RNA testing cannot be used to predict infectivity of HEV in meat products

**Lunch & Poster Viewing 2 / 26****Prevalence of *Francisella* and other tick-borne pathogens in ticks across Germany**

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*Francisella tularensis* is the causative agent of the zoonotic disease tularemia and can affect more than 250 animal species, including rabbits, rodents and birds. The disease, also known as rabbit fever, is characterised by various clinical signs depending on the route of infection, and ranging from flu-like symptoms to severe forms of pneumonia. *F. tularensis* ssp. *holarctica* is the most clinically relevant subspecies in European countries, including Germany. Transmission to humans can occur through consumption of contaminated water or food, by contact with infected animals or by arthropod bites. Ticks play a particularly important role in transmission of tularemia in Germany. Therefore, we aimed to perform a nationwide study to evaluate the prevalence of *F. tularensis* ssp. *holarctica* in ticks across Germany. In addition, *Francisella*-like endosymbionts and other pathogens (such as *Rickettsia* spp. and *Borrelia* spp.) are commonly found in ticks and their prevalence in ticks was also investigated in this study. Ticks from all German federal states were collected and analysed in a newly implemented multiplex qPCR, specifically targeting *F. tularensis* ssp. *holarctica* and *Francisella*-like endosymbionts. We also used a commercial multiplex qPCR assay for the detection of nine tick-borne pathogens, including *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Babesia duncani*, *Rickettsia* spp. and *Anaplasma phagocytophilum*.

## Lunch &amp; Poster Viewing 2 / 66

## Unveiling the phylogenetic relationships of Central European *Culicoides* species by analysing their mitochondrial genome

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*Culicoides* biting midges (Diptera: Ceratopogonidae) are considered the vectors of bluetongue and Schmallenberg viruses. Morphological species identification within this genus is often unreliable or even impossible. Current molecular tests, based on PCR and DNA sequencing of selected DNA regions, help in distinguishing many, but not all, described species and their haplotypes. However, genetic variations within and between these species make species determination uncertain, and phylogenetic relationships and systematic classifications remain unclear in many cases.

To identify suitable markers for genetic species identification and clarify phylogenetic relationships, comparative analyses of the complete mitochondrial genome of important Central European *Culicoides* species have been initiated. So far, 11 complete genomes of the *Obsoletus* Group, six of the *Pulicaris* Group, and six from other *Culicoides* species have been sequenced. For annotation and analysis, only one *Culicoides* mtDNA sequence (*C. arakawae* from Japan) is available in GenBank, although mtDNA sequences from other nematocerans might also be useful.

All *Culicoides* sequences analysed so far have an AT-rich region in their mitochondrial genomes, with inverted repeats in the *Obsoletus* and *Pulicaris* Groups. A second AT-rich region with inverted repeats is found in all *Obsoletus* Group species except *C. dewulfi*, confirming its systematic distinction from the group. Ongoing analyses aim to resolve open systematic questions.

**Lunch & Poster Viewing 2 / 69****ProBioHuhn – Examining Antimicrobial Susceptibility of *Escherichia coli* and *Campylobacter* spp. in Organic Broiler Farms**

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While multi-resistant bacteria can also be detected in organic broiler flocks, studies indicate an overall lower proportion compared to conventional broiler flocks.

To examine possible relations between different chicken types, outdoor access and occurrence of resistant bacteria in organic broiler, we collected longitudinal data on antimicrobial susceptibility of commensal *Escherichia coli* and thermotolerant *Campylobacter* spp. on thirteen German organic broiler farms. All farms are sampled over four fattening periods via chick papers, boot swabs and cloacal swabs from first day of life to slaughter. Antimicrobial susceptibility is tested via broth microdilution and compared to results from German conventional broiler husbandry. Interim results show high percentage of fully susceptible *E. coli* throughout the fattening period (69-79%) and a low number of multi-resistant isolates compared to conventional broilers at the age of slaughter. ESBL-producing *E. coli* have been found in 9 of 48 examined flocks on six different farms, mostly after chickens were provided with outdoor access. Among *Campylobacter* spp., *C. jejuni* accounted for 78% and *C. coli* for 22% of isolates. *C. jejuni* showed a higher share of fully susceptible isolates at age of slaughter compared to isolates from conventional broilers (37.5% vs. 21.7%). For *C. coli*, we observed a tendency of a lower number of identified resistances compared to conventional isolates but the overall number of isolates is still small.

**Lunch & Poster Viewing 2 / 79****Establishing an Epidemic Panel integrating the Human-Animal-Environment Interface for assessing infectious pathogens in Côte d'Ivoire.**

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Epidemic panels are population-based longitudinal cohort studies able to rapidly and adaptively survey a relevant population group on diverse pathogens for infection frequency and burden to provide estimates for projections. Worldwide, there is a lack of epidemic panels and in particular integration with One Health surveillance efforts is rarely included. Here, we are developing the study design for a planned epidemic panel with an integrated One Health approach in Côte d'Ivoire. In the first instance we will examine the feasibility of assessing burden of infectious diseases with this epidemic panel and link to relevant environmental and animal data. We intend to design a longitudinal cohort recruiting 2000 individuals in the pilot region with a participatory approach through consultations with multiple partners in the region. We will employ a multi-stage sampling process targeting individuals, their animals in households as well as wild animals and their environment. We will conduct on-site health screening, standardized questionnaires including anthropological determinants, and laboratory analyses to detect and characterize a wide range of pathogens. In putting our study design forward we hope to generate relevant input from the community with the aim of developing a participatory study protocol as well as identifying facilitators and obstacles for implementing this one-health cohort study at the community level.



**Lunch & Poster Viewing 2 / 92****Metarhizium pemphigi as a promising strategy for the containment of TBEV natural foci****Author:** Alexander Lindau<sup>1</sup> ; Katrin Facht-Lehmann<sup>1</sup>; Markus Grünke<sup>1</sup>; Ute Mackenstedt<sup>2</sup><sup>1</sup> *University of Hohenheim, Department for Parasitology*<sup>2</sup> *University of Hohenheim, Department of Parasitology***Corresponding Author:** alexander.lindau@uni-hohenheim.de

This research project investigated the potential use of the entomopathogenic fungus *M. pemphigi* to eradicate a tick-borne encephalitis (TBE) “hotspot”. The fungus was originally isolated from an-infected tick at the Department for Parasitology (Hohenheim) in 2005. It was demonstrated in 2016 that the fungus kills ticks (Wassermann et al., 2016). Fungal conidia were applied to cotton wool and spread monthly in cardboard rolls. The cotton wool is used by rodents as nesting material, thus exposing juvenile tick stages inside the rodent burrows to *M. pemphigi*. A reduced seroprevalence of TBEV antibodies in rodents served as indirect proof of the effectiveness of the fungus application. The study spanned the years 2021 to 2023, with a seroprevalence of approximately 15% in rodents in 2021 prior to the application of the fungus. Following the application in 2022, no TBEV antibodies were detected in the rodents examined. Additionally, ticks were collected in the hotspot and tested for TBEV. While no TBEV was detected in ticks in 2022, one female tested positive in 2023. However, sequence analyses showed that this isolate differs from the TBE strain already known from the TBE hotspot investigated. This demonstrates that TBEV was eliminated in the local tick population by the application of *M. pemphigi*. However, it is not possible to prevent the introduction of new TBE viruses. The high turnover of wild animals in the hotspot may be a contributing factor to this new introduction.

**Lunch & Poster Viewing 2 / 101****Studying the impact of cgMLST software selection on allelic distance estimates across bacterial pathogens**

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Surveillance of bacterial pathogens commonly relies on the in-silico method of core genome multilocus sequence typing (cgMLST) to obtain detailed insights into the genetic relationships, enabling outbreak and infection chain investigations.

Several cgMLST software with different principles and input requirements exist. Some of which utilize k-mers and perform allele calling on raw reads, while others rely on scanning for known alleles in an assembly of interest. Due to differences in their implementation, cgMLST software can yield differing allele calls, affecting downstream analyses and potentially impacting decision making and policies on a public-health level.

In order to study the impact of cgMLST tools on downstream analyses and genomic cluster detection, we compared allele calls from four cgMLST software (one read-based: MentaLiST; and three assembly-based: chewBBACA, hash-cgMLST, and Ridom SeqSphere+) for identical sets of samples originating from a range of bacterial pathogens of high importance for public-health.

Our analyses indicate that the choice of the software and its parameters for cgMLST analysis can influence the final allelic distance estimates. Thus, comprehensive documentation and description of the cgMLST schemes, methods, and their parameters, as well as detailed information on distance matrix calculation and quality control, should be considered in downstream analyses, and routinely included in reports containing conclusions derived from cgMLST analyses.

**Lunch & Poster Viewing 2 / 131****Virtual One Health Modules**

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The integration of human, animal and environmental health, known as the One Health approach, is essential for tackling complex global health problems. Training in the One Health approach is critical in equipping healthcare professionals with interdisciplinary knowledge and skills to address health problems across species and environmental boundaries. Traditional training models face barriers such as geographical, financial and time factors that limit access to essential training.

Blended learning, which combines online media with synchronized sessions, offers a solution to these challenges. It provides a flexible, inclusive and interactive learning experience that is tailored to different learning needs. The virtual One Health modules are an example of this approach. Aimed at Master's and PhD students, they offer courses on One Health's core competences, the impact of climate change and One Health and the global threat of antimicrobial resistance.

These modules utilize the strengths of online and synchronous sessions. Online components, including interactive lectures, forums and virtual labs, allow students to engage with the material at their own pace and location. Real-time webinars and synchronous sessions complement these activities and encourage face-to-face interaction, teamwork and practical application of concepts. This hybrid model enhances understanding and supports a global network of professionals committed to the principles of One Health.

Feedback from participants emphasizes the effectiveness of blended learning, which provides an in-depth, interactive and flexible experience. Students appreciate the opportunity to balance their studies with their personal commitments and benefit from the various educational tools. The global reach of the online components ensures that a wider audience is reached, even in remote or underserved areas.

To summarize, blended learning and interactive online tools significantly improve the accessibility and impact of One Health education. By overcoming traditional barriers and offering a diverse, engaging learning environment, these methods prepare a diverse, globally distributed cohort of health professionals. This approach democratizes access to critical knowledge and fosters a global community ready to address and solve complex health problems within the One Health framework.

**Lunch & Poster Viewing 2 / 139****Promoting transparent and systematic use of evidence in policy decision-making on One Health**

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**Introduction:** Recent global challenges, such as the COVID-19 pandemic and climate crisis, underscore the complex links between human, animal and ecosystem health, and the need for evidence-based decision-making. These events have led to a surge in One Health initiatives aimed at enhancing effectiveness of health interventions. However, these initiatives have not consistently been translated into policy for the population's benefit. The Evidence-Informed Policy Approaches for One Health (OH-EVI) project aims to enhance One Health and knowledge translation capacities, promoting systematic and transparent use of evidence in One Health policy-making in partner countries.

