Paradigm Shifts for Global One Health
Greater resilience requires transformation and integration

Book of Abstracts

International symposium
23-25 April 2024
Wageningen, The Netherlands

ERRAZE@WUR

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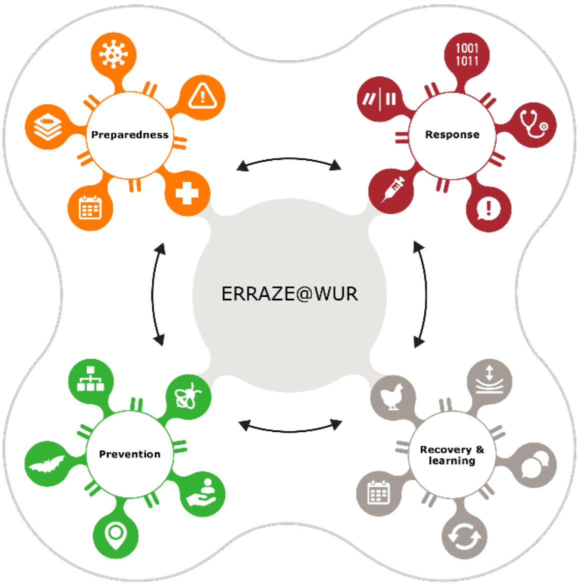

Introduction

In recent years, the concept of Global One Health has gained significant traction as the approach to understanding the interconnectedness of human, animal, and environmental health. However, even though this concept is widely supported, bringing it to practice is notoriously challenging. Plus, we still face growing concerns over emerging infectious diseases, environmental degradation, and the escalating impact of climate change on health. Hence it is time for a paradigm shift in Global One Health work and thinking.

At the heart of this imperative is the recognition that our current approach to health is anthropocentric, often prioritising human health outcomes at the expense of other species and the ecosystems that support them. At best we aim for healthy animals so that their health can underpin human health. The consequences of this narrow focus leaves us facing more wide spread, impactful and increasingly complex health crises.

To truly achieve health for all species, we must reevaluate our priorities and adopt a more inclusive approach that considers the health and well-being of every living organism on our planet. This necessitates a shift towards primary preventive measures aimed at mitigating the human-made impact on ecosystems and animals, thereby minimizing the risk of future health crises.

Central to this paradigm shift is the recognition that the health of humans, animals, and the environment is inherently interconnected, and that safeguarding the health of one necessitates safeguarding the health of all. It requires a divergence from siloed approaches to health and the adoption of interdisciplinary collaboration that integrates insights from ecology, epidemiology, veterinary science, and public health. It also dearly needs a more committed inclusion of the fields of the humanities and social sciences; critical to making the required changes to governance systems and ways of living on, and making use of, our planet.

As we confront the urgent need to prevent health crises before they emerge, we are compelled to ask ourselves: How should our approach be changed to make the paradigm shift towards health for all species? We welcome our symposium participants to help us think about that question.

For the organisers
Joukje Siebenga, Programme Lead ERRAZE@WUR, Wageningen University & Research (WUR),
Joke van der Giessen, Programme Lead ZOOVER, the Dutch National Institute for Public Health and the Environment (RIVM), and
Wim van der Poel, WUR representative of the Global One Health Research Partnership (GOH-RP)

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08.30	**Conference registration at Omnia, Wageningen Campus**	
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10.00	• Fast scalable diagnostics for the detection of avian influenza	Marleen Voorhuijzen (WUR)
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Day 3 (part 2) | 25 April Omnia, Wageningen Campus

13.10 – 13.50	**PhD presentations – 10 minutes**	
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13.20	• A comparative analysis of the dendritic cell response upon exposure to different rabies virus strains	Keshia Kroh (Erasmus Medical Center)
13.30	• Lateral genetic transfer driving co-evolutionary interactions of phages of probable industrial importance and their hosts across the Escherichia, Listeria, Salmonella, Campylobacter, and Staphylococcus Domains	Saba Kobakhidze (Iv. Javakhishvili Tbilisi State University)
13.40	• Prevalence and Characterization of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Dogs Admitted to a Veterinary Hospital in Vienna	Pia Saria (University of Veterinary Medicine Vienna)
13.50 – 14.00	*Switching rooms*	
	Workshop 1. One Health Collaborations **Get out of your head and into the process.** by the Centre for Unusual Collaborations More information – next page	**Workshop 2. One Health Collaborations** **What makes or breaks an interdisciplinary project?** by Knowledge, Technology and Innovation of WUR More information – next page
15.30 – 15.45	Tea break	
15.45 – 17.15	**Workshop 3. Using visualisation in One Health research.** **How to make your results accessible to a wide audience.** More information – next pages	**Hybrid session 4. Global One Health networking.** **Connect beyond the meeting and share opportunities.** More information – next pages
17.15 – 18.30	Closing remarks followed by drinks	

Day 3 | Workshops – page 1

How to establish a good and productive One Health collaboration?

One Health collaborations often entail working with people who have different training backgrounds, and who use different jargon.

It might also be difficult to define a shared approach to the workplan. We may have different methods, or different approaches to designing a study. That way, we may end up working in parallel, rather than together.

Finally, even though working towards "health for all species" may seem straightforward, we may have different perspectives on specific project goals. In fact…, are we sure we share the same goals for the project? And what value systems do we each use? Complex inter- and transdisciplinary collaborations require a specific set of skills and attention to the process on top of the content.

We *all* know about these challenges, yet we are often lost in translation, and fail to get productive co-creation off the ground.

To help One Health researchers come to good collaborations, we offer 2 parallel and distinct approaches to this challenge.

Workshop 1 One Health Collaborations Workshop Option 1: Get out of your head and into the process

The Centre for Unusual Collaborations (CUCo - part of the EWUU Alliance) provides training to help establish complex collaborations. In this workshop Sylvia Brugman and Meghann Ormond offer us a sneak peek into some of the training methods used by CUCo to get out of our head and into the process. During this interactive and active workshop we learn to facilitate creating a safe space in a fun way. Not only useful in the setting we'll be in during the GOH symposium, but you can use this approach in future collaborations, too. The insights will help you understand the challenges you may have in collaborations or team work.

Workshop 2 One Health Collaborations Workshop Option 2: What makes or breaks an interdisciplinary project?

An important premise for interdisciplinary work is to understand how science works. What is a discipline? How have they emerged and changed over time? What is interdisciplinarity, and why is it important? Are there differences between natural-technical sciences and social science? In this workshop Cees Leeuwis and Harro Maat from the Knowledge, Technology & Innovation (KTI) group address these fundamental questions to help you reflect on your own discipline, and how this creates barriers as well as opportunities for interdisciplinary collaboration. We will also discuss strategies that may be used to create conducive conditions for interdisciplinary work.

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3. Using visualisation in One Health research

After having created actionable results in your complex One Health project, how can you achieve optimal impact from your hard work? This workshop will provide you with tools to communicate complex research findings in visualisations to a diverse audience. The techniques that you will learn during this workshop can be useful not only to you, but also to a wide variety of the end-users of your work.

Good visual representation will make it easier for end-users (e.g., citizens, policymakers, researchers, NGOs) to understand and implement your research recommendations. This workshop therefore contributes to knowledge transition and ensures that your research findings will be translated into policy and practice.

4. Global One Health networking session – Connect for a Healthy Planet

Moderated by Dr Annabelle Daburon (Wageningen Centre for Innovation Development), Ms. Jackline Owili and Ms. Michou Weimar (Wageningen University)

Immerse yourself in a dynamic and hybrid experience to finish the Global One Health symposium with. This side-event will help expand the network of Global One Health practitioners, to people beyond those who are in Wageningen. The session will offer a platform for emerging professionals in the field of Global One Health to showcase their work, connect beyond institutional boundaries, and engage in thought-provoking debates. This side event will be presented in hybrid format. This gives us the opportunity to unite various communities, such as PhD candidates connected to the European One Health Association (EOHA, formerly the Med-Vet-Net Association), World Food Forum Transformative Research Challenge prize winners and GOH short course alumni.

The session comprises two integral components.

- A networking and profiling opportunity that utilises an online mapping tool will allow participants to present their current One Health projects through video, PowerPoint, or written documents. The map will require some preparatory work by participants, and will be viewable before the side event. It will serve as a dynamic platform for interaction, comments, and discussions.
- An interactive online session will explore ideas in One Health, encouraging participants to share viewpoints, learn about the latest developments, and identify resources and opportunities for funding and training. Facilitated through Zoom, the session will be interactive and engaging, by mentimeter quizzes and the promotion of the WFF Transformative Research Challenge and other relevant opportunities. Don't miss this chance to be part of an enriching exchange that will leave you inspired and better connected within the vibrant landscape of Global One Health, beyond Europe.

Meet the Keynotes

Wim van der Poel

Wim van der Poel is professor at Wageningen University & Research. His current research interests include: Emerging Veterinary Viruses, Zoonotic Viruses and One Health. In these areas our research has primarily been focussing on the detection and characterization of viruses in different animal species and sources including food and environmental matrices.

His work has focussed on the development of new tools, methods and procedures to study infection dynamics in susceptible hosts and host populations including reservoirs. Through this type of research infection and transmission levels can be elucidated and it also enables studies of virus epidemiology in the broader ecosystems. Research in the field of One Health was based on collaboration between human and veterinary medicine but has been extended to multidisciplinary approaches including much more health related research disciplines.

Carmen Bullón

Carmen Bullón is a legal officer with a Masters in EU law and a specialized postgraduate training in international trade. In 2006 she commenced her work at FAO, at the Development Law Branch of the legal office. As a legal officer she has provided support to more than 70 countries on different areas related to One Health and sustainable agrifood production. At FAO she has a range of responsibilities including guiding legal revision and drafting processes at the national and regional levels, supporting FAO’s normative work on matters related to food and agriculture legislation, and participate in the development of Legislative studies and other knowledge products. Prior to working at FAO she gained experience in public administration.
Recently, Carmen has collaborated with the other Quadripartite Organizations (WHO, WOAH and UNEP) in the development of a One Health Legislative Assessment Tool for antimicrobial resistance (AMR). In her spare time, Carmen researches the role of legislation for One Health.

Frank Aarestrup

Frank Aarestrup is a veterinarian with a PhD in bovine mastitis microbiology, who has dedicated his research career to addressing this challenge. Initially, his research mostly focused on the association between the use of antimicrobial agents in farm animals and the emergence and spread of antimicrobial resistance. As it became increasingly clear that bacteria do not respect traditional borders and that increased globalization means a problem in one country becomes a problem for all countries, the focus has shifted towards global spread of initially foodborne, but now also other pathogenic bacteria. More recently, the focus has shifted to utilizing the possibility in genomic sequencing and global data exchange for improved global surveillance of AMR and potentially all pathogens. The research done by Frank, now in the role of Professor and Head of his Research Group at the Danish Technical University, has contributed to international standards for the detection and monitoring of AMR and impacted how antimicrobial agents are used worldwide.

Olli Vapalathi

Olli Vapalahti is a specialist in Clinical Microbiology and a professor of zoonotic virology at the University of Helsinki. Here he runs a research unit of Viral Zoonoses together with 6 other PIs at the Medical and Veterinary Faculties. Olli is also involved in diagnostic work at Helsinki University Hospital Diagnostic Centre. The research Olli does focuses on targeting particularly sero- and molecular eco-epidemiology, disease associations, diagnostics, and host-virus associations of zoonotic and arboviruses. Recently there was some divergence towards a certain coronavirus.

Andrew Cunningham

Professor Andrew Cunningham has worked at the Zoological Society of London since 1988, initially as veterinary pathologist for London Zoo and Whipsnade Zoo (1988-2001), as Head of Wildlife Epidemiology and latterly as Deputy Director of Science. Since 2001, Andrew has developed a vibrant wildlife disease research group investigating infectious and non-infectious disease threats to wildlife conservation, including the drivers of disease emergence and zoonotic spill-over.

His truly remarkable research and publication record underpins his roles as European specialist in zoological medicine and a diplomate of the European College of Zoological Medicine, specialising in Wildlife Population Health. His current projects include investigating the emergence, spread, impact and mitigation of disease threats to wildlife conservation, understanding the role of anthropogenic environmental change on wildlife disease emergence and identifying factors which lead to zoonotic spill-over. Since its inception in 2021, he has been a member of the WHO/WOAH/FAO/UNEP One Health High Level Expert Panel.

Joke van der Giessen

Joke van der Giessen is a veterinarian and specialist in veterinary microbiology and parasitology. Currently she is top-expert in Emerging Zoonoses Prevention at the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM). She is also a member of the technical advisory group One Health WHO Europe and chair of the national signalling forum zoonoses as part of the zoonoses crisis structure.
Joke’s main fields of interest are zoonoses at the animal-human interface and One Health surveillance, including data sharing and risk assessment to prevent public health emergencies. She will tell us more about the development of a One Health signalling surveillance to response structures in the Netherlands.

Abstracts

Controlling and eliminating zoonotic diseases

Session chair: Natalie Vinkeles Melchers (WUR)

In order to prevent transmission, control, and eventually eliminate (re)emerging zoonotic diseases, it is imperative that experts of animal, human, and environmental sectors collaborate with a common goal. With a changing environment and ever closer interactions between humans and animals, zoonotic outbreaks will occur, and have potentially increased pandemic potential.

With an increased pandemic potential is crucial to develop and test, and subsequently be able to select the most suitable and effective mitigating measures and control strategies for different situations. Such measures include several approaches, including vaccination strategies, hygiene or biosecurity measures, travel or transport bans, and even culling of (potentially) affected animals. In view of the limited financial and human resources, it is essential to develop effective and efficient interventions with direct impact on restraining further disease transmission. Geospatial or statistical/mathematical risk mapping or forecasting models could provide some insight for the decision-making of policy-makers, as well as innovative epidemiological tools for improved control interventions, including for the hard-to-reach populations.

In this session we welcome abstracts that address the current state of the art in the field of control measures of infectious disease, including disease modeling, effective and innovative control interventions, and other public health, animal health, and/or environmental health factors that may assist in controlling and eliminating zoonotic diseases.

Keynote: Embracing Global One Health against emerging zoonoses

Wim HM van der Poel
Wageningen Bioveterinary Research, Wageningen University & Research

The Covid-19 pandemic has shown that better preparedness for emerging zoonosis is a major issue. We need to develop more knowledge on the pathogen animal-human interface and on the environmental factors affecting the emergence and spread of zoonoses, to enable forecasting important zoonotic transmission and outbreak events. For better pandemic preparedness we therefore need a Global One Health (GOH) approach to emphasize the interdependence of human health under pressure of globalization with the health of animals, plants, and sustainable ecosystems from a global perspective. Embracing such a GOH approach means including multiple disciplines, both natural sciences and social sciences, to seek transnational solutions for improving the health of humans, animals, and plants, and ultimately, the sustainability of the ecosystems of system Earth. It also means adopting a systems approach to reveal and try to explain the main cause-effect relationships of emerging zoonoses. Important factors include the loss of biodiversity, changing farming practices, increased travel and transports, global warming, and wildlife trade. For emerging zoonoses preparedness innovative methods for pathogen detection and characterisation are anticipated and should be employed without delay. In addition, a data structure should be implemented to enable efficient and simple data management and use of multiple data sources to perform real-time data driven analysis and monitoring of emerging pathogens and epidemics. Mechanistic models can be developed to evaluate the effectiveness of intervention measures for the reduction of emerging zoonoses risks.

A spatial kernel model for risk mapping and source detection of airborne disease.

Benincà E 1), Spekken DL 1), Vermeulen L 1), Limaheluw J 1), de Baar J 2), van der Schrier G 2), Swart A 1)

1) Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
2) Royal Netherlands Meteorological Institute, De Bilt, The Netherlands

Transmission of airborne pathogens from a source to humans is dependent on the location and properties of the source, and on distance-dependent probability of transmission. In addition other risk factors may play an important role, such as meteorological conditions (e.g. precipitation or wind) and environmental variables that may affect the probability of transmission (e.g. land-use).
Here we present a source-finding model for airborne pathogens. The core of the model is a spatial transmission kernel, which estimates the probability of infection as a function of the distance from the source, as well as estimating the locations of sources, based on the spatial distribution of human cases. However, in its basic form, this approach has some limitations, since risk factors cannot be included. In this work, we improve and extend the current framework of the model by including high resolution maps of meteorological and environmental factors into the spatial transmission kernel.
The aim of the model is two-fold: 1) given the sources of infection, identify the areas with a higher risk of infection, 2) given the infected cases identify the most probable source locations.
Both the identification of the risk areas and the identification of the source locations are assessed using a Bayesian framework. The model has been tested with synthetic data and will later be applied to real epidemiological case data.

Keywords: Riskmapping, Source tracing, Airborne transmission, Kernel based model, Bayesian inference

Uncovering mechanisms behind the spatial-temporal emergence of Usutu virus in the Netherlands in a multi-host context

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Problem statement: The Netherlands has recently seen the emergence of both West Nile and Usutu virus. Arboviruses circulating in wildlife species form a highly complex disease system. To understand transmission dynamics in such systems, high quality data on demographics, environment, and epidemiology are crucial but challenging to collect. By bringing together a multitude of data sources in a Bayesian inference framework, we aim to uncover which factors have shaped the spatio-temporal patterns of Usutu virus emergence in the Netherlands and how different host types contributed to this.
Methods: We developed a temperature-dependent, age-structured stochastic metapopulation model to simulate Usutu virus transmission between Culex pipiens sl. mosquitoes, blackbirds, and possible reservoir birds in the Netherlands. Random Forest models were fitted to predict relative abundance of birds and mosquitoes using mosquito trap count data (n=1544), blackbird point count data (n>500,000) and environmental predictors. Blackbird dispersal was parameterised by analysing ring-recovery data for their home range in breeding and non-breeding season as well as for natal and breeding dispersal. Using an Approximate Bayesian Computation approach, the model was fitted to multiple summary statistics based on several sources of surveillance data, including PCR and antibody-tested live blackbirds, and dead blackbird counts of which a subset was PCR-tested. We performed model comparison and parameter estimation to uncover which biological mechanisms were best supported by the data.
Results: Assuming blackbirds are the only relevant host species, we found that variation in environmental suitability and pre-existing immunity were insufficient explanations of the observed emergence pattern. While blackbirds are the most severely affected host species and the main target of surveillance, other bird host species, which do not die from infection and disperse further, contributed significantly to the spread of Usutu virus. Lastly, alternative overwintering routes, additional to vertically infected diapausing mosquitoes were needed to reproduce the observed multi-annual dynamics. The best fitting model was used to estimate spatiotemporal variation in R0 in the Netherlands and reconstruct the emergence across space and time.
Conclusions: Using a highly data-driven modelling approach, we identified several mechanisms that have shaped the emergence of Usutu virus, including the contribution of reservoir bird species. This framework allows us to evaluate the spatial spread and multi-year infection dynamics, features which have rarely been studied for Usutu and related arboviruses. It can be adapted to study other mosquito-borne pathogens, enhance surveillance programmes and evaluate the potential impact of future change scenarios on arbovirus transmission.

Keywords: mathematical modelling, arbovirus, Usutu virus, birds, mosquitoes

Challenging presumptions of guilt: shifting the paradigm on the role of animals in zoonotic disease dynamics in complex emergencies

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Problem statement: In complex emergencies, defined by environmental breakdown and disintegration of state services, the interaction between humans and animals in a changed environment affects vulnerability to zoonotic diseases with pandemic potential. Moreover, disease surveillance, testing and other mitigating and control measures are often unavailable. While climate change and geopolitical challenges increases the risk of further disasters, humanitarian resources are becoming increasingly scarce, and may be unavailable altogether in remote locations.

As animals are seen as disease 'vectors', they are generally excluded from relief camps and other humanitarian assistance, however evidence on their role during emergencies and impact on human health remains scarce. Those most vulnerable to disasters, conflict and displacement are also those most vulnerable to disease, regardless of the emergency, in particular communities living in marginal areas, highly dependent on agriculture and livestock. During disasters, communities themselves are the first responders, and animals are often the only moveable asset, essential to their lives and livelihoods. The trade-offs between retaining access to animals for nutrition, food security, livelihoods and health are however not well understood.

Method or Approach: Using a qualitative multisite case study methodology, in-depth interviews and focus group discussions were conducted in Sindh province in Pakistan with livestock keepers who had experienced disasters and displacement, supplemented with semi-structured observations. In addition, key informant interviews were conducted with (animal) health practitioners, humanitarian responders, and authorities to discuss policies and responses to animals in disasters, displacement, and veterinary public health.

Results: During emergencies, biological and environmental factors affected the risk of disease, through damage to ecosystems and built environments. Crowded and unsanitary camps increased the risk of water and food-borne diseases, while animals were not facilitated in relief camps. Instead, the displaced herded their animals elsewhere, resulting in communities and households splitting up, with herders unable to access relief services. Lacking resources to protect themselves, attend health services, or buy fuel to boil water and milk, structural political and socio-economic barriers influenced their risk of disease, offset through retained access to nutrition through their animals, and ability to restart their lives following the disaster.

Conclusions: People in complex emergencies had to make difficult decisions between keeping their animals for food security and nutrition, or accessing humanitarian assistance. Facilitating communities and their animals during disasters, will support more effective zoonotic disease prevention and control, while supporting lives and livelihoods. This requires more inclusive policy approaches to address interspecies health inequalities.

Keywords: Zoonoses, complex emergencies, social determinants of health, health inequalities, Pakistan

A quantitative microbial risk assessment for Toxoplasma gondii infections in The Netherlands and Europe

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Toxoplasma gondii is an important zoonotic protozoan parasite in Europe, with a high disease burden largely owing to cases of congenital toxoplasmosis. People mainly become infected either via the ingestion of oocysts shed by felines or by tissue cysts in a wide range of meat products. To develop effective intervention strategies, it is important to know the relative importance of the different sources of infection. An aim of the European project TOXOSOURCES was to identify the most important sources of T. gondii infection in nine European countries by quantitative microbial risk assessment (QMRA).
The QMRA food chain model consists of several steps, for which data has been gathered and models established: 1) Data on the prevalence of T. gondii infection in livestock and wildlife were collected from the literature and an age-dependent model was developed to obtain prevalence estimates at slaughter or hunting age. 2) Data on food habits and other exposure behavior was collected using an online consumer survey in the Czech Republic, Denmark, France, Germany, the Netherlands, Norway, Poland, Portugal, and Spain. This questionnaire is specifically tailored to QMRA needs by addressing: uncertainty, variation over individuals, handling of non-consumers, specific questions on risky behaviour. 3) Processing information for generic and country-specific meat products were collected from handbooks, recipes, and product label information. 4) Previously developed models on the effect of heating, freezing and salting on the viability of T. gondii were improved, and updated with new data from literature. 5) In the final step, a dose-response model quantified the probability of infection.
Two forms of Toxoplasma gondii are considered: 1) bradyzoites, transmitted via meat products, 2) oocysts transmitted via soil on vegetables, and direct ingestion of oocysts via soil (e.g. from gardening). Infection is lifelong and provides immunity to re-infection. We incorporate this by modeling a large number of individuals, and track the ages at which the first infection occurred, after which the individual is protected to further infections.
We will describe the model, highlighting the innovative aspects, and conclude with a risk ranking of products.

Keywords: Toxoplasma, QRMA, risk-assessment, modelling, zoonoses

Understanding and predicting the spread of Phlebotomine sand flies in Europe

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Climate change influences the transmission of vector-borne diseases by affecting the distribution and survival of disease vectors. Numerous diseases are transmitted by phlebotomine sand flies (SFs), including Leishmaniasis. Several major sand fly-borne diseases (SFBDs) are responsible for high global disease burdens and high socio-economic costs. In Europe, 22 known SF vector species are largely confined to the Mediterranean Basin, yet global warming is predicted to drive the spread of SFs to large areas of Europe in the 21th century, an effect likely to be exacerbated by anthropogenic variables. However, the constraints to the geographic distributions of SFs are not well understood. This study aims to increase the understanding of the drivers of the spatial distributions of SFs. To achieve this, we use species distribution modelling (SDM) to assess the role of climate, land-use and socio-economic drivers in shaping the geographic distributions of all endemic SF vectors in Europe. With this knowledge, we predict future hotspots of SFs in Europe. Our predictions are spatially explicit, scenario-based, and informative for surveillance efforts.

Keywords: Climate change, land-use, Phlebotomine sand flies, species distribution modelling

ERRAZE zoonotic framework - intervention & simulation model for disease outbreaks in the Netherlands involving wildlife, livestock and vectors.

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Imulation modeling is a useful tool when faced with an outbreak with severe economic or zoonotic potential. It is one of the few ways in which governments can get quantitative feedback on the effectiveness of different proposed interventions (e.g., closing farms, restricting access to recreational areas, lockdowns), and can weigh that effectiveness against the negative effects that the intervention has on business and the population.

To build these simulation models, model capabilities need to be designed and coded, and data sources need to be collected and curated. These processes are best done prior to an outbreak as they can take considerable time. Within the larger ERRAZE Pandemic Preparedness framework of Wageningen University & Research, we focus on preparing a generic outbreak simulation model suitable for wildlife and livestock diseases.

To simulate outbreaks that can handle the scale of the Netherlands, with its ±190 million livestock animals and millions of wildlife animals and birds, we use SimInf, a stochastic metapopulation differential equation framework that uses the R language as an interface to compile parallel C code.

Here we present our efforts thus far: we have tailored SimInf to the Dutch setting with several design decisions that allow for (1) farms to communicate through transport, (2) farms and wildlife in the environment around farms to communicate through the shedding of virus particles or the local movement of vectors in a non-stochastic layer, (3) the diffusion of virus particles or vectors to neighboring grid cells and (4) the migration of wildlife between grid cells.

To test the simulation model, we are working on case studies such as Bluetongue virus and Swine Influenza, which include data layers such how the wind influences the dispersion of bluetongue virus by midges and how pigs are traded across the Netherlands.

In the coming years we will continue to consolidate, document, and extend this model, as well as work on generating anonymized data layers for trade, so that all components of the model can be open sourced.

Keywords: Outbreak Simulations, Intervention modelling, Netherlands livestock-wildlife interface, ERRAZE

Making One Health happen: designing suitable legal, governance and policy frameworks

Session chair: Harro Maat (WUR)

Over the past years a myriad of reports were written with recommendations on "how to prevent the next pandemic". A key recommendation in many of these reports is "Operationalise the One Health approach". To accomplish this, One Health professionals need to be able to collaborate, share information and align their actions. The institutional, governance, policy and legal frameworks in which they work need to enable their collaboration. In practice they frequently experience contradictions or incompatibilities in for example mandates, goals, timelines or priorities for different sectors.

In this session we welcome presentations that help identify hurdles to the One Health approach, and approaches to mitigating and taking away these hurdles.

Keynote: Why is legislation relevant to One Health? The work of the FAO Development Law Service

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Legislation can serve as a catalyst for advancing the implementation of a One Health approach. Appropriate legal frameworks can establish effective and transparent coordination mechanisms across institutions, fostering sustainable collaboration across sectors. They also foster an accountability framework by recognizing clear roles and responsibilities for public and private actors.
Recognizing this significance, the Quadripartite Guide to implementing the One Health Joint Plan of Action at the national level emphasizes the role of legislaton in institutionalizing intersectoral governance at the national level. It recommends countries to conduct a thorough analysis of their national legislative landscape as an initial step towards effective One Health implementation.
However, important questions persist regarding how governance and legislation can effectively foster a regulatory environment conducive to One Health interventions. Drawing lessons from the realm of antimicrobial resistance can provide valuable insights. Since the approval of the Global Action Plan for Antimicrobial resistance in 2015, many countries have established governance mechanisms for multistakeholder collaboration, yet their success varies significantly.