**Methods:** OH-EVI employs a participatory and multisectoral approach, and is implemented in Croatia, Ghana and Nigeria. Each country will form multi-stakeholder, cross-disciplinary teams to prioritize a national One Health problem through stakeholder engagement, literature review, and problem tree analysis. The teams will use relevant toolkits to identify and synthesize the best available evidence on intervention options and facilitate stakeholder dialogues to contextualize key implementation considerations, aligning with national resources, culture, and values.

**Expected Outcomes:** The OH-EVI project will provide example cases for policy development and implementation, fostering long-term collaboration between scientists and policy-makers within and across the three countries, bridging the gap between technical expertise and policy-making. Each country will develop evidence briefs for policy, addressing high-priority issues for the benefit of the population.

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**Companion animals as carriers of Enterobacteriaceae with mobile colistin resistance genes - Implications for pet owners' health?**

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Since 2015, mobile colistin resistance genes (*mcr-1* to *mcr-10*) have been found mainly in *Enterobacteriales* in livestock, with limited presence in humans. We investigated the emergence of *mcr* genes and/or their bacterial hosts among companion animals.

From 2009 to 2023, 12,288 Gram-negative bacterial isolates from companion animals were screened for *mcr-1* to *mcr-9* (95.7%) or to *mcr-10* (4.2%) using two multiplex PCR protocols. *Mcr*-positive isolates were sequenced and analysed for multi locus sequence types (ST), antimicrobial resistance genes, and plasmids. Putative plasmid sequences were verified using BLAST.

*Mcr* genes were found in 73 isolates: *Enterobacter* spp. (2.8 %), *Klebsiella* spp. (1.0 %), and *E. coli* (0.3 %). *Mcr-9.1/9.2* (67.1 %; 95.9 % on a 270-320-kb IncHI2 plasmid) was most prevalent, followed by *mcr-1* (27.4 %; variants 1.1., 1.2, 1.26, 1.37; 55.0 % on a 35-kb IncX4 plasmid), *mcr-10.1* (4.1 %), and *mcr-4.6* (1.4 %).

Forty-two isolates co-expressed ESBL genes: *blaSHV-12* (n=31), *blaCTX-M-9* (n=8), *blaCTX-M-1* (n=3), *blaCTX-M-15* (n=3), and *blaCTX-M-3* (n=1); 28 isolates carried carbapenemase gene *blaOXA-48*. Dominant clones included ST506 and ST114 (*Enterobacter* spp.) and ST1196 (*E. coli*).

The origin of *mcr* genes in isolates from companion animals remains elusive. The frequent occurrence of IncHI2 plasmids carrying *mcr-9* among OXA-48 positive isolates of ST1196, ST114, and ST506, which are highly distributed among humans, significantly contributes to the global spread of *mcr-9*.

**Lunch & Poster Viewing 2 / 164****Lot Quality Assurance Sampling as a novel approach for AMR surveillance in livestock**

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In the German livestock sector, antimicrobial resistance (AMR) monitoring is evaluated at the national level within the framework of the zoonoses monitoring. Lot Quality Assurance Sampling (LQAS) is a statistical approach that could classify AMR prevalences as high or low at a regional level. Based on small sample sizes compared to prevalence estimates, this would allow for evidence-based local interventions. Therefore, we investigated the applicability of LQAS in the field of AMR using indicator *E. coli* from fecal samples of broiler chickens.

In a representative sample of 1,404 German farms, one random *E. coli* isolate per sock swab was tested for susceptibility to 14 antibiotics or antibiotic combinations. In addition, samples were cultured on 4 selective media to isolate resistant *E. coli*.

The prevalence of non-wild-type strains (EUCAST ECOFFs) detected in our study demonstrates comparability with national surveillance data. Evaluation at the regional level shows clear differences in AMR prevalence for several antibiotics, including cefotaxime and colistin, in the random *E. coli* and even clearer in the selectively isolated *E. coli*, highlighting the need for regional AMR surveillance.

Using appropriate thresholds, in silico LQAS simulations based on small sample sizes were able to identify regions of increased AMR prevalence with high specificity and sensitivity. Thus, in poultry, LQAS may complement AMR surveillance of indicator *E. coli* in a resource-efficient manner.

**Lunch & Poster Viewing 2 / 172****Extended-spectrum  $\beta$ -lactamase and carbapenemase-producing *Klebsiella pneumoniae* in different environments.****Authors:** Kabiru O. Akinyemi<sup>1</sup>; Kehinde O. Amisu<sup>1</sup>; Rebecca O. Abegunrin<sup>1</sup><sup>1</sup> *Department of Microbiology, Faculty of Science, Lagos State University, Ojo, Nigeria***Corresponding Author:** kabiru.akinyemi@lasu.edu.ng

*Klebsiella pneumoniae* is a fast-spreading pathogen in human and animal worldwide. It continues to exhibit increasing resistance to broad-spectrum  $\beta$  lactams (ES $\beta$ L) and carbapenem with negative clinical outcomes. This study investigated the carriage of ES $\beta$ L and carbapenemase genes in *K. pneumoniae* from different sources. A total of 1,889 samples; 440 humans clinical from public hospitals; 373 animal, and 86 effluent samples from abattoirs and farms were collected in Lagos State between May 2019 and April 2023. Samples were processed and isolates were subjected to an in vitro susceptibility test. Detection of ES $\beta$ L, Carbapenemase and virulence genes was carried out by RT-PCR using specific gene makers. The prevalence of 4.5% (65/1440), 4.3% (16/373) and 10.5% (9/86) *Klebsiella pneumoniae* was recorded from humans, animals and environmental sources. All the isolates were multiple drug-resistant exhibiting 13 distinct profiles. Interestingly, co-coexistence of one or more of carbapenemase genes (*blaIMP*, *blaVIM*, *blaOXA-48*, and *blaNDM-1*) and ESBL/AmpC genes; *blaSHV*, *blaTEM*, and *blaCTX-M*) occurred in 20/24 of the *K. pneumoniae* (11 human and 9 animal strains) exhibiting hyper-virulent genes *wabG*, *rmpA*, *uge* and *magA*. The study highlights the importance of molecular surveillance for tracking emerging extended-spectrum beta-lactam and carbapenem-resistant *K. pneumoniae* from human and animal sources. There is a need to safeguard the transmission from animal to humans through the food chain.

**Lunch & Poster Viewing 2 / 114****The occurrence of pathogenic *Escherichia coli*, *Salmonella* and antibiotic-resistant *Staphylococcaceae* in *Apodemus flavicollis* and *Apodemus sylvaticus* in Lower Saxony, in 2019**

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Rodents are well known as reservoir hosts and vectors for a variety of viruses. In contrast, the occurrence of bacterial zoonotic pathogens is less well studied. Thus, we aimed to determine if bacterial pathogens such as Shigatoxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC) and *Salmonella* spp. occur in yellow-necked mice (*Apodemus flavicollis*) and wood mice (*A. sylvaticus*) collected 2019 in Lower Saxony, Germany. Moreover, we aimed to determine if these species harbour antibiotic-resistant members of the family *Staphylococcaceae* that might puzzle within the spread of antimicrobial resistance.

Using enrichment and realtime PCR on intestinal samples, the STEC pathotype was detected in three and the EPEC pathotype in seven of the 410 yellow-necked mice. Contrarily, STEC and EPEC were detected in two and one of the 42 wood mice respectively. Enteroaggregative and enteroinvasive *E. coli* were not detected nor were *Salmonella*. The samples also contained *Mammaliococcus sciuri* (N=4) and *M. fleurettii* (N=2) resistant to seven antimicrobial agents.

The low occurrence of STEC and EPEC as well as the absence of *Salmonella* or antibiotic-resistant *Staphylococcus* spp. is indicating a limited capacity of the tested mice to act as reservoir host or vector. The inclusion of ecological data as well as other years and regions are the next steps for a thorough view on mice as potential vectors for these bacterial pathogens.



## Lunch &amp; Poster Viewing 2 / 179

## Antimicrobial resistance patterns and characterization of emerging beta-lactamase-producing *Escherichia coli* in camels from Northern Kenya

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**Background:** The misuse and overuse of antimicrobials in clinical and agricultural settings have increased interactions between these agents and microbes in humans, livestock, and the environment. Camel husbandry practices in Kenya's Arid and Semi-Arid Lands (ASALs) may facilitate exposure to antibiotics, the development of antimicrobial resistance (AMR), and the spread of zoonotic microbes. The growing camel population, driven by changes in land use and increased demand for camel products, raises concerns about the spread of Extended Spectrum Beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*).

**Objective:** This study aimed to assess the antimicrobial susceptibility profile and characterize beta-lactamase-producing *E. coli* isolated from camels reared in extensive and intensive production systems in Kenya's ASALs.

**Methods:** Fecal swabs were aseptically collected from 304 camels, including 137 reared by pastoral communities (extensive production system) and 167 from ranches (intensive production system). Samples were processed, and 123 *E. coli* isolates were recovered. Antimicrobial susceptibility was tested, and beta-lactamase genes, including ESBLs, were identified using molecular techniques.

**Results:** Among the 123 *E. coli* isolates, the highest resistance was observed to Cefaclor (28.46%), followed by Cefotaxime (16.26%) and Ampicillin (9.76%). Four ESBL-producing *E. coli* isolates harboring blaCTX-M-15 and blaCTX-M-27 genes were identified, associated with phylogenetic groups B1, B2, and D. Multiple non-ESBL blaTEM gene variants, including blaTEM-1 and blaTEM-116, were also detected. No statistically significant difference in AMR prevalence was found between intensive and extensive production systems ( $p = 0.61$ , 95% CI).

**Conclusion:** This study highlights the presence of diverse ESBL and non-ESBL-producing *E. coli* in camels across different production systems. The findings underscore the importance of a One Health approach to managing AMR in camel production, particularly in ASALs, to better understand transmission dynamics and promote responsible antimicrobial use.