From a regulatory perspective, the health of this inquiry lies in understanding what is One Health and the resulting obligations, whether substantive, procedural or behavioral, for both public and private actors. Additionally, crafting effective legislative strategies to support One Health interventions, requires identifying actionable legal elements, guided by the definition and principles delineated by the One Health High Level Expert Panel (OHHLEP). This holistic approach ensures that legal frameworks not only recognize the interconnected nature of human, animal, and environmental health but also provide clear guidance on roles, responsibilities, and collaborative mechanisms necessary for effective implementation. The FAO Development Law Service supports the implementation of a One Health approach to different complex health threats under the purview of FAO, including antimicrobial resistance, biosecurity, wildlife management, zoonosis and epizooties management and emergency preparedness and response, to name a few. This presentation will provide an overview of this work, discussing key challenges and implementation strategies. It will propose solutions and raise new questions to engage both legal and non legal professionals in recognizing the potential role of legislation in advancing One Health.

One Health: think big, act small

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In its simplest definition, One Health is 'about managing problems at the interface between eco-system health, animal health and human health.' However, 'management' of public values, at different interfaces, worldwide, interconnected, is far from a simple task. It asks for a clear narrative, integrative thinking, and operalisation of shared values. While veterinarians have been educated for more than 200 years as protectors of human and animal health by providing services for food safety and security, public health, animal health and welfare; human doctors specialized more and more, more recently advocating integrative approaches and 'life style medicine'. Nature does not have doctors for 'disease', such as biodiversity loss, asking for other actors. As Runhaar states (Editorial in Earth System Governance, April 2024; The governance of biodiversity recovery: From global targets to sectoral action) :'The ongoing loss in nature and biodiversity due to human activity, exacerbated by climate change, is one of the most pressing sustainability challenges today. Mainstreaming biodiversity targets in, for instance, the food sector, is considered an effective governance approach, because it can directly address the driving forces of biodiversity loss. However, scientific literature attests little progress. A main problem is that biodiversity targets are often traded off against sectoral goals.' Our food system, and the animals in it, from many different perspectives, including the health of humans, domesticated animals, wildlife and nature, plays a pivotal role in health for all. Those who manage our health should at every level, public and private, think big and integrative, to guide and accommodate actors in the field to exert their more focused work on a daily basis.

Operationalizing Global One Health – a tool for the screening of proposals based on One Health criteria

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Objectives: Our objectives align with the overarching goal of operationalizing One Health, to design impactful tools for directing resources and investments towards OH. We aim to address the challenge posed by funding deficiencies by bringing attention to the importance of taking into account health across its different dimensions in projects. We aim to valorize proposals that take into account OH in their goals and activities in the eye of investors.

Our primary objective is to develop a comprehensive evaluation tool specifically tailored to assess proposals based on One Health criteria, thereby ensuring a rigorous and systematic approach to investment decision-making in this critical field.

Method: The tool we propose is a One Health proposal screening (Excel) workbook. It is designed to assess the impacts, synergies, and trade-offs of Global One Health proposals submitted to investors, across different aspects and dimensions of health in a comprehensive manner. The finished tool should operate at two different levels, providing users with options for both a quick individual assessment and a more in-depth collective evaluation.

The users will be analyzing the impact of a proposal on 66 different dimensions through 8 aspects of One Health: Project OH-ness, Governance OH-ness, Human health, Animal health, Plant health, Ecosystem health, Environmental health, and Earth system health. The tool also fosters reflection on eventual trade-offs and synergies between these different aspects.

For quick assessments, individuals in the screening team fill out the Excel workbook independently. The in-depth assessment involves workshops with investors and top contestants, facilitated by an external facilitator. The team collectively votes on the proposal's impact on dimensions. The facilitator aggregates scores and guides group discussions on trade-offs and synergies, encouraging brainstorming among team members.

For both approaches, a final visual summary in the form of spider diagrams and synergy/trade-off tables provide insights into proposal performance.

Results, conclusion: By April 2024, the individual approach of the tool will be fully developed, with ongoing peer-reviewed revisions for customization and continuous improvement. An oral presentation during the symposium could be an ideal way of demonstrating it to an audience and gathering various feedback, using a practical case and simulating the process together from start to finish.

Next steps for this project task is to develop the collective assessment method, define a communication strategy for the product, gathering additional feedback from investors and adapt our tool accordingly.

Keywords: operationalization, proposal screening tool, investors

Coping with complexity: a comparative study of Oak Processionary Moth adaptation the Netherlands, England, and Flanders

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Aim: To address climate change threats to human health, public health adaptation (PHA) strategies are necessary. PHA strategies are designed to reduce adverse health impacts and enhance resilience to climate change and related consequences. However, the operationalization of PHA strategies remains underexplored. A better understanding of the development of PHA strategies may enhance the ability of professionals', policymakers, and others in the field of One Health and climate change to develop and implement PHA strategies. In this study, we investigate one specific manifestation of PHA; public health adaptation to the Oak Processionary Moth (OPM). We provide an overview of the development of OPM adaptation from the start of OPM infestation till now, in the Netherlands, England, and in Flanders, to compare how stakeholders deal with complexity in the development of OPM adaptation. Method: A document study was conducted, analysing 62 policy documents, articles, and website texts, from 1991 to 2023. Drawing on environmental policy analysis frameworks by Hisschemöller and Dunn, key concepts including problem definitions, knowledge, values, and stakeholder involvement in decision-making processes were examined to understand how complexity is addressed. Results: In all three areas the human health impact of the OPM was the reason for the start of OPM adaptation. Stakeholder involvement grew over time, and roles became increasingly clear. In the Netherlands, national networks undertook a leading role, whereas in England, forestry parties predominantly managed adaptation efforts, and in Flanders, provincial authorities coordinated OPM adaptation. Increased knowledge about OPM management resulted in a broadening of the problem definition and revisions of management strategies, with some OPM management strategies being prohibited by law in Flanders. Additionally, changing values shaped OPM adaptation policy development, with an initial focus on human health and ecology shifting towards broader considerations including biodiversity, social factors, and economic dimensions. Conclusion: The complexity of OPM adaptation is increasingly acknowledged across all areas. This is reflected by recommendations of national OPM networks to discontinue harmful OPM management strategies, expanded stakeholder engagement, and recognition of diverse values. However, stakeholders such as health experts and citizens, remain marginally involved, with their perspectives potentially neglected. Examining complexity in PHA shows that the participation of a wide range of stakeholders and the development of knowledge can contribute to the inclusivity of diverse values into adaptation strategies. Nevertheless, PHA requires (social) learning, including discussion and integration of competing values into PHA policy processes.

Keywords: Public health adaptation, complexity, values, stakeholder involvement, Oak Processionary Moth

Multi-sectoral approaches for AMR governance: Stakeholder interviews in the Netherlands on policy, practice and research from a One Health perspective

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Aim: Antimicrobial resistance (AMR) is a problem of the global commons, whose resolution depends on coordination of collective global strategy through employing effective governance approaches and models. In order to address the challenges a One Health approach poses for AMR governance, the DESIGN project aims to analyse AMR governance from a One Health perspective in five high-, middle- and low-income countries in Europe, North-America, Africa and Asia. These insights will be used to co-design robust integral policy and practice with decision-makers and key stakeholders working on AMR across human, animal and environmental settings.
Methods: The DESIGN study uses both a national context analysis - including a situational, institutional, legal and impact analysis - and a comparative framework between the five countries. Each country research team performs in-depth interviews with key informants working on AMR policy and practice in human, animal and environmental health settings. Building on insights from these interviews, recommendations for policy and practice will then be co-created with participants from all sectors. This presentation will highlight key insights from the Dutch stakeholder interviews.
Results: In the Netherlands, national collaboration between the human, animal and - to a lesser extent - the environmental health sectors are in place. The human-centred perspective dominates AMR control and policy, with a focus on multi-resistant bacteria. Preliminary results show that effective One Health collaborations in the Netherlands requires structural participant engagement, increased communication and exchange of information on AMR between the sectors. There is a need for shared unity of language, clear goals and objectives to work towards an inclusive One Health collaborating culture.
Conclusions: We offer insights into how Dutch actors working on AMR collaborate and how integrated One Health governance can be stimulated by designing policies and practices around a holistic picture of AMR that emphasises the interdependency between animals, humans and the environment.

Keywords: Antimicrobial resistance (AMR), AMR policy, AMR governance, One Health collaborations

One health preparedness and response for mosquito-borne viruses: a stakeholder- and social network-analysis in the Netherlands

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Background: The emergence of mosquito-borne viruses (MBVs) in Europe emphasizes the need for preparedness and response plans. This requires knowledge integration and collaboration across the human, animal, vector, and environmental health domains, aligning with the One Health approach. Despite the importance of a One Health approach, engaging stakeholders from each domain remains challenging. This study aims to identify stakeholders in the field of preparedness and response to MBVs in the Netherlands and map collaborations, knowledge- and information-sharing between these stakeholders, their domains and governance levels. In addition, we aim to identify bottlenecks in these networks and uncover underlying reasons.
Methods: This study combined stakeholder- and social network analysis. Stakeholders were identified through document analysis and snowballing. Semi-structured interviews were conducted with eligible stakeholders. Stakeholders' collaborations, dependencies, and their roles in MBV preparedness and response were discussed. Interviewees who indicated they did not have an active role in MBV policy currently, were provided the opportunity to answer questions based on their experiences related to '(zoonotic) infectious diseases' or 'healthy living environment'. Interview transcripts were coded to identify collaborations and information- and knowledge sharing between stakeholders. Stakeholders were categorized into domains (animal, vector, human, environment, other) and governance levels (international, national, regional, local, other). Networks were visualized and analysed using Cytoscape and R.
Results: Stakeholder analysis identified 87 stakeholders who influence or are (likely to be) influenced by MBV preparedness and response. Of those stakeholders only 47 were recognized as currently having an active role in collaborations or information and knowledge sharing related to mosquitoes and MBVs. The networks of interactions between these stakeholders, their respective domains and governance levels showed underrepresentation of the environment domain, as well as of the regional and local stakeholders. The Human, Animal and Vector domain and the national level were most represented in the network. Transcript analysis revealed low urgency for MBVs among environmental and regional/local stakeholders as an underlying reason for their underrepresentation in the stakeholder network. Analysis and visualisation of the networks for the other two themes ("healthy living environment" and "(zoonotic) infectious diseases") did show multiple connections with environmental and regional/local stakeholders.
Conclusion: The underrepresentation of the environment domain, regional and local stakeholders in the MBV preparedness and response network underlines the remaining challenge of including all relevant stakeholders. We recommend utilising existing collaborations, identified in this study, and central stakeholders to overcome these bottlenecks.

Keywords: Vector Borne Diseases, One health, Intersectoral Collaboration, Preparedness and response

Curbing the silent AMR pandemic

Session chair: Mike Brouwer (WUR)

The growing global spread of antibiotic resistance is an urgent risk for human and animal health. Reflecting the One Health character of the problem, any human or animal can become carrier of resistant bacteria and any environment can become a potential source for spread, depending on the organism of interest. Due to the complex epidemiology, measuring the impact of antibiotic resistance is a real challenge.

In this session we welcome presentations on the role of monitoring in various One Health compartments and the role of novel detection techniques, studies on the epidemiology of microorganisms and mobile genetic elements, interventions into the spread of AMR, and molecular mechanisms for resistance.

Keynote: Different suggestions for improving Global One Health surveillance of AMR

Frank Aerestrup

Technical University of Denmark

Ionophore coccidiostats: risk of co-selection of antimicrobial resistance

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Objective: Today’s intensive broiler production is highly dependent on in-feed ionophore coccidiostats to manage parasitic infections caused by protozoa of the genus Eimeria. Ionophores do not only exhibit antiparasitic activity but are also antimicrobial against Gram-positive bacteria. The latter has generally not been considered a risk to human health, as ionophores are not used in human medicine and transferable resistance was presumed not to exist. This view is changing, however, since Norwegian research identified a two-gene operon (narAB) that confers resistance to narasin, salinomycin, and maduramicin and showed that ionophores drive the co-selection of vancomycin resistance in enterococci*.

Method: To assess the prevalence of ionophore resistance and its co-occurrence with other resistances in the Netherlands, we collected antimicrobial susceptibility data of 137 isolates of Enterococcus faecium and faecalis from poultry products of conventional and organic origin. The resistance profiles were statistically analysed and subsequently a selection of 20 isolates was DNA sequenced and analysed for the presence of resistance genes.

Results: The results showed that salinomycin resistance was highly prevalent, with almost 50% of the enterococci from conventional origin showing a resistant phenotype. Statistical analysis showed a significant correlation between the occurrence of ionophore resistance and resistance to medically important antibiotics (MIA’s). DNA sequencing revealed the presence of narAB in all phenotypically resistant isolates. Plasmid reconstruction showed the co-occurrence of narAB and genes conferring resistance against MIA’s.

Conclusions: The results imply that the application of ionophores potentially promotes transmission of resistance to MIA’s by co-selection. Therefore, current management practices with respect to ionophore use in poultry may need to be reconsidered.

*Naemi et al. Front Microbiol. 2020;11:104

Keywords: ionophores, antimicrobial resistance, enterococci, co-selection

One Health MRSA surveillance: the Dutch experience

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Aim: In 2018 a joined One Health livestock (LA)-MRSA surveillance in animals and humans in the Netherlands was started with a consortium of the Public Health Institute RIVM, the veterinary and food institute Wageningen Bioveterinary Research (WBVR), Wageningen Food Safety Research (WFSR), and the Netherlands Food and Consumer Product Safety Authority (NVWA) to monitor the nature of MRSA transmission between these two sources.
Approach: Every year another livestock sector was sampled. Sampling was done at farms including farmers and persons working on the farm. In addition, LA-MRSA from former research projects were included as well as isolates from meat and caeca samples collected during the national monitoring of antimicrobial resistance in animals and food. Human LA-MRSA were obtained from national surveillance via Type-Ned MRSA. Genomes were analysed using next-generation sequencing (NGS). MRSA genogroup 0398 (LA-MRSA) isolates collected were compared to LA-MRSA isolates of the national human surveillance using whole-genome multi-locus sequence typing (wgMLST). In addition, resistance and virulence genes were compared.
Results: A high MRSA farm prevalence was observed on finishing pig farms (76%), whereas the prevalence was lower on veal calf farms (25%), dairy farms (6%) and MRSA was not found on broiler farms. NGS data from 1770 LA-MRSA from Type-Ned MRSA and 811 animal-related isolates were collected and compared.
The wgMLST minimum spanning tree (MST) showed that some animal-related isolates were closely related to human ones. None of the MST branches contained animal isolates only. One branch only comprised of LA-MRSA from humans, and these isolates were often positive for the virulence factor Panton Valentine Leucocidin (PVL). PVL-positive LA-MRSA originating from animals were not found. However, two PVL positive isolates from two other MST branches were derived from persons that reported contact with pigs.
The multiresistance gene cfr was detected in an isolate from one pig farm and in seven isolates from the national human MRSA surveillance. wgMLST comparison of these cfr-positive LA-MRSA showed no relatedness, although two plasmids were genetically similar.
Conclusions: As there were no clusters containing animal isolates only and some animal-related isolates were closely related to human isolates, it is likely that transmission between animals and humans occurred. In addition, (indirect) transmission between different animal species also occurred as some isolates differed by less than 16 wgMLST alleles.
OneHealth surveillance of LA-MRSA is important to monitor genomic epidemiology of LA-MRSA in animals and humans.

Keywords: LA-MRSA, NGS, livestock, surveillance, OneHealth

Prevalence of Colistin-Resistant Escherichia coli in Humans and Poultry in Indonesia

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Background: Colistin (polymyxin E) is a last-resort antimicrobial for treating infections with multidrug-resistant bacteria, classified by WHO as highly prioritized critically important for human medicine. In Indonesia, data on colistin resistance in human Escherichia coli isolates are scarce. The presence of colistin resistance in E. coli from poultry led to a ban on use in livestock in 2020. Colistin is suspected to be routinely used in humans in the community, with unclear indication. The ban provides an opportunity to assess its impact on colistin resistance in both humans and animals, and to investigate transfer of colistin resistance between animals and humans. The current project aims to estimate the prevalence of colistin-resistant E. coli from humans and layer chickens in Indonesia three years after the ban by the Ministry of Agriculture.
Method: Between July and November 2023, we collected rectal swabs from healthy individuals from the community (n=710) in primary healthcare centers from three districts (2/district) in Central Java, Indonesia. Additionally, we visited 60 small-scale layer farms in the same region (20 farms/district) to collect boot swabs and rectal swabs from farmers and their family members (n=134). Isolated E. coli were screened for colistin resistance by using a spot test on CHROMagar™ COL-APSE medium.
Results: The rectal swab samples yielded 673 E. coli isolates from community, 148 E. coli isolates from farmers, whereas boot swabs from 60 layer farms yielded 900 E. coli isolates, representing 15 isolates per farm. The prevalence of colistin resistance was 3.2% in both humans in the community (18/556 – additional testing is ongoing) and layer chickens (29/900). The latter had a prevalence of 2.5% (10/400) prior to the ban. There was no record or observation of colistin use in both primary healthcare centers and layer farms included in this study.
Discussion: The detection of colistin-resistant E. coli in both human and layer chicken populations in Indonesia, despite the colistin ban, suggests potential persistence of resistance mechanisms, possibly originating from prior usage or transmission. However, the absence of documented colistin use in both primary healthcare centers and layer farms highlights the necessity for further investigation into potential pathways of resistance dissemination, such as environmental contamination or residual resistance. Moving forward, conducting genomic characterization to study transmission dynamics and comparing pre- and post-ban isolates from the same region will offer valuable insights into the effectiveness of policy implementation and help to inform future strategies to combat antimicrobial resistance in both human and animal settings.

Keywords: Colistin-resistant Escherichia coli, prevalence, Indonesia, one health, antimicrobial resistance

Various Mobile Genetic Elements Involved in the Dissemination of the Phenicol-Oxazolidinone resistance Gene optrA in the Zoonotic Pathogen Streptococcus suis: a Nonignorable Risk to Public Health

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The rapid increase of phenicol-oxazolidinone (PhO) resistance in Streptococcus suis due to transferable resistance gene optrA is a matter of concern. However, genetic mechanisms for the dissemination of the optrA gene remain to be discovered. Here, we selected 33 optrA-positive S. suis isolates for whole-genome sequencing and analysis. The IS1216E element was present in 85% of the optrA-carrying contigs despite genetic variation observed in the flanking region. IS1216E-optrA-carrying segments could be inserted into larger mobile genetic elements (MGEs), including integrative and conjugative elements, plasmids, prophages, and antibiotic resistance-associated genomic islands. IS1216E-mediated circularization occurred to form the IS1216E-optrA carrying translocatable units, suggesting a crucial role of IS1216E in optrA spreading. Three optrA-carrying MGEs (ICESsuAKJ47_SSU1797, plasmid pSH0918, and prophage USsuFJSM5_rum) were successfully transferred via conjugation at different transfer frequencies. Interestingly, two types of transconjugants were observed due to the multi locus integration of ICESsuAKJ47 into an alternative SSU1943 attachment site along with the primary SSU1797 attachment site (type 1) or into the single SSU1797 attachment site (type 2). In addition, conjugative transfer of an optrA-carrying plasmid and prophage in streptococci was validated for the first time. It concluded that the optrA-carrying mobilome in S. suis includes ICEs, plasmids, prophages, and antibiotic resistance-associated GIs. IS1216E-mediated formation of optrA-carrying translocatable units played important roles in optrA spreading between types of MGEs, and conjugative transfer of various optrA-carrying MGEs further facilitated the transfer of optrA across strains, highlighting a non-ignorable risk to public health of optrA dissemination to other streptococci and even to bacteria of other genera.

Keywords: Streptococcus suis, optrA, horizontal gene transfer, IS1216E, mobile genetic elements

Early detection and characterisation of new emerging pathogens

Session chair: Wim van der Poel (WUR)

Emerging infectious diseases (EID) are defined as infectious diseases that are newly recognized in a population or have existed but are rapidly increasing in incidence or geographic range. Such pathogens are often not detected in the standard surveillance systems in place in different countries However, once they are found, we need to understand as quickly as possible what their potential is for infecting different species, transmitting among and between species, and causing serious disease. The risk for human health needs to be assessed as soon as possible, and in many cases the risk for animals and the ecosystems in which they live as well.

Research challenges include the development of effective surveillance systems or tools that allow for the detection of previously unknown or underrecognized pathogens, and the implementation of accurate, fast and sensitive detection methods. What will be the best sampling strategies and what will be the best matrices to focus on? Once pathogens are detected, they need to be characterized to elucidate their relevance in causing diseases and their ability to spread within and between species. For this session we welcome presentations on the state of the art of the detection of newly emerging pathogens, and for characterizing them.

Keynote: Early and late alarms: Detecting potential emerging viral threats in clinical and field samples

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Shotgun metagenomics for the detection of foodborne zoonotic pathogens

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Detection of foodborne pathogens at their source could prevent human infections. Shotgun metagenomics is a method to analyze all DNA in a sample, irrespective of the origin. Therefore, this method could be used to detect pathogens that are difficult to culture, or as a method for untargeted detection. By applying shotgun metagenomics on food and animal samples, we aim to detect pathogens and improve food safety. To validate shotgun metagenomics for the detection of protozoal parasites on raw vegetables, we spikes lettuce with Cryptosporidium parvum and Giardia duodenalis DNA. We were able to detect the C. parvum DNA at a level of 100 pg per 1 mg plant DNA and G. duodenalis at 1 pg per 1 mg plant DNA. To support the meat inspection process, we analyzed the serum of sick and healthy cattle collected at an abattoir. With shotgun metagenomics, we were able to detect pathogens that were already confirmed by culture methods, but we also detected a virus and a intracellular bacterium that are not culturable by commonly applied methods. In both studies, we encountered many false positive results and we detected pathogens that could not be present in the sample. In addition, the limit of detection is likely inferior compared to targeted detection methods, such as qPCR. With these studies, we explored the potential of shotgun metagenomics for the detection of zoonotic pathogens and found that application is possible, but with very strict bioinformatic analyses and interpretation of the results.

Keywords: Shotgun metagenomics, detection, pathogen, zoonosis

Early detection and characterization: fieldable sequencing detected a novel mpox clade 1 virus causing an outbreak in Kamituga, DRC

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Objective: Emerging infectious diseases typically originate from rural areas, having access to state-of-the art technologies can be challenging in these settings. Advances in sequence technologies have allowed for the mobility required to perform small scale Nanopore sequencing experiments in such resource low-settings. Local scientists however still have to be trained to perform the sequencing and the subsequent initial analysis and interpretation of the sequence data. Here, we trained local scientist from Rwanda and DRC to perform amplicon-based sequencing and subsequent data analysis and interpretation.
Method: Since September 2023 there has been a sharp increase in the number of mpox virus cases in South Kivu, DRC. During the training 10 samples collected at the Kamituga hospital in the first month of 2024 were subjected to amplicon-based whole genome sequencing, using a Mpox amplification scheme specifically designed for Mpox Clade IIb viruses to generate 2,500 bp amplicons. Sequencing libraries were prepared and sequenced using the Native Barcoding Kit 24 V14 using a R10 flow cell.
Results: Near-complete MPXV sequences were generated from 6/10 patients and all were classified as Clade I. Phylogenetic analysis was done including 94 complete genome reference mpox sequences from Africa available from GISAID and two sequences from the recent 2022 global mpox outbreak. The new sequences clustered with currently published Clade I sequences, but were distinct from all other Clade I sequences from DRC, suggesting the ongoing outbreak in South Kivu results from a separate introduction. The 6 sequences have several SNPs differences between the genomes which suggests ongoing circulation of this outbreak strain for some time already. Performance of the diagnostic primers was evaluated and showed that the region targeted by the primer and probe sequences which are being recommended by the CDC to discriminate between MPXV lineage I and II is deleted in this novel clade I strain.
Conclusion: We obtained 6 near-to-complete genome sequences of a novel Clade I mpox virus obtained during an amplicon-based sequencing training in Rwanda to train local scientists from Rwanda and DRC. These viruses contain a gap in a similar region compared to Clade II viruses, leading to a target failure of the Clade I specific PCR recommended by the CDC. In addition, the results suggest a recent introduction separate from other circulating variants. Altogether this study shows why there is an urgent need to train local scientists and apply whole genome sequencing for the early detection and characterization of emerging pathogens.
Keywords: Whole genome sequencing, mpox, emerging viruses

Development of novel diagnostics for monitoring and surveillance of zoonotic diseases using mosquitoes for xenosurveillance

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To be well prepared for outbreaks of new and emerging zoonotic pathogens, early detection and characterization of the zoonotic organism plays an important role. This involves a multipronged approach, including sampling, broad detection and targeted detection. Effective sampling requires knowledge about the (type of) sample in which the zoonotic organisms are most likely to be present, and whether these are representative for zoonotic organisms circulating in different animal species. Effective broad or 'untargeted' detection requires optimization of DNA/RNA extraction protocols, enrichment of DNA/RNA of the target organisms, sequence analysis and bioinformatics. Targeted detection requires fast (on-site) detection tools, performed on-site for each type of sample, and knowledge of the odds of detection compared to an 'untargeted' screening approach.
In this presentation we will focus on the field collection and downstream analyses of blood from blood-fed mosquitoes as sample type. For our project, we first aimed to optimize sampling of blood-fed mosquitoes using several trap designs in different habitat types (farm, wetland and peri-urban), followed by downstream DNA and RNA analyses to identify blood host species, and to assess the presence and diversity of bacterial and/or viral DNA/RNA in the blood of sampled mosquitoes. Based on our results, we will present a workflow for detection of potential zoonotic pathogens in blood-fed mosquitos captured in three different habitats. In collaboration between WUR and RIVM a Wikipedia like flow scheme has been set up for this sample type which will be expanded to other types of samples. Preliminary data on experience with and primary outcomes using the above approach will be presented and discussed.

Keywords: mosquitos, blood, zoonotic, metagenomics, detection

Designing surveillance systems for early detection of highly pathogenic avian influenza in wild birds to reduce the risk of incursion into poultry farms

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Aim: HPAI viruses have been circulating all year round among migratory and resident wild birds in the Netherlands, which has resulted in infections in commercial poultry becoming widespread. Dutch farmers culled about 6.9 million poultry birds between January 2021 and May 2023 following HPAI outbreaks. Prevention and control costs are sharply increasing in recent years; estimated at 55 million euros in 2022. Wild birds are the main sources of HPAI infections in Dutch poultry farms. Early warning of HPAI should, therefore, focus on surveillances of HPAI viruses in wild birds. An effective surveillance system is critical for the early warning system long-term sustainability. This study aims at evaluating the effectiveness of alternative output-based surveillance systems in wild birds as early warning for HPAI infections in poultry farms.
Methods: The effectiveness of surveillance systems can be improved by conducting targeted surveillance at high-risk areas during high-risk seasons. We evaluated the effectiveness of alternative systems by estimating the early detection surveillance sensitivity (EDSS). EDSS is a function of four parameters: proportional relative risk (PRR), population coverage (sample size), temporal coverage (target timeframe for the system to effectively detect infections (sampling frequency)) and detection sensitivity of the test/analytical method (derived from a scenario tree model). We stratified Netherlands into low-, moderate- and high-risk areas based on relative risk of incursions into poultry farms (PRR).
Results: Poultry farms located in high- and moderate- risk areas are 6.60- and 2.98-times more likely to be infected with HPAI than those farms located in low-risk areas, respectively. The corresponding PRRs are 0.095, 0.282 and 0.623 for low-, moderate- and high-risk areas, respectively. Our tentative results show that the spatial HPAI risks for wild birds and poultry farms are similar (a crude risk map for wild birds (which is under refinement) resembles to the poultry risk map).
Which species, where and when to sample? Samples should be collected from identified "High risk" wild bird species among dabbling ducks, geese and swan species, which are associated with high risk of HPAI incursions into poultry farms. Following the PRR estimates, the distributions of samples are 10%, 28% and 62% for low-, moderate- and high-risk areas, respectively. Based on temporal risks, 73% of samples should be collected during winter (September-February) and the remaining 27% during breeding season.
Conclusions: The effectiveness of surveillance systems can be improved by collecting samples from high-risk wild bird species while accounting for the spatial and temporal risks for poultry farms.