## Lunch &amp; Poster Viewing 2 / 173

**Assessing knowledge, attitudes, and practices related to human-wildlife interactions and zoonotic disease risk using a One Health approach in a Brazilian Atlantic Forest community.**

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Traditional communities in Brazil's Atlantic Rainforest are in close contact with wildlife, increasing their risk of zoonotic disease. Understanding their knowledge, attitudes, and practices (KAP) is vital for developing effective prevention strategies. This study assessed the KAP of Castelhanos, a rural community at Paraná State, southern Brazil, using a semi-structured questionnaire. Conducted between September 2022 and 2023, the survey involved 150 participants aged 18 and older. Data were analyzed using descriptive statistics and Pearson's chi-squared test for associations. About 23.3% of respondents reported wild animals, such as bats and rodents, entering their homes in the past year, with bats specifically noted by 21.3%. Around 32% of respondents had occupations involving animal contact, primarily in banana plantations. In terms of knowledge, 78.7% believed wildlife trade could influence disease transmission, while 64.7% recognized potential transmission through wildlife. 52.6% acknowledged risks in slaughtering animals with open wounds, though they were unsure of specifics. The most cited animals in disease transmission were ticks (97.4%), bats (68%), and rodents (13.3%). Residents living in the community for under 10 years were significantly ( $p < 0.001$ ) more likely to have been bitten by bats. Findings of different levels of knowledge, attitudes and practices related to risk of zoonotic disease provide insights for prevention using the One Health approach.

**Lunch & Poster Viewing 2 / 181****Monitoring of zoonotic diseases in Dzanga Sangha Protected Areas (DSPA) in 2023– Central African Republic (CAR)**

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**Background:** Zoonoses are infectious diseases transmitted naturally between animals and humans. More than 60% of human infectious pathogens are considered zoonotic, and originate from wildlife and domestic animals.

Novel zoonotic diseases, like SARS-CoV2 are of special concern and their emergence is often associated with biodiversity hotspots under anthropogenic influence.

The Dzangha Sangha Protected Areas (DSPA), located in the South-West of Central African Republic (CAR), is considered a biodiversity hotspot, and provides critical resources to the local population who largely rely on hunting and gathering. This creates the possibility of pathogen spillover and highlights the need for a One Health approach to monitoring and addressing zoonotic diseases that threaten both public health and biodiversity. In 2018, WWF-CAR and Robert Koch Institute (RKI) set up a One Health monitoring program of zoonotic diseases in the DSPA. The goal of this initiative was to help gain insight into occurring pathogens, and raise awareness of zoonotic diseases in the local population. The aim of this study was to monitor the health of wildlife for the early detection and prevention of possible zoonotic diseases transmission to the local population living in and around the DSPA.

**Materials and Methods:** Our team consisting of trackers, rangers, hunters, and vets, actively searched the forest for carcasses of all wildlife between January and November 2023. We collected samples of carcasses by necropsy for fresh carcass, and swabbing in cases of old carcasses in RNAlater and stored -20 °C. Then we carried out DNA extraction on these carcasses using the Qiagen Viral RNA mini kit 250 following the manufacture's protocol. We further performed PCRs on aliquots using published protocols for the filovirus (ebola, marburg), orthopoxvirus (Mpox), and Bacillus anthracis (Anthrax) and observed our results in 1,5% agarose gel.

**Results:** A total of 10 wild animals belonging to 5 different species were identified; Gorilla gorilla gorilla (n = 3), Loxodonta Cyclotis (n = 4), Manis Gigantea (n = 1), Syncerus caffer nanus (n = 1), Tragelaphus Eurycerus -(1). All the samples tested negative for filovirus, orthopoxvirus, and Bacillus anthracis.

**Conclusion:** The continuous monitoring of zoonotic diseases in the DSPA is crucial for providing data that supports local One Health initiatives.

**Lunch & Poster Viewing 2 / 182****Characterization of the Staphylococcus aureus complex (SAC) from bat species in Nigeria**

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**Introduction**

The Staphylococcus aureus complex (SAC), comprising *S. argenteus*, *S. schweitzeri*, *S. singaporensis*, *S. roterodami*, and *S. aureus*, have been implicated in human infections, except *S. schweitzeri*, a commensal in non-human primates and bats from sub-Saharan Africa. Some members of the SAC (*S. aureus*, *S. argenteus*, *S. schweitzeri*, and *S. roterodami*) have been recovered from faecal samples of the straw-coloured fruit bat in Obafemi Awolowo University, Nigeria. However, faecal samples from roosting sites and not individual bats were analyzed. This study investigated the distribution, antibiotic susceptibility and genetic diversity of the SAC from bat species in Osun State.

**Materials and Methods**

Pharyngeal samples were obtained from insectivorous bats (*Hipposiderous* sp and *Nycteris* sp) and pharyngeal and anal samples from fructivorous bats (*Epomorphorus* sp and *Eidolon helvum*). Identification of SAC was determined by MALDI-TOF MS, PCR detection of the *S. aureus*-specific thermonuclease (*nuc*) and the non-ribosomal peptide synthase (NRPS) genes. Antibiotic susceptibility testing was conducted by VITEK 2 using EUCAST clinical breakpoints. Molecular characterization included PCR detection of PVL, human IEC genes and the staphylococcal protein A (*spa*) typing.

**Results and Discussion**

Anal (n=212) and pharyngeal (n=278) samples of bats in Osun State, Nigeria, were analyzed. The SAC (n=236) comprised of *S. aureus* (n=171; 72.5%), *S. argenteus* (n=33; 13.9%), and *S. schweitzeri* (n=32; 13.6%). Over 95% of the SAC were susceptible to tetracycline, cotrimoxazole, and 86% to penicillin. Sixty-six *spa* types, including 41 new types, were identified. The predominant *S. aureus* *spa* type was t15966 (45/171; 26.3%). One human-related *S. aureus* genotype (t4690-ST152), confirmed as MRSA was detected from a pharyngeal sample of *E. helvum*. All the isolates (except the MRSA isolate) were negative for the PVL and IEC genes. These findings highlight the genetic diversity of the SAC in bats, and the detection of a human-associated MRSA lineage suggests the potential for reverse zoonosis.

**Lunch & Poster Viewing 2 / 38****Capacity of environmental and intestinal bacteria in phytate dephosphorylation and inositol catabolism**

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Phytate is the primary storage form of phosphorus in plants and abundant in seeds. Phytases produced by soil or intestinal bacteria are assumed to continuously release *myo*-inositol (MI), which is a readily available growth source for many bacterial species in the environment and in the gut. Animal food is often supplemented with microbial phytases to foster the dephosphorylation of phytate and thus to increase the availability of phosphate. We here address the prevalence, the interdependencies, and the metabolic activities of commensal bacteria involved in phytate dephosphorylation and inositol degradation.

To this end, we combined a systematic genomic survey of all bacterial species producing phytases with an experimental culturomics approach of the intestinal microbiome from piglet. Analyses on all taxonomic levels revealed that homologs of the three classes of phytases are not ubiquitously present in bacteria, but are mainly found in Pseudomonadota, Synergistota, and Bacteroidota. Nearly 25% of 25,105 species with validated genome sequence harbor at least one gene that encodes this enzyme, and 9% carry both a phytase and the enzymes to degrade MI. Sequencing of ~300 MI-positive commensals taken from piglet revealed a predominance of the genera *Aneurinibacillus*, *Bacillus*, and *Paenibacillus*. Growth assay of synthetic microbiota with phytase producing bacteria and MI utilizing commensals are currently performed to reveal the interdependencies within the swine gut.

## Lunch &amp; Poster Viewing 2 / 63

**Rat hepatitis E virus natural infection in pigs in Spain: a large-scale cross-sectional study**

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Rat hepatitis E virus (ratHEV) is an emerging zoonotic cause of acute and chronic hepatitis. While contact with rats, the primary reservoir of the virus, has been suggested as a potential transmission route, there is only one documented case who reported it, making the transmission pathways of this virus unclear. Recently, ratHEV was detected in faeces from pigs, raising concern about the potential susceptibility of this species to the virus. In this context, we aimed to determine whether pigs are naturally infected by ratHEV. A cross-sectional study was conducted on extensively raised pig populations in southern Spain. Serum samples from 1861 animals belonging to 64 farms, collected between 2015 and 2017, were retrospectively analysed. The presence of ratHEV RNA was assessed in pooled serum samples using a multiplex qPCR targeting two specific genomic regions. Viral RNA was detected in two (0.4%) out of the 466 pools analysed. One pool tested positive for both targets, while the other showed a signal for only one target. Our results confirm that pigs are susceptible to ratHEV, thereby expanding the known host range of the virus and raising concern about the possibility that pigs intended for human consumption could contribute to ratHEV transmission. Although the low prevalence suggest that this species do not play a relevant role in the maintenance of ratHEV, a preliminary evaluation of ratHEV in pig products should be conducted in Europe to better understand virus transmission dynamics.

**Lunch & Poster Viewing 2 / 65****A participatory approach for the establishment of a One Health cohort study in Côte d'Ivoire****Authors:** Filipe Dias<sup>1</sup>; Oby Wayoro<sup>2</sup>; Léonce Kouadio<sup>3</sup>; Fabian Leendertz<sup>1</sup>; Fee Zimmermann<sup>1</sup><sup>1</sup> *Helmholtz Institute for One Health*<sup>2</sup> *Centre Hospitalier Universitaire de Bouaké; Helmholtz Institute for One Health*<sup>3</sup> *Centre Suisse de Recherche Scientifique; Université Peleforo Gon Coulibaly; Helmholtz Institute for One Health***Corresponding Author:** filipe.dias@helmholtz-hioh.de

Sub-Saharan Africa continues to experience emerging infectious disease threats. We intend to establish a One Health cohort study placed around the Tai National Park in Côte d'Ivoire, a hotspot for disease emergence. An initial stage in developing One Health interventions with communities involves the engagement of different stakeholders. Participatory study design leads to greater acceptability, trust and engagement in the local population due to more collaboration and communication, which builds a sense of ownership. Local communities are often undervalued stakeholders, even if they have local knowledge and can help design appropriate approaches. We show the importance of involving local communities and authorities from the very beginning, resulting in a truly participatory study design. We visited nine communities where we organized meetings with traditional leadership structures and local authorities. As a result, we collected information, opinions, concerns and suggestions. We identified social and cultural norms and beliefs, gaps in knowledge, rumors and misinformation. This was the first step, which will be followed by participatory workshops focused on the details of the study design. Ultimately, the cohort will be part of a sustainable long-term One Health surveillance system, which will allow for the early detection of infectious diseases, support epidemic management and give broader insights into determinants of infectious diseases.

**Lunch & Poster Viewing 2 / 73****Evaluation of methods to reliably detect influenza virus in wastewater**

**Authors:** Till Fretschner<sup>None</sup>; René Kallies<sup>None</sup>; Birgit Walther<sup>None</sup>; Edgar Elon Zeisler; Steffi Scheller; Andreas Aurenz

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Monitoring influenza viruses in wastewater samples provides a valuable tool for public health assessment. In this study, we investigate the presence of influenza virus A and B gene fragments in wastewater systems using reverse transcription-polymerase chain reaction (RT-PCR) methods. To effectively detect gene fragments, we tested different concentration methods and compared digital droplet PCR (ddPCR) with quantitative RT-PCR (RT-qPCR) for sensitivity and accuracy. A critical component of our methodology was a spike experiment, which allowed us to evaluate the methods in a controlled environment. Various concentration methods showed different levels of efficiency in recovery of influenza A and B. Furthermore, the limit of detection for the ddPCR was significantly lower than for RT-qPCR. However, both PCR Systems are suitable for detecting Influenza A and B in wastewater.