Keywords: Avian influenza, Early warning, HPAI, Poultry, Surveillance, Wild bird

Human infections with Eurasian Avian-like swine influenza virus A(H1) in The Netherlands coincidentally detected in routine respiratory surveillance systems.

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Human infections with swine influenza A viruses are a notifiable disease in the Netherlands. Only a limited number of human cases are known to date in The Netherlands, often detected due to severe symptoms and hospitalization with subsequent influenza diagnostics and subtyping. No monitoring systems are in place to specifically aim to detect such cases of swine influenza virus infection, for example in specific risk groups. As symptoms might be very comparable to other respiratory diseases, especially for milder cases, the true number of human infections and zoonotic potential of swine influenza viruses might be underestimated.

The National Influenza Center (NIC) in The Netherlands is situated at the Dutch National Public Health Institute (RIVM) and Erasmus Medical Center and coordinates several methods to monitor influenza like-illness and circulating influenza viruses. This includes sentinel surveillance at general practitioners (in collaboration with Nivel, Utrecht), community surveillance (Infectieradar) and subtyping of representative influenza virus positive clinical specimens from diagnostic laboratories or by request from diagnostic laboratories.

We have identified three human cases, two A(H1N1)v and one A(H1N2)v, in 2020-2023 of swine influenza A virus infections among these different activities of NIC/RIVM. The three cases were detected by coincidence and not as part of surveillance or diagnostics related to direct exposure to pigs. The patients experience mild to moderate symptoms, and all fully recovered. Two of the cases had no direct contact with pigs and source of the infection remained unknown. For one case, close contact to pigs had taken place shortly before start of symptoms and SIV positive pigs were identified and whole genome sequencing revealed highly similar strains.

A swine influenza surveillance system at pig farms was initiated in 2022. This surveillance showed that 77% of the 90 participating pig farms tested positive for SIV and that subtypes are H1N1 and H1N2. Sequencing analyses showed clustering of the observed strains with the two human cases with unknown source, indicating that SIV viruses with zoonotic potential are endemic in The Netherlands.

This work shows that there might be an underestimation of the number of human cases of swine influenza virus infection in The Netherlands and that infections seem possible without direct contact with infected pigs. Surveillance of swine influenza viruses at pig farms allows monitoring of circulating subtypes and comparison of strains detected in human cases.

Keywords: swine influenza virus, surveillance, zoonosis

Experimental models for characterization of emerging vector-borne viruses

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Arthropod-borne (arbo)viruses pose an increasing risk to veterinary and public health due to the increasing contact between wildlife, livestock and humans, climate change, intensified international transport, and geographic expansion of vectors that transmit medically important arboviruses. The World Health Organization (WHO) recognizes that arboviruses are expected to induce outbreaks in animals and humans at an unprecedented frequency and magnitude in the coming years. In the Netherlands, several arboviruses have been detected in recent years, including the zoonotic mosquito-borne Usutu virus (USUV), West Nile virus (WNV), and the tick-borne encephalitis virus (TBEV), as well as the veterinary bluetongue virus (BTV). In addition, other zoonotic arboviruses such as Rift Valley fever virus (RVFV) are high on the priority lists of public health and veterinary institutes.

In order to rapidly respond to (newly) emerging arboviruses, it is essential to understand the impact these viruses may have in animals and/or humans. The aim here is to the develop and characterize experimental model systems to gain this much needed understanding.

Our approach was to develop complex cell systems to study 3 key barriers in arbovirus dissemination and disease. First we developed an endothelial barrier model to study arbovirus dissemination. Second we developed a placenta barrier model to study invasion of the placenta as a risk during pregnancy. Third we developed a blood-brain barrier model and neuronal cultures to study routes of neuroinvasion. Our results show that these models can be used to asses virulence of distinct arboviruses from different families. In addition, these models allowed us to study potential differences in routes of entry across these barriers, as well as key host responses involved in the differential ability of genetically distinct viruses, to cross these barriers.

In conclusion, the models developed here, can be used in rapid risk assessments of newly emerging viruses, to elucidate the primary target organs and disease severity before an outbreak, without the need for extensive epidemiological studies.

Keywords: arbovirus, in vitro models, dissemination, placenta, central nervous system

Striking the right balance: healthy environments, resilient and productive agrifood systems

Session chair: Marion Kluivers Poodt (WUR)

Nature, agriculture and the human living environment are becoming increasingly intertwined. With the growing demand for food, the footprint of our production systems as well as the length of supply chains is growing too, putting pressure on the functioning of our natural systems. This pressure is further increased by other manmade changes such as climate change. It is widely acknowledged that this results in increased risks of emerging pathogens and zoonotic spillovers.

In this session we welcome presentations that provide pathways towards a balance in on the one hand maintaining and restoring biodiversity and ecosystems, and on the other hand productive, safe and healthy food systems. Special attention is to be given to the One Health approach to minimizing the pressure / risk of zoonotic infectious diseases.

Keynote: One Health: the biodiversity-agriculture nexus

Andrew Cunningham

The Zoological Society of London

Contributions of livestock production animals to ambient ammonia and particulate matter in a livestock dense area

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Objective: This study assessed how differences in emissions and geographical distribution of different production animals (veal calves, dairy cows, laying hens, broilers, other poultry, sows, fattening pigs, goats) affect the spatial patterns of ambient concentrations of ammonia (NH_3) and particulate matter (PM).
Methods: The study area (40 x 48 km, the Netherlands) covered an intensive livestock production area with approximately 2000 livestock farms, densely populated residential areas and large and small nature areas. Based on emitting livestock farms, the widely used Dutch dispersion model STACKS that accounts for deposition was used to calculate ambient PM and NH_3 concentrations for 102381 receptor points on a 100 x 100 m grid. Sensitivity analyses (SA) were performed for model assumptions.
Results: Livestock production elevated local levels of NH_3 and PM. Relative contributions of production animals to the spatial patterns of NH_3 and PM varied, driven by geospatial distribution of farms. The distribution of farm contributions to the concentration on receptor points in rural areas were characterized by high contributions of a few farms nearby and considerable smaller contributions by many farms further away. In nature areas (at the borders of the study area), concentrations were mainly driven by a large number of farms on a greater distance in the absence of farms in the direct vicinity. SA showed that the size of the model inclusion area for farm sources affected concentrations in nature at the borders of the study area significantly but concentrations in livestock dense areas minimally.
Conclusion: Reduction of air concentration of NH_3 in nature requires a general reduction of livestock emissions, instead of reduction from local sources alone.

Keywords: ammonia; particulate matter; livestock

Risk for high-pathogenicity avian influenza virus infection in commercial poultry farms at the micro landscape scale, The Netherlands, 2014-2022.

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Aim: Currently circulating strains of the Goose/Guangdong lineage of highly pathogenic avian influenza (HPAI) H5 virus affect domestic and wild birds and occasionally mammals and humans. Efforts in the Netherlands aim at keeping poultry free of HPAI-infections and outbreaks in poultry have mostly been attributed to introduction of infection via an environment around poultry farms contaminated by infected wild birds. The aim of this study was to identify factors that are associated with the introduction of HPAI virus in poultry and develop risk maps for HPAI virus infections.

Methods: Data on wild bird population densities, landscape and production data around all poultry farms in the Netherlands was collected (c. 90 variables). HPAI-infected and non-infected poultry farms were identified for the period 2014-2022. We used the data to build multiple univariable and multivariable logistic regression models to make inferences on risk factors associated with introduction of HPAI-infection in poultry. In addition, we developed machine learning (ML) models to identify geographical hotspots for HPAI infection in the country.

Results: Turkey and duck farms were more at risk for infection than chicken farms. Some of the landscape characteristics investigated by GIS in a radius of 500 m of the poultry farm were strong (indirect) indicators of risk: distance of the poultry house to water bodies, the area of water and the area of grassland around the farm were associated with an increased risk for infection in poultry, whilst an increasing area of trees or forest around the farm had a protective effect. The regression and ML models agreed in the identification of higher counts of wild waterbird species in the neighborhood of poultry farms to be associated with the risk of infection in poultry. The best performing ML model was used to build a risk map for infection of HPAI for poultry in the Netherlands. Hotspots for infection were geographical areas where there is a combination of water bodies, grasslands and high densities of waterfowl.

Conclusion: In addition to poultry species, distance to and the area of the water bodies in the vicinity of farms as well as the area of grassland are significant risk factors for HPAI-infection in poultry. These landscape characteristics around a farm provide an attractive environment for wild waterbird species, which is reflected in the identified hotspots. These results can be used to develop targeted surveillance and/or avoid placing new farms or relocate existing farms in high-risk areas.

Keywords: Epidemiology, Heat Maps, Infectious diseases, Zoonoses

Enhancing zoonotic disease prevention: Using behavioural insights to look at compliance in farmers

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Objectives: Zoonotic diseases, threatening human and animal health, demand proactive interventions. Strategies and interventions such as education, regulation, incentives, and vaccination aim to equip farmers with preventive measures to the threat of zoonotic outbreaks. However, effective prevention requires a certain number of stakeholders to adhere to advised measures. That is why understanding the behavioural factors driving effective intervention adoption is crucial for ensuring compliance of farmers and prevention of zoonoses.

The paper's objective is to enhance zoonotic disease prevention by evaluating the effectiveness of interventions on farmers' compliance to zoonoses threat preparedness.

Method: A literature review and analysis were conducted to assess important behavioural drivers of compliance to the application of zoonotic preparedness interventions, harnessing the knowledge provided by psychological behaviour change theories and farmers' behaviour in contexts of zoonotic threat and other contingencies. The Theory of Planned Behaviour (TPB) (Ajzen, 1991) was used as a theoretical framework. The identified interventions were categorized into four groups: awareness and knowledge interventions, social influence and collaboration interventions, economic incentives and resources, and regulatory and policy frameworks. Example of interventions from each category were applied to the TPB framework to assess their effectiveness.

Results, conclusion: Based on the literature reviewed and through the use of the TPB framework applied to interventions, farmer's attitude came out as one of the main drivers of behavioural change in farmers in this context. Insights from the study inform an integrated approach to farmer's compliance to preventive interventions, emphasizing collaboration with key stakeholders such as fellow farmers, friends, family, and veterinarians, the consideration of past experiences, local contexts, participative methods, shared responsibility, and strategic communication.

The next steps involve validating these guidelines for farmers' compliance with zoonotic threats preparedness in future research. This aims to confirm the significance of the main behavioural drivers identified and enable their integration into epidemiological models, thereby enhancing preparedness and outbreak predictions.

The presentation will include figures used in the paper, providing a visually appealing summary of our findings. Through clear visuals and concise text, the presentation will effectively communicate the study's objectives, methodology, results, and implications, inviting engagement and discussion at the symposium.

Keywords: Zoonoses, Behaviour change, preventive interventions, compliance

Integration of farmers’ and experts’ knowledge in livestock disease management in Kisumu County, Kenya.

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Problem statement: The interactions between humans and animals present a major global health risk disrupting agrifood and healthcare systems and carrying implications for food security and animal health. These health risks include newly emerging zoonotic diseases, highlighting theoretical need for resilient livestock management and effective biosecurity measures. International health agencies, governments, and scientific communities are working to improve disease management systems, recognizing the critical role of land use in shaping disease dynamics and transmission pathways. Efforts are underway to identify potential breeding spots for new zoonotic pathogens and implement biosecurity measures to mitigate spillover events, underscoring the importance of sustainable land management practices. Rural communities have substantial local knowledge rooted in experience with a variety of common diseases, including zoonoses, and play a crucial role in addressing these health threats. Using Kenya as an empirical context, this study aims to investigate the challenges and opportunities for integration of local (farmers’) and expert knowledge in disease management.

Methods: This study investigates the potential opportunities for integrating local with expert knowledge in livestock disease management using the social practices approach, which focuses on the everyday practices of groups of farmers. Rooted in practice theory, the study employs a qualitative case study design and participatory methods to examine the socio-technical, agroecological, and biomedical connections within farmers’ disease management practices. This includes fodder and grazing management practices that influence animal health and resilient livestock systems. Fieldwork was conducted from August 2023 to November 2023. The methodology involved semi-structed interviews with livestock farmers to gain insights into their local coping mechanisms for managing diseases on their farms. Additionally, interviews were conducted with experts such as County animal health workers, community-based organisation workers, veterinary workers, and animal health researchers who are involved in livestock health. Non-participant observations were also conducted with farmers, extension workers, and veterinary workers to better understand their knowledge and perceptions about disease management.

Preliminary findings: Farmers heavily rely on local coping methods due to challenges in accessing adequate animal healthcare support, including limited veterinary services, lack of awareness of disease transmission, high treatment costs, and insufficient resources. Agricultural extension workers and animal health service providers play crucial roles in disseminating disease management practices, offering training, and conducting awareness campaigns. However, they face obstacles like transportation issues and understaffing, hindering their ability to reach rural communities efficiently.

Conclusion: Livestock disease management is a complex issue that necessitates collaboration among farmers, veterinary professionals, and agricultural extension agents. Study findings offer valuable insights for developing new ways to ensure timely disease diagnostics and implementation of context-specific biosecurity measures. These efforts are important for enhancing food security and promoting sustainable livestock management practices. Therefore, collaborative initiatives such as community awareness campaigns on disease identification, knowledge exchange and trainings, and investing on more qualified animal health care service providers will considerably improve the resilience of Kisumu County's livestock farming communities to disease outbreaks. This, in turn, contributes to resilient agrifood systems and enhances overall food security situation.

Keywords: Agrifood-system, Zoonoses, Spillover, Resilient livestock system, Food security.

Assessing the Urban Wildmeat Value Chain in Lagos, Nigeria.

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Urban wildmeat value chains represent a vital protein and livelihood source intertwined with cultural traditions and complex market systems. Against the backdrop of escalating zoonotic disease concerns linked to wildmeat, this research aimed to unravel the structure, governance, species composition and actor characteristics of the urban wildmeat value chain in Lagos, West Africa's most populous metropolitan city. This study employed a cross-sectional study design incorporating qualitative and quantitative approaches. Over a 4-month period, structured questionnaires were administered to 257 respondents across 15 towns in Lagos Metropolis. These were retailers (n=100), hunters (n=55), processors (n=68), and wholesalers (n=34). Also, key informant interviews were conducted, participant observations recorded and geographic data captured. Findings revealed 5 main nodes: hunter, processor, wholesale, retail and consumer nodes; and 4 major external sources of in-flow. The consumer node showed an overlapping with the other nodes. Hunter nodes played pivotal role as product origin, representing a first line of exposure to any potential health risks. The governance structure showed a blend of traditional and formal mechanisms. 88.1% of actors were motivated by income generation, with 55.6% of them having a household size of 6 to 10 persons. Women accounted for 64.2% of all actors, predominated at processor and retail nodes. A total of 35 traded wildlife species were identified by the study, from which 26 were mentioned by study participants as species they traded. These were grasscutters (100%), duikers (100%), porcupines (94.9%), African giant pouched rats (90.7%), monitor lizards (84%), and ball pythons (82.8%). Pottos, buffalos, tortoises and house snakes were the least traded (≤ 7.0% each). Species mentioned as frequently traded have previously been documented in studies globally, as reservoir hosts of zoonotic pathogens. This research provides a nuanced understanding of the Lagos urban wildmeat value chain, paving the way for evidence-based interventions to address conservation challenges and zoonotic spillover risks in Lagos and beyond. We recommend the integration of formal governance systems into the value chain to aid monitoring and regulation. Stakeholder engagements and public health interventions should be node-specific and gender-sensitive, targeting men at the hunter node, and women at other nodes due to their prominent roles. Relevant authorities should provide sustainable alternatives to populations who depend on wildmeat for nutrition and livelihoods, incorporating solutions such as cheaper access to livestock protein sources, and agribusiness start-up support for actors who exit wildmeat trade. Lastly, we recommend a review and update of the conservation status of African wildlife by relevant bodies, to capture current conservation realities foisted by wildmeat trade.

Keywords: Wildmeat trade, Value chain, Pathogen reservoirs, Conservation, Zoonotic Spillover

From One Health signalling, surveillance to response

Session chair: Eelco Franz (RIVM)

Timely response to emerging pathogens asks for integrated signalling and surveillance across the borders of the different domains. Especially for threats with pandemic potential, timely response is essential. Challenging issues are sharing signals and what kind of data can be shared between domains in One Health surveillance systems.

In this session, we welcome examples of One Health surveillance systems including pitfalls, hurdles and challenges. Also, we welcome presentations of methods for prioritization methods for pathogens or One Health surveillance methods and results of these studies. In addition, collaborations between sectors with examples from zoonotic signals or surveillance systems and how this was handled at the public health site will be welcomed.

Keynote: Pandemic prevention and pandemic preparedness, what is in the name?

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Most emerging infections originate from animal reservoirs indicating that knowledge of these reservoirs, transmission pathways and risk factors for zoonotic infections in humans are of paramount importance to prevent or mitigate human exposure to these pathogens. Of the risk factors identified, human behavior is one of the key elements that drives zoonotic disease emergence. Multidisciplinary collaborations combining expertise from different domains are needed to assess and mitigate the zoonotic risks. The Netherlands is a densely populated country and includes aquatic and terrestrial wildlife, migratory birds, and an intensive livestock production system. The Netherlands experienced various major zoonotic outbreaks of both animal and public health concern, which warranted timely signaling to response of zoonotic outbreaks in close collaboration between the medical, veterinary, wildlife, vector control, and food safety professionals. This led in 2011 to the National Zoonoses Structure, where a multidisciplinary signaling group meets monthly and evaluates new zoonotic signals from animal and human populations. When urgent upscaling to response is organised. Again Covid-19 showed the importance of pandemic preparedness, and many international joint action plans and initiatives are currently in progress. Prevention to avoid exposure of humans to zoonotic pathogen threats is the first element in pandemic preparedness plans but still under lighted. In 2022, the Dutch national plan of action to strengthen zoonoses policy was launched and new initiatives to improve the zoonoses signaling to response structures were endorsed. Since zoonotic influenza is still a priority, One Health surveillance initiatives to systematically detect, share and assess the risks are currently being developed including a shared genomic database for animal and human influenza viruses to timely assess zoonotic influenza risks. One Health collaborations demands multidisciplinary approaches and tools to assess the risks. Moreover, responses for zoonotic agents with pandemic potential such as zoonotic influenza are currently being updated. Pandemic prevention is a key element in the chain of pandemic preparedness and national and international collaborations in a One Health approach on all levels should be enforced.

The Role of Poultry Feed in a Major Salmonella Outbreak Linked to Eggs

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Introduction: Salmonella outbreaks are a major cause of foodborne outbreaks in the Netherlands. This study describes one of the largest known outbreaks of Salmonella Enteritidis (SE) in the Netherlands, aiming to identify its source.

Methods: National Salmonella surveillance registries were used to identify SE patients. Since 2019, all SE patient isolates are typed using whole genome sequencing. Outbreak cases were defined as SE patients belonging to one of two outbreak clusters (A and B) based on core-genome Multilocus Sequence Typing (cgMLST) with a cluster cut-off of ≤5 alleles, since June 2023. A case-control study, with questionnaires on egg consumption, was conducted. Controls were randomly sampled from the population registry (matched on age, sex and municipality). Trace-back and trace-forward investigations were carried out by the Netherlands Food and Consumer Safety Authority (NVWA) based on case questionnaire data and non-human isolates belonging to the outbreak clusters based on WGS.

Results: By February 2024, 154 outbreak cases were identified, with a median age of 41.5 years and an equal gender distribution (75 males and 79 females). The case-control study found cluster A (n=103) primarily associated with barn egg consumption (OR 5.9; 95% CI 2.2-16.0; $p<0.001$) and cluster B (n=51) with organic eggs (OR 38.0; 95% CI 5.5-261.7; $p<0.001$). While SE isolates from 12 laying hen farms were genetically linked to the outbreak, no common traceable relationship could be established, suggesting multiple potential sources. Furthermore, an isolate from a packing station and four isolates from dried eggshells (two originating from eggshell milling companies, and two from animal feed producers) were genetically linked to the outbreak clusters. Investigations revealed that eggshells were probably inadequately treated before being processed in poultry feed. Further tracing efforts revealed a potential circular transmission chain involving contamination of laying hen farms through the contaminated poultry feed.

Conclusion: The findings indicate that the outbreak strains likely have spread through contaminated eggshells utilized in poultry feed across multiple laying hen farms in the Netherlands. Inadequate treatment of these shells underscores systemic vulnerabilities. Measures such as channeling eggs from infected farms and improving raw materials control for animal feed have been implemented to mitigate the outbreak. Companies had to halt eggshell supply until they demonstrated adequate treatment procedures. Despite source identification, the outbreak strains may still be present in laying hen farms not yet identified. Additional cases are therefore anticipated, emphasizing the need for sustained vigilance and intervention measures.

Keywords: Salmonella, Disease Outbreaks, Case-Control, whole-genome sequencing, One health

In the Congo Basin, South Kivu, Drcongo, the implementation of a one health approach through the establishment of a community-based epidemiosurveillance network combining actors from the 3 health sectors

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The Kahuzi Biega Park in South Kivu, DR Congo, boasts exceptional biodiversity in the heart of an area subject to major demographic and socio-economic constraints. Anthropogenic activities exert pressure on the park's flora and fauna resources and are a source of ecosystem degradation, which represents a major health risk factor for both the surrounding and more distant populations. Deforestation and the trade and consumption of bushmeat increase the interaction between wild fauna, domestic animals and human populations, which increases the risk of "spill-over" and therefore the risk of a pandemic. It should be noted that the Congo Basin has already seen the emergence of several major pandemics, the most recent of which is Ebola haemorrhagic fever. However, the security of the area is threatened by various armed groups, and logistical difficulties make certain sites difficult to access.

As a result, it is difficult to carry out regular, detailed deep-surveillance in this area, even though the risks are real. In order to avoid detecting a major new epidemic too late, but also to improve the speed and acceptance of the response by the populations concerned in the first place (the Ebola response mechanisms, for example, have experienced difficulties in this respect, particularly in North Kivu, with several attacks recorded against response agents and even the death of a doctor), Vétérinaires Sans Frontières Belgique (VSF-B), Médecins du Monde Belgique (MDM/B) and Action pour le Développement des Milieux Ruraux (ADMR) have set up a community-based epidemiosurveillance network in three health areas bordering the Kahuzi Biega park. The system is based on local committees working with private veterinarians, human health professionals and park and environment department staff. During this pilot phase, the aim is to assess how this combined community approach can ensure sensitive and early detection, to characterise and then define and finally assess the effectiveness of the most rapid and appropriate response possible in a context that is particularly constrained by its geography and insecurity. The initial findings after 2 years of implementation are already providing some answers, as illustrated by the case of the skin disease known as "Eleki", a disease that has been reported in the area for over 4 years and is now being diagnosed under the impetus of the provincial One Health platform and its local deconcentrations, with the support of VSF-B, MDM/B and ADMR.

Keywords: Spill-over, participatory epidemiosurveilance, livelihood, insecurity, inaccesibility

One Health innovative sampling to understand the ecology and spread of Usutu virusand West Nile virus in the Netherlands, 2016-2022

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Outbreaks of emerging arbovirus infections are increasing worldwide. In Europe, several arboviruses, including West Nile Virus (WNV) and Usutu virus (USUV) show a growing geographical spread and an increase in cases in recent decades. Extensive molecular and serological surveillance of live wild birds, dead birds, chickens and mosquitoes was therefore set up in 2016 to monitor the introduction and spread of a selection of high-risk arboviruses in the Netherlands. In addition, the risk of arbovirus infections for humans (bird ringers) and predators (wild carnivores) in close contact with wild birds, was investigated.

Mortality in captive and wild birds, as well as wild mammals, is reported through a citizen science-based alerting system. A selection of fresh carcasses is further investigated by the Dutch Wildlife Health Centre. Live wild birds are sampled (throat swab, cloaca swab, ticks, feather and serum) in collaboration with bird ringers of the Dutch Centre for Avian Migration and Demography. In addition, a network of mosquito trapping sites was set up in July 2020. Serum from chickens from petting zoos around WNV outbreak locations was also collected. A cross-sectional serological screening of bird ringers was conducted between May and September 2021. Samples were tested for USUV and WNV RNA using RT-PCR and full genome sequencing when positive. Flavivirus antibodies in serum were detected using protein microarray and FRNT.

Endemic Usutu virus circulation was first detected in 2016, with continued enzootic presence in subsequent years. Through phylogenetic analyses, we show co-circulation of USUV lineages Europe 3 and Africa 3, with continued enzootic presence of the USUV lineage Africa 3. Indeed, USUV lineage Africa 3 has also been found during winter, in live wild birds and hibernating mosquitoes. Local West Nile virus (WNV) was found in 2020 in a live Common Whitethroat, followed by detection in other passerine birds, mosquitoes and humans. In 2021, seroconversions in petting zoo chickens showed continued WNV circulation. This was further confirmed in 2022, when West Nile lineage 2 virus was detected in a Grey Heron with a partial sequence closely related to sequences from the 2020 outbreak. Multiple USUV infections in blood donors and in bird ringers, as well as in wild carnivores, show that USUV also infects mammals in the Netherlands, although symptoms appear to be mild or absent.

We show that a One Health framework for research and surveillance of WNV and USUV can serve as early warning for human infections, and provides deeper insights in flavivirus ecology and spread.

Keywords: Usutu virus, West Nile Virus, One Health, zoonotic, surveillance

One Health approach on coronaviruses – Investigating virus diversity in reservoir and sentinel animals

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Coronaviruses (CoV) are a family of RNA viruses that can infect multiple animal species, including humans, with great potential to diversify and cross species barriers. It is believed that 70% of viruses that can infect humans have zoonotic origin. It is therefore not surprising that all known human CoVs (HCoVs) have evolved from animal spillover events, including the three highly pathogenic Severe Acute Respiratory Syndrome (SARS) CoVs that have emerged from bats in the last 20 years. Notably, the most recent SARS-CoV – SARS-CoV-2 – has been shown to cause clinical and unapparent disease, and even mortality in several animals cohabitating with humans (e.g. farmed minks, zoo lions, and snow leopards). However, the data on common cold HCoVs ability to cause disease in animals is scarce and represents an important gap-of-knowledge. Indeed, canine-like recombinant CoVs have been identified as causative agents of several human infections. Considering that all CoVs have reservoirs in different animal species, it is therefore crucial to determine the diversity and transmission dynamics of CoVs in circulation within sentinel/reservoir animals and assess their zoonotic potential. In this study, we aim to identify circulating CoVs in sentinels (pets and farm animals) and reservoir animals (bats and rodents) by targeting species-specific CoVs and HCoVs (including SARS-CoV-2) to assess the spillover potential of CoVs from the human to the animal population. We will also determine if different CoVs circulating in people during the SARS-CoV-2 pandemic may have elicited antibody responses in cohabitating animals at corresponding time points when specific SARS-CoV-2 strains were circulating in the human population. Preliminary results of the prevalence of anti-SARS-CoV-2 neutralising antibodies in sentinel animals – dairy sheep form Portugal – indicate that SARS-CoV-2 virus cross-species transmission was established through contacts between people and sheep during the SARS-CoV-2 pandemic. Neutralisation activity towards the SARS-CoV-2 delta strain (the most similar to the wild type Wuhan strain) was found, while neutralisating antibody escape was observed in all evaluated omicron strains. The fast mutations rates of SARS-CoV-2 and occurrence of new virus variants have been shown to have influenced its transmissibility to different animal species as well. Using animals cohabitating in close contact with humans as sentinels represents great potential for more comprehensive virus surveillence for identifying potential high-risk areas, and informing evidence-based strategies for disease prevention and control.

Keywords: coronavirus, One Health, sentinels, reservoirs, virus diversity

Monitoring of asymptomatic persons exposed to highly pathogenic avian influenza virus in the Netherlands

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Aim: Since autumn 2021 outbreaks of highly pathogenic avian influenza virus (HPAI) A(H5N1) clade 2.3.4.4b occur in poultry and wild birds. Incidentally, wild mammals are infected and mutations related to adaptation to replication in mammalian cells have been found. Worldwide, a few human cases with this clade have been reported. Passive monitoring of symptomatic persons exposed to HPAI already existed in the Netherlands. To monitor transmission of HPAI to humans more closely, we added active monitoring of asymptomatic exposed persons.
Methods: Municipal health services were supplied with sampling kits and are asked to visit locations where an HPAI A(H5N1) outbreak in poultry was confirmed, to introduce the active monitoring to potential participants. Asymptomatic persons who are exposed to poultry infected with HPAI without sufficient protection measures are asked to sample themselves with a throat and nose swab and a finger prick for blood sampling. The nose-throat samples are tested by PCR at the RIVM for influenza virus type A and HPAI A(H5N1) specifically. In case of a positive PCR result, an additional nose-throat sample is obtained and coalescent sera 3-4 weeks post-exposure for serological analyses.
Results: The monitoring started on 13th November 2023. Since then, five HPAI outbreaks in poultry occurred in The Netherlands. From all five outbreaks, a large majority of asymptomatic persons exposed to poultry infected with HPAI A(H5N1) participated in the active monitoring and have sent in samples. All nose and throat swabs tested negative for influenza A virus and HPAI A(H5N1) specifically.
Conclusion: Our results show that active monitoring of highly exposed persons to HPAI using self-sampling in the Netherlands is feasible. Although the risk of human infection with HPAI is currently low, this monitoring helps to follow unexpected transmission of HPAI to humans closely. This contributes to be prepared for a timely response in case of a human infection with HPAI in the Netherlands, which is crucial for pandemic preparedness.

Keywords: Highly pathogenic avian influenza, Influenza A(H5N1), monitoring, self-testing

The relationships between urban green space and the abundance of rats and their zoonotic pathogens

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Urban greening has become an increasingly popular strategy to improve human health in cities. However, urban greening may also influence wildlife living in cities. The effects of urban greening on the abundance of wildlife and the prevalence and diversity of their zoonotic infectious pathogens are not thoroughly investigated. Therefore, we examined the relationships between the amount of urban green space and the abundance of wild brown rats (Rattus norvegicus) and their pathogens.
In our study, we examined 222 systematically trapped brown rats and 185 rats retrieved from pest controllers from three cities in the Netherlands, collected in 2020-2021. We tested them for 18 zoonotic pathogens (Bartonella spp., Leptospira spp., Borrelia spp., Rickettsia spp., Anaplasma phagocytophilum, Neoehrlichia mikurensis, Spiroplasma spp., Streptobacillus moniliformis, Coxiella burnetii, Salmonella spp., methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamase (ESBL)/AmpC-producing Escherichia coli, rat hepatitis E virus (ratHEV), Seoul orthohantavirus, Cowpox virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Toxoplasma gondii and Babesia spp.) with methods consisting of (q)PCR/RT-PCR, enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA) and bacterial culture. Using generalized linear mixed models, we modelled the relationships between pathogen prevalence and diversity and urban greenness. We included both rat-specific and location-specific variables in these pathogen models. The amount of green space per location was calculated using the Normalized Difference Vegetation Index (NDVI).
We observed positive relationships between the relative abundance of rats and both greenness (NDVI) and different proxies for food resources (restaurants, waste items, and petting zoos). We detected 13 different zoonotic pathogens. Rats from greener urban areas had a significantly higher prevalence of Bartonella spp. and Borrelia spp., and a significantly lower prevalence of ESBL/AmpC-producing E. coli and ratHEV. Rat age was positively correlated with pathogen diversity while greenness was not related to pathogen diversity. Additionally, Bartonella spp. occurrence was positively correlated with that of Leptospira spp., Borrelia spp. and Rickettsia spp., and Borrelia spp. occurrence was also positively correlated with that of Rickettsia spp.
Our results show an increased rat-borne zoonotic disease hazard in greener urban areas, which for most pathogens was driven by the increase in rat abundance rather than pathogen prevalence. This highlights the importance of keeping rat densities low and investigating the effects of urban greening on the exposure to zoonotic pathogens in order to make informed decisions and to take appropriate countermeasures preventing zoonotic diseases.

Keywords: brown rats, zoonoses, urban areas, greening, abundance

PhD presentations

Quantifying Antimicrobial Use on medium-scale broiler farms in West-Java, Indonesia

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Introduction: Indonesia has a large broiler industry, marked by extensive antimicrobial use (AMU) according to empirical evidence. However, a good system to collect quantitative data of on-farm AMU is lacking. Accurate quantification is crucial for effective interventions in antimicrobial stewardship (AMS). This study aims to develop a reliable and easy applicable monitoring method for on-farm AMU, and test it in a pilot study across medium-scale Indonesian broiler farms.

Methods: As part of the larger CORNERSTONE project, this study collected baseline AMU data from medium-scale broiler farms in West Java, Indonesia, from 2019 to 2022. Following the initial baseline data collection, additional AMU data were gathered from the same farms over another 4-6 production cycles during an intervention study between 2022 and 2024. Farms were selected through convenience sampling based on size and location. By collecting used antimicrobial product packages and using written log-sheets, the study recorded the amount of antimicrobial used per farm per production cycle (averaging 30 days). Following the AACTING guideline, "Quantification of veterinary antimicrobial usage at herd level and analysis, communication, and benchmarking to improve responsible usage," the study analyzed the most suitable AMU indicator for the farms.

Results: The baseline study covered 98 production cycles on 19 farms (4-6 cycles per farm). Dosages of the same antimicrobial varied significantly between farms, e.g., enrofloxacin, where dosages ranged from 0.0017 mg/kg to 203 mg/kg live weight. On average, broilers were exposed to 10 days of antimicrobial treatment per production cycle. Of the applied antimicrobials, 60.8% belonged to the Highest Priority Critically Important Antimicrobials (HPCIAs). Different AMU indicators (dose-based, treatment frequency-based, or mass-based) resulted in varying farm rankings based on AMU levels. In the second data collection round (intervention study), a 10% reduction in HPCIAs was observed using the AMU indicator "Number of Treatment Days."

Conclusion: The greatly varying dosages that were used per farm could lead to considerable over- or under-estimations of AMU per production cycle. The wide variation might be due to the mostly lacking (veterinary) professional oversight. Quantifying AMU using the Number of Treatment Days seems most suitable for a setting comparable to medium-scale broiler farms in Indonesia. Our findings of reduction in HPCIA highlights the importance of quantitative data, to observe trends and enables benchmarking. This supports antimicrobial stewardship on farms, ultimately resulting in reduction of antimicrobial resistance.

Keywords: Antimicrobial-Resistance (AMR), (veterinary) antimicrobial-use, poultry, Indonesia, AntimicrobialStewardship (AMS)

Identification of a novel immune suppression target for MD vaccine development

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Keywords: MDV, LORF1, herpesvirus, immune suppression, vaccine development

Reduction and Genetic Variability of Campylobacter in Free-Range Broilers Using Feed and Water Additives in a Production Setting

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Aim: This study investigated the efficacy of selected feed and water additives in reducing Campylobacter contamination in broilers' caeca within a commercial free-range farm and investigated genetic strain variations of Campylobacter isolates.
Methods: Inside a Danish commercial free-range farm in 2022, broilers were divided into five groups and given a standard diet (control); feed supplemented with 2% biochar (Charcoal Feed Granules for Poultry); feed added supplemented with 0.125% probiotic-like product (Saccharomyces cerevisiae fermentate, Original XPC™); feed supplemented with 15% oat hulls; drinking water supplemented with 0.2% organic acid (Selko®-pH E). Each group comprised 28 broilers in separate cages within the farmhouse that accommodated 6,000 birds with access to an outdoor area from around day 36. Half of the broilers were slaughtered on day 36, and the remaining were slaughtered on day 59. Air, floor, and water samples were investigated for the presence of Campylobacter, while caecum samples were evaluated quantitatively. The experiment was repeated thrice, and 47 Campylobacter isolates were whole-genome-sequenced (WGS).
Results: All broilers were found to be naturally infected with Campylobacter at both slaughter time points across the rotations. Campylobacter was consistently detected in air samples between days 22 and 33 before outdoor access. In three flocks fed Biochar and oat hulls, Campylobacter in caecum was reduced on day 59. The study identified five distinct Campylobacter sequence types (STs) in chickens, including two newly discovered types (ST12339 and ST12340). ST52 was also found; this ST was also isolated from most humans recorded with campylobacteriosis in Denmark in the same period. Additionally, ST45 and ST475 were found to be prevalent. No specific pattern of STs was associated with the tested groups, but the predominant ST differed between slaughter days, before access outdoors, and within the same rotation; furthermore, two different STs were isolated from one caecum sample (ST45 and ST52).
Conclusions: Under field conditions, the biochar and oat hulls exhibited promising potential in mitigating Campylobacter contamination in free-range broilers. Moreover, the observed genetic variability of Campylobacter within the same rotation and group of chickens highlights that the ST isolated from cloacal swabs at slaughter is not necessarily the only ST present in the flock. However, further research is imperative to verify the efficacy of these additives.

Keywords: Food-borne, Zoonosis, Intervention, Campylobacter, WGS

A critical role for host-derived cystathionine-β-synthase in Staphylococcus aureus-induced udder infection

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Cystathionine-β-synthase (CBS) catalyzes the first step of the transsulfuration pathway. The role of host-derived CBS in Staphylococcus aureus (S. aureus)-induced udder infection remains elusive. Herein, we report that S. aureus infection enhances the expression of CBS in mammary epithelial cells in vitro and in vivo. A negative correlation is present between the expression of CBS and inflammation after employing a pharmacological inhibitor/agonist of CBS. In addition, CBS achieves a fine balance between eliciting sufficient protective innate immunity and preventing excessive damage to cells and tissues preserving the integrity of the blood-milk barrier (BMB). CBS/H2S reduces bacterial load by promoting the generation of antibacterial substances (ROS, RNS) and inhibiting apoptosis, as opposed to relying solely on intense inflammatory reactions. Conversely, H2S donors alleviate inflammation via S-sulfhydrating HuR. Finally, CBS/H2S promotes the expression of Abcb1b, which in turn strengthens the integrity of the BMB. The study described herein demonstrates the importance of CBS in regulating the mammary immune response to S. aureus. Increased CBS in udder tissue modulates excessive inflammation, which suggests a novel target for drug development in the battle against S. aureus and other infections.

Keywords: Staphylococcus aureus; cystathionine-β-synthase; udder infection; blood–milk barrier

Mapping the highly pathogenic avian influenza landscape using mathematical models for cell culture dynamics

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Problem statement: Highly pathogenic avian influenza (HPAI) H5-viruses are circulating in wild birds and are repeatedly introduced from wild birds to poultry. The H5N1 clade 2.3.4.4b virus caused the largest outbreak ever recorded in Europe in 2021-2022. HPAI viruses can rapidly alter their virulence by reassortment or mutations. Viruses causing only mild disease in poultry may remain undetected, increasing the risk of virus spread to other farms, wild birds and mammals. Animal experiments are used to identify virulence for each host species which limits the amount of HPAI variants that can be monitored. Mammalian in vitro models have been used extensively to characterize human influenza viruses however avian in vitro models are still lacking [1]. Therefore, we have established an avian in vitro model to characterize HPAI viruses which can be easily adapted to other species including mammals.

Method: The amount of infectious virus particles and amount of living cells is measured over time on chicken embryonic fibroblasts and duck embryonic fibroblasts for seven Dutch HPAI index cases (H5N8-2014, H5N8-2016, H5N6-2017, H5N8-2020, H5N1-2020-C, H5N1-2021-AC, H5N1-2021-AB) and one wild bird isolate (H5N1-2022-BB). To describe the population dynamics of the chicken and duck cells and the different HPAI viruses, we used a simple ordinary differential equation (ODE) model that divides the cell population in susceptible (S), infectious (I) and dead (D) compartments. The transmission rate and infectious period were calculated for each virus and cell type combination.

Results & Conclusions: Interestingly, the H5N1 viruses showed high transmission rates and long infectious periods compared to the other HPAI viruses. The highest transmission rate was observed for the H5N1-BB genotype. This genotype was mostly circulating in charadriiforms species and caused a limited number of outbreaks in poultry [2]. Furthermore, transmission rates and infectious periods were in general longer on duck cells then on chickens cells. Dependent on the virus, mortality in chickens usually occurs within 2-3 days while mortality in ducks occurs after 4-5 days or can even be absent for some HPAI viruses. This results in a lower transmission rate and infectious period for chickens compared to ducks similar to the output of the in vitro model. This indicates the potential of in vitro models to predict species specific transmission rates which can be useful for risk assessment of HPAI in poultry. Finally, we are expanding the in vitro model to human and porcine cells and are developing methods for high-throughput characterization of HPAI viruses.

Keywords: HPAI, in vitro models, mathematical models, virus characterization, risk assessment

Fast scalable diagnostics for the detection of avian influenza

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For the early warning toolbox for pandemic preparedness and response, rapid, at-location and cost-effective diagnostic tools are of paramount importance. These tools can, amongst others, be applied for the detection of possible zoonotic viruses, in or around the agri-food system for early identification of hotspots for spill-over.
Within the ERRAZE (Early Recognition and Rapid Action in Zoonotic Emergencies) program of WUR rapid diagnostic assays using reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) were developed for the specific detection of genetic material of avian influenza (AI) subtypes in swab samples (trachea and cloaca) from infected birds. Additionally, a diagnostic pipeline was designed for future rapid design and production of new rapid, at-location molecular tools to monitor a broad range of zoonotic viruses.

Keywords: RT-LAMP, Avian Influenza, at-location diagnostics, early identification

A species-independent lateral flow microarray immunoassay to detect WNV and USUV NS1-specific antibodies in serum

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Objective: Arboviruses such as West Nile Virus (WNV) and Usutu Virus (USUV) are emerging pathogens that circulate between mosquitoes and birds, occasionally spilling over into humans and horses. Current serological screening methods require access to a well-equipped laboratory and are not currently available for on-site analysis. As a proof of concept, we propose here a species-independent lateral flow microarray immunoassay (LMIA) able to quickly detect and distinguish between WNV Non-Structural 1 (NS1) and USUV NS1-specific antibodies. Method: A double antigen approach was incorporated into the LMIA whereby WNV NS1 and USUV NS1 antigens were used as both capture and detection antigens. Firstly, the LMIA was optimized with respect to both the concentration of capture antigen spotted on the LMIA membrane and the amount of detection antigen conjugated to detector particles. Optimized LMIAs were then used to test a larger serum panel and results were compared to other serological methods. All tested sera were collected from humans, horses, European jackdaws (Corvus monedula), and common blackbirds (Turdus merula) and confirmed to be positive or negative for WNV and USUV antibodies. Using a Receiver Operating Characteristics (ROC) curve, WNV and USUV LMIA results were compared to "gold standard" WNV or USUV neutralization tests, respectively. Results: Optimization of the concentration of capture antigen and the amount of detection antigen conjugated to detector particles indicated that maximizing both parameters increased assay sensitivity. Upon screening of a larger serum panel, the optimised LMIA showed significantly higher spot intensity for a homologous binding event than for a heterologous binding event. Using a ROC curve, WNV NS1 LMIA results in humans, horses, and C. monedula showed good correlation when compared to "gold standard" WNV FRNT90. The most optimal derived sensitivity and specificity of the WNV NS1 LMIA relative to corresponding WNV FRNT90-confirmed sera were determined to be 96% and 86%, respectively. Although the sample size was smaller, USUV NS1 LMIA results in T. merula also showed good correlation when compared to USUV FRNT90. Conclusions: While further optimization is required, this study demonstrates the feasibility of developing a species independent LMIA for on-site analysis of WNV, USUV, and other arboviruses. Such a tool would be useful for the on-site screening and monitoring of arboviruses in relevant species found in the Netherlands and in more remote and/or low-income regions around the world.

Keywords: Arboviruses, Rapid Diagnostics, One Health, Lateral Flow Assay (LFA), Serology

Association between antimicrobial usage and resistance in livestock and antimicrobial resistance among human Salmonella, Campylobacter and E. coli infections

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Problem statement and aims: Antimicrobial usage (AMU) promotes antimicrobial resistance (AMR) in bacterial populations. The Netherlands has substantially reduced AMU in livestock since 2009; however, it is not clear whether this reduction has influenced AMR levels among human infections. Here, we assessed the associations between AMU and AMR in livestock, and AMR in human infections caused by non-typhoidal Salmonella and Campylobacter (both zoonotic pathogens), and Escherichia coli from urinary tract infections (UTI).

Methods: An ecological registry-based study was conducted using AMU and AMR data in livestock and human infections from various Dutch national surveillance programmes. Study periods were selected according to the availability of data in each pathogen. Associations were studied per homologous antimicrobial-class, using multivariable logistic regression and correlation analysis. For Salmonella and Campylobacter, use of other antimicrobials in livestock was accounted for potential co-selection. Known travel-related cases were excluded if data was available. For Salmonella, the most common serotypes (S. Enteritidis and S. Typhimurium and its monophasic variant) were included, while for Campylobacter, the species C. jejuni/coli were included. Associations were studied per animal reservoir, serotype or species. For E. coli, only isolates from UTI samples in primary care were analysed, and besides use of other antimicrobials in livestock, age, gender, and AMU in humans were accounted for. In all pathogens, a one-year lag was explored to determine relations between distinct populations.

Results: For Salmonella, mainly positive associations were found between livestock AMU and human AMR Typhimurium/monophasic-variant infections, and between AMU and AMR among Typhimurium/monophasic-variant isolates from broilers/pigs. No significant correlations were found between AMR in broiler/pig and human isolates. For Campylobacter, AMU in livestock was inversely associated with AMR among human infections. No significant associations were observed between AMU and AMR among broiler Campylobacter isolates, while positive correlations were found between AMR in broiler and human isolates. For E. coli, several positive/negative associations between livestock AMU and human AMR were observed, but associations were generally stronger within human or animal populations.

Conclusions: Overall, the association between AMU in livestock and AMR in human zoonotic infections is ambiguous and varies according to pathogen, subtype, and animal reservoir. Confounding concerns are human infections resulting from imported meat and travel. The inverse association observed for Campylobacter stresses that reducing AMR may not be enough to tackle increasing AMR in humans. The marginal association between livestock AMU and human AMR in E. coli UTIs provides further evidence that the zoonotic spread of E. coli causing UTIs is limited.

Keywords: Antimicrobials, salmonellosis, campylobacteriosis, Escherichia coli, One Health.

Publication Trends, Research, and Policy Needs Regarding the One Health Approach and Vector-Borne Diseases (VBDs): A Systematic Review.

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Introduction: Vector-borne diseases (VBDs) have a significant global health impact, causing a wide variety of diseases that impacts both humans and animals. In addition, disease transmission is closely linked to the environment. The One Health approach encourages interdisciplinary collaboration between sectors. Here, we performed a systematic review to analyse trends in VBD research in relation to the One Health approach since 2000, to summarize directions for the research agenda, and address challenges and opportunities in One Health policy in the VBD space. Furthermore, an open-access database was constructed for further research in this field.
Methods: A comprehensive search of online databases (i.e., PubMed, Scopus) was conducted to identify relevant studies published between 2000 and 2023. Eligible studies were screened and selected based on inclusion and exclusion criteria (e.g., at least two pillars of the One Health approach). Out of 30,000 screened articles, a total of 13,000 articles met the inclusion criteria. Variables included were type of VBD, VBD research topics, geographical distribution, and study methodologies/interventions.
Results: A significant increase in publications of One Health articles was identified between 2000-2023. We found an increasing number of One Health publications on generating evidence on innovative strategies to reduce population resilience and surveillance against VBDs. Furthermore, increased trends in the inclusion of the environment as a pillar in VBD have taken place. A major challenge identified by the review is unstructured communication, collaboration, and coordination of knowledge between experts, as well as finding funding for overarching One Health programmes.
Conclusion: This systematic review provides a comprehensive overview of trends in VBD research and the application of the One Health approach over the past two decades. The findings underscore the evolving landscape of VBDs and the importance of interdisciplinary collaboration in addressing these complex health challenges.

Keywords: Vector-Borne Diseases, One-Health Approach, Anthropod

Long-read shotgun metagenomics as a One Health tool in support of the quality control of pharmaceutical products

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Aim: Non-sterile water is used for dilution or reconstitution of orinasal and cutaneous pharmaceutical preparations. Currently, following the European Pharmacopeia (Ph. Eur.), for quality control, solely aerobic culture-based plating methods for determination of colony forming units (CFU) are used, with a 200 CFU/ml threshold for compliance, without obligation to identify the bacteria encountered. Therefore, potential (opportunistic) pathogens and their antimicrobial resistance genes (ARG) will largely remain elusive. Shotgun metagenomics allows sequencing of all genetic information within a sample enabling the detection and identification of any microorganism and their ARG. Despite this technology already proving its added-value in many One Health fields, this was not yet the case for the quality control of medicinal products, which was the aim of this study.
Materials and methods: First, a defined mock community containing five species with ARG was spiked in PBS at 200 CFU/mL in total, and two sampling methods were tested, i.e. centrifugation and filtration. qPCR was used to verify the presence of the spiked species in the DNA extracts. Extracted DNA was analysed using long-read sequencing, followed by KMA for taxonomic and ARG identification, and potential linking of the ARG to its host. Next, this workflow was tested on five water samples collected in the context of the official monitoring programme (2 conform and 3 non-conform) and compared to the conventional Ph. Eur. methodology. Additional identification of the CFU by MALDI-TOF was performed.
Results: The spiked samples used for the workflow development fell within the expected CFU/mL count. All spiked species were detected by qPCR. Taxonomic analysis of the long-read sequences identified all 5 spiked species, regardless of the sampling method used. With centrifugation more ARG were detected and possible ARG-host links were correctly identified. Subsequently, the workflow with centrifugation was tested on five water samples, identifying microorganisms in all samples. If MALDI-TOF could identify the CFU (which was not the case for one non-conform sample), the metagenomic-based results corresponded. Several ARG were identified, which are currently under investigation.
Conclusion: The added-value of the developed metagenomics workflow comprises identification of bacterial species, even at low abundance, and when not identified with MALDI-TOF after culture, characterization of ARG and linking these with their host. Therefore, it can be a complementary tool to the Ph. Eur. and used to motivate manufacturers to be alert for unwanted bacteria that might colonize their water purification systems, minimize product recalls, and consequently protect the consumer's health.

Keywords: Metagenomics, bioburden testing, AMR and pathogens detection, monitoring medicinal products

A pan-orthohantavirus preclinical human lung xenograft mouse model

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Orthohantaviruses are emerging zoonotic viruses that can infect humans via the respiratory tract. There is a clear unmet need for an in vivo model to study orthohantavirus infection in physiologically relevant tissue and to determine the broad reactivity of novel orthohantavirus countermeasures. Here, we describe the use of a human lung xenograft mouse model to study the permissiveness for different orthohantavirus species and to assess its utility for preclinical testing of pan-orthohantavirus therapeutics. Following infection of xenografted human lung tissues, distinct orthohantavirus species differentially replicated in the human lung and subsequently spread systemically. The different orthohantaviruses primarily targeted the endothelium, respiratory epithelium and macrophages. Treatment of these mice with a virus neutralizing antibody could block orthohantavirus infection and dissemination. This model will facilitate progress in the fundamental understanding of pathogenesis and virus-host interactions for orthohantaviruses. Furthermore, it is an invaluable tool for preclinical evaluation of novel candidate pan-orthohantavirus intervention strategies.

Keywords: orthohantavirus, xenograft, preclinical, in vivo model, pan-orthohantavirus therapeutics

Pathogen monitoring in Konik horses in a nature reserve in the Netherlands

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Surveillance of infectious pathogens is crucial to control the health of domestic animals, wildlife, and humans. Active disease surveillance systems are usually used for livestock, while, for wild and free-living animals passive surveillance is the most common. Surveillance of these animal populations is valuable, as it contributes to determine the presence of pathogens that are important for both veterinary and public health. At the Oostvaardersplassen (OVP) nature reserve in the Netherlands, the health and pathogen presence of free-living large herbivores have been actively monitored since the late 1990s. Our study aimed to estimate seroprevalence of pathogens in free-living Konik horses and evaluate the current monitoring system at OVP.

We used data obtained from yearly serological surveys (2011 to 2023) and faecal analyses (1999 to 2023) of Konik horses at OVP for 13 pathogens. Animals were sampled during annual culling for health monitoring and population control. Initial sample sizes were estimated based on a 25% design prevalence (DP). Yearly apparent seroprevalences (AP) were estimated. Probability of freedom from infection was estimated if all sampled animals tested negative.

The findings indicate presence of Equine herpesvirus 1 and 4 (EHV-1 and EHV-4), and bacteria Salmonella enterica serovars Dublin (SDu) and Typhimurium (STy). EHV-1 was detected in 1 horse in 2012 (AP 16%, [95% CI: 3-56]) and EHV-4 in 2 horses in 2017 (AP 33% [95% CI 9-70]). Due to insufficient sample sizes between 2012 and 2018 it was not possible to determine freedom from infection for these pathogens. Variable AP was observed for SDu (0 to 75%) and STy (0 to 25%). Equine Infectious Anaemia Virus (EIAV), West Nile Virus (WNV) and Equine Influenza (H3N8) were tested only in 2012, 2013, 2016 and 2018, and not detected. However, for all these pathogens sample size was insufficient to determine freedom of infection. Strongyloides westeri, Parascaris spp., and Strongyle-type eggs were observed in all examined faecal samples, while Oxyuris spp., Paranoplocephala spp. and Anoplocephala spp. were not observed.

This study offers insights into the pathogens present in Konik horses at OVP, which may be relevant to other natural areas. Due to the limited sample size, it is recommended that the number of animals sampled should be updated. Additionally, in the context of One Health and surveillance, it may be beneficial to re-introduce testing for certain pathogens into the monitoring system, such as WNV, utilizing horses in the area as possible sentinel species for emerging pathogens.