These methods were applied to wastewater collected from four wastewater treatment plants in Germany (November 2023 to October 2024). Gene fragments were detected for both influenza A and B during the entire flu season.

In parallel, we are developing RT-PCR assays for the detection of influenza C gene fragments and the subtyping of influenza A subtypes H1, H3, H5, H7 and H9, as well as the B/Yamagata and B/Victoria lineages of influenza B. This advanced molecular characterization will help to improve our understanding of circulating strains and their changes over time.



**Lunch & Poster Viewing 2 / 80****Establishing an Epidemic Panel integrating the Human-Animal-Environment Interface for assessing infectious pathogens in Côte d'Ivoire**

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Epidemic panels are population-based longitudinal cohort studies able to rapidly and adaptively survey a relevant population group on diverse pathogens for infection frequency and burden to provide estimates for projections. Worldwide, there is a lack of epidemic panels and in particular integration with One Health surveillance efforts is rarely included. Here, we are developing the study design for a planned epidemic panel with an integrated One Health approach in Côte d'Ivoire. In the first instance we will examine the feasibility of assessing burden of infectious diseases with this epidemic panel and link to relevant environmental and animal data. We intend to design a longitudinal cohort recruiting 2000 individuals in the pilot region with a participatory approach through consultations with multiple partners in the region. We will employ a multi-stage sampling process targeting individuals, their animals in households as well as wild animals and their environment. We will conduct on-site health screening, standardized questionnaires including anthropological determinants, and laboratory analyses to detect and characterize a wide range of pathogens. In putting our study design forward we hope to generate relevant input from the community with the aim of developing a participatory study protocol as well as identifying facilitators and obstacles for implementing this one-health cohort study at the community level.

**Lunch & Poster Viewing 2 / 88****Method development for the surveillance of antimicrobial resistance (AMR) in wastewater**

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Infectious diseases caused by multidrug resistant pathogens are a serious threat to Public Health causing millions of deaths globally. Therefore, the WHO highlights the need for antimicrobial resistance (AMR) surveillance. In 2024, Council and European Parliament reached an agreement to implement AMR wastewater surveillance in the urban wastewater treatment directive. The AMELAG (wastewater monitoring for epidemiological situation assessment) project aims at establishing wastewater surveillance in Germany by developing reliable detection methods using influent wastewater. For AMR-pathogen combinations relevant to the Public Health sector, especially carbapenemase-producing Enterobacterales (CPE), a screening procedure is set-up complemented by biochemical and molecular methods for confirmation. Whole genome sequencing is used to investigate AMR genes and to investigate phylogenetic relationships with clinical samples. Both spread plating and filtration-based analyses revealed bacterial counts between 104-105 colony forming units (CFU)/mL for E. coli, which is in line with the current literature regarding orders of magnitude. Thus, overall E. coli counts might serve as a baseline parameter to classify less abundant AMR-pathogen combinations such as CPE. The currently used methods are promising regarding future implementation of a One Health-inspired wastewater-based AMR surveillance, which is still in its infancy.

**Lunch & Poster Viewing 2 / 90****Investigation of a piroplasm outbreak in a herd of horses in Southern Germany**

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Equine piroplasmosis is a tick-borne disease caused by the protozoan parasites *Babesia caballi* and *Theileria equi*, transmitted by hard ticks (Ixodidae). It causes symptoms such as fever, anaemia, jaundice, and, in severe cases, can be fatal.

In October 2022, 18 out of 34 horses in a herd in the district of Rastatt were infected with at least one of the two pathogens and three of them had to be euthanized due to severe symptoms.

The aim of this case study was to determine the prevalence of the two pathogens in this horse herd and in the local tick population. Therefore, blood samples were collected in September 2023 and again in March 2024, and molecularly and serologically tested for both pathogens. Additionally, ticks were collected from the horses' pastures from November 2022 until May 2023 and examined for Piroplasms via PCR.

Molecular testing of the blood samples showed infection rates for *B. caballi* of 53%/26% (2023/2024) and of 71%/65% (2023/2024) for *T. equi*. Serological testing revealed infection rates of 74%/88% for *B. caballi* and 62%/65% for *T. equi*. In total over 1000 ticks were collected and 965 were identified as adult *Dermacentor reticulatus*. *B. caballi* was detected in 7 ticks from a pasture which had been used prior to the onset of symptoms of the horses in 2022.

These findings highlight the rising importance of these two parasites, which are not yet endemic in Germany but could become so due to the increasing distribution of their vector *D. reticulatus*.

**Lunch & Poster Viewing 2 / 91****Investigations of the interactions of zoonotic pathogens in humans and animals (SHIP-NEXT Module One Health): Data collection and experiences from 3 years of animal examinations in private households**

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Zoonoses have not been sufficiently studied on population level due to the complex interactions between pathogens, humans, animals and the environment. It is important to understand the dynamics between the contacts of humans and animals contributing to disease transmission, which occurs most frequently in private households. We offer participants of the population-based cohort Study of Health in Pomerania (SHIP-NEXT) living in households with dogs, cats, poultry or pigeons the opportunity to take part in a One Health module. Data collection (2021-2025) includes veterinary examinations, biosamples, interviews, and self-administered questionnaires (SAQ). Of 2.810 (16.06.2024) SHIP-NEXT participants, 986 (35.1%) pet owners reported to have contact with animals such as dogs, cats, poultry and pigeons in their private household. So far, the veterinary team examining 732 animals by visiting 401 pet owners at home; 93 pet owners conducted only an interview, 36 a SAQ, and 52 both interview and SAQ. Housing situation and animal husbandry on site pose a unique challenge carrying out the study in a study-compliant setting. The range of veterinary examinations and the equipment required for them also have a major influence on the procedure on site. The experiences could be used to optimize the planning of future One Health studies in private households. In this way, a profound understanding of the interaction between humans, pets, livestock and the environment could be achieved.

## Lunch &amp; Poster Viewing 2 / 98

**Land-use as an influence factor on *Borrelia burgdorferi* s.l. prevalence and genodiversity in ticks from Central Germany**

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Ticks are significant vectors for zoonotic pathogens. In Europe, *Ixodes ricinus* plays a major role due to its prevalence and versatile host preference. Among these pathogens are bacteria from the *Borrelia burgdorferi* s.l. group, comprising over twenty genospecies. Both the vector and the reservoir host of *B. burgdorferi* depend on habitat structure, influenced by land use. This study analyzed *B. burgdorferi* s.l. in ticks along a land use gradient in Central Germany. In total, 1,896 ticks were collected from 25 grassland and 25 forest sites from Biodiversity Exploratories in Hainich-Dün region. Ticks were tested through qPCR for *Borrelia* spp. Multi-locus sequence typing was performed on *Borrelia*-positive ticks to identify sequence types (ST). Three tick species were identified: *I. ricinus*, *I. frontalis*, and *Dermacentor reticulatus*. *Borrelia* DNA was detected in 210 of ticks (11.1%), all from *I. ricinus*. The prevalence was identical for female (30.8%) and male adults, and lowest in nymphs (10.2%). Ticks from grasslands were significantly more infected (29.4%) than those from forests (6.4%). Genotyping of 184 samples revealed 4 genospecies (*B. afzelii*, *B. garinii*, *B. valaisiana*, and *B. burgdorferi* s.s.) with 59 known and 41 previously unknown ST. *Borrelia* genotypes demonstrate high genetic variability, promoting high intraspecific diversity. Preliminary modelling revealed that increased land and forest use may reduce biodiversity but increase *Borrelia* prevalence and diversity.

**Lunch & Poster Viewing 2 / 99****Utilizing high-throughput bioinformatics for the integrated genomic surveillance of bacterial pathogens**

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Integrating genomic analyses with epidemiological data remains a key factor in providing comprehensive surveillance on the emergence and evolution of microbial pathogens. In order to support our federal health authorities, we are developing a set of modular computational workflows for the surveillance of bacterial pathogens with high relevancy to public-health.

We hereby utilize open-source state-of-the-art bioinformatics software to provide in-depth analytical workflows for selected microbial pathogens. With the requirements for genomic surveillance continuously evolving, our modular pipelines can be easily extended or updated to reflect newest developments. Following the guidelines for sustainable software development, subworkflows may further be exchanged or adapted according to latest computational and microbiological challenges.

Our automatized workflows range from genomic reconstruction and characterization, to the evaluation of pathogenicity based on resistance and virulence genes, including the assessment of relatedness, which in turn allows valuable insights into complex clusters and evolutionary dynamics.

Combined with a centralized infrastructure, these open-source tools will support the development of a continuous integrated genomic surveillance on a national level. Our pipelines will hereby help to streamline the processes of data handling and computational analyses, as well as enable reporting on national and international levels for public-health authorities.

**Lunch & Poster Viewing 2 / 102****Behaviours towards antibiotics among farmers and households owning livestock in Ghana: a mixed-methods approach**

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Antimicrobial resistance (AMR) is a serious concern, driven by extensive antibiotic use in livestock husbandry. Globally, livestock antibiotic consumption is expected to rise by 67% by 2030. We aimed to analyse behaviours towards antibiotics among farmers and households with animals in Ghana using a mixed-methods approach.

An observational study with a triangulation design was conducted in Asante Akim North District. Quantitatively, 415 of 1,114 surveyed households owned livestock, and data were analysed using STATA 17. Qualitatively, semi-structured interviews with eleven farmers were conducted at veterinary shops and with an informal veterinarian using a customer exit strategy, and data thematically analysed using NVivo 14.

Quantitative results showed that 43.3% of participants used traditional herbal medicines, and 65% gave antibiotics. High socio-economic groups were more likely to access veterinary services, and semi-urban residents were more likely to purchase antibiotics from drugstores. Qualitative results found that farmers could describe symptoms they observe when administering antibiotics. Many use traditional herbal mixtures before or instead of antibiotics. During the rainy season, antibiotics are used more frequently for prevention.

In conclusion, triangulating results provides a comprehensive understanding of livestock owners' behaviours towards antibiotics. These findings can inform the development of targeted interventions to promote responsible antibiotic use.