Keywords: monitoring, surveillance, animal health, free-living konik horses, nature areas, Oostvaardersplassen

Drivers of infection with Toxoplasma gondii genotype type II in Eurasian red squirrels (Sciurus vulgaris)

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Background: In September 2014, there was sudden upsurge in the number of Eurasian red squirrels (Sciurus vulgaris) found dead in the Netherlands. High infection levels with the parasite Toxoplasma gondii were demonstrated, but it was unclear what had caused this increase in cases of fatal toxoplasmosis. In the present study, we aimed to gain more knowledge on the pathology and prevalence of T. gondii infections in Eurasian red squirrels in the Netherlands, on the T. gondii genotypes present, and on the determinants of the spatiotemporal variability in these T. gondii infections. The presence of the closely related parasite Hammondia hammondi was also determined.
Methods: Eurasian red squirrels that were found dead in the wild or that had died in wildlife rescue centres in the Netherlands over a period of seven years (2014–2020) were examined. Quantitative real-time polymerase chain reaction was conducted to analyse tissue samples for the presence of T. gondii and H. hammondi DNA. Toxoplasma gondii-positive samples were subjected to microsatellite typing and cluster analysis. A mixed logistic regression was used to identify climatic and other environmental predictors of T. gondii infection in the squirrels.
Results: A total of 178 squirrels were examined (49/178 T. gondii positive, 5/178 H. hammondi positive). Inflammation of multiple organs was the cause of death in 29 squirrels, of which 24 were also T. gondii polymerase chain reaction positive. Toxoplasma gondii infection was positively associated with pneumonia and hepatitis. Microsatellite typing revealed only T. gondii type II alleles. Toxoplasma gondii infection rates showed a positive correlation with the number of days of heavy rainfall in the previous 12 months. Conversely, they showed a negative association with the number of hot days within the 2-week period preceding the sampling date, as well as with the percentage of deciduous forest cover at the sampling site.
Conclusions: Toxoplasma gondii infection in the squirrels appeared to pose a significant risk of acute mortality. The T. gondii genotype detected in this study is commonly found across Europe. The reasons for the unusually high infection rates and severe symptoms of these squirrels from the Netherlands remain unclear. The prevalence of T. gondii in the deceased squirrels was linked to specific environmental factors. However, whether the increase in the number of dead squirrels indicated a higher environmental contamination with T. gondii oocysts has yet to be established.

Keywords: Toxoplasmosis, zoonoses, parasite, oocyst, sentinel, monitoring, squirrel, microsatellite typing

Factors associated with zoonotic literacy across socio-demographic groups

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Introduction: Zoonotic literacy is the knowledge, awareness and behavior of civilians regarding zoonoses. It enables people to prevent and act upon zoonotic infections. Identifying sociodemographic factors associated with zoonotic literacy provides targets for interventions.
Objective: The aim of the study is to identify factors that are significantly associated with zoonotic literacy across socio-demographic groups in order to improve knowledge, awareness and zoonotic risk-averse behaviors.
Method: Data was collected in 2022 using an online survey of a nationally representative sample of residents in the Netherlands. A multivariable logistic regression analysis, accounting for multiple hypothesis testing, was applied to assess whether there were significant associations between socio-demographic factors and attitudes towards zoonosis prevention.
Results: A total of 2,039 respondents completed the survey. Men, people with an educational level of primary education and pre-vocational secondary education [VMBO], people with limited language and computer skills and immunocompromised people reported significantly more risky behaviors. No significant associations were found for pregnant women, dog and cat owners, those with an intermediate level of education and those who do have contact with farm animals.
Conclusion: Certain sociodemographic groups display significantly riskier attitudes towards zoonoses. In order to improve the population's ability to prevent zoonotic infections, these groups could be considered as target groups to enhance zoonotic literacy.

Keywords: Zoonotic literacy, Risk-prevention, Health literacy, One Health

Antimicrobial use in Dutch pig farms: risk factors and associated diseases

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Objectives: Antimicrobial use (AMU) has decreased significantly in Dutch pig farms since 2009. However, this decrease has stagnated in recent years, with relatively high AMU levels still persisting during the weaning phase. Focusing on weaners, this study aimed at investigating which diseases are associated with i) overall AMU, ii) use/not use, and iii) total consumption of specific antimicrobial classes. Moreover, we investigated which farm characteristics were associated with iv) those diseases requiring AMU, v) total AMU and vi) use of specific antimicrobial classes.
Method: In 2020, cross-sectional data from 154 Dutch pig farms were collected, including information on AMU, disease aetiologies for group treatments and farm characteristics. Associations between disease occurrence and AMU (overall and per class) were assessed using multivariable generalized linear regression models. Subsequently, mixed-effects conditional Random Forest analysis was used along with the "binomialRF" algorithm for variable selection to identify farm characteristics correlated with each relative disease and AMU outcome independently.
Results: Group treatments for respiratory and musculoskeletal/neurological diseases (MNDs) along with individual treatments in weaners were significantly associated with their total AMU. Tetracyclines and penicillins were most frequently used to treat the former two aetiologies respectively. Main farm characteristics that overlapped between the disease and AMU models were: PRRS vaccination in sucklings (risk for respiratory conditions and total AMU), having fully slatted floor in weaners (risk for MNDs, total AMU and use of macrolides-lincosamides), longer lactation period (protective against MNDs, respiratory conditions, total AMU, use of tetracyclines and trimethoprim-sulphanamides and their quantities and use of penicillins). In addition, being a biological farm protected against use of specific antimicrobial classes as well as incidence of MNDs. The same was found for free-sow systems during lactation.
Conclusions: Respiratory conditions and MNDs in weaners are currently the main culprits of AMU in Dutch pig farms and their control should be prioritized by targeting farm characteristics that improve biosecurity and animal welfare. The non-parametric nature of the risk models developed here provides opportunities for developing tailor-made interventions for a given farm that seeks to reduce AMU. Furthermore, factors overlapping between disease and AMU models are candidates for future mediation analysis to broaden our understanding of the causal pathways involved.

Keywords: Epidemiology , Control measures, Antibiotics, Animal welfare, Biosecurity

A comparative analysis of the dendritic cell response upon exposure to different rabies virus strains

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Objectives: Rabies is a zoonosis most commonly transmitted by the bite of an infected dog. It is considered a neglected tropical disease, accounting for approximately 60.000 annual deaths, most of which occur in impoverished communities. While current post-exposure prophylaxis can reliably prevent the disease, it is often not available to dog bite victims in these areas. Particularly striking about natural rabies virus (RABV) infections is the fatality rate of nearly 100 % once neurological symptoms arise. The observed lack of immune response is considered to play an important role, but the processes responsible for this phenomenon remain poorly understood. Our hypothesis is that RABV fails to activate dendritic cells (DCs), preventing the initiation of a robust adaptive immune response.
Methods: We exposed human monocyte-derived dendritic cells (moDCs) in vitro to two highly pathogenic street RABV strains (silver-haired bat RABV (SHBRV) and dog bite-associated RABV (dogRV)), as well as the attenuated vaccine-based RABV strain SAD-P5/88 Potsdam (SAD-P5). The latter strain was included, since previous studies indicate that attenuated strains cause stronger immune activation compared to pathogenic strains.
Results: A low percentage of moDCs was found susceptible to SHBRV and SAD-P5, but not dogRV. In line with susceptibility to infection, only moDCs exposed to SHBRV and SAD-P5 showed a slightly more activated and migratory phenotype than mock-exposed moDCs. However, this was only observed at a high MOI of 5, and did not result in greater T cell proliferation in co-culture experiments. To investigate whether RABV-exposed moDCs were actively suppressed, we stimulated them with LPS following exposure to virus. Interestingly, moDCs exposed to SHBRV and SAD-P5 at high MOIs showed increased activation following LPS stimulation when compared to mock-exposed moDCs. The LPS-induced activation of RABV-exposed moDCS was not impaired, suggesting that exposure to RABV does not actively suppress the moDC response.
Conclusions: Overall, our data indicate that in vitro RABV exposure of moDCs does not induce an antiviral state. Since DC activation is crucial for initiating an adaptive immune response, it will be of interest to further investigate if the activation of specific DC populations upon RABV infection can play a role in novel post-exposure prophylaxis strategies.

Keywords: Zoonoses, Rabies, Neglected Tropical Diseases, Antiviral Immunity, Immune Evasion

Lateral genetic transfer driving co-evolutionary interactions of phages of probable industrial importance and their hosts across the Escherichia, Listeria, Salmonella, Campylobacter, and Staphylococcus Domains

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The utility of different bacteriophage (phage) formulations has been a particular focus for alternative human and animal therapeutic measures, as well as for food safety and environmental safety practices, combatting the growing crisis caused by antimicrobial resistance. In this light, for health and environmental safety precautions, understanding the role of lateral genetic transfer (LGT) in the co-evolutionary interactions of phages of probable industrial importance and their hosts is essential to rationalizing especially those phage applications that extend to microbially rich environments. In this in silico study, we provide strong evidence for LGT-driven co-evolutionary interactions between the host species and specific virulent versus temperate phages, both considered elsewhere to be potentially effective therapeutic or biocontrol agents. In the in silico experiments, we applied a large array of genetic recombination detection algorithms, embedded in the SplitsTree and RDP4 software packages, to detect and elucidate LGT between multiple Escherichia, Listeria, Salmonella, Campylobacter, and Staphylococcus phages and their hosts' prophages. PHASTER and RAST were applied to identify prophages across their host genomes and to annotate LGT-affected genes with unknown functions respectively. In addition, PhageAI was applied to gain deeper insights into the lifestyle history of the recombined phages identified. The inferences (bootstrap values: 91.3-100; fit: 91.433-100) obtained from the split decomposition analyses, coupled with the Phi test-resulted probability estimates ($p = 0.0$-$2.836E$-12), and the RDP4-generated statistics ($P \leq 0.05$), exhibited collectively strong evidence for LGT events between these phages and their host species prophages. In the Escherichia, Listeria, Salmonella, and Campylobacter virulent phages and their host species, LGT involved mainly the several phage genes linked to the synthesis of hypothetical proteins. For certain L. monocytogenes strains, specific Listeria virulent phages appeared to have served at least as the donors of the gene encoding for basal promoter specificity factor. The LGT events, involving the Staphylococcus temperate phages and S. aureus prophages, were determined to have affected the large genetic clusters encompassing multiple phage genes associated with this species morphogenesis, infectivity, virulence, and specificity to different hosts. Our study strongly suggests that LGT-driven phage-prophage interactions, occurring across the Escherichia, Listeria, Salmonella, Campylobacter, and Staphylococcus domains, can sometimes lead to unprecedented trajectories of the co-evolution of both industrial phages and their hosts especially in microbially rich environments.

Keywords: lateral genetic transfer, antimicrobial resistance, bacteriophage, phage-prophage interactions

Prevalence and Characterization of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Dogs Admitted to a Veterinary Hospital in Vienna

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Extended-Spectrum β-Lactamase (ESBL)- producing Enterobacteriaceae are classified by the World Health Organization (WHO) as pathogens of critical priority for development of new antibiotics because of their clinical importance. Companion animals may act as reservoirs of ESBLs, posing potential risks of infection for humans and animals. To assess the situation in Vienna, 88 faecal samples from dogs visiting the small animal hospital at the University of Veterinary Medicine of Vienna were collected. The samples were streaked on selective media containing 2 µg/ml cefotaxime. Species identification was carried out using MALDI-TOF. The isolates were tested for phenotypic resistance via the combination disc test according to EUCAST standards. An antibiotic disc containing meropenem was added to screen for resistance to carbapenems. VITEK® 2 was used to verify disc diffusion results and screen for other phenotypic resistances. Whole genome short-read sequencing was performed to identify sequence types and screen for antibiotic resistance genes as well as virulence determinants. Among the 88 samples, 13 isolates of ESBL-producing E. coli were isolated (prevalence of 14.8 % (95% CI = [8.1 - 23.9]). The β-lactam antibiotic resistance genes identified most often were blaCTX-M-1 and blaCTX-M-15. Multiple other phenotypic and genotypic AMR were discovered, including resistances against quinolones, aminoglycosides and amphenicols. No carbapenem resistance was detected. 54 % of ESBL isolates were classified genotypically as multi-drug resistant. Virulence profiling revealed over 160 different virulence factors that included adherence, iron/heme uptake and exotoxins. Three extraintestinal pathogenic E. coli (ExPEC) isolates belonging to the sequence types ST38, ST131 and ST141 were identified, the latter two of them carrying virulence genes encoding exotoxins including hemolysin. This study demonstrates the concerning situation of AMR among companion animals, with dogs potentially acting as transmission sources for humans, other animals and the environment. Antimicrobial stewardship, One Health surveillance programs and further scientific research are required to combat the progression of this global crisis.

Keywords: antimicrobial resistance, companion animals, phenotypic resistance, WGS, virulenc

Poster abstracts

Poster abstracts: Making One Health happen: designing suitable legal, governance and policy frameworks

A curricula review of global one-health graduate programmes

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Aim:
This study examines 43 One Health (OH) graduate programmes offered worldwide and highlights the uniformity and variety therein. Due to the recent global prominence of the OH approach, efforts are underway to develop mechanisms and operationalize collaboration between humans, animals, plants, and environmental health systems. One of such is the mushrooming graduate programmes training the future OH workforce. Evidence from the few localized studies that have analysed the curricula of these programmes indicates thematic inconsistencies and underrepresentation. The present study, therefore, provides insights into the nature of OH graduate education to inform appropriate policy frameworks and decisions.
Method:
From December 2022 through April 2023, the following terms ‘ONE HEALTH MASTERS COURSES’, and ‘ONE HEALTH MASTERS PROGRAMMES’ with variations of ‘AFRICA’, ‘NORTH AMERICA’, ‘ASIA’, ‘AUSTRALIA’, ‘EUROPE’, ‘GLOBAL’ were searched via Google and Bing. Data on programme title, curricula contents, years to completion, host universities, country and continent were collated, and programmes without access to these details were excluded. An exception was made to include two bachelors degrees programmes.
Results:
Forty-three programmes satisfied all criteria to be included in this study. Contents of curricula clustered into 12 sub-divisions including ‘principles and concepts of OH’ (100%), ‘epidemiology and biostatistics’ (91%), ‘major branches of OH’ (86%), ‘internship/externship/research project’ (81%), ‘infectious diseases, zoonoses, and surveillance’ (84%), ‘risk analysis and crises management’ (58%), ‘food safety (39%), microbiology, immunology, and allied’ (39%), ‘communication’ (35%), ‘ethics’ (30%), ‘economics, policy, and management’ (58%) and ‘others (58%). Infectious disease, public/global health (84%), veterinary institutions (48%), North America, and Europe (81%) are the most represented theme, institution and region respectively. Varying mode of delivery, time to completion and thematic focus demonstrated heterogeneity of the analyzed programmes. Though the dominance of infectious diseases and public/global health in the curricula largely unified these programmes, aligning the curricula content with the OH core competencies was challenging.
Conclusions:
Global OH graduate programmes are heterogeneous, and the central theme of infectious diseases and public/global health was the chief basis for their uniformity, rather than the OH core competencies. Though this ensures interdisciplinary cross-talks, it inadvertently sidelines other non-infectious diseases professionals. Therefore, thorough pedagogical analysis and curricula mapping are required to examine and inform how these programmes align with the OH core competencies. This also calls for policy and legal frameworks to standardize OH education and trainings and ensure a degree of uniformity in the global OH workforce.

Keywords: One Health, Education, Master programme, Curricula, Core competencies

The GIFTS-AMR global research agenda - Strategies & natural medicinal products to prevent and treat infections to reduce antimicrobial resistance with the primary focus on health promotion

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Aim/Objectives
To describe the global research agenda on Traditional, Complementary & Integrative Healthcare (TCIH) strategies & natural medicinal products to prevent and treat infections and reduce antimicrobial resistance (AMR) primarily focusing on health/resilience promotion. In addition, to describe the connection of this agenda to two global AMR research agendas and suggested advocacy actions.
Approach/Method
The global research agenda was developed by the GIFTS-AMR (Global Initiative for Traditional Solutions to Antimicrobial Resistance) project group, a JPIAMR 10th call funded, globally organized, growing network of TCIH and AMR/infectious diseases research institutes, researchers in both human and veterinary medicine and global/regional policymakers.
Results
Four GIFTS-AMR research themes linked to 14 research priorities, prioritized research projects for the next 10 years and contributions to two global AMR research agendas are presented in a table. https://www.b2match.com/e/research-on-tcih-strategies/components/34631/biYz4tsQRpZA
Conclusion
The research agenda provides the strategic basis for the GIFTS-AMR network to collaborate on research proposals and funding applications and provides information on TCIH for policymakers, researchers, healthcare professionals (organizations) and patients (organizations) at national, regional and global level.

Keywords: One health, complementary medicine, integrative medicine, antimicrobial resistance, resilience

Integrating One health into public policies: a One health policy screening tool

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To enhance policy actions and health management with regard to the cumulative impacts across environmental, animal and human health, many potential trade-offs should be taken into account when prioritizing one action over another. Also, there are specific requirements for integrative and cross-sectoral as well as multilevel governance with respect to cross-disciplinary capacity, stakeholder acceptance and other implementation conditions.

Aims

To develop a One Health policy screening tool providing an overview as input to the political designation of balanced policy priorities that is the responsibility of EFA. The aims of the tool are (1) to inform a reconsideration of the government’s weighing of One Health aspects, as well as (2) to assess the policy proposal’s integrative capacity for One Health. The tool supports the identification of potential policy impacts or lacking consideration of a range of One Health core aspects, and a rapid appraisal of the current integrative level of One Health aimed policies.

Methods

A literature study was conducted to identify authoritative frameworks for six core components of One health: human, animal, environmental health, circular economy,ecosystem and biodiversity, and biosphere and climate. These were integrated into a turntable model, an indicator index and a checklist.

Results

In order to identify policy proposal relevant OH impacts we developed a simple turntable model linking proposed measures to OH aspects with issues and indicators in separate rings. Since there is overlap in issues and impacts across different core OH aspects, e.g. AMR, food safety, welfare, land use, waste management, the separate rings that can be turned independently, making AMR relevant to multiple core aspects. Key aspects, issues and indicators are listed in an elaborate One health index. The One health index and turntable model are aids to a checklist for policy screening of specific proposals, on policy scope and One health relevance; screening of possible impacts; and joint deliberation and prioritization.

Conclusions

The tool can be customized to serve politicians and policymakers at multiple governance levels. It provides input to political policy prioritization and policy formulation. A periodical revision of the tool is recommended to comply with latest scientific evidence.

Keywords: One Health, policy screening, prioritization, deliberation

ZoNoH - Preventing zoonoses in Kenya by fostering collaboration in the food system

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Risks of zoonotic pandemics are increasing worldwide. In Kenya, zoonoses prevalence remains extremely high – not only threatening human and animal life, but also posing a significant socio-economic burden. Operationalising One Health remains a major challenge at local levels and local One Health governance mechanisms are scarce globally. Meanwhile, little has been done to bridge food system approaches to health-related topics, besides food and nutrition security. Finally, social sciences remain underrepresented in One Health literature. The ZoNoH project aims to bridge these gaps. In line with the Kenya national One Health strategic plan, ZoNoH will support two Kenyan County One Health Units (COHU), which are local platforms bringing together One Health key stakeholders from one county. A hands-on service is being designed with the aim of equipping COHUs to better manage zoonoses in their food systems, hence contributing to preventing future pandemics. The service offers three specific objectives: 1) to foster One Health and Food Systems operationalization, 2) make existing data (at global, national, and local levels) on zoonoses impact on local food systems available to COHU members and 3) support with the co-creation of contextualised zoonoses management strategies. Through an iterative process the building blocks of the service are being outlined by an interdisciplinary team. It comprises: a scoping step, where a zoonotic challenge will be prioritised by COHU members; an analytical step, where the impacts of the selected zoonotic challenge on the food system and in the different One Health dimensions will be explored to foster a holistic understanding of the current context; an identification of the leverage points in the food system; an exploration of the possible scenarios to evolve towards a desirable future and a prioritisation of the desired pathways of change; and the elaboration of a concrete action plan to operationalize this pathway. Each step comprises of a combination of workshops and desk research, tapping into multiple disciplines. The service will be peer-reviewed and tested before piloting with two COHUs. The service aims to support One Health governance mechanisms in practice. By fostering a more inclusive and coordinated response to a concrete One Health challenge, i.e. a zoonotic challenge, ZoNoH will pave the way in supporting local One Health governance organisations. It will support Kenya as a pioneer in this field and open new perspectives to operationalise One Health in the food system to the benefit of humans, animals and our environment.

Keywords: One Health governance, food system, zoonosis, decision support, Kenya

Untangling the governance of public health aspects of manure in the Netherlands

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The Netherlands is one of the most densely populated countries in terms of people and livestock and is the second largest exporter of agricultural products worldwide. As a result, the Netherlands has a manure surplus. Excess application of manure can lead to environmental problems. Therefore, manure needs to be treated and discharged. Manure can contain zoonotic pathogens, but whether exposure to manure and manure treatment also poses a risk to public health is still unknown. This study analyzed the regulations, relevant actors, and responsibilities in the complex system of manure and public health in the Netherlands. Interviews and system mapping have demonstrated interlinkages between environmental, economic, and health aspects. Constraints and opportunities for public health protection have been identified. This study reveals the complexity of the Dutch manure policy, its scattered responsibilities, the challenge to deal with uncertainties, and, most importantly, the need for a microbial risk assessment in order to adequately communicate and manage possible risks to protect the health of animals, the environment, and people.

Keywords: agricultural policy, manure treatment, risk management, system mapping

Inspiring future One Health initiatives: lessons learnt from the One Health European Joint Programme

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Problem statement

Modern, multifaceted health issues require a multidisciplinary – One Health – approach. This is acknowledged at both scientific and policy levels. However, whilst One Health research continues to yield promising outcomes, the operationalisation of One Health tools and knowledge, and the institutionalisation of One Health seem to proceed at a slower pace.

With this in mind, the One Health European Joint Programme (One Health EJP) consortium was launched in 2018, under the EU Research and Innovation Programme Horizon 2020, to address challenges associated with foodborne zoonoses, antimicrobial resistance, and emerging threats through a One Health approach.

Approach

The One Health EJP brought together 44 partners from across Europe: food safety, animal health and public health laboratories and institutes with reference functions, mandated from their respective national authorities or ministries. The partners joined forces to collaboratively perform scientific and integrative projects and activities, and aligned their actions along the prevent-detect-response axis. The crucial step from outcomes to impact was guided by a defined strategy of dissemination and interaction with stakeholders (national authorities, EU agencies, and international organisations).

Results

In less than six years, the One Health EJP produced a remarkable amount of new scientific knowledge (including more than 200 publications). The One Health approach, however, transcends mere scientific projects, demanding us to look into our own way of working. To reinforce this multi-sectoral attitude, initiatives such as short-term missions and education and training activities were organised. Particular attention was paid to translating science into policy, by reaching out to the relevant stakeholders with several dedicated interaction tools (e.g., targeted reports, workshops). After the funding period of the One Health EJP, most partners continue the work of exploiting its results, notably providing support to the MedVetNet Association.

Conclusions

Despite its limitations (e.g. limited duration), the experience of a major programme like the One Health EJP can serve as a guide for future One Health initiatives. During the One Health EJP, strong relations with key stakeholders were built that are now maintained by the MedVetNet Association. Of particular interest is the way that the One Health EJP promoted the operationalisation and institutionalisation of One Health, by supporting experts keen to take a multi-disciplinary approach (bottom-up approach), and at the same time advocating at the higher policy level (top-down approach). An experienced European network is ready to further develop the One Health approach.

Keywords: operationalisation, institutionalisation, dissemination, cross-sectoral collaboration

How Much Titanium Dioxide Is Present In Products We Orally Consume? A Systematic Review

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Aim: Although titanium dioxide has long been considered a safe and inert material, it has been the subject of controversy in recent years. In 2021, the European Commission decided to ban its use as a food additive in the European Union - a decision expected to be echoed in other regions and industries. The aim of this review was to provide a comprehensive summary of the available information on the concentration of titanium dioxide in products that we orally consume, such as food, food supplements, pharmaceuticals and personal care products. By looking at more than one source of exposure, an effort was made to move towards a more holistic approach to risk assessment - a perspective borrowed from the exposome paradigm, which strives to measure the totality of an individual's exposures over their lifetime, including titanium dioxide.
Method: A literature search was performed in Web of Science, Scopus and Google Scholar. The resulting data was analysed using a hierarchical Bayesian model, from which estimates of the median concentration of titanium dioxide were obtained in each category of products, at four different levels of aggregation.
Results: A total of 52 studies were retrieved from the literature, covering 632 products. The overall median concentration was estimated at 756 (575 - 974) mg/kg, though there was considerable heterogeneity between categories. At the highest level, estimates ranged between 628 mg/kg in food and 2,141 mg/kg in food supplements. For individual products, concentrations of up to 51,000 mg/kg were found.
Conclusion: A considerable amount of data was retrieved from the systematic review process, which should prove to be useful for any following risk assessments of titanium dioxide. However, it also emphasised the lack of sufficient and representative data in certain product categories. This might prove to be a challenge when aiming to comprehensively assess the combined risk of multiple sources of exposure to titanium dioxide in the future.

Keywords: titanium dioxide, E171, nanomaterials, exposome, hierarchical models

Poster abstracts: Striking the right balance: healthy environments, resilient and productive agrifood systems

Role of glyphosate in selection of tolerant bacteria in human samples from Thailand

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Glyphosate, a widely used herbicide, has the potential to impact microorganisms, but its influence on antibiotic resistance (AR) in bacteria remains understudied. The objective of our study was to evaluate the impact of glyphosate exposure on microbial diversity and AR in bacteria isolated from the digestive tracts of Thai farmers, focusing on Gram-negative bacteria (GNB). Human fecal samples from Thailand, grouped by level of glyphosate exposure, were cultured on MacConkey (McK) agar with or without 7 g/L glyphosate. Antibiotic susceptibility and glyphosate minimum inhibitory concentration (MIC) of isolated strains were assessed using disk diffusion and liquid microdilution methods respectively. A total of 547 isolates were isolated from 112 human fecal samples. Glyphosate medium predominantly selected Escherichia coli, Klebsiella pneumoniae, and Citrobacter freundii, commonly isolated on non-selective media. Glyphosate MICs ranged from 2 to 32 g/L. K. pneumoniae species had the highest median MIC (16 g/L). AR rates were not significantly different between NE and E groups. In contrast, six MCR- and/or ESBL-producing E. coli strains were isolated from glyphosate-exposed group, while only one colistin-resistant K. pneumoniae strain was isolated from a sample not exposed to glyphosate. This study highlighted the putative effect of glyphosate selection of clinically relevant pathogens, which can lead to changes in microbial diversity. While we couldn't establish a direct link between antibiotic resistance (AR) and glyphosate exposure, the isolation of colistin-resistant and/or ESBL-producing strains among glyphosate-exposed farmers prompts further investigation into a potential connection between glyphosate exposure and resistance to colistin and/or cephalosporin in future studies.

Keywords: glyphosate, MIC-glyphosate, antibiotic resistance, Thailand, one health

Resilience of the food supply chain towards food safety shocks

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Aim
Food supply chains are constantly challenged by food safety hazards entering the chain, leading to large economic losses to manage the food safety contaminations effectively. To date, the HACCP approach is mostly used to manage food safety. To become more pro-active, the impact of food safety threats on the supply chain can be investigated with the concept of resilience. The resilience concept is used in several domains, but – to date- has not yet been used in the food safety domain.
This study aimed to develop the resilience concept for food safety management in supply chain, and to demonstrate this concept quantitatively in two food safety cases related to the pork supply chain.
Methods
The resilience concept applied in other domains was studied to derive a framework that can be applied to food safety. Once developed, the food safety resilience concept was applied to two case studies related to food safety shocks in the pork supply chain, one microbiological (Salmonella spp.) and one chemical one (dioxin contamination). The resilience of the chain as well as the potential costs associated with the incident, using several monitoring and/or other intervention strategies were compared with the aim to determine the optimal measures to manage the food safety contamination throughout the chain. For dioxins, the focus was on monitoring, whereas for Salmonella spp other interventions were considered as well. A stochastic simulation model based on the Dutch pork supply chain was used in both cases. Cost and effectiveness of the various interventions were estimated in a quantitative way, with the focus on reducing the impact of the shock.
Results
Based on the general resilience concept, the resilience of the supply chain, using food safety as an indicator, was defined as: the ability to provide safe food within a reasonable time after a food safety threat or shock. The resilience takes both the severity of the shock into account as well as the time to fully recover or to reach a new equilibrium. The performance deviation due to the shock is used to measure this food safety resilience.
Results for the Dioxin case showed that it is optimal to collect and analyze samples at more than one control point along the pork supply chain to control dioxins, in particular the combination of collecting samples at feed level and the fat melting stage is the preferred option. For the Salmonella case and investigated interventions, results showed that for chains with low or medium risk profiles, more attention should be paid to increasing resilience of pigs towards Salmonella spp. infection. For supply chains with maximum risk profile, the focus should be on improving the performance of the slaughterhouse, such as careful evisceration, logistic slaughtering.
Conclusions
The effectiveness of food safety management can be improved by applying the resilience concept. We demonstrated that enhancing resilience performance of the pork supply chain can contribute to the safe pork supply.

Keywords: food safety; resilience; dioxins; Salmonella spp; modelling

Zoonotic diseases across fragmented land use types: The role of small mammals

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The Netherlands is considered vulnerable for emerging infectious diseases due to high human and livestock population density and intensive land conversion that has led to a mosaic of three dominant land-use types: farmland, urban areas, and forest fragments. These land use types differ in small mammal species assemblages, which at the same time alters inter-and intra-species interactions, contact rates and consequently their community competence. This is particularly relevant for small mammals, which are often resilient to land conversion and are known to be suitable hosts for many zoonotic pathogens. Despite accumulating evidence showing the association between land use change and zoonotic disease emergence, the underlying mechanisms that facilitate transmission are still not well understood. To better understand how three fragmented land use types influence the prevalence of zoonotic pathogens in small mammals, we investigated how farmland, urban, and forest areas impact the hazard of zoonotic pathogens through changes in species assemblages.

In the summer of 2022 and 2023 small mammals were collected using 100 Heslinga live traps per plot in 22 locations across The Netherlands. Following a block design, each location contained all three contiguous land use types: mixed forest, cattle fields, and the green public area of cities (66 plots in total). A small tissue biopsy was obtained from all caught animals from the tip of the ear to corroborate the species by molecular techniques and to screen for zoonotic pathogens (Anaplasma spp, Neoerlichia spp, Rickettsia stenos, Borrelia spp, Bartonella spp). In total, 1.590 individuals were captured from 9 different species. Preliminary results showed that Apodemus sylvaticus, Clethrionomys glareolus and Crocidura russula were highly abundant species in two or more habitat types, while Sorex species were less common and never observed in urban environments. The majority of zoonotic pathogens were present in all habitat types, however pathogen prevalence was generally lower in urban environments. With the exception of C. russula, which showed lower pathogen prevalence compared to the other species, host species relative contribution to the community competence depended on the habitat and pathogen type. Together these preliminary results suggest that land use changes can modify the community competence of small mammals by shifting species assemblages, however changes in disease hazard are dependent on pathogen type.

Keywords: Species, assemblages, land-use, competence, small mammals

Contributions of livestock production animals to ambient ammonia and particulate matter in a livestock dense area

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Objective This study assessed how differences in emissions and geographical distribution of different production animals (veal calves, dairy cows, laying hens, broilers, other poultry, sows, fattening pigs, goats) affect the spatial patterns of ambient concentrations of ammonia (NH3) and particulate matter (PM).
Methods The study area (40 x 48 km, the Netherlands) covered an intensive livestock production area with approximately 2000 livestock farms, densely populated residential areas and large and small nature areas. Based on emitting livestock farms, the widely used Dutch dispersion model STACKS that accounts for deposition was used to calculate ambient PM and NH3 concentrations for 102381 receptor points on a 100 x 100 m grid. Sensitivity analyses (SA) were performed for model assumptions.
Results Livestock production elevated local levels of NH3 and PM. Relative contributions of production animals to the spatial patterns of NH3 and PM varied, driven by geospatial distribution of farms. The distribution of farm contributions to the concentration on receptor points in rural areas were characterized by high contributions of a few farms nearby and considerable smaller contributions by many farms further away. In nature areas (at the borders of the study area), concentrations were mainly driven by a large number of farms on a greater distance in the absence of farms in the direct vicinity. SA showed that the size of the model inclusion area for farm sources affected concentrations in nature at the borders of the study area significantly but concentrations in livestock dense areas minimally.
Conclusion Reduction of air concentration of NH3 in nature requires a general reduction of livestock emissions, instead of reduction from local sources alone.

Keywords: ammonia; particulate matter; livestock

Rice blast control drives yield benefits from variety mixture in rice: a global meta-analysis

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Rice blast, caused by the fungus Pyricularia oryzae, is the most important disease of rice globally. Variety mixture has proven potential to reduce the incidence of rice blast and increase yield, but the effectiveness of variety mixture as a disease control method varies, and the general effectiveness and yield benefits of this agroecological practice have not been demonstrated. Here, we present a global synthesis of experimental data on the effect of variety mixture on rice blast. Based on 1255 disease observations from 38 research publications, the relative yield in mixture (yield in mixture divided by yield in monocrop) increased with disease incidence in the monocrop, for hybrid rice from 0.92 to 1.06 as disease incidence in the monocrop increased from 0 to 100%, and for glutinous rice from 0.16 to 0.30. Variety mixture reduced the odds of disease by 68% on average. The average reduction in disease odds was substantially greater in blast-susceptible glutinous rice varieties (80%) than in more blast-resistant hybrid rice varieties (34%), but still substantial and significant in the latter. The disease control effect of fungicides and variety mixture were additive. Blast-resistant hybrid rice varieties became much more susceptible to blast at high than low fertilizer N input but the disease controlling effect of mixture was unaffected by fertilizer N input. The results show that variety mixture is an effective agroecological practice, particularly for the cultivation of indigenous varieties with high market value but low blast resistance.

Keywords: rice variety mixture, yield benefit, disease control, pesticide reduction,

Chemical and microbial food safety hazards in sludges and their fate in circular food systems.

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Residual sludge streams, such as domestic sewage sludges or industrial sludges, have the potential to be used in circular food systems, considering their nutrient content. These streams are available in large volumes and have relatively low value and limited valorisation options. However, when considering the use of these sludges in circular food systems, attention should first go to how the application of these sludges affects food safety. Food safety hazards, including pathogens and chemical contaminants, may accumulate, and new, unknown food safety hazards may emerge. This project aims to gain insight into food safety hazards in sewage and industrial sludges and assess how these behave in selected circular food production systems like seaweed and insects. Domestic sewage sludge from four locations in the Netherlands, four sludges from the food industry and one aquaculture sludge was collected and analysed for the presence of chemical contaminants (i.e., heavy metals, dioxins, pesticides, short chain PFAS and drug residues) and microbial hazards (i.e., bacterial pathogens and antibiotic resistance by ESBL- and carbapenemase producing bacteria). During the conference, we will highlight the results of the selected sludges when used for insect rearing and the fate of selected chemical and microbial hazards in this food production system. The results of this study show that varying amounts of chemical and microbial hazards are present in the studied sludges. With these results, we provide knowledge on the potential impact of the application of sludges on food safety in future circular food systems, to ultimately safeguard safe products for consumers.

Keywords: Food safety, circular food systems, microbial hazards, antimicrobial resistance.

Poster abstracts: Controlling and eliminating zoonotic diseases

Species-independent assessment of neutralization titers by a rapid and kinetic lateral flow test following a natural infection or a vaccination

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Objectives: The lateral flow diagnostic platform is a user-friendly Point-of-Care test that can be applied anywhere and yields a qualitative signal. Recent improvements in lateral flow test technology include the application of an array of printed spots, allowing detection of five or more biomarkers simultaneously, and the analysis of raw data by a real-time video reader that records the increasing color intensity of each spot of the array. Kinetic analysis of interactions is possible as well, which would allow to calculate quantitative levels of the target biomarkers. To investigate whether a lateral flow test (LFT) can be transformed into a kinetic and quantitative test format the procedure of running the test and reading the spot intensities real-time has been applied to detect and analyze neutralizing antibodies against SARS-CoV-2. It is generally accepted that neutralizing antibodies are highly predictive of immune protection.
Approach: An array of spots (20 nL per spot) was printed on a nitrocellulose membrane consisting of duplo-spots of the following capture ligands: angiotensin-converting enzyme-2 (ACE2) receptor, the receptor binding domain (RBD) of the SARS-CoV-2 Spike Protein (SP) and the S1 part of the SP. A conjugate of carbon nanoparticles with the S1 part of SP was prepared as the colored label in the LFT. This would allow a double antigen approach for the RBD and S1 spots making the test immunoglobulin class- and species-independent. A software script was written in Python to analyze the data.
Results: Non-immune sera showed bright ACE2 spots and no spots for RBD and S1, whereas sera from infected or vaccinated persons showed various patterns of spots from weak to no ACE2 spots to clear or very intense RBD and S1 spots. Real-time recording of the spot intensities over time yielded additional information on interaction kinetics. A study to analyze whether, based on the kinetic data of the LFT, an algorithm can be found with a high correlation to neutralization titers obtained by the gold standard test (PRVNT), is ongoing.
Conclusions: The lateral flow technology is well suited for the real-time analysis of interactions between binding pairs such as the SARS-CoV-2 RBD and its human counterpart the ACE2 receptor. The kinetic format of the LFT, especially when using the double antigen approach, opens new possibilities; in addition to assessing a concentration, kinetic data also reveal information on the overall affinity of binding pairs. This will result in added value diagnosis as compared to endpoint assays such as the ELISA.

Keywords: lateral flow test, multibiomarker, kinetic data, neutralizing antibodies, neutralization titer

Integrating social sciences in preventing and responding to zoonotic diseases outbreaks

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Aim

Zoonotic diseases occur due to pathogen spillover from animals to humans, and a subsequent increase in human disease cases. Although much technical knowledge is available to prevent and control zoonotic diseases, social science expertise is less commonly integrated. Social science expertise is required to prevent and mitigate zoonotic diseases outbreaks, for example, by understanding and modifying human behavior. This was recognized after the 1918 influenza pandemic and amplified during the recent COVID-19 pandemic. The potential contributions of social sciences are currently underutilized. The objective of this review was to map the literature on the integration of social science expertise in preventing and responding to zoonotic disease outbreaks, for identifying knowledge gaps.

Methods

We conducted a scoping review following the PRISMA-ScR guidelines. As basis for the review, we developed a conceptual framework to help understand the contribution of social science expertise during zoonotic disease outbreaks. The framework comprised of three components: outbreak drivers, outbreak management and post-outbreak effects. The retrieved studies were mapped in relation to these components, and particularly, regarding their contributions to zoonotic disease prevention, preparedness, response, and recovery. Articles were searched in SCOPUS, Web-of-Science, and CAB Abstracts.

Results

After de-duplication, and title and abstract reviewing, 833 articles were eligible for full-text screening, and finally, 764 articles were retained for data extraction. Rabies (127 articles), brucellosis (65) and avian influenza (58) were the most studied zoonoses. Ethiopia (rabies), India (rabies), USA (swine influenza, rabies), and Australia (Hendra virus) were the main focus countries. About 50% of the studies applied insights from behavioral sciences, mainly employing the ‘Knowledge, Attitude and Practices’ (KAPs) framework. Most of these studies also assessed associations, not causality, between KAPs and socio-economic characteristics, which limit the policy relevance of the findings. Wildlife trade and (religious) festivities were the most studied socio-economic disease drivers. About 70% of the studies focused on disease prevention, mainly towards understanding stakeholders’ KAPs and risk perceptions. Relatively, more studies were also retrieved regarding disease prioritization (preparedness) and evaluation of risk communication and media coverages (recovery/learning). However, scientific reporting of integration of social science expertise in responding to zoonoses is limited. Most impact assessment studies performed simple cost estimation for evaluating disease burdens, without assessing the broader socio-economic impacts of outbreaks.

Conclusions

Rigorous and comprehensive analyses of economic, social and environmental drivers and impacts of zoonosis outbreaks, as well as their preventing and mitigating strategies, are limited but required for informed decision making to effectively control zoonotic diseases.

Keywords: Epidemics, Preparedness, Prevention, Response, Social science, Zoonosis

Setting-Specific Spread: Contributions of Indoor Spaces to Overall SARS-CoV-2 Transmission

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Problem statement/aim: In the context of pandemic preparedness, the effective design of non-pharmaceutical intervention (NPI) policies is crucial to mitigate the adverse impacts of future pandemics. Indoor settings are important sources of transmission for respiratory pathogens, and the infection patterns depend on the frequency and the context of between-human interactions. Therefore, the overall risk of individuals is based on the settings in which they spend their time and the transmission risk associated with these specific settings. However, an incomplete understanding of the contribution of different indoor settings to overall transmission still hampers the assessment of the impact of different NPI strategies. This research project aims to untangle the contribution of various indoor settings to the overall transmission of respiratory pathogens and how this is influenced by NPIs in indoor public spaces, and illustrate it for the recent SARS-CoV-2 pandemic in the Netherlands.

Method/approach: We construct a modeling framework that combines granular epidemiological and behavioral data to quantify the contribution of indoor spaces to overall transmission and the impact of NPIs in the public domain. For the setting-specific transmission risk, we use indoor transmission models (SSO-mod, SamenSlimOpen) accompanied with offspring distributions per setting based on the Netherlands contact-tracing data. On the top level, we build a stochastic agent-based model, SSS-mod, in which agents have daily schedules based on their demographic characteristics (age, gender, income) and interact with each other in different settings (e.g., homes, schools, workplaces, restaurants). In a specific setting, the expected number of new infections caused by an infectious individual depends on the distributions of setting-specific risk. Then, those infected follow an extended SEIR-type model with parameter values informed by the literature. Using a Bayesian inference framework, the model is calibrated to real-world epidemiological data for different waves of the SARS-CoV-2 pandemic in the Netherlands.

Results: The simulation results illustrate the contribution of diverse indoor settings to the overall transmission for different socio-economic groups (e.g., high vs. low-income populations, smaller vs. larger household sizes) across different NPIs (e.g., work from home vs. from office, school closures). The calibrated model will be used to project the impact of NPIs across different epidemiological contexts (i.e., prevalence, population-level protection, variants) for different risk groups by counterfactual scenarios.

Conclusions: SSS-mod is a unique model that is especially developed for the investigation of specific NPIs targeting indoor spaces. Understanding the contribution of different indoor settings, hopefully, enhances our preparedness for subsequent outbreaks.

Keywords: epidemiology, agent-based modeling, mobility, behavior, SARS-CoV-2

Understanding farmers’ and other stakeholders’ behaviour as a key towards effective and efficient interventions for stimulating animal and public health.

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Effective and efficient interventions are needed to stimulate farmers to take biosecurity measures on their farms, for example for controlling and eliminating zoonotic diseases. For the development of these interventions, it is important to understand which behavioural factors determine the choices of farmers and other stakeholders involved with regard to animal health control. The COM-B model of Michie et al. (2011) can be helpful to elucidate this. COM-B refers to Capability (knowledge and skills), Opportunity (social and physical context) and Motivation (values, norms and beliefs). To be effective, interventions should be tailored to the behavioural factors that determine the farmers’ and other stakeholders’ choices. In a study concerning further reduction of antibiotics use in livestock farming in the Netherlands, we chose this approach to explore why public and private instruments applied over the past decade to reduce antibiotic use in livestock farming have often, but not always led to the desired reduction of antibiotics. The key question of the study was whether current antibiotic reduction policies and the resulting behavioural interventions address all or only some of the behavioural factors that influence the achievement of further reduction of antibiotic use. We also examined which behavioural factors interventions should target, to achieve further reduction and/ or refinement of antibiotics use.

To answer the question, a literature research was conducted and in-depth interviews were performed with sector representatives, coaches, veterinarians and other stakeholders. The main conclusion was that many interventions to further reduce antibiotic use have so far mainly focused on increasing awareness and the knowledge and skills of livestock farmers and veterinarians (the behavioural determinant related to capability) and applying social pressure (the behavioural determinant related to the social context). However, interventions focused on the physical context (e.g. the farm situation) under which livestock farmers and veterinarians have to operate and on their motivation are still largely missing. For this reason, new interventions should target these behavioural determinants. This can be done, for example, by highlighting examples of farms at which delaying or not deploying herd/flock antibiotic treatment has the desired result.

A comparable approach could be useful for the development of effective and efficient interventions for controlling and eliminating other zoonotic health threats.

Keywords: behavioural factors, tailor-made interventions, livestock farming, reduction antibiotic use

Generation of immortalized swine bone marrow derived macrophages for development of the next generation of ASF DISC vaccine

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Keywords: ASF, macrophages, cell lines, vaccine

The value of multivariate indicators of resilience in early warning for infectious disease outbreaks: a West Nile Virus case study

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Background and aims
Signalling when an infectious disease outbreak is about to start is crucial to efficiently prevent the transmission and reduce the costs of interventions. The use of resilience indicators to anticipate upcoming epidemics has been investigated in a number of studies. Unlike current early-warning systems, resilience indicators are generic and do not require model building. These typically rely on the loss of resilience of the system prior to an epidemic, leading to an increasing time to recover from external perturbations. This can be detected by a rising autocorrelation and variance in incidence time series. However, to reach a sufficient sensitivity, long, high-resolution time series are necessary. This hampers the use of resilience indicators in practice. Here, we investigate the use of multiple variables as a solution to these challenging data requirements.
Methods and results
We used West Nile virus as a case-study. As WNV infects various species, the spread of the virus can be signalled in data of multiple species: mosquitoes as vectors, birds as enzootic hosts, and humans and horses as dead-end hosts. We used a multi-host SIR-type model to simulate, for each vertebrate host, prevalence and seroprevalence time series, and prevalence time series for mosquitoes. We then used different data reduction techniques such as PCA and MAF to calculate multivariate indicators of resilience. We found that when combining multiple time series, the length of each time series can be reduced down to half of the length of univariate time series to reach the same performance of the early warning system. Additionally, including seroprevalence time series improved the performance compared to scenarios where only prevalence time series were included.
Implications
Overall, these results indicate that combining multiple data sources can help overcome the challenges of data requirements for resilience indicators. Furthermore, it has identified seroprevalence data as an additional data source to improve the sensitivity of resilience indicators. The comparison of several scenarios showed that enzootic species are the most informative to signal an upcoming epidemic. Future studies should use real-world data to confirm these findings and estimate the sensitivity, specificity and lead time of multivariate resilience indicators.

Keywords: Early-warning, resilience, epidemiology, infectious disease modelling, vector-borne diseases

Preparedness for the transmission of pandemic viruses in the food chain

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Aim: Governments and the food industry make major efforts to ensure food safety throughout the global supply chain and support food availability. Experiences with Coronavirus disease 2019 (COVID-19) have re-emphasized the need for preparedness in many sectors, including the food sector. This study analyzes the potential introduction and transmission of pandemic viruses via the food chain and hypothesizes which new food safety issues could arise.
Methods: Two scenarios, a gastrointestinal virus and a respiratory virus, were explored. Possible risks and economic costs of introduction and transmission, regulatory and analytical needs, and control strategies associated with such scenarios are described.
Results: Overall, if a pandemic virus associated with the food chain was to occur, our preparedness is currently lacking given our potentially limited knowledge of introduction and transmission pathways, as well as access to methods to detect the viral presence and infectivity and model the transmission of the pathogens, even though the economic and societal impact of such a scenario is likely extensive. The food and fomite component could be easily neglected or underestimated in a future pandemic. On the other hand, better tools to prove the lack of food chain transmissibility of a pandemic virus could also prevent unnecessary economic losses across the sector. In the event of a foodborne pandemic virus, food safety testing would provide a clear purpose to detect deviating batches, obtain monitoring data, and assess compliance to hygiene criteria; however, providing complete safety through enforcement criteria is demonstrated to be economically unfeasible; therefore, other control interventions will be needed.
Conclusions: Our findings are important for food business operators and governments to understand the possible analytical needs, opportunities, and caveats for food safety testing. Narrowing the knowledge gaps on introduction and transmission, and improvement of analytical feasibility is required to benefit our preparedness against the emergence and spread of future foodborne pandemic pathogens.

Keywords: food; pandemic virus; scenario; foodborne virus; control strategies

Risk assessment of human exposure to Highly Pathogenic Avian Influenza after vaccination of poultry

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Problem Statement:Continuous circulation of highly pathogenic avian influenza virus (HPAIV) in European wild bird populations leads to year-round incursions in poultry, posing a threat to human health. Vaccination, when resulting in most chicken having a sufficient hemagglutination-inhibition assay (HI-titre), reduces transmission in transmission experiments. This may stop transmission but it also might obscure outbreaks in poultry, because of less infected birds and clinical protection, thus increasing human exposure to zoonotic HPAIV. Thus, it is crucial to understand the conditions under which this occurs.
Methods:Dynamic simulation models were employed to assess HPAIV outbreaks in poultry under various scenarios, parameterized with experimental and observational data. The impact of vaccination on time-until-detection, probability of major outbreaks, and human exposure for individual farms was evaluated. These outcomes were integrated into a between-farm model, allowing for the calculation of human exposure on national level for simulated outbreaks.
Three scenarios were explored: 1) 'Partial Sufficient Titre', where a portion of birds has sufficiently high HI-titres is clinically protected and has reduced infectivity; 2) 'Waning of Immunity', wherein HI-titres diminishes over time resulting in a loss of protection; and 3) 'Reduced Clinical Signs', all birds have reduced clinical signs but only birds with sufficient HI-titres experience reduced transmission.
Results: In scenarios 1 and 2, vaccination delays detection times due to reduction in transmission, but decreases human exposure. Scenario 3 shows a trade-off between clinical protection and transmission reduction and human exposure. For instance, if transmission is not reduced but only 50% of birds die, exposure within a single flock can increase by a factor of 1.5, while if 50% of birds also experience reduced transmission, the exposure will be less than without vaccination. On the other hand protection against transmission decreases the probability of major outbreaks and thus between farm transmission. Vaccination slows the virus's spread within farms and lowers mortality in birds with a sufficient HI-titre, prolonging detection times. Weekly active surveillance enhances detection speed and identifies otherwise undetected outbreaks, reducing human exposure. Conversely, for unvaccinated farms, active surveillance offers minimal benefits as outbreaks are promptly detected passively.
Conclusions: poultry vaccination reduces human exposure by curbing virus transmission. However, clinical protection due to preventive vaccination may inadvertently lead to increased exposure, but this can be mitigated by active surveillance.

Keywords: zoonosis, spill-over, chicken, pandemic, monitoring

Cost-effectiveness of selected control measures against Hepatitis E in the Dutch pig supply chain

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Hepatitis E (HEV) can lead to liver disease in humans. Although mostly self-limiting, severe HEV infections can occur in immunosuppressed adults or pregnant women. The consumption of pig meat is a main contributor to the total disease burden caused by HEV. The first aim of this study is to estimate the Disability-Adjusted-Life-Years (DALY) due to HEV in pig meat by simulating HEV through the entire supply chain, including the farm, transport, lairage, slaughtering, processing and consumption stages. The second aim of this study is to challenge the model with several types of control measures at farm level and to estimate their cost-effectiveness.

The first four stages (farm, transport, lairage, slaughtering) are modelled using a Susceptible-Exposed-Infected-Recovered (SEIR) model. For the last two stages, pig meat and liver products are divided into six categories commonly consumed by Dutch consumers: three categories bought raw to be prepared at home (i.e., pig meat, pig minced meat and sausages, and pig liver), and three categories mostly bought ready-to-eat (RTE), consumed as snack or with bread (i.e., cold pig meat, liver products, and dried sausages). Depending on the product category, different ways of heating and storing, leading the reduction of infectious HEV genome copies, are assumed. Furthermore, the model is challenged by four selected control options at the pig farm: the cleaning of driving boards, the use of predatory flies, the use of rubber mats on the floor, and the vaccination of finishing pigs. The cost-effectiveness of these control measures is estimated by estimating the costs per avoided DALY.

The prevalence of infected pigs at slaughter age estimated by our model is 26.2%. Due to the limited time spend in the transport and lairage stage, this prevalence does not change significantly during these stages. Cross-contamination during slaughtering is also limited for HEV, resulting in a prevalence of infected carcasses of 25.9%, with 6.6% of liver, and 7.0% of blood samples contaminated. This prevalence leads to an estimated 21 DALYs per year, with 68% attributable to dried sausages and 25% attributable to RTE liver products. The costs per avoided DALY were approximately €0.5, €2.4, €2.4, €7.5 million for the cleaning of driving boards, the use of predatory flies, the use of rubber mats, and the vaccination of pigs respectively.

To conclude, when taking into account the disease burden in the Netherlands only, this would indicate that none of the control measures are cost-effective unless these measures are also effective against other pathogens.

Keywords: disease burden of zoonoses, control measures, safe food systems, economics

Evaluating control measures against Highly Pathogenic Avian Influenza spread between Dutch poultry farms: preventive culling and bucket sampling

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Problem Statement:
In the epidemic of highly pathogenic avian influenza (HPAI) in Dutch poultry in 2003, there was extensive between-farm transmission in two poultry-dense areas. Because the application of minimum European control measures in these areas was not sufficient to prevent transmission, preventive ring culling was also carried out on a large scale.
Epidemiological modelling based on parameter estimates from the 2003 epidemic predicts that the current risks of spread are much reduced compared to twenty years ago. On the one hand, still one area, in the Gelderland valley, is identified where sustained between-farm transmission is to be expected if only minimum European control measures are applied. On the other hand, the predicted size of future epidemics in this high-risk area are much reduced compared to twenty years ago.
Over the period September 2021 to September 2022, 57 HPAI outbreaks occurred in commercial poultry in The Netherlands. Fourteen of these outbreaks occurred within the high-risk area in the Gelderland valley, and around most of these outbreaks, 1-km preventive ring culling was applied. The pattern of occurrence of the 57 outbreaks turned out to be compatible with the model predictions.
Against this background, policy makers asked, amongst other, the following two questions:

1. According to the epidemiological model, what is the currently expected effect of preventive culling within a radius of 1 km in the area in the Gelderland valley?
2. To what extent could the sampling of dead poultry (bucket sampling) in a zone around outbreak farms, speed up the detection of outbreaks and thus reduce transmission risks compared to the existing, mainly passive, surveillance?

Method:
Each one of the two questions required its own type of modelling analysis:

1. Model simulation of between-farm transmission for different control scenarios.
2. Modelling within-farm HPAI spread and detection.

Results:
1. The model predicts that preventive culling within 1 km in the Gelderland valley will reduce the average duration of an epidemic of between-farm transmission from 51.4 to 43.9 days and the average total number of outbreak farms from 14.6 to 9.7. However, this comes at a price, as the expected total number of culled farms is 44.8 with preventive culling, compared to 14.6 farms without preventive culling.
2. Bucket sampling on poultry farms can detect an HPAI outbreak on the farm earlier than the existing surveillance system. The predicted reduction in the average time between introduction and detection is approximately 30 percent.
Conclusion:
Both bucket sampling and preventive culling are relevant intervention measures for HPAI control in Dutch poultry.

Keywords: vian influenza, poultry, preventive culling, bucket sampling, transmission

ROADS to Health

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Problem statement and aim:
The spread and emergence of new pathogens from zoonotic origin intensify the likelihood of a health crisis. COVID-19 has uncovered shortcomings of national and global mitigation strategies. In Austria, control measures were at first set up lacking validated backgrounds, exacerbating uncertainty and mistrust among the population. In response, the project "ROADS to Health" aims to develop a technological decision-support tool that aims at facilitating holistic and enhanced pandemic management from a societal perspective. The tool offers potential measures for the selection of decision makers in a health crisis event and provides the possibility to adapt interventions to the given situation and anticipated scenarios. The objective is to systematically evaluate valuable lessons learnt from COVID-19 and enhance the implementation of integrated control measures.