**Lunch & Poster Viewing 2 / 113****Multidrug resistance and reduced susceptibility to biocides in the pandemic ST398 methicillin-susceptible *Staphylococcus aureus* (MSSA) from Austria****Authors:** Adrienn Gréta Tóth<sup>1</sup>; Olga Makarova<sup>2</sup><sup>1</sup> *Centre for Bioinformatics, University of Veterinary Medicine, Budapest, Hungary*<sup>2</sup> *Centre for Food Science and Veterinary Public Health, VetMedUni Vienna***Corresponding Author:** [olga.makarova@vetmeduni.ac.at](mailto:olga.makarova@vetmeduni.ac.at)

Methicillin-susceptible *Staphylococcus aureus* (MSSA) of the sequence type (ST) 398 is a pandemic lineage that is associated with invasive infections, and its incidence has been increasing globally. Here, we report the isolation and characterisation of MSSA ST398 of an unknown spa type from a household in Austria for the first time. The isolate was phenotypically resistant to clindamycin and erythromycin, and intermediately resistant to ciprofloxacin. Additionally, it showed reduced susceptibility to biocides benzalkonium chloride and chlorhexidine. Whole genome sequencing (WGS) revealed the presence of multiple antimicrobial resistance genes (ARGs), including those mediating resistance to biocides. The clindamycin/erythromycin resistance gene *ermT* was located on a plasmid. The StauST398 prophage contained *scn*, a marker of human adaptation, and genes encoding hemolysins. The phylogenetic analysis of 274 WGS of MSSA ST398 from four continents revealed that the isolate clustered most closely with French strains of both human and animal origin, almost all of which contained the phage-associated *scn* gene. Our findings point at the possible circulation of a pandemic multidrug-resistant *S. aureus* with potentially mobile ARGs and reduced susceptibility to biocides in the community and a spillover into animals, calling for an improved One Health surveillance.



**Lunch & Poster Viewing 2 / 120****Perceptions And Understanding Of Antibiotics And Antimicrobial Resistance Among Community Members In Ghana: A Mixed-Methods Approach**

**Authors:** Elisenda Cama i Gibernau<sup>None</sup>; Maresa Neuerer<sup>None</sup> ; Mawuli Leslie Aglanu; Alexander Amponsah; Albrecht Jahn; Valérie R Louis; John Humphrey Amuasi; Aurélia Souares

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Antimicrobial resistance (AMR) is a significant global health threat. It is predicted that Western sub-Saharan Africa would be most affected due to lack of medicine access, limited awareness, and poor policy enforcement. Using a mixed-methods approach, we aimed to analyse perception and understanding of antibiotics and AMR among community members in Ghana.

An observational study with a triangulation design was conducted in Asante Akim North District. Quantitatively, 1,114 households were surveyed using double-stage random sampling, and data were analysed with STATA 17. Qualitatively, semi-structured interviews with nine community members were conducted at chemical shops and pharmacies and analysed thematically using NVivo 14.

Quantitative analysis found that 61.7% of the sample knew the term “antibiotic,” while only 34.2% were aware of AMR, from which 73.4% did not understand its transmission and consequences. Qualitative results showed that participants distinguish different antibiotics through specific words in the local language and according to symptoms. However, except for one interview partner, they do not have experience with AMR and are not aware of the concept.

To conclude, triangulating all results provide valuable insights into the perception of antibiotics and AMR. These findings can serve for developing a targeted intervention to address AMR and promote the responsible use of antibiotics.

**Lunch & Poster Viewing 2 / 121****Composition and dynamics of mosquito vector communities in a German peatland****Author:** Patrick Gutjahr<sup>1</sup>; Franziska Tanneberger<sup>2</sup>; Mandy Schäfer<sup>1</sup><sup>1</sup> *Friedrich-Loeffler-Institut – Federal Research Institute for Animal Health (FLI), Institute of Infectology*<sup>2</sup> *Greifswald University, partner in the Greifswald Mire Centre***Corresponding Author:** patrick.gutjahr@fli.de

Due to drainage and soil degradation, drained peatlands contribute about 7% to Germany's annual GHG emissions. To curb emissions, rewetting these landscapes is urgent. However, wet peatlands provide a wide array of mosquito breeding sites yet little is known about these mosquitoes, their ecology and vector potential. Rewetting practices combined with the influence of climate change on the spatial and temporal distribution of mosquitoes and mosquito-borne pathogens may entail unknown health risks. To elucidate these risks, we monitor mosquito communities via adult and larval sampling in combination with climate and water data collection in a fen complex in north-eastern Germany since 2023. Our monitoring sites include both wet and drained peatlands as well as human settlements. Our data shows a more pronounced seasonality of vector abundance in wet settings due to a wide availability of temporal water bodies leading to a dominance of flood water mosquitoes. We did not find Culicidae to form more diverse communities in natural or near natural conditions but rather that dominant species differ. However, which species dominates a wet site can vary even on a small spatial scale while drained sites are more uniform. The dominance variation is associated with variations in breeding water conditions and may be influenced through land use. Thus, our findings may help to manage mosquito vectors in peatlands through targeted land use while restoring ecosystem services and biodiversity.

**Lunch & Poster Viewing 2 / 127****Legionella genotype map**

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Despite their significant public health impact, the relationships between environmental sources and human Legionella infections are poorly understood due to the lack of validated detection methods for complex water matrices and insufficient comparative data across water types. Climate change is anticipated to exacerbate Legionella proliferation due to increased and sustained high temperatures and expanded water reuse. Traditional laboratory methods provide delayed responses, necessitating the exploration of next-generation sequencing (NGS) combined with PCR amplification directly from environmental samples. This pilot project aims to enable rapid, accurate characterization of Legionella within 1-3 days using prototype genomic tools and software. An NGS-based method, similar to amplicon-based sequencing of SARS-CoV-2 genomes, will be developed to target specific Legionella genomic regions for genotyping, as 16S rDNA sequencing alone is inadequate for sub-species classification. The methodology will be detailed in a technical report, along with necessary PCR primers and software. Following its establishment, the method will be applied to routine samples from the Lower Saxony State Health Office (NLGA) to create a Legionella genotype map, with potential extension to other federal states. Regular and project-specific samples from surface and freshwater sources will form the basis of this map.

**Lunch & Poster Viewing 2 / 133****Investigations of the interactions of zoonotic pathogens in humans and animals (SHIP-NEXT Module One Health): Toxocara species infections in pet dogs and cats, Pomerania, Germany.**

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*Toxocara canis* and *Toxocara cati* are nematode infections of dogs and cats that are transmissible to humans. *Toxocara* spp. are important zoonoses with around 1/5 of the human population estimated to be seropositive. Up-to-date knowledge of *Toxocara* spp. prevalence in pet dogs and cats, ownership practises that promote pet infection, and pet-owner interactions that facilitate transmission to humans is critical for disease management. Here, we sought to determine estimates for these parameters in Pomerania, Germany.

We analysed pet fecal samples collected as part of the population based cohort study, SHIP-Next OH (Study of Health in Pomerania, One Health), for the presence of *Toxocara* spp. eggs using both classical parasitological and molecular methods. Accompanying interview and questionnaire data were analysed to characterise husbandry and human-pet interaction behaviour.

We found *Toxocara* spp. eggs in the feces of 24 dogs from 246 sampled (9.8%; 95%CI: 6.6%-14.1%) and 23 of 206 cats (11.2%; 95%CI: 7.6%-16.2%). Pet owners reported husbandry behaviours that may increase the risk of infection in their pets, such as free access to outdoor areas, kennelling, and not de-worming their pets. They also reported close contact with their pets (a known risk factor for human infection) such as kissing their pet's fur and/or nose and allowing the pet to sleep in the bed.

Taken together, these results provide valuable up-to-date estimated prevalence, husbandry and animal-interaction information.

**Lunch & Poster Viewing 2 / 148****The Political and Institutional Changes surrounding One Health implementation in Ghana****Author:** Ana Maria Perez Arredondo<sup>1</sup><sup>1</sup> *Center for Development Research (ZEF) Bonn University***Corresponding Author:** aper@uni-bonn.de

In Ghana, fundamental changes in public health management, brought attention to the One Health (OH) Approach, leading to the formation of a Technical Working Group (OHTWG) to develop a OH policy and strategy for Ghana.

This work is intended to offer helpful insights into the implications of the OH policy for different health actors and their contributions.

The framework for analysis combined two methodologies for Political Economy Analysis, which were applied to the case of Ghana. The first step was to identify the relevant legislation that enabled multi-sectoral collaborations, followed by a systematic analysis of the collective action capacities, the strategic base, the strategic development, and the expected outcomes of the OH policy.

The information reviewed was obtained from interviews and questionnaires applied to stakeholders which were directly or indirectly involved in the policy development. Other sources of information were published documents, grey literature, and reports from news and government agencies.

Results show that the legislation in Ghana endorses institutional silos at the levels of policy implementation, planning, and evaluation. Nevertheless, a set of policy action plans are bridging between sectors to promote disease control and surveillance. The incentives for adopting the OH approach were related to achieving institutional goals in terms of multi-sectoral collaboration and meeting the commitments made to the International Health Regulations 2005 and the Health Security Agenda. A broad number of stakeholders were identified and categorized into (1) policy development actors, (2) technical, financial or operational support actors, and (3) non-mobilized actors.

The OH policy has the general goal of advancing Health Security in Ghana and has specific programs of action in terms of reducing the burden of infectious and zoonotic diseases, promoting healthy environments, and controlling for disease emergence. The strategies for policy change are led by the OHTWG and involve continuous advocacy of stakeholders and promotion of the OH approach.

**Lunch & Poster Viewing 2 / 150****The role of the One Health UBA's in-house research project "Advancing the New European Bauhaus" (AdNEB)**

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In its in-house research project "Advancing the New European Bauhaus" (AdNEB), the German Environment Agency (UBA) aims to develop recommendations for climate resilient, healthy, climate and environmentally friendly urban spaces.

Conceptionally, AdNEB offers a new framework, the model of threefold inner city development, which advocates integrated planning of building/housing, mobility and green/blue infrastructures in cities. This model can be a key building block in unlocking the potential of One Health for improving cities, as it focusses on interactions between the state of the built and natural urban environment and human health.

From an empirical point of view, AdNEB is augmenting Urban One Health research by a socio-economic context, e.g. by investigating the extent to which environment-related health resources and risks vary according to social groups. For example, data from the German Environmental Survey (GerES V) revealed that in urban areas youths with lower socio-economic status live more often near roads with heavy traffic, compared to those with higher socio-economic status. We aim to further quantify how residential pollutants vary by degree of equalized income or migration history among children and adolescents in Germany.

With these conceptional and empirical insights, AdNEB provides a reliable knowledge base for policy-makers, administrators and urban planners so that they can design cities in Germany in a way that benefits from the One Health idea.

**Lunch & Poster Viewing 2 / 156****Molecular characterisation of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* among children and chicken in rural Korogwe, Tanzania**

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Extended-spectrum beta-lactamase (ESBL) producing *Klebsiella* spp. are a global concern due to the overuse of antibiotics in humans and animals. Although studies have focused on ESBL prevalence in Tanzanian children, few have assessed *Klebsiella* in chickens as a potential transmission source. This study employs a one-health approach to assess ESBL producing *Klebsiella pneumoniae* (KP) and antibiotic resistance in humans and chickens in rural Tanzania.

This cross-sectional study, conducted from February 2019 to July 2020, collected stool samples from children under five with and without diarrhea at Magunga Hospital. Chicken feces were sampled from farms within Korogwe District. Strains were confirmed using VITEK2 System. Antimicrobial susceptibility was tested with disc diffusion, and WGS was performed using the NextSeq500 Illumina.

Among 258 asymptomatic children and 259 children with diarrhea, 16 (6%) and 32 (12%) tested positive for ESBL-KP, respectively. Additionally, 54 (7%) chicken samples were positive. All isolates were resistant to beta-lactams and cephalosporins but sensitive to carbapenems. WGS identified ST17 as the most common sequence type and 12 closely related human and chicken isolate clusters.

The study demonstrates ESBL-KP presence in both human and chicken populations, with closely related strains. Chickens may act as a reservoir for ESBL-KP transmission to humans. Implementing measures using a one-health approach is crucial to control this pathogen's spread.

**Lunch & Poster Viewing 2 / 169****The Impact of Donkey Bush Slaughter on Animal Welfare, Environmental Health, and Public Health in Kenya****Author:** James Kithuka<sup>1</sup><sup>1</sup> *Brooke East Africa; University of Nairobi***Corresponding Author:** james.kithuka@thebrookeeastfrica.org

The unauthorized slaughter of donkeys in Kenya for meat and skins has devastated donkey populations and harmed communities that depend on them for livelihoods. Donkeys play a vital role in transportation, agriculture, and resilience, especially in arid and semi-arid regions. The surge in demand for donkey skins, driven by Traditional Chinese medicine, has led to unsustainable slaughter. Between 2009 and 2019, Kenya's donkey population dropped from 1.8 million to 1.1 million. With a reproduction rate of just 1.4% compared to a slaughter rate of 5.1%, donkeys were on the brink of extinction by 2024, prompting the closure of four legal slaughterhouses in 2022.

Despite these closures, illegal bush slaughter persists, with severe consequences for animal welfare, the environment, and public health. This illegal activity causes immense cruelty, environmental contamination from carcasses, and the spread of zoonotic diseases like anthrax and brucellosis. The sale of uninspected donkey meat in markets poses a significant health risk to consumers.

Efforts by Brooke East Africa and others have resulted in a resolution from African Union Member States to protect donkeys and enforce a moratorium on the donkey skin trade. A coordinated ecological, epidemiological, human well-being, and animal welfare approach involving stronger law enforcement, public awareness, and global cooperation is essential to combat this crisis and protect both donkeys and public health.



## Lunch &amp; Poster Viewing 2 / 171

## Anthropogenic Impact on Macaque Gut Microbiomes: A One Health Perspective on Human-Primate Interactions

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The COVID-19 pandemic underscored the importance of monitoring zoonotic spillovers at human-wildlife interfaces. In response, epidemiologists and ecologists have increasingly recognised that a comprehensive understanding of disease ecology, especially within the context of natural ecosystems and biodiversity, is essential for effective infectious disease management. Macaques are susceptible to natural and anthropogenic risk factors, which can profoundly disrupt gut microbiome-pathogen interactions in disturbed environments. This study investigates how anthropogenic exposure affects gut microbiomes and zoonotic disease risks in southern pig-tailed macaques (*Macaca nemestrina*) from two habitats in Perak, Malaysia: an urban mangrove park (high human interaction) and an oil palm plantation-rainforest area (low human exposure). Using metagenomic analysis, the research entailed a baseline gut microbiota profile, revealing dysbiosis in both populations. Urban macaques exhibited reduced microbial diversity and the presence of *Helicobacter macacae*, an enterohepatic bacterium related to *Helicobacter pylori*. *Giardia* antigen was also detected in the urban population. In contrast, macaques in the natural agricultural-forest habitat showed higher microbial diversity, indicating healthier gut ecosystems. These findings substantiate previous research linking urbanization to diminished microbiome diversity and higher pathogen prevalence. This study highlights the significance of the One Health approach in monitoring zoonotic risks and promoting collaborative efforts to safeguard both wildlife and public health in shared environments.

**Lunch & Poster Viewing 2 / 178****Molecular characterization of Extended Spectrum  $\beta$ -Lactamase (ESBL)-producing *Escherichia coli* among children and farm animals in Agogo, Ghana**

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Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is a growing global healthcare threat due to its resistance to multiple antibiotics. A One Health approach, examining various reservoirs, is crucial in addressing this challenge, yet limited data exists on ESBL-producing *E. coli* among children and farm animals in Ghana. This study aimed to characterize the genetic diversity of ESBL-producing *E. coli* and other antimicrobial-resistant (AMR) genes among children and farm animals in Agogo, Ghana.

A total of 435 fecal samples from children and 357 from farm animals were collected. ESBL-producing *E. coli* was screened using the Vitek 2 compact system and confirmed via double-disk synergy testing. PCR was used to identify ESBL genes, and 117 isolates underwent whole-genome sequencing.

ESBL carriage rates were 40.9% in children and 36.0% in farm animals. The CTX-M 15 gene was prevalent in both children (92.4%) and farm animals (99.1%).

Whole-genome sequencing identified over 70 antibiotic-resistance genes and 40 sequence types. Besides the CTX-M 15 gene, other beta-lactam genes (*bla*<sub>EC</sub>, *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub>) were found. Additional AMR genes included resistance to tetracyclines, aminoglycosides, and quinolones. The most common sequence type was ST617.

The study emphasizes the genetic diversity of ESBL *E. coli*, highlighting their potential as reservoirs for spreading AMR genes, crucial for healthcare and veterinary practices in Ghana.

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## Computer vision and artificial intelligence as emerging diagnostic tools for highly accurate classification of clinical fungi

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### Background

Artificial intelligence (AI) based on deep-learning (DL) approaches enables computers to learn from large datasets to replicate human intelligence. DL circumscribes a diverse set of algorithms, which have demonstrated state-of-the-art performance in biomedical applications for accurate classification of image data. In the advent of a perpetual AI revolution taking place at an intangible pace, microbial diagnostics utilizing DNA and proteins as primary data sources to characterize fungal pathogens, would greatly benefit from computer vision to increase precision, reduce costs, and to further democratize accurate diagnostics in low-income countries. The methodology offers a supplementary (developmental) diagnostic opportunity also universally expandable to prokaryotes, to contribute to a successful long-term implementation of the One Health approach.

### Methods

We developed a supervised learning classifier based on convolutional neural networks, with pre-sets of transfer learning, to identify fungal pathogens directly from high-resolution petri-dish image data. Core of the dataset is the yet largest and most precisely photographed set of medical fungi, derived from the Atlas of Clinical Fungi encyclopedia. The tested image subset comprised >100 species as a proof of principle.

### Results

Utilizing different transfer learning approaches, and building on neural network architectures, such as VGG16 or Efficientnet trained on millions of random images, we successfully classify all target pathogens with a varying degree of accuracy, and depending on experimental settings, of up to >95%. Our results successfully benchmarks a simple, powerful method, and is the yet most comprehensive study in microbial diagnostics utilizing computer vision to date.

### Conclusion

The AI revolution will enhance and open possibilities for clinical diagnostics at a yet unseen pace. We intend to further train and fine tune our classifier on all known fungal pathogens and opportunists to create a functional tool for identification of clinical fungi.

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## Progress towards predicting significant changes in wastewater microbial communities: A machine learning approach using time series data

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Changes in the human microbiome can be an indicator of the onset of an infectious disease such as sepsis whereas fluctuations of the microbes observed e.g. in wastewater might indicate a disease outbreak in the respective catchment area. The capacity to distinguish these significant changes from naturally occurring fluctuations in the microbiome could facilitate the early detection of potentially harmful diseases. We aim to take a step toward this prediction of microbial abundance trends based on analysis of 16S rRNA data. To this end, we apply Long Short-Term Memory (LSTM) models to publicly available long-term microbial time series data from two healthy subjects. To increase the explanatory power of the model, Shapley Additive Explanations (SHAP) is used for feature significance analysis.

Thus far, the model has demonstrated superior performance compared to other tested model architectures, including VARMA and Random Forest. It has also exhibited promising results in predicting the normal abundances in a sewer in the Ruhr area. Further testing of additional architectures is planned. Overall, the aim is to provide a treatment advantage to physicians and patients by providing a system for the early detection of potential health risks. In an environmental context, a machine learning-based early warning and monitoring system could assist with decision-making in the event of public health concerns.

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## Exploring the human gut microbiome from over 500 thousand genomes: Insights into functional potentials and biodiversity via Dense Deep Clustering

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We compiled 509,610 metagenome-assembled genomes (MAGs) and their metadata to create human gut microbiome fingerprints, distinguishing functional potential by country of origin and disease condition. We selected 14,082 metagenomes from the HumanMetagenomeDB, recovering over 300,000 MAGs. Our database included 154,000 MAGs from Pasolli et al. (Cell, 2019) and 60,000 from Nayfach et al. (Nature, 2019). Metadata was unified into a standardized dataset, and MAGs were dereplicated into 6,794 species. Gene annotation was performed using MuDoGeR and Prokka. After one-hot encoding of MAGs from adult gut samples, we had 426,648 MAGs and 40,424 non-redundant genes. An autoencoder neural network with dense layers and ReLU activation was applied, followed by DenMune clustering to capture the metagenomic fingerprint. We analyzed MAG distribution in the new feature space by taxonomy, geographical origin, and host medical condition. The embedded space revealed different density profiles, capturing relevant microbiome metadata. These profiles led to new findings, such as distinct profiles between samples from healthy subjects and cancer patients (PERMANOVA < 0.05). The embedded space also separated libraries by country, demonstrating robustness. MAG clusters based on functional potential identified taxonomical groups diverging from their majority cluster, indicating differences in microbial strains. Our findings could be the basis for developing predictive models for health and disease.

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## From Variance to Unity: A NFDI4OneHealth Data Infrastructure Proposal

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The increasing complexity of global health challenges necessitates a comprehensive and integrated approach, recognizing the intricate interdependencies between human, animal, and environmental health. This proposal seeks to establish a robust national research data infrastructure that bridges these domains, facilitating seamless data integration and collaborative research.