Approach:
This measure matching tool is developed through an interdisciplinary collaboration of Austrian partners, leveraging expertise from global public health, crisis management, social sciences, policy support and technology. The outcome of ROADS will be the concept and demonstrator of a decision-support tool integrating data from COVID-19 but focussing on adaptability in future outbreak scenarios. In the post project phase, the tool consequently needs to be further developed by continuously integrating data from diverse sources, including human health records, animal health databases, and environmental monitoring systems to be primed in the One Health perspective. A cross-sectoral advisory board supports and facilitates the further sharpening of project goals.

Results:
As the project is still in the research phase, only preliminary results can be reported. So far, Austrias strategic, tactic, and operational objectives in response to the COVID-19 pandemic were analysed. These findings create the basis for the analysis of decreed control measures and their expected as well as unexpected and often negative side effects. After the evaluation and processing of specifically relevant measures, the tool will be conceptualised and consequently tested and validated through use cases, including e.g. zoonotic outbreaks.

Conclusion:
The development of this measure matching tool represents the potential for a significant advancement in the field of infectious disease control. By integrating data from multiple sectors and applying advanced analytics, the tool should support evidence-based decision-making and targeted interventions. This tool will present aspects of a cost-benefit estimation of certain measures and will lead to effectively addressing the complex dynamics of disease transmission. Moving forward, continued investments in innovative approaches like "ROADS to Health" are essential for strengthening global health security and mitigating the impact of (zoonotic) disease outbreaks.

Keywords: control measures, infectious diseases, digital tooling, social sciences, data utilization, interventions

Using drivers and transmission pathways to identify SARS-like coronavirus spillover risk hotspots

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The emergence of SARS-like coronaviruses is a multi-stage process from wildlife reservoirs to people. Here we characterize multiple drivers—landscape change, host distribution, and human exposure—associated with the risk of spillover of zoonotic SARS-like coronaviruses to help inform surveillance and mitigation activities. We consider direct and indirect transmission pathways by modeling four scenarios with livestock and mammalian wildlife as potential and known reservoirs before examining how access to healthcare varies within clusters and scenarios. We found 19 clusters with differing risk factor contributions within a single country (N = 9) or transboundary (N = 10). High-risk areas were mainly closer (11-20%) rather than far (< 1%) from healthcare. Areas far from healthcare reveal healthcare access inequalities, especially Scenario 3, which includes wild mammals and not livestock as secondary hosts. China (N = 2) and Indonesia (N = 1) had clusters with the highest risk. Our findings can help stakeholders in land use planning, integrating healthcare implementation and One Health actions.

Keywords: zoonotic risk, viral emergence, deforestation, host diversity, pandemics, scenario analysis

Metabolic regulation of taurine on Streptococcus uberis-induced udder infection

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Streptococcus uberis (S. uberis) is an important pathogen causing mastitis, which causes continuous inammation and dysfunction of mammary glands and leads to enormous economic losses. Most research on infection continues to be microbial metabolism-centric, and many overlook the fact that pathogens require energy from host. In this perspective, we uncovermetabolic reprogramming during host immune responses is associated with infection-driven inflammation, particularly when caused by intracellular bacteria. Taurine, a metabolic regulator, has been shown to effectively ameliorate metabolic diseases. We evaluated the role of taurine in the metabolic regulation of S. uberis-induced mastitis. Metabolic profiling indicates that S. uberis exposure triggers inflammation and metabolic dysfunction of mammary glands and mammary epithelial cells. Challenge with S. uberis upregulates glycolysis and oxidative phosphorylation in MECs. Pretreatment with taurine restores metabolic homeostasis, reverses metabolic dysfunction by decrease of lipid, amino acid and especially energy disturbance in the infectious context, and alleviates excessive inflammatory responses.These outcomes depend on taurine-mediated activation of the AMPK mTOR pathway, which inhibits the over activation of inflammatory responses and alleviates cellular damage. Thus, metabolic homeostasis is essential for reducing inflammation. Metabolic modulation can be used as a prophylactic strategy against mastitis.

Keywords: mastitis, Streptococcus uberis, taurine, metabolic regulation

Coupled individual-level heterogeneities in vector borne diseases

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Coupled individual-level heterogeneities in vector borne diseases
When modelling disease transmission on a population level, often population averages are used. However, individual hosts will not behave ‘average’ but will show large heterogeneities. These individual heterogeneities make some hosts more important to transmission compared to others. Individual heterogeneity can play an important role in transmission dynamics on the population level. Especially the risk of introduction, detectability, generation time and transmission rate can be strongly influenced by a few individuals. Both biotic and abiotic drivers can drive this heterogeneity by altering the susceptibility of individuals and change the course of a subsequent viremia and immune response. By driving individual level heterogeneity, environmental drivers could potentially influence epidemiologic outcomes on the population level.
Many experiments have been performed to investigate the effect of a specific driver on a specific aspect of heterogeneity of a host. However, environmental drivers rarely affect only one aspect of heterogeneity. Instead, they will drive multiple heterogeneities in a coupled manner. For example, food shortage can alter the behavior of individuals, while at the same time it can cause stress and influence the immune response. The immune response can in turn be a driver of heterogeneities in infectiousness and the severity of disease. This way positive feedback loops can arise in which the effect of a driver, via coupling, strengthens its original effect. As a result, the effect of coupled individual level heterogeneities can be much larger than one would expect based on experiments only looking at singular aspects.
The focus of this research is to investigate coupled individual level heterogeneities in vector-borne disease systems. We aim to gain a better understanding of how coupling of these individual level heterogeneities influences transmission dynamics on a population level by evaluating the influence on introduction risk, detectability and exponential growth rate.

Approach
Using West Nile Virus (WNV) as a case-study, we will map all the known drivers of heterogeneity and how they are coupled. We will identify positive feedback loops and how they are related to environmental drivers. A stability analysis will be performed to assess the stability of alternative states of disease presence. This will be done for a situation that takes coupling of heterogeneity into account and for a situation without coupling.

Keywords: Coupled heterogeneities, vector-borne disease, modelling, epidemiology

Safety and immunogenicity of the live-attenuated candidate vaccine ‘hRVFV-4s’ against Rift Valley fever in healthy adults

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Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic bunyavirus that causes severe and recurrent outbreaks across the African continent and the Arabian Peninsula. Human infections result from either infected mosquito bites or contact with body fluids or tissues of infected animals and are characterized by a self-limiting febrile illness, though a minority of cases develop severe disease. The overall case-fatality rate is about 1-2%. No licensed vaccines for human use are yet available. Due to its ability to cause severe epidemics and the absence of effective countermeasures, RVFV is included on the World Health Organization (WHO) Blueprint list. A four-segmented RVFV candidate vaccine (hRVFV-4s) is being developed for human use by a consortium of six partners. Pre-clinical data in various animal models have demonstrated that a single dose regimen of the hRVFV-4s vaccine combines full protection without any safety signals. Here we report the preliminary data of a First in Human Phase I clinical trial in healthy volunteers in Belgium. Neither significant safety issues were observed in any of the individuals in any dose group, nor vaccine virus shedding in urine, saliva and semen was detected. Moreover, robust anti-nucleocapsid antibody and T-cell responses were observed in all dose groups and a strong dose dependent neutralizing antibody response was induced.

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Keywords: Rift Valley Fever virus, vaccine, humans, clinical trial, mosquito

Streptococcus pasteurianus as an Emerging Zoonotic Pathogen: Virulence, Reservoirs, Serotype, Antimicrobial resistance, and Epidemiology

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Streptococcus pasteurianus is increasingly recognized for its zoonotic potential, causing severe infections across a wide host range, including humans and various animals. There are no reports about the gene-deletion mutagenesis, virulence factors, reservoir niches, serotyping, or animal infection models for this important zoonotic pathogen. Our group was the first to confirm that this bacterium can cause meningitis in pigs and is a new pathogen of swine streptococcosis.
Objectives: This study aims to elucidate the virulence factors, identify potential animal reservoirs and serotypes, and establish a reliable detection method for this pathogen.
Approach: We developed an infection model using three-week-old ICR mice to study the pathogenesis. Comparative genomic and transcriptome analyses were conducted to identify virulence factors. We also investigated the presence of S. pasteurianus in potential reservoirs, such as the tonsils and hilar lymph nodes of healthy pigs, and developed a multiplex PCR method for detection.
Results: Comparative genomic and transcriptome analyses identified several virulence factor candidates. Our findings confirmed that the capsule is a crucial virulence factor for S. pasteurianus. The strain WUSP067, isolated from a diseased pig with meningitis, exhibits a high identity in the fucose utilization cluster with Streptococcus suis, contributing to its pathogenicity. Tonsils and hilar lymph nodes of healthy pigs were identified as potential reservoir niches, with a positive rate of S. pasteurianus of 9.35% (104/1112) in healthy pig tonsils. Additionally, most strains displayed multi-resistance, with integrative and conjugative elements being primary vectors for antimicrobial resistance gene spread. Genetic diversity was observed in the capsule gene cluster, with at least five serotypes identified.
Conclusions: Our study provides valuable information about the pathogenetic characteristics and antimicrobial resistance of S. pasteurianus and essential technical support for epidemiological research.

Keywords: Streptococcus pasteurianus, Zoonotic Pathogen, Virulence, Antimicrobial resistance, Epidemiology

Poster abstracts: Curbing the silent AMR pandemic

AMR hazards: Carbapenem-resistant bacteria isolated from imported food products in the Netherlands

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Aim: Global transmission of antimicrobial resistance (AMR) by international food transport can be a risk for human health. With the biggest harbour in the European Union (EU), the Netherlands monitors imported food products from outside the EU, such as seafood, seaweed and herbs for the presence of AMR as part of the national AMR monitoring program. Carbapenems are used as last resort treatment against severe infections, therefore carbapenemase-producing Enterobacterales (CPE) form a threat to humans. Carbapenemases have a broad spectrum of activity including aminopenicillins and cephalosporins, which are used in some countries as prophylactics or as growth promotors in aquaculture. CPE can be selected for by usage of these antibiotics or presence in the environment. Between 2017-2023, imported food products were actively monitored for the presence of carbapenem-resistant E. coli, Enterobacter and Klebsiella by selective culturing. The results are presented here.
Methods: Carbapenemase-producing bacterial strains were isolated from imported food product samples by a non-selective enrichment and selective isolation on CHROMID CARBA plates. Species identification was performed using MALDI-TOF. Susceptibility testing was performed by broth microdilution according to ISO standards using European standardised antibiotic panels (Sensititre©, ThermoFisher Scientific). Illumina Short- and ONT long-read sequencing and (hybrid) assembly were used for genomic characterization.
Results: Between 2017 and 2023 fourteen carbapenemase-producing isolates were found in imported food products. In total 1815 seafood products, 179 seaweed products, and 1138 herb samples were analyzed, yielding twelve carbapenemase-producing isolates from shrimp and tilapia (from Asia), one isolate from samphire (from the Middle-East), and one isolate from coriander (from Africa). Out of the fourteen isolates, thirteen were identified as Enterobacter cloacae. Genotyping revealed that the isolates obtained from coriander and samphire encoded blaIMI-6 and blaIMI-16, whilst the strains from seafood encoded blaFLC-1, blaIMI-1, blaIMI-2, blaIMI-17, blaNDM-1, blaNDM-5, and blaOXA-48. One isolate from seafood was identified as Escherichia coli, encoding blaNDM-1. Resistance genes were present on IncFII plasmids, IncC plasmids or the chromosomally integrated EcloIMEX mobile genetic element, while in two isolates, the method of integration is still under investigation.
Conclusion: Between 2017 and 2023, we isolated and characterized 14 carbapenem-resistant bacterial strains from imported seafood, seaweed and fresh herbs. It is unknown what the attribution is of food products for human colonization or infection in the silent AMR pandemic. However, these findings underline the importance of monitoring imported (sea)food products for antibiotic-resistant bacteria to be aware of potential microbial hazards and routes for introduction.

Keywords: Aquaculture, Import, Food, AMR, CPE

Various Mobile Genetic Elements Involved in the Dissemination of the Phenicol-Oxazolidinone resistance Gene optrA in the Zoonotic Pathogen Streptococcus suis: a Nonignorable Risk to Public Health

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The rapid increase of phenicol-oxazolidinone (PhO) resistance in Streptococcus suis due to transferable resistance gene optrA is a matter of concern. However, genetic mechanisms for the dissemination of the optrA gene remain to be discovered. Here, we selected 33 optrA-positive S. suis isolates for whole-genome sequencing and analysis. The IS1216E element was present in 85% of the optrA-carrying contigs despite genetic variation observed in the flanking region. IS1216E-optrA-carrying segments could be inserted into larger mobile genetic elements (MGEs), including integrative and conjugative elements, plasmids, prophages, and antibiotic resistance-associated genomic islands. IS1216E-mediated circularization occurred to form the IS1216E-optrA carrying translocatable units, suggesting a crucial role of IS1216E in optrA spreading. Three optrA-carrying MGEs (ICESsuAKJ47_SSU1797, plasmid pSH0918, and prophage USsuFJSM5_rum) were successfully transferred via conjugation at different transfer frequencies. Interestingly, two types of transconjugants were observed due to the multi locus integration of ICESsuAKJ47 into an alternative SSU1943 attachment site along with the primary SSU1797 attachment site (type 1) or into the single SSU1797 attachment site (type 2). In addition, conjugative transfer of an optrA-carrying plasmid and prophage in streptococci was validated for the first time. It concluded that the optrA-carrying mobilome in S. suis includes ICEs, plasmids, prophages, and antibiotic resistance-associated GIs. IS1216E-mediated formation of optrA-carrying translocatable units played important roles in optrA spreading between types of MGEs, and conjugative transfer of various optrA-carrying MGEs further facilitated the transfer of optrA across strains, highlighting a non-ignorable risk to public health of optrA dissemination to other streptococci and even to bacteria of other genera.

Keywords: Streptococcus suis, optrA, horizontal gene transfer, IS1216E, mobile genetic elements

Evaluating the relevance of antibiotics added to semen extenders for dogs - characterization of the native semen microbiome of breeding dogs.

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Antimicrobial resistances (AMR) are a great threat to national and global health and have been causing over 1.27 Mio deaths in 2019 alone. Mostly arising from excessive or wrong antimicrobial use (AMU) in both, humans and animals, and lots of AMR genes nowadays being present in the environment AMR can be only targeted effectively within a holistic One-Health context. For this all fields, including animal breeding, need to identify ways to reduce AMU. Despite various efforts to achieve this on national and EU level, AMU is till common in the animal breeding sector even without clear indication. AMU in semen extenders is hereby especially problematic because the resulting non-therapeutic application of antimicrobial substances may contribute to the development of AMR in the female genito-urinary tract. In dogs AMR have been identified at alarmingly high rates in the genito-urinary tract. At the same time, for dogs no semen extenders without added antimicrobial substances are available commercially. It is often argued that this was necessary to minimise the risk of transmission of potentially genito-uropathogenic microbiota but to our knowledge there are no empirical studies giving prove to this assumption. Investigating and characterising the microbiome in the semen of 60 male breeding dogs with both, classical culture-based methods and targeted amplicon sequencing we aim to re-evaluate this risk and the necessity of AMU in semen extenders in dog breeding. Pre-liminary culture-based results show that about 43 % of the semen samples showed only low colony counts of different bacterial species and another 9 % moderate colony counts of only one species (Mycoplasma cynos). On the other hand, 48% of the samples showed high colony counts (> 105 colony forming units/ml semen) of Mycoplasma cynos or an undescribed Mycoplasma species. was identified in 30 % of the samples showing low to moderate colony counts and in one case even a high colony count. Critical bacterial species like Streptococcus canis, Mycoplasma canis or Ureaplasma canigenitalium were identified in 26 %, 13 % and 4 %, respectively only in low colony numbers. However, in one sample, which also showed high colony counts of an undescribed Mycoplasma species, Streptococcus canis and Escherichia coli, were found in high and moderate colony counts, respectively. These pre-liminary results already suggest a targeted AMU in semen extenders, based on identification of potentially genito-uropathogenic microbiota and sensitivity testing, rather than the currently practised general addition of antimicrobial substances to semen extenders.

Keywords: antimicrobial resitance, dog breeding, microbiome analysis, semen extenders

Colistin resistance in feces from livestock in the Netherlands

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Introduction: The spread of multidrug-resistant (MDR) bacteria is emerging worldwide. Infections by MDR bacteria can be treated with colistin, a last-resort antibiotic designated as "highest priority critically important antimicrobial for human medicine" by the World Health Organization. The discovery of plasmid-mediated mobile colistin resistance (mcr) genes raised global concerns as these genes can spread by horizontal transfer. For a joined One Health surveillance of livestock in the Netherlands, samples are taken of different livestock farms every year. To be able to screen for colistin resistance in Escherichia coli (E. coli), a selective cultivation and isolation method as well as a multiplex qPCR method for mcr-gene detection were developed. Fecal samples from veal calves and sheep were screened using these methods.

Methods: A selective cultivation method for feces was developed consisting of a two-step enrichment in buffered peptone water with 2 µg/ml colistin sulphate. Enrichments were incubated two times three hours at 37°C and subsequently plated on selective CHROMID® Colistin R agar. Isolates were identified using MALDI-TOF. Antimicrobial susceptibility testing was performed by broth microdilution according to ISO standards. Primers and probes were developed for screening of enrichment cultures for mcr-1 to mcr-10 using multiplex qPCR.

Results: Applying the cultivation method on 256 fecal samples from 172 veal farms in 2022 resulted in the isolation of eight colistin resistant E. coli from seven farms. Four of these isolates contain the mcr-1 gene. Additionally, qPCR screening resulted in four mcr-1 positive samples in three other farms. This resulted in a colistin resistance prevalence of 5.8% in veal farms in 2022. The screening of 153 fecal samples from 153 sheep farms in 2023 resulted in one chromosomal colistin resistant E. coli and one mcr-4 gene positive sample with qPCR screening, a colistin resistance prevalence of 1.3%.

Conclusion: To monitor colistin resistance in livestock, both a sensitive cultivation method and a multiplex PCR screening method were successfully developed. Applying these methods showed the infrequent presence of colistin resistance in fecal samples from veal and sheep. The results in veal calves confirm earlier findings (MARAN) about the continuous low-level presence of mcr-positive E. coli in fecal samples from livestock. To our best knowledge, this is the first report of colistin resistance in sheep in the Netherlands. Although the use of colistin in livestock animals is low, it is important to continue monitoring of colistin resistance in livestock considering the potential risk of spread to humans.

Keywords: AMR, colistin, E. coli, livestock

Plasmid Mediated Fluoroquinolone Resistance in Broilers

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Background: Antibiotic resistance is a major threat to the health of both humans and animals. Fluoroquinolones are potent, broad-spectrum antibiotics that are highly important in both human and animal health. Fluoroquinolone resistance is caused by acquired genes found on plasmids and mutations of the chromosome that result in a modified target protein. Plasmids play a major role in the dissemination of acquired antimicrobial resistance and can cause resistance to transfer between animals, humans and the environment. The sale of antibiotics, including fluoroquinolones in the animal sector has reduced over the last few years. In the Netherlands, the sale of fluoroquinolones in 2020 had decreased by 87.6% compared to 2011. Despite this, resistance to fluoroquinolones is still commonly found in Escherichia coli from cecal samples of broilers in the Netherlands and the rest of Europe. In 2021, 28% of E. coli isolates from broilers in the Netherlands were resistant to ciprofloxacin. This is compared to the low prevalence observed in pigs (0.3%) and white veal calves (9.3%). There is a lack of recent information regarding the genetic cause of this persisting fluoroquinolone resistance. It is important to understand the molecular epidemiology of fluoroquinolone resistance to implement effective control strategies.

Objectives: The main objective of this study is to retrospectively, determine the prevalence of plasmid mediated genes in E. coli from broilers in the last two decades and determine the genetic background of the detected plasmid mediated genes.

Method: E. coli isolates from the routine antimicrobial resistance monitoring of broilers in The Netherlands have been used in this retrospective study. Epidemiological cutoff values published by EUCAST were used to select isolates with non-wild type susceptibility to ciprofloxacin (>0.06 mg/L) and nalidixic acid (>8 mg/L) from 2022 to 2004. These isolates were further screened by PCR for the presence of qnrA, qnrB and qnrS, as these are the most common plasmid mediated resistance genes found in broilers.

Results: Plasmid mediated resistance to fluoroquinolones in E. coli isolated from broilers has increased over the past 20 years. The most prevalent plasmid mediated genes that cause this resistance are qnrS and qnrB.

Conclusions: Fluoroquinolone resistance in broilers is mainly caused by mutations in the chromosome, which modifies the targets of fluoroquinolones. However the role of plasmid mediated resistance to fluoroquinolones has increased over the last 20 years. Further research is needed to elucidate the molecular epidemiology of the plasmids that carry these antibiotic resistance genes.

Keywords: AMR, Fluoroquinolone resistance, qnr, Plasmid mediated resistance, Broilers

The impact of antibiotic growth promoters (AGPS) on broiler chickens' health and environment in kibaha town council – Tanzania

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Antibiotic growth promoters (AGPs) in Tanzania is administered in the poultry production system to increase profits and efficiency and to reduce keeping time and production costs of the birds, albeit at the expense of the environment and adverse health effects to human, particularly through the spread of antimicrobial resistance (AMR) pathogens. Because of that, a cross-sectional survey was conducted at Kibaha Town to assess awareness and effects of using AGPs on both broiler chicken and environmental health, purposely for guaranteeing safe animal source foods (ASF) to consumers. A structured questionnaire was administered through face to face interview to 40 broiler chicken keepers, where qualitative and quantitative methods were used to collect data. Results showed that all respondents (n=40) had no diseased chickens in their houses, attributable to AGPs use, broiler boost and some hygienic practices. AGPs were administered by mixing with food (47.5%; n=19) or drinking water (52.5%; n=21) during the first week (45%; n=18), after first week (25%; n=10), after second week (12.5%; n=5) or continuously regardless of the withdrawal period (17.5%; n=7). The respondents administered AGPs themselves (62.5%; n=25) while 37.5% (n=15) had neither considered dosage nor the number of broilers kept in their poultry houses. Only 42.5% (n=17) of respondents knew about the negative effects of using AGPs whereas 57.5% (n=23) had no knowledge at all. Of these respondents 67.5% (n=27) sold the litter to vegetable growers and fish ponds owners as organic manure while 25% (n=10) randomly dumped it, only 7.5% (n=3) safely dumped it in shaded pits to avoid environmental contamination. It's concluded that broiler chickens are fed AGPs to maintain birds' health, particularly in intensive animal production, and their wastes are used as organic manure for agricultural fields and fish ponds production, posing risks to human, animals and environmental health. However, the AGPs use cannot act as a substitution for good hygiene management and hence, further studies on "One Health Approach" to analyze effects of AGPs use in the shared environment are currently needed.

Keywords: Poultry, AMR pathogens, organic manure, environment.

Poster abstracts: Early detection and characterisation of new emerging pathogens

Virus detection and characterization using sequencing approaches, virus-specific whole-genome detection and broad surveillance procedures

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To be well prepared for outbreaks of virus threats, early detection and characterization of new and emerging viruses is extremely important.
Accurate characterization of a known virus is important for estimating the risk of a circulating virus. Whole-genome analysis, achieved through deep sequencing, is a common method. However, challenges arise when environmental, host, or bacterial DNA/RNA is abundant, leading to sequencing failures. This means the selection of the appropriate starting material is important and in addition, a proper virus pre-enrichment of the material is crucial. Subsequently, enrichment of the target DNA/RNA can be accomplished via target-specific PCRs or a random amplification (Sequence-Independent, Single-Primer Amplification (SISPA)-PCR) followed by specific probe capturing before completing sequencing. We have developed protocols based on these techniques for several viruses, including Hepatitis E virus, Influenza, SARS-Cov-2, Bluetongue virus, African swine fever virus and West Nile virus originating from all different kinds of starting materials.
To deal with unknown or unexpected virus variants, we have created a Veterinary Probe Capture Set (VetCap). This set includes probes derived from a large range of viruses derived from the virus disease lists of WOAH, DISCONTOOLS, EmZoo and our WBVR institute (diagnostic) list. With these VetCap set we were able to detect all the included viruses. For each selected target virus a combination of specific probes was selected to be included in the VetCap probe set. As mentioned earlier, a well-optimized pre-enrichment is essential. This has been executed for different significant matrixes, including faeces, organ tissues, water, blood, serum, respiratory swabs, dust or air samples. To enlarge the amount of genetic material a SISPA-PCR was performed. The sensitivity of this newly developed probe set has been tested using a variety of archived viruses from the list.
The development of the above tools has enabled rapid characterisation of a known emerging virus through whole-genome sequencing. Furthermore, the VetCap is a comprehensive tool for capturing and identifying a broad range of viruses in veterinary settings, both animal and environment derived.

Keywords: virus, characterization, Whole-genome sequencing,

Description of a novel Class II aroA associated with transposable elements in Klebsiella spp.

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Glyphosate, the most used herbicide in the world, prevents plant and bacterial growth by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This study aimed to investigate the molecular mechanisms of glyphosate tolerance in nine Klebsiella sp. strains isolated from human fecal samples from Thailand, which display high minimum inhibitory concentrations (MIC) values (16-32 g/L) by whole genome sequencing. DNAs were extracted using EZ1 and then sequenced using both Illumina MiSeq and Oxford Nanopore MinIon technologies. EPSPS protein sequences were extracted and classified according to the species degree of tolerance to glyphosate, using existing classifications. All Klebsiella sp. strains analyzed had a class I EPSPS enzyme. Among these strains, four carried an additional EPSPS enzyme, classified as a class II enzyme. This novel aroA gene was identified to be carried by an IncFIIK conjugative plasmid on a composite transposon, which also harbored a qacA-like gene encoding resistance to antiseptics. Interestingly, a BLAST search on NCBI revealed only 73 hits with 100% coverage and identity to our novel sequence, found in strains of different genera (Klebsiella, Enterobacter, Escherichia, Pseudomonas, and Achromobacter). These strains were isolated from various types of samples, mostly from clinical human samples, and predominantly originated from Asian countries. The comparison of the genetic environment of the class II aroA gene between strains isolated in our study and those found in NCBI shows that this gene is located on a similar transposon carried by different types of IncF plasmids. Additionally, it was also found in the chromosome of Achromobacter sp. and Enterobacter hormaechei strains. Since our Klebsiella sp. strains and most of the strains found carrying this gene are of Asian origin, and as class II EPSPS sequences were introduced into plants to make them resistant to glyphosate, we collected food samples from various origins, emphasizing Asian origins, and looked for the presence of this undescribed gene. Only one Klebsiella pneumoniae, with glyphosate MIC of 64 g/L, carrying the same class II EPSPS was isolated from the Boesenbergia pandurate plant from Thailand. The class II aroA was located on the same transposon found in our study in an IncFIB plasmid. Overall, our study highlighted the vertical and horizontal transfer of a novel class II aroA in association with a qacA-like gene among various Enterobacteriaceae spp. in clinical and environmental samples. Moreover, various questions arise about the selection of this gene, which may be attributed to glyphosate or other agents such as antiseptics.

Keywords: Glyphosate-MIC, glyphosate, EPSPS, aroA, Thailand.

A species-independent lateral flow test to detect Rift Valley fever virus antibodies in serum

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Objective: Several arboviruses, viruses that spread by using arthropod vectors, pose an increasing risk for human and animal health and outbreaks have major consequences for the individual farmer, local communities, and local and national governments. Currently PCR and ELISA are diagnostic methods of choice, however pen-side tests would help the farmer and veterinarians to confirm or exclude presence of (arbo)virus pathogens at short notice. Therefore, the aim was to develop a capillary flow based immunodiagnostic assay (lateral flow test; LFT) able to detect bunyavirus specific antibodies, using Rift Valley fever virus (RVFV) as a model pathogen. Furthermore, by applying a double-antigen format the test would be both immunoglobulin class- and species-independent.
Approach: Antigen involved in viral RNA encapsidation, the so called nucleocapsid protein (N), was produced in E.coli and the protein was purified using affinity chromatography. For the lateral flow assay, the antigen was coupled to carbon nanoparticles and also sprayed onto nitrocellulose membrane; a double-antigen approach. This assay was subsequently tested with sheep, calf, goat and human immune sera (150 in total), and compared with the commercially available competitive RVFV assay from IDvet (ID Screen® Rift Valley Fever Competition Multi-species). A species-independent double-antigen N-ELISA to detect antibodies against RVFV was developed as well and was compared to both assays using the same set of sera.
Results and conclusions: Both the LFT and the ELISA showed a 100% correlation with the commercially available assay from IDvet. Importantly, in this setup no background signals were observed. This lateral flow test format can also be used to detect specific antibodies against other pathogenic viruses. In addition, the assay qualifies as a platform to develop a DIVA test differentiating infected from vaccinated animals.

Keywords: Lateral flow test, Rift valley fever virus, Antibodies, Species independent

Temporal and spatial variability of bat alphacoronaviruses in Common Pipistrelle Bats (Pipistrellus pipistrellus) in Utrecht, the Netherlands

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Background: A large variety of both Alpha- and Betacoronaviruses can be detected in bats. Detected coronavirus RNA prevalence is usually high: detection rates of 20% are common. Little is known about factors driving variation in bat coronavirus prevalence. Viral prevalence is likely influenced by the yearly cycle of bat contact structure changes, including seasonal factors, such as the presence of immunologically naïve pups. Common Pipistrelle Bats, an abundant bat species in the Netherlands, display three large changes in contact structure annually: (i) formation of maternity colonies of 50-100 bats (pre-parturition), (ii) birth of pups (post-parturition) and (iii) formation of a hibernaculum of over 1,000 bats (pre-hibernation). We hypothesized that the presence of immunologically naïve pups in the post-parturition period increases virus prevalence.
Methods: We determined virus prevalence for three maternity colonies and for their mass hibernaculum, in Utrecht, The Netherlands. Virus prevalence was based on coronavirus RNA detection in faeces via pan-coronavirus PCR. We tested our hypothesis using a Generalized Linear Mixed Model. Detected coronaviruses were further investigated by sequencing both a conserved (440 nt Rdrp) and a variable genomic region (860 nt S1).
Results: When all faecal samples were combined, alphacoronavirus prevalence was indeed significantly higher post-parturition (29.3%, 95% CLs: 24.1-35.2%; n=256) than pre-parturition (13.8%, 95% CLs: 10.7-17.7%; n=377) and in the hibernaculum (14.6%, 95% CLs: 11.6-18.3%; n=425; GLMM = 0.00696, $p<0.05$). However, viral prevalence did not increase consistently in each colony. Variants were generally more homologous within maternity colonies than between them. Moreover, the occurrence of recombination was detected.
Conclusions: An overall increase in alphacoronavirus prevalence was detected in the post-parturition period. However, as the colonies exhibit different changes in prevalence over the parturition periods, further research is needed to understand both the movement ecology of Common Pipistrelle Bats and the dynamics of alphacoronaviruses in these maternity colonies.

Keywords: bats, coronaviruses, viral epidemiology, spatial dynamics

Naïve camelid single-domain antibody phage display library: A toolbox for identifying functional antibodies for varied applications

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Single-domain antibodies (sdAb) are defined as single VH domains capable of antigen binding, while VHHs or nanobodies are natural sdAbs derived from camelid heavy chain-only antibodies (HCAbs). VHHs retain full antigen binding capacity, and are advantageous as compared to conventional antibodies due to their unique properties such as small size, excellent solubility, superior stability, quick clearance from blood and deep tissue penetration. These distinctive characteristics make them useful tools in a diverse array of applications such as biosensing, affinity-capture, protein crystallization, molecular imaging, diagnosis and therapeutics. Immunization and library generation for each target is an expensive and time-consuming process. Furthermore, there is growing societal concern about the use of animals for such purposes. Therefore, we have generated a naïve VHH phage display library from the blood sample of 47 animals (23 llamas, 11 camels and 13 alpacas) using the golden gate cloning approach. The library size is 4 × 109 independent clones with a high percentage (92) of VHH insertion. The library was validated by screening a wide range of targets (from plant to animal antigens) for target specific VHHs, and the diversity was confirmed by sequencing of the clones. Diverse sets of clones were obtained for different targets as ascertained by generation of the sequence logo plots. We could retrieve binders for most of the recombinant proteins or virus-like particles, or inactivated viruses. However, we did not obtain suitable VHHs against complex molecules such as lipopolysaccharides or crude antigen preparations. Out of the binders for 7 antigens, clones identified for 2 targets had affinities in the nanomolar range as assessed by biolayer interferometry measurement. This approach allows the rapid identification of binders for any potential antigen, including pathogens (bacteria or virus) or toxins, and complements the approach of obtaining the binders from the libraries generated after llama immunization. Furthermore, it allows rapid VHH isolation in case of a novel pandemic.

Keywords: VHH, Phage display, Naive library, antibodies, affinity

European human granulocytic anaplasmosis is caused by a genetic variant of Anaplasma phagocytophilum ecotype I

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Ixodes ricinus, widely distributed across Europe, serves as a carrier for numerous pathogens with implications for both medical and veterinary significance. Among these, Anaplasma phagocytophilum is of particular concern, as it can lead to human granulocytic anaplasmosis (HGA). In Europe, A. phagocytophilum ecotype I, known as a host generalist, is considered zoonotic and transmitted through bites of I. ricinus. Despite its ubiquitous presence in ticks and wildlife, the number of clinical HGA cases remain rare in Europe. Here, we isolated and cultivated A. phagocytophilum from a Slovenian HGA patient, and sequenced its complete genome. In addition, we sequenced a fragment of the groEL gene from the blood of HGA patients from France (n=5) and Poland (n=20). Genetic comparison of whole genomes (n=13), multilocus sequence typing (n=420), and a single genetic locus (groEL, n=817) revealed that the pathogenic variant forms a small, but distinct cluster within A. phagocytophilum ecotype I, which probably circulates among Ixodes ticks and carnivores. Our findings indicate that the most abundant A. phagocytophilum ecotype I variants, which are found in deer and livestock, are probably not zoonotic. These findings provide a straightforward explanation for the low abundance of HGA cases in Europe. We propose that the geographic distribution and the enzootic cycle of the pathogenic A. phagocytophilum ecotype I variant in Europe should be investigated further to assess the public health risk of HGA in Europe.

Keywords: Anaplasma phagocytophilum, whole genome sequence, HGA, groEL, MLST, Ixodes ricinus

Assessing Surveillance of Wildlife Diseases by Determining Mammal Species Vulnerability to Climate Change

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Climate change is one of the drivers of wildlife-borne disease emergence, as it can affect species abundance and fitness, host immunocompetence, and interactions with pathogens. To detect emerging wildlife-borne diseases, countries may implement general wildlife-disease surveillance systems. Such surveillance exists in the Netherlands. However, it is unclear how well it covers host species vulnerable to climate change and consequently disease emergence in these species. Therefore, we performed a trait-based vulnerability assessment (TVA) to quantify species vulnerability to climate change for 59 Dutch terrestrial mammals. Species' vulnerability was estimated based on the magnitude of climatic change within the species' distribution (exposure), the species' potential to persist in situ (sensitivity), and the species' ability to adjust (adaptive capacity). Using these vulnerability categories, we identified priority species at risk for disease emergence due to climate change. Subsequently, we assessed the frequency of occurrence of these priority species compared to other mammal species examined in general wildlife disease surveillance during 2008–2022. We identified 25% of the mammal species to be highly exposed, 24% to be highly sensitive, and 22% to have a low adaptive capacity. The whiskered myotis and the garden dormouse were highly vulnerable (i.e., highly exposed, highly sensitive, and low adaptive capacity), but they are rare in the Netherlands. The Western barbastelle, the pond bat, and the Daubenton's myotis were potential adapters (highly exposed, highly sensitive, and high adaptive capacity). Species vulnerable to climate change were relatively poorly represented in current general surveillance. Our research shows a comprehensive approach that considers both exposures to climate change and ecological factors to assess vulnerability. TVAs, as presented in this study, can easily be adapted to include extra drivers and species, and we would therefore recommend surveillance institutes to consider integrating these types of assessments for evaluating and improving surveillance for wildlife-borne disease emergence.

Keywords: life-history traits, mammals, surveillance, wildlife health, pathogens, zoonoses

Poster abstracts: From One Health signaling, surveillance to response

Towards a common database to improve wildlife disease surveillance in Europe

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Over 70% of the zoonotic emerging infectious originate from wildlife. Wild animals are also present in urban areas, that are unique ecosystems that provide ample opportunity for zoonotic pathogen transmission between wildlife, domestic animals and humans. Urban areas can therefore form hotspots of increased zoonotic disease risk. The different disease dynamics in urban areas ask for an improvement of wildlife disease surveillance. To facilitate wildlife disease surveillance in urban areas, we first need to have a comprehensive overview of zoonotic pathogens studied in urban wildlife species to determine how to set-up this surveillance and to help prioritize surveillance efforts.

We therefore created a database, based on a systematic literature review in Embase focused on zoonotic pathogens in ten common urban wildlife mammals in Europe, namely brown rats, house mice, wood mice, common voles, red squirrels, European rabbits, European hedgehogs, European moles, stone martens, and red foxes. Search terms included keywords related to (1) the Latin species name and any equivalent English names, AND (2) terms for ‘zoonosis’, and also (1) AND an elaborate list of important zoonotic pathogens created by the Dutch government. We included all articles published before 01-01-2023. Taken all together, we screened 7,403 articles of which 1,047 articles were included. The data of the articles that were included in the database was amongst others: pathogen species, tissue(s) sampled, laboratory methods used, mammal species studied, total number of animals tested, number of positive tested animals, country, and trapping location type.

In total, 186 zoonotic pathogen species were described, including 88 bacteria, 42 helminths, 19 protozoa, 22 viruses, and 15 fungi. Most of these pathogens were only studied in one single animal species. Taking the Netherlands as an example, we show how this database can be used by other countries to identify wildlife disease surveillance gaps on a national level. In the Netherlands, only four percent of all potential host-pathogen combinations have been studied.

In conclusion, this database comprises a comprehensive overview that can guide future research on wildlife-borne zoonotic diseases both on a European and national scale. Even taking into account that some pathogens are relatively species-specific, many European countries have no (accessible) data on zoonotic pathogens in these relevant animal species. To be better prepared for emerging wildlife-borne zoonoses, we need to strengthen wildlife disease surveillance across Europe. Sharing and expanding this database provides a solid starting point for future European-wide collaborations to improve wildlife disease surveillance.

Keywords: Wildlife, surveillance, review, database, urban

24-h diel cattle- and sheep-seeking behavior of mosquitoes in the Netherlands: input for arbovirus transmission modelling

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Background: Mosquito feeding behavior is critical to our understanding of how zoonotic vector-borne diseases are transmitted amongst livestock and humans. As such, vector-host density and biting-rate are important parameters in arbovirus transmission modelling. Parameter estimates are mostly extrapolated from indirect measurements in the field using traps like the Mosquito Magnet (MM). Knowledge concerning real interaction between mosquitoes and livestock remains poorly documented.
Aim: To assess hourly mosquito-activity on cattle and sheep in the Netherlands over the 24-hour diel in the mosquito-active period using direct aspiration. Furthermore, we wanted to evaluate mosquito data collected with the MM by correlating them with data collected by aspiration from cattle and sheep at the same time.
Methods: Mosquitoes were collected with mechanical aspiration from a cow and sheep in pasture during 60 observation days in the period May-October. Mosquitoes were aspirated for 5 min per whole hour of each host at the same time by a collector. MM were operated for one hour per whole hours at the same time as aspiration of mosquitoes was done from hosts.
Results: Daily median number of collected mosquitoes using the MM was approximately 40 and 100 times smaller than the number collected from sheep and cows, respectively. In total 40.5% and 13.5% of mosquitoes collected from the cow and sheep were blood fed, respectively. Only 1.7% of the mosquitoes collected by the MM were blood fed. For cows and sheep most of the mosquito-activity was around sunset (SS). Peak collections were observed within the first 30 min following SS, with peak collections at 15 min post SS. On the contrary, the MM collections (one hour sampling time) provided a different overview on mosquito activity, with higher number of mosquitoes collected between 2 and 5 hours post SS. The overall number of mosquitoes collected between 1 hours before and after SS with the MM were much lower than the collections made from the cow or the sheep. Exploration of the effect of temperature, humidity and light intensity on the numbers of mosquitoes collected, indicates apparent higher number of mosquitoes collected when temperature ranged between 15 and 20 °C, humidity 40-60% and low light intensity.
Conclusions: Important estimates of mosquito feeding characteristics were obtained, indicating considerable differences between livestock hosts. MM-collected mosquito data should not be used in arbovirus transmission modelling, because of considerable bias.

Keywords: mosquito, ruminants, biting rate, Mosquito Magnet, 24-h diel

Evaluation of One Health integrated WGS-based surveillance of foodborne diseases

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Aim Near real-time integrated monitoring of whole genome sequences (WGS) of Listeria monocytogenes, Salmonella and Shiga toxin-producing Escherichia coli (STEC) from food and human patients has been implemented in the Netherlands since 2019. We here evaluate the effectiveness of WGS-based One Health surveillance with regard to source-finding in food-related outbreak investigation and preventing further illness.

Approach Clinical isolates are obtained by the National Institute for Public Health and the Environment (RIVM) from microbiological laboratories for national infectious disease surveillance purposes. Commissioned by The Netherlands Food and Consumer Products Safety Authority (NVWA), food isolates are obtained by Wageningen Food Safety Research (WFSR) within the framework of monitoring programs. Assessment of the core genome multilocus sequence typing (cgMLST) is done using Ridom SeqSphere+.

Results Identifying joint patient-food clusters and their subsequent investigation has led to finding the source of the contaminated food products in at least nine clusters of L. monocytogenes with a minimum of five human cases per cluster. Control measures taken during an investigation always led to a reduction in cases with that particular strain, in most cases even leading to elimination of that strain from subsequent surveillance data. Within the Salmonella surveillance, the shared database did not lead to direct source finding, but was in several occasions informative for the possible food source. Moreover, matches between human and non-human STEC isolates are rare and detected clusters contain up to a maximum of only four human cases.

Conclusions The integration of WGS data from clinical and food isolates clearly enabled the identification of (direct and indirect) links between food and cases of listeriosis and salmonellosis. Although such a link gives a clear indication of the source of the outbreak, it is important to validate this link via subsequent investigation at food producers and epidemiological data confirming actual consumption of the implicated products by human cases. Once transmission of a specific strain from a food product or production facility to consumers is sufficiently established, the NVWA can act based on the statement in the General Food Law that food should be safe for consumption. Differences in the added value of One Health surveillance between the three pathogens could be due to persistence or reoccurrence of strains versus new introductions, type of reservoir, sampling schemes, under-recognized sources, multiple-strain contamination of one food product or location, ease of laboratory detection, and availability of isolates for WGS typing.

Keywords: One Health surveillance systems , Whole Genome Sequencing, collaborations between sectors

Learning from the Past: Cholera Epidemics and Public Health Responses during the 19th Century

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The past century has witnessed unprecedented progress against communicable diseases with the development of antibiotics, effective vaccines, efficient surveillance systems and the overall improvement of health infrastructures. However, unfettered globalization and unpredictable agrifood systems pose a constant threat to the health of nations due to the emergence of new infectious diseases. How can societies prepare to avoid or mitigate the impact of sudden epidemics? Which public health responses should be prioritized and on which basis?

This research proposes learning from the past experience of Europe with cholera, a deadly disease that caused a large number of epidemics in the continent during the 19th century. It uses archival sources to track the spatial and temporal spread of all epidemics occurring in (historical) Germany during the 19th century for 900 locations ranging from small communities to large urban centers, as well as their respective rural hinterlands. Then, I use these data to examine how (and whether) communities mitigated the demographic impact of cholera. I consider different policy responses (e.g. quarantines) as well as information about the local hydro-geological environment and various public health infrastructures, such as waterworks, sewerage systems and hospitals.

The preliminary results show that early public health responses were, at best, mixed. On the one hand, the extremely stringent measures implemented to isolate goods and individuals and disinfect them created unrest early on. Also, the regulation of the day-to-day life to promote social distance triggered protests, which in turn made public health policies less effective as individuals did not comply with them. Clearly, the disconnection between policy makers and the working classes created an unproductive backlash. On the other hand, an effective disease registration and surveillance system allowed localities to have an updated and accurate record of the state of the outbreak. This, in turn, allow for the implementation of isolation measures to curb the spread of the epidemic. In addition, access to sanitary and health care infrastructures contributed to mitigate the impact of the epidemics (although not always).

Ultimately, cholera shook the economic, social and demographic foundation of the German society. An overreliance on mobility and trade restrictions led to tensions between local elites and the population. Lack of communication with the population and uncertainty about this disease worsened the impact of the epidemics, which would ultimately be controlled with the cooperation of all classes.

Keywords: Pandemic prevention and preparedness, Past Lessons, Prioritisation methods, Epidemics, Cholera

Long term monitoring of vectors:

National Action Plan for the strengthening of the Zoonotic Disease Policy in the Netherlands

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In 2022, the Dutch Action Plan for strengthening the zoonotic disease policy was initiated. One of the actions was for the Centre for Monitoring of Vectors (CMV), a part of the Netherlands Food and Consumer Product Safety Authority (NVWA), together with Centre for Zoonoses and Environmental Microbiology (Z&O) of the National Institute for Public Health and the Environment (RIVM), to set up a Knowledge Platform for Vectors and vector-borne Infectious diseases. The Platform (KPVI) aims to prevent the emergence and spread of (zoonotic) diseases by monitoring vectors and detecting pathogens early and responding swiftly to outbreaks. Vector related knowledge relevant for developing guidelines to policy makers is generated with partners and shared via the Knowledge Platform with interested parties such as provinces, municipalities and citizens.

Most projects related to monitoring mosquitoes, midges, or ticks typically span the duration of a funded PhD or post-doctoral program. The advantage of a centralised governmental entity conducting vector monitoring is the ability to build upon a valuable longitudinal database. Since its establishment in 2009, the CMV has expanded its monitoring capacity and amassed a growing database. This data can be utilised for assessing the impact on vector populations on environmental changes, such as climate change, climate adaptation measures, and nature restoration in order to be better prepared for emerging zoonotic outbreaks. In addition, vector monitoring is valuable for the evaluation of control measures against vectors.

To further strengthen the Dutch zoonosis preparedness, the CMV plans to increase its monitoring activities and explore innovative methods for analysing and automating incoming data streams. We are looking to cooperate with stakeholders who have shared interests in monitoring environmental changes and their impact on biodiversity, including vector populations and their relation to zoonoses. This may include parties involved in climate adaptation initiatives, such as urban greening, the national programme for rural areas, or developments in the animal industry. Interested stakeholders can range from e.g. municipalities, waterboards, animal husbandry organisations to research institutes and universities. Collaboratively, we aim to establish long-term monitoring programs and facilitate data and knowledge sharing between stakeholders via the Knowledge Platform.

Keywords: vectors, monitoring, knowledge platform, zoonoses, surveillance

Towards a Dutch One-Health data sharing platform

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Within UNITED4Surveillance (U4S) and the Wageningen Common Data Solutions (WCDS) projects, we aim to develop a One-Health (OH) data sharing platform. U4S is a large European project with over 40 partners within 24 EU countries of that is led by the RIVM. The main goal is to improve the OH-Surveillance system in each individual country, during and after which there is an exchange in lessons learned between all parties. Within this project the focus will be on the improvement of genomic data sharing between the RIVM and WBVR. In addition, the WCDS project focuses on genomic data sharing between WFSR and WBVR. Together these projects will create a OH Surveillance system, as we combine our veterinary genomic data (WBVR) with genomes from patients (RIVM) and from the food sector (WFRS).

To get a good overview of the current OH-Surveillance system and the other possible participants a stakeholder analysis and system mapping were done. In addition, a pilot will be set up in which we link the genomic databases of foodborne pathogens (Campylobacter and Salmonella) of RIVM, WFSR, and WBVR. As all three institutes are in the process of adopting an iRODS based data storage system. We set out to create one server to host an iRODS instance for the OH-Surveillance database that can be accessed by all parties. Moreover, we will streamline our protocols to work on shared data, share selections of metadata, and get similar assembly standards, this will enhance the (machine) interoperability of our data, making in more FAIR. To accomplish this, regular contact moments of different expert groups, i.e., bioinformaticians, microbiologists, and epidemiologists, or all together are organized.

The stakeholder analysis and system mapping have revealed a broad network of interested parties. Furthermore, it has identified additional parties that we could link to the database at a later stage. However, we currently have the core established by linking these three institutes. The involved parties are very motivated to create this shared database, but there are challenges concerning the sharing of metadata. Furthermore, the sharing platforms, including the iRODS based, are rapidly developing. This calls for a dynamic process that remains open to new solutions, aiming to find one that will be beneficial and usable for all parties involved over an extended period.

Keywords: One-Health, Surveillance, Genomic data, Data-sharing, foodbourne pathogens

Exploring Life History Strategies: Unravelling the Interplay of Immune Defence, Signalling Dynamics, and Reproductive Output in Male Black Grouse

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Investing in immune defences is crucial for animals but excessive investments may waste limited resources, affecting other physiological, morphological, and behavioural functions and thus potentially threatening the animals' survival and reproduction. Accordingly, there are resource trade-offs between immune defences and other functions. The honest signalling hypothesis predicts that certain ornamental and behavioural signal traits reflect the quality of the host, such as its immune defences and reproductive potential, and therefore variables related to signalling, immune defences and reproduction should co-vary. Integrating these hypotheses is crucial for unveiling how species' life-history strategies evolve to balance self-maintenance and reproduction under various environmental conditions.

Male black grouse (Tetrao tetrix), as lekking species, undergo intense intrasexual competition for leks, where only a few lek holders have the chance to attract females for successful reproduction, providing a good animal model to investigate how birds strategically allocate resources between signalling traits, immune defences, and reproduction.

In our study, we investigated whether certain signals, such as darker feathers, larger size and more frequent vocalizations, were correlated with better individual quality, which is manifested as higher immune defences and greater reproductive success in male black grouse, in line with the predictions of the honest signalling theory. Conversely, if the resource trade-offs are pronounced, a larger investment in these signalling traits might lead to lower immune defences and reproductive output. In addition, we tested how a Sindbis virus (SINV) infection affected these relationships between signalling traits, immune defences, and reproduction.

Our results indicated indeed that a larger investment in certain ornamental and behavioural signalling traits was positively correlated with higher immune defences and better reproductive outcomes, even though the relationship between immune defences and reproduction output was lacking. SINV infection was not correlated with immune defences and did not affect reproduction output. These results underscore that some signalling traits reflect individual male black grouse quality, which is in line with the honest signalling hypothesis rather than the resource trades-off hypothesis.

Our study contributes to a deeper understanding of how avians develop different life history strategies to balance self-maintenance and reproduction in certain environmental conditions.

Keywords: honest signalling hypothesis, ecological immunology, Sindbis virus, black grouse

Results and lessons from ten years One Health livestock surveillance in the Netherlands (2013-2023)

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Objectives

Livestock can be a reservoir of various zoonotic pathogens and thus plays an important role in their dissemination. Examples are Salmonella, Campylobacter and Shiga-toxin producing E. coli. Humans can become infected by these pathogens through direct contact with livestock, contact with a contaminated environment and consumption or handling of contaminated food. In addition to zoonoses, antimicrobial resistance (AMR) is another important public health threat for which livestock plays a role in transmission. Surveillance of zoonotic pathogens and AMR in livestock is vital to obtain information about prevalence and characteristics of these pathogens. In addition, EU member states are obligated to collect and report data on zoonotic agents to the European Food Safety Authority. In the Netherlands, it was decided to report annual prevalence data based on active surveillance in one specific livestock sector per year. The objectives of the surveillance program are to monitor trends in the prevalence and types of zoonotic pathogens in livestock and people in contact with livestock, to analyze risk factors and to provide input data for risk assessment.

Approach

Since 2013 this program has been carried out as a collaboration between RIVM, NVWA and WFSR. Up to 2023, eight livestock sectors were investigated. In addition to sampling of the animals, samples were also collected from livestock farmers, family members and employees. Pathogens were selected based on importance for public health and relevance in the specific livestock sector. Campylobacter, ESBL-producing E. coli, Salmonella and STEC have been analysed in all or most of the investigated livestock sectors.

Results

The highest Campylobacter prevalence was found in cattle (dairy, beef and veal calves) with farm level prevalence of 85-95%. ESBL-producing E. coli was most prevalent in poultry. The prevalence of ESBL-producing E. coli was equal in participating humans and the general Dutch population. The prevalence of Salmonella was highest in finisher pigs while low in ruminant sectors, with the exception of veal calves. STEC prevalence was highest in small ruminants. In asymptomatic human participants from dairy goat and sheep farms, STEC carriage was high (5-10%) and on four farms human and animal isolates matched in serotype and stx genes.

Conclusions

This surveillance program has shown the variations in prevalence of multiple zoonotic bacterial species as well as antibiotic resistant E. coli among different livestock sectors. The program has provided important baseline information for, among others, source attribution and risk assessment.

Keywords: surveillance, livestock, zoonoses, antimicrobial resistance

The impact of the living environment on infectious diseases: a scoping review

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Objectives or Aim
To identify associations between the living environment and health, specifically infectious diseases by updating a previously conducted scoping review.
Approach or Method
A scoping review was conducted to identify studies that examine associations between the living environment and infectious diseases. The WING database was used. This database will be used as a starting point for the new literature search as it includes all relevant literature on the topics of living environment and health from 2015 – 2021 found in EMBASE, PUBMED, SCOPUS or PSYCHINFO.
The same search query will be used and focus on studies published from 2021 to 2024. Furthermore, the same inclusion criteria will be used. Studies must refer to infectious diseases and well-being; make explicit reference to a relation between human health and the living environment; be relevant for or able to be translated to the Dutch context. Studies were excluded if they focus on; indoor housing environment/quality; animal studies; individual social environment (e.g. social support or peer-pressure), unless there was a specific link to the living environment; systematic or scoping reviews; only symptoms without the clear presence of an infectious agent. Labels that were used regarding living environment were air quality, green space, blue space, livestock dense areas, population density and urbanization, climate, and land-use change. Infectious disease were labelled as and viral, parasitic or bacterial diseases.
Results
The previous scoping review (e.g. from the WING database) showed 94 articles that addressed infectious diseases in relation to the living environment. Most studies were conducted in the United States, China, the Netherlands and Italy. Two types of labels were used to classify the articles: environmental and health labels. The most used labels were green space, urbanization/urban densification, air quality and blue spaces. There seemed to be evidence for associations between the living environment and infectious diseases.
Conclusions/discussion
Results from the previous scoping review have not (yet) been published. However, we aim to reproduce this study and update the results including more recent studies. We would love to hear your thoughts, advice and/or remarks regarding themes, search query or labelling that should also be included, search query or databases that should be used in addition.

Keywords: Living environment, infectious disease, One Health