NFDI4OneHealth will increase interoperability and integrate various data standards from different fields, leveraging existing efforts within the NFDI framework, including NFDI4Health, NFDI4Microbiota, NFDI4DataScience, NFDI4Biodiversity, and NFDI4Earth. This synergy will enable the creation of machine-actionable data repositories, standardized data models, and advanced analytical tools capable of handling the high variance in data types inherent to One Health research. Emphasizing FAIR (Findable, Accessible, Interoperable, Reusable) data principles, NFDI4OneHealth will ensure that data is optimally managed and utilized. Key challenges such as developing interoperable One Health data models and implementing combined time series and multilevel multi-factor analyses across diverse research areas will be central to this initiative. The interdisciplinary approach will enhance data handling capabilities, crucial for rapid responses to global health crises. The NFDI4OneHealth will provide a comprehensive platform, ultimately contributing to a more sustainable and resilient global health ecosystem.

## Session 8 / 39

**West Nile virus infection in mosquitoes from Berlin, 2023**

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West Nile virus (WNV) is an arbovirus that is maintained in nature through a transmission cycle involving birds and mosquitoes. Zoonotic human infection can lead to West Nile Fever and West Nile neuroinvasive disease. Since 2018, WNV is endemic in eastern Germany including Berlin. Here, we aimed to get insight into urban areas that favour WNV amplification in Berlin.

We collected mosquitoes in Berlin at five sites (< 1 km distance): a sponge city site (S), a residential courtyard (RB), a park-like residential area (RA), a park-like cemetery (C) and a natural conservation area (N). Mosquitoes were collected for four consecutive nights once per month from Jun-Sept 2023, identified morphologically and tested for WNV infection by qPCR.

In total, 13.627 mosquitoes were collected, with highest densities at N (n=4202) and C (n=3927), and lowest at RB (n=669). For all sites, the main vectors of WNV were the most prominent (up to 92%). WNV was detected in 78 out of 1625 mosquito pools. While mosquito density peaked in July, WNV infection rates peaked in August, with the highest minimum infection rate (MIR) at C (2.08 %) and RA (1.46%), and surprisingly low MIR at N (0.26% in August). All detected WNV strains contained unique Berlin-specific nucleotide variants and emerged from local amplification.

An unexpected high WNV infection rate in mosquitoes at RA and C in Berlin in 2023 was found, compatible with infection rates detected during outbreak situations in other European countries.

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## Quo vadis West Nile virus? – Auguring viral expansion through wild bird monitoring

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West Nile virus (WNV) is an arthropod-borne flavivirus with almost worldwide distribution and an increasing number of human infections in Europe in recent years. WNV circulates in an enzootic cycle between *Culex* mosquitoes and birds with occasional transmission to dead-end hosts like humans, where it can cause severe disease and even death in some cases.

In 2021 and 2022, more than 2300 blood and 3100 sets of organ samples were collected from birds by members of the wild bird-associated zoonoses network (WBA-Zoo) in order to monitor the distribution of WNV and the closely related Usutu virus (USUV) in the German (wild) bird population. Specimens were analysed by molecular and serological methods.

Both WNV and USUV increased in circulation in 2022 compared to 2021. This enabled WNV, hitherto considered to be enzootic only in the central-east of Germany, to emerge e.g. further north in Hamburg. Phylogenetic analyses revealed a continuous dominance (95% of generated sequences) of one subcluster of WNV lineage 2, which has maintained the infection process over the last five years. New serological data also confirm a low level of enzootic transmission of the virus outside the endemic regions, without detection of acute infections in animals or humans so far.

In conclusion, WNV is “on the move” and wild bird surveillance constitutes a suitable early-warning system for potential zoonotic spillover events in a One Health approach.



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## **Spatial and temporal analysis of West Nile Fever in Germany using a process-based epidemic model**

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Since the first autochthonous transmission of West Nile Virus was detected in Germany, it has become endemic in several parts of the country and has been spreading due to the attainment of a suitable environment for vector occurrence and pathogen transmission. Increasing temperature associated with a changing climate has been identified as a potential driver of mosquito-borne disease in temperate regions. We developed a process-based epidemiological model driven by environmental and epidemiological data. Functional traits of mosquitoes and birds of interest were used to parameterize our model appropriately. Air temperature, precipitation, and relative humidity were the key climatic forcings used to replicate the fundamental niche responsible for supporting mosquito population and infection transmission risks in the study area. An inverse calibration method was used to optimize our parameter selection. The epidemiological model was used to simulate spatial and temporally explicit vector population, basic reproductive number ( $R_0$ ) maps showing dynamics of WNV infection on birds, number of infected birds and humans across Germany. Epidemiological data for human infections sourced from Robert Koch Institute and animal cases collected from the Animal Disease Information System (TSIS) of the Friedrich-Loeffler-Institute were used to validate model-simulated transmission and infection rates. This study presents a path for developing an early warning system for vector-borne diseases driven by climate change.

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## Experimental Infection of Mosquito Larvae: A Novel Approach for Vector Competence Studies

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Vector competence studies are a valuable method in arthropod-borne virus (arbovirus) research, as they can provide knowledge about transmission routes and virus-vector-interactions. Due to the geographical co-circulation of arboviruses, studies on co-infections in mosquitoes have become increasingly important, but are difficult to implement because of the low feeding and survival rates of mosquitoes under laboratory conditions. An alternative method could be the experimental infection of mosquito larvae, resulting in the emergence of infected adults that can be used for further infection studies.

A total of 300 *Culex pipiens* biotype *molestus* larvae were placed for 24 hours on mammalian cells (Vero, n=150) or mosquito cells (C6/36, n=150) infected with Usutu virus (USUV). Larvae were then raised to adults. 15 days after infection, saliva was collected from all living females. Mosquito bodies and saliva were then investigated for USUV RNA via RT-qPCR.

The results showed that the mosquito larvae acquired USUV from the infected cells and were very susceptible to an infection. A total of 19/22 (Vero) and 39/40 (C6/36) adult females were infected with USUV. Viral RNA was also detected in the saliva samples of 17/22 (Vero) and 31/40 (C6/36) animals. An experimental infection of mosquitoes in the larval stage thus offers the opportunity to produce infected adult animals, which can be used for vector competence studies to investigate viral interactions in the mosquito vector.

**Keynote V / 184**

## **Prevention of health risks through the OH approach**

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## Micropollutants and trace substances as quality control parameters for wastewater-based epidemiology

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Since the coronavirus pandemic, the focus has shifted to wastewater-based epidemiology (WBE) for monitoring pathogens in the population. In line with the One Health approach, the German Infection Protection Act (2023) and the revision of the Urban Wastewater Directive (2024), a research field of health data generation by wastewater and environmental samples has been emerging.

In Germany, the project AMELAG, Wastewater Monitoring for Epidemiological Situation Assessment, is currently implementing the establishment of the WBE at 170 wastewater treatment plants. One significant challenge in the interpretation of quantitative data sets is posed by site-dependent factors and fluctuations in flow rate and wastewater composition. Consequently, normalisation must be carried out with further parameters, such as the daily flow rate, ammonia content or control viruses (surrogate viruses). Furthermore, municipal wastewater contains a plethora of chemical substances, such as pharmaceuticals, food ingredients and human metabolites.

The presentation will provide an overview of the suitability of various micropollutants and trace substances as normalisation parameters in various wastewater treatment plants. The results indicate that carbamazepine and caffeine yield more favourable outcomes than diclofenac and isolithocholic acid in the process of normalisation. The analysis of different normalisation models shows that such human markers are suitable for normalising WBE data.

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**Isolate- and metagenome-based detection of antimicrobial resistance in *Klebsiella pneumoniae* from wastewater**

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*Klebsiella pneumoniae* (KPN) is a significant healthcare-associated pathogen responsible for severe infections. The rise of antimicrobial resistance (AMR) in these bacteria poses a critical challenge due to their rapid acquisition of multidrug resistance genes (MDR). Wastewater (WW) surveillance emerges as an effective method for monitoring pathogens, offering community-level insights, acting as an early warning system, highlighting environmental reservoirs, and being a cost-effective approach for continuous monitoring of trends. Within the AMELAG project our research involved collecting and culturing WW samples to isolate KPN strains at the Umweltbundesamt, Germany. We performed genome reconstruction and resistance screening with short and long read sequencing. This approach identified various resistance patterns, like MDR genes and their host organism. Nanopore sequencing allowed the assembly of complete chromosomes and plasmids, accurately pinpointing resistance genes. Metagenomic sequencing of the same WW samples enabled detection of the broader microbiome and its resistome, allowing comparison of MDR genes in a metagenomic context to specific isolates. The integration of isolate-based genomic detection and metagenomic sequencing provides valuable insights into the dynamics of AMR in WW. Thus offering valuable insights into community-level AMR trends and environmental reservoirs, highlighting its potential as a critical complementary component of AMR monitoring frameworks.

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## Identification and full genome sequencing of sandfly-borne phleboviruses using a newly established capture-based next generation sequencing approach

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Sandflies are vectors of phleboviruses that cause febrile illness and neuro-invasive infections in humans. We recently discovered five novel viruses in sandflies in Kenya indicating that sandfly-borne phleboviruses circulate beyond the Mediterranean. Here, we aimed to develop a capture-based next-generation sequencing (NGS) approach for phlebovirus genome sequencing.

For specific capture and enrichment of sequences in NGS libraries, a bait set was designed based on sandfly-borne phlebovirus genomes. The bait set was established using infectious cell culture supernatants from eleven phleboviruses and tested using phlebovirus-positive sandfly specimens collected in Kenya.

Complete genomes were retrieved for all eleven reference viruses with mean genome coverages ranging from 378 to 440,018 reads. Further, coding-complete sequences of four viruses with mean genome coverages ranging from 334 to 9764 reads were retrieved from the sandfly specimens whereas conventional NGS was not successful. Sequence analyses showed the presence of Embossos, Bogoria and Kiborgoch viruses, as well as the detection of three previously unknown viruses, the latter showing 25-31% nucleotide distances to viruses of the Salehabad serocomplex, as well as to Embossos and Bogoria viruses. We successfully established a capture-based NGS approach for known and previously unknown sandfly-borne phleboviruses. Our findings further expand the genetic diversity of sandfly-borne phleboviruses circulating in Kenya.

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## Today's challenges demand modern solutions: innovative pipelines for reliable antimicrobial resistance surveillance within a One Health framework

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Antimicrobial resistance (AMR) is a global threat and a major focus of the World Health Organization. Multidrug-resistant *Enterobacterales* such as *Escherichia coli* and *Klebsiella pneumoniae* are among the most critical bacterial pathogens, making AMR surveillance crucial not only for human and livestock sectors but also for the environment and wild animals.

Our group applies a global One Health approach, addressing all these sectors to detect the dynamics of pathogen transmission and contribute to outbreak identification. To achieve this, we combine state-of-the-art genomic and phenomic techniques, employing them on a variety of different samples including water, soil, migratory birds, and feces from humans and wildlife in sub-Saharan Afrika and northeast Germany.

To investigate these samples, establishing a standardized and innovative pipeline is essential. The *InnoDia* project is developing reliable sampling strategies alongside methods for preprocessing, bacterial cultivation, and DNA extraction. This includes a biobanking approach for long-term storage. An automated metagenomic pipeline will be established as well. The innovative methods being developed will combine specificity and sensitivity even during the upscaling process to a high-throughput system.

In summary, the developed innovative pipelines will be a valuable tool for global AMR surveillance, providing a standardized sample process for detecting multidrug-resistant bacteria within a global One Health framework.

## Session 9 / 10

## A One-Health Rapid Diagnostic Pipeline for Emergency Transboundary Infectious Disease

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It has become apparent that the ease of travelling, global warming, and changes in the environment accelerate the spread of infectious diseases of zoonotic origin. International collaboration is essential for controlling disease epidemics, and as the COVID pandemic has shown, rapid diagnostics are one of the main pillars. The Pipeline for Rapid Diagnostics of Emergency Transboundary Infectious Diseases (PREPARE-TID) project targets infectious agents of three types: an ongoing neglected epidemic, an ongoing emerging epidemic, and an ongoing elimination drive. The aim is to develop the necessary in vitro diagnostic tools for preparedness needed in case of the emergence of infectious agent X. PREPARE-TID is a multidisciplinary research consortium drawn from 16 European and 4 international research organizations and enterprises. Biomedical researchers are working alongside epidemiologists, clinicians, veterinarians, and software engineers to implement a pipeline from a mobile suitcase sequencing laboratory with a simple bioinformatics to a fieldable rapid nucleic acid extraction procedure, a mobile smartphone linked molecular point-of-care and a multiplex PCR one-health surveillance platform, and a one-health digital platform. These are providing innovative diagnostic solutions for cross-border health threats at international level. PREPARE-TID increases the accessibility to novel diagnostics for detection of pathogens with pandemic potential. The consortium delivers point-of-care and mobile diagnostics, which can be easily deployed within advanced surveillance programs based on a comprehensive digital “One Health” platform, and mass produced in case of an epidemic or pandemic.



**Session 10 / 128**

## Targeting viral and host factors against zoonotic RNA viruses

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Zoonotic diseases caused by RNA viruses have gained huge public interest since the SARS-COV-2 pandemic. Currently, avian influenza viruses (AIV) of Subtype H5N1 are being detected in many different mammalian species worldwide, which may increase the chance of a virus adaptation to humans. Most of these viruses show already different degrees of resistance against the common antiviral drugs. In 2023, the highly pathogenic Nipah Virus, a member of the genus Henipavirus which has been reported to infect pigs and humans since 1998 with fatality rates of 40-75%, re-emerged and caused human cases in India. Up to now there are no drugs or vaccines available against Nipah virus. Both RNA viruses could potentially play a role in future pandemic outbreaks which would require therapeutic interventions. To counteract these pathogens, we are exploiting antiviral drug combinations that not only rely on viral targets, but also on host factors. We have therefore combined different nucleoside analogues with pyrimidine synthesis inhibitors targeting enzymes which are essential for the intracellular generation of CTP to achieve exquisite synergy against virus propagation. AIVs of H5 subtypes as well as different porcine influenza viruses with zoonotic potential have been tested in vitro for their sensitivity against different drug combinations. So far, we found all tested strains to be sensitive to drug treatments at varying degrees. Similar results could be found for Cedar virus, a low pathogenic henipavirus. To further validate this drug efficacy in vivo, animal trials in ferrets with H5N1 are currently ongoing. In conclusion, this approach may lead to new therapeutic strategies against important zoonotic pathogens with decreased chance of antiviral resistance.

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## Analyzing the Zoonosis Risk Along the Bushmeat Value-Chain in the Democratic Republic of the Congo

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Wild animals hunted and sold as bushmeat are a vital protein source in some African regions but also pose a risk as potential reservoirs of pathogens transmissible to humans. Previous research on bushmeat has primarily focused on the zoonotic origin of HIV and related retroviruses such as Simian T-lymphotropic virus (STLV). However, for acute disease-causing pathogens like Ebola virus and Mpox, the connections have mostly been epidemiological.

To enhance our understanding of zoonotic risks associated with bushmeat in the Democratic Republic of the Congo, we studied the trade chain from Salonga National Park to rural and urban markets. We collected 716 tissue and swab samples from various species including primates, rodents, ungulates, pangolins, and civets. These samples were analyzed using PCR systems targeting coronaviruses, filoviruses, retroviruses, orthopoxviruses, and anthrax-causing *Bacillus cereus biovar anthracis* (BCBVA). PCR-positive samples underwent further analysis through target enrichment, whole genome sequencing, and phylogenetic placement.

Our findings confirmed the presence of known pathogens such as STLV and, for the first time, identified agents capable of causing acute disease, including orthopoxviruses and BCBVA. The next phase of our research will correlate these findings with local hunting practices, meat processing, transportation, and consumption habits. Engaging with local communities to discuss these results will be crucial for insights into risk perception, historical outbreak records, and countermeasures previously employed. This community-engaged approach will enhance in-country pandemic preparedness and underscore the need for integrating social sciences to bolster pandemic prevention at the source.

## Session 10 / 136

## A strain typing approach to investigate the transmission barrier of TSE field isolates

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Transmissible spongiform encephalopathies (TSEs) encompass human and animal diseases with infectious, hereditary or sporadic aetiology, including Scrapie in small ruminants, Chronic Wasting Disease (CWD) in cervids and bovine spongiform encephalopathy (BSE) in cattle. The pathognomonic spongiform lesions and amyloid plaque accumulation in the central nervous system are caused by the host encoded prion protein (PrPC) converting into a pathologic isoform (PrPSc). Different phenotypical characteristics of PrPSc indicate the presence of prion strains. Besides a strain's distinct PrPSc and lesion profile, it is important to define its ability to overcome the transmission barrier and adapt to a new host. BSE easily overcomes the transmission barrier of several species including humans. However, for Scrapie and CWD prions the infectious interspecies potential is not yet fully understood. Therefore, we aimed to investigate the transmission barrier of several Scrapie and CWD field cases, with BSE as reference. All isolates were intracerebrally inoculated into the well-established TgshpIX mouse model, which overexpresses ovine PrPC. The resulting phenotype was characterized by using biochemical, histopathological and immunohistochemical methods. This study will help to further characterize the infectious nature of different TSEs and evaluates the potential threat of cross-species transmission between wildlife, livestock and humans due to environmental contamination.

## Session 10 / 89

**Genomic context of SARS-CoV-2 in farmed mink outbreaks in Spain during pandemic: unveiling host adaptation mechanisms**

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After the onset of the SARS-CoV-2 pandemic, mink susceptibility to the virus has been well reported. During the 2020-2021 season, 117 respiratory samples from 18 farm outbreaks were sequenced and analyzed. To obtain the complete genome, we used an in-house approach combining SISPA amplification with the ARTIC protocol. Specific primers targeting the spike protein were used to complete the sequences of the main antigen. The binding kinetics between mink spike protein and various ACE-2 receptors were measured using Biacore technology. Antigenic characterization involved pseudovirus neutralization and competition assays.

We obtained 68 complete SARS-CoV-2 sequences and 6 complete spike sequences from 5 different lineages. Sequencing revealed numerous non-lineage-defining mutations. Outbreaks were associated with groups of mutations rather than specific lineages especially in the ORF1a polyprotein and spike protein. Genomic analysis of spike proteins from different outbreaks revealed mutations in key antigenic sites, such as Y453F and Y453F plus M869T in lineage B.1.1777, and N501T with F486V, N501T, D796H in lineage B.1. These changes, previously unassociated with these lineages, were present in various variants of concern. Functional analysis showed these mutations increased affinity for the mink ACE-2 receptor, demonstrating host adaptation. However, these spikes did not significantly impact vaccinated individuals' serum. The spread of SARS-CoV-2 in farmed mink is a potential environment where the rapid transmission can facilitate the emergence of new variants.

Session 10 / 7

## Lymphocytic choriomeningitis virus: A neglected zoonotic pathogen

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Lymphocytic choriomeningitis virus (LCMV) is a globally distributed zoonotic arenavirus. In humans, LCMV infection can cause meningitis and encephalitis, while prenatal infections can lead to hydrocephaly, birth defects or miscarriage. In New World primates (NWP), LCMV causes callitrichid hepatitis. Despite the global distribution of its reservoir host, the house mouse (*Mus musculus*), and the severity of disease caused by infection, LCMV is under-reported.

Here, we investigate the outbreak and ongoing localized presence of LCMV in captive NWP and wild house mice in a zoo in Germany. LCMV was not detected in any mice collected in the zoo in 2009, but in about half of all 280 tested mice sampled between 2021-2023. For the first time, two lineages of LCMV (lineages I and II) were found in the same population of wild mice, but only lineage II in diseased NWP. Additionally, more than 2,100 small mammals from seven European countries (2005-2023) were screened for LCMV RNA. A single LCMV lineage I positive house mouse from the Netherlands and a novel, divergent lineage of LCMV was detected in two wood mice (*Apodemus sylvaticus*) from Germany.

The re-emergence and localized persistence of LCMV in wild rodents in Germany may have significant implications for public health. Moreover, the newly discovered virus lineage indicates a greater diversity of susceptible hosts, which may influence the distribution and transmission of LCMV.

Session 10 / 93

## Biofilm formation and persistence of various anthrax causing bacteria on leaves

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*Bacillus cereus* biovar *anthracis* (*Bcbva*) is a novel anthrax causing agent infecting animals in African rainforest areas. Human infections were not yet confirmed, but exposure is likely due to hunting and consumption of 'bush meat' and was also evidenced by seroprevalence studies in humans living in affected regions.

It is still largely unknown how animals get infected with *Bcbva*. One route of infection might be similar to that of *Bacillus anthracis* via spores released into the soil by dead animals. However, also monkey species living only in trees get regularly infected by *Bcbva*. Thus, we assume that carrion flies are able to transmit the disease and indeed *Bcbva* has been confirmed in carrion flies. These flies could potentially spread *Bcbva* and its spores from infected carcasses to leaves and fruits which in turn get consumed by various monkey species.

In our study, we use *in vivo* models to show that *Bcbva* is capable of persisting and even replicating on leaves and fruits as biofilm.

To gain insight into biofilm formation, we use confocal laser scanning as well as electron microscopy. Multiplication and spore formation of *Bcbva* are confirmed by quantification methods such as colony forming unit calculation and quantitative PCR. At last, we aim to characterize and quantify biofilm matrix composition by labeling with specific fluorescent markers and photometric measurement.

**Keynote VI / 175**

## **Environmental DNA and RNA for outbreak investigation and disease ecology**

